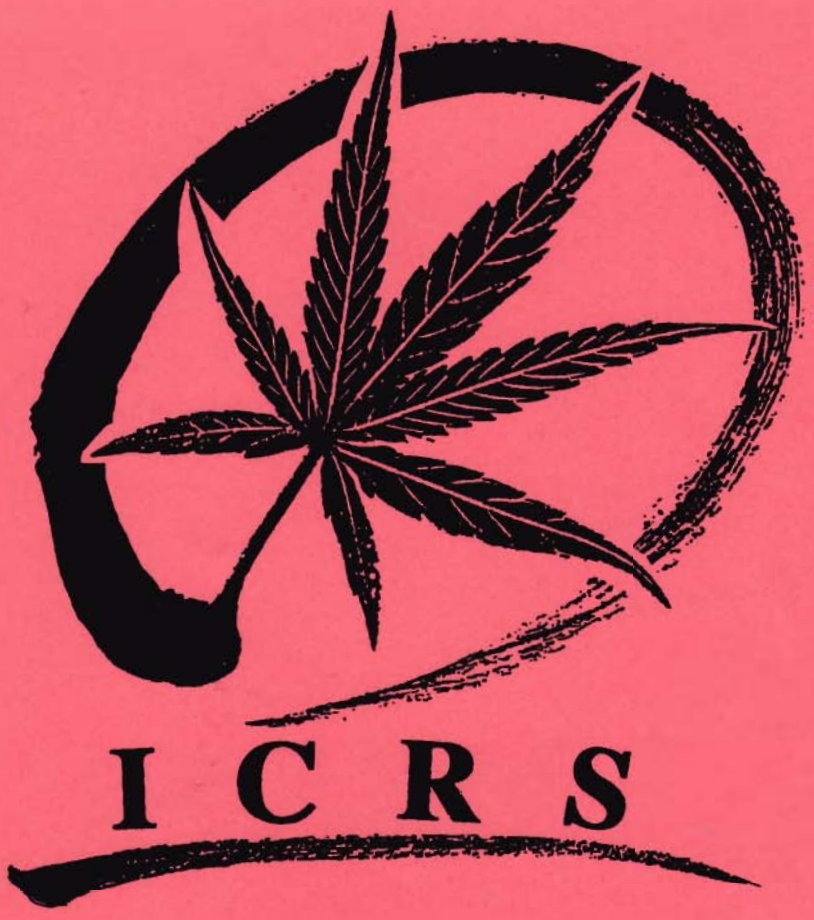
Allyn Howlett



15TH ANNUAL SYMPOSIUM ON THE CANNABINOIDS

Hilton Clearwater Beach Hotel
Clearwater Beach, Florida, USA

JUNE 24 – 27 2005

Program and Abstracts

Department of Psychology
University of Vermont
Burlington VT 05405 USA

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2005 SYMPOSIUM OVERVIEW

Friday June 24th	Saturday June 25th	Sunday June 26th	Monday June 27th
0820 – Welcome			
0830 – 0945 Structure Activity Relationship Studies and New Pharmacological Tools (5 Orals)	**0800 – 0915** Nervous Functions and Animal Models of Neuropsychiatric Disorders (5 Orals)	**0800 – 0915** Human and Clinical Studies (5 Orals)	**0800 – 0915** Reward and Substances of Abuse (5 Orals)
	0915 – 0945 – Coffee and Continental Breakfast	**0915 – 0945 – Coffee and Continental Breakfast**	**0915 – 0945 – Coffee and Continental Breakfast**
0945 – 1015 – Coffee and Continental Breakfast	**0945 – 1015** Nervous Functions and Animal Models of Neuropsychiatric Disorders continued (2 Orals)	**0945 – 1100** Human and Clinical Studies continued (5 Orals)	**0945 – 1030** The Endocannabinoid System in the Eye (3 Orals)
1015 – 1230 Endocannabinoids, Biosynthesis and Metabolism (9 Orals)	**1015 – 1100** The Endocannabinoid System in Non-Mammalian Organisms (3 Orals)		
	1100 – 1200 Cannabinoids and Pain (4 Orals)	**1100 – 1200** Cardiovascular and Gastrointestinal Functions (4 Orals)	**1030 – 1200** Neuroprotection and Neurodegenerative Disorders (6 Orals)
1230 – 1400 – Lunch /	**1200 – 1400 – Lunch /**	**1200 – 1400 – Lunch /**	**1200 – 1330 – Lunch /**

Free Time	Free Time	Free Time	Free Time
		1315 – 1400 **NIDA Talk for Students**	
1400 – 1545 Cannabinoid Receptor Structure, Regulation and Signal Transduction (7 Orals)	**1400 – 1545** **Poster Session / Coffee Break**	**1400 – 1545** **Poster Session / Coffee Break**	**1330 – 1430** **Business Meeting**
		1545 – 1715 Advanced Therapeutic Applications: Emesis, Obesity, and Smoking Cessation (6 Orals)	**1430 – 1530** Cancer (4 Orals)
1545 – 1815 **Coffee Break / Poster Session**	**1545 – 1715** Cannabinoids in the Immune System (6 Orals)		**1530 – 1815** **Coffee Break / Poster Session**
1745 – 1815 **Controversial Issues Session #1**		**1715 – 1800** Control of Reproduction (3 Orals)	**1745 – 1815** **Controversial Issues Session #2**
	1730 – 1830 **Kang Tsou Memorial Lecture**		
		1800 – 1830 **Young Investigator Award Ceremony and Talk**	
2015 – Dinner	**2015 – Beach Banquet / Dinner**	**2015 – Dinner**	**2000 – Award Banquet**

POSTER SESSIONS

FRIDAY 1545 – 1815	SATURDAY 1400 – 1545	SUNDAY 1400 – 1545	MONDAY 1530 – 1815
Structure Activity Relationship Studies and New Pharmacological Tools (7 Posters)	Endocannabinoids, Biosynthesis and Metabolism (10 Posters)	Cannabinoid and Pain (6 Posters)	Human and Clinical Studies 1 and 2 (8 Posters)
Cannabinoid Receptor Structure, Regulation and Signal Transduction (17 Posters)	Nervous Functions and Animal Models of Neuropsychiatric Disorders (12 Posters)	Cannabinoids in the Immune System (10 Posters)	Control of Reproduction (4 Posters)
Vannilloid-Cannabinoid Interactions (5 Posters)	The Endocannabinoid System in Non-Mammalian Organisms (2 Posters)	Cardiovascular and Gastrointestinal Functions (8 Posters)	Reward and Substance of Abuse (8 Posters)
			The Endocannabinoid System in the Eye (2 Posters)
			Neuroprotection and Neurodegenerative Disorders (7 Posters)
			Cancer (4 Posters)

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2005 Symposium on the Cannabinoids

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Hilton Clearwater Beach Resort
Clearwater Beach, Florida

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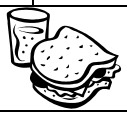
15th Annual Symposium On The Cannabinoids

2005 Program

Registration June 23, 2005 (1600-2000)

Day 1
Friday, June 24th

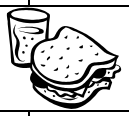
0820-0830	***OPENING REMARKS***		
Structure Activity Relationship Studies and New Pharmacological Tools ➢*Chairs: John Huffman / Ruth Ross*			
0830	Huffman, J.W., Szklennik, P.V., Almond, A., Bushell, K., Hurst, D.P., Reggio, P.H., Selly, D.E., Cassidy, M.P., He, H., Wiley, J.L., and Martin, B.R.	3-Phenylacetylindoles, a New Class of Cannabimimetic Indoles	1
0845	Pertwee, R.G., Stevenson, L.A., Ross, R.A., Price, M.R., Wease, K.N., and Thomas, A.	Tetrahydrocannabivarin is Markedly more Potent as an Antagonist of R-(+)-WIN55,212 and Anandamide Than of Δ^9-Tetrahydrocannabinol in the Mouse Isolated Vas Deferens	2
0900	Lunn, C.A., Lavey, B.J., Kozlowski, J.A., Hipkin, R.W., Lundell, D.J., Fine, J., and Bober, L.	Triaryl Bis-Sulfones as a New Class of Cannabinoid CB_2 Receptor Inhibitors: Identification of a Lead and Initial Biological Characterization	3
0915	Martin, B.R., Wiley, J.L., Beletskaya, I., Sim-Selley, L.J., Dewey, W.L., Razdan, R.K.	Pharmacological Characterization of Water-Soluble Cannabinoids in Mice	4
0930	Muccioli, G.G., Wouter, J., Poupaert, J.H., and Lambert, D.M.	2-Thioxoimidazoilidinones and Imidazolidinediones Derivatives: Inhibition of FAAH and Binding to CB_1 Cannabinoid Receptor	5
0945-1015	**Coffee and Continental Breakfast** 		
Endocannabinoids, Biosynthesis and Metabolism ➢*Chairs: Bela Szabo / Tiziana Bisogno*			
1015	Tsuboi, K., Sun, Y-X., Okamoto, Y., Araki, N., Tonai, T., and Ueda, N.	Molecular Cloning and Characterization of N-Acylethanolamine-Hydrolyzing Acid Amidase	6

1030	Morishita, J., Okamoto, Y., Tsuboi, K., Ueno, M., Sakamoto, H., Maekawa, N. and Ueda, N.	Expression Analysis of N-Acylphosphatidylethanolamine-Hydrolyzing Phospholipase D in Rat Brain Regions	7
1045	Thorpe, A.J., Cravatt, B.F., and Lichtman, A.H.	Mice Lacking Fatty Acid Amide Hydrolase Demonstrate Increased Cross-Tolerance to THC Following Suchronic Anandamide	8
1100	Felder, C.C., Moore, S.A., Chesterfield, A.K., Schober, D.A., Kidd, S.R., Nomikos, G.R., Ying, B-P., and Schaus, J.M.	Pharmacological Characterization of the Anandamide Transporter – The Missing Link in Endocannabinoid Neurotransmission	9
1115	Kaczocha, M., Hermann, A., Glaser, S.T., and Deutsch, D.G.	Anandamide Uptake and its Inhibition in Rat Basophilic Leukemia Cells	10
1130	Barker, E.L., Bardell, T.K., Yates, M.L., and McFarland, M.J.	Anandamide Uptake Via Endocytosis in Neuronal and Non-Neuronal Cell Lines	11
1145	Glaser, S.T., Gatley, S.J., and Gifford, A.N.	Imaging of the In Vivo Accumulation of [^{3}H] Anandamide and its Metabolites in the Mouse Brain	12
1200	Cascio, M.G., Ligresti, A., Pryce, G., Kulasegram, S., Beletskaya, I., De Petrocellis, L., Saha, B., Mahadevan, A., Bisogno, T., Baker, D., Martin, B.R., Razdan, R.K., and Di Marzo, V.	Novel Selective Inhibitors of Anandamide Re-Uptake with Anti-Spastic Activity in a Mouse Model of Multiple Sclerosis	13
1215	Szabo, B., Urbanski, M.J., and Di Marzo, V.	The Role of 2-Arachidonylglycerol in Retrograde Signalling	14
1230-1400	**Lunch / Free Time** 		
Cannabinoid Receptor Structure, Regulation and Signal Transduction ➢*Chairs: Tiziana Rubino / Chris Breivogel*			
1400	Rubino, T., Viganò, D., Castiglioni, C., Premoli, F., Zippel, R., and Parolaro, D.	Role of the RAS/ERK Pathway in the CB_1 Receptor Trafficking Following Chronic Cannabinoid Exposure	15
1415	Brown, A.J., Ueno, S., Suen, K., Dowell, S.J., and Wise, A.	Molecular Identification of GPR55 as a Third G Protein-Coupled Receptor Responsive to Cannabinoid Ligands	16
1430	Sim-Selley, L.J., Cassidy, M.P., Sylvester, J.L., He, H-J., Urban, N.H., Tao, Q., Martin, B.R., and Selley, D.E.	Agonist-Stimulated Signaling, Trafficking and Desensitization of CB_1 Receptors in a Cultured Cell Model	17
1445	Monory, K., Massa, F., Blaudzun, H., Marsicano, G., and Lutz, B.	The Role of Different Neuronal Populations in the Pharmacological Actions of Δ^9-Tetrahydrocannabinol	18
1500	Breivogel, C.	Beta-Arrestin2 Affects CB_1/G-Protein Coupling	19
1515	Shim, J-Y., Cowsik, S.M., Rani Grace, R.C., and Howlett, A.C.	Structural Determination of the Third Interacellular Loop and the 8th Helix of the CB_1 Cannabinoid Receptor	20
1530	Reggio, P.H., Nebane, N.M., Lynch, D.L., and Song, Z-H.	A CB_1 Lipid Face V6.43A/I6.46A Mutation Completely Separates the Binding Pockets of SR141716A and WIN55212-2 vs AEA, CP55940 and HU210: Implications for Ligand Entry into CB_1	21
1545-1815	**Coffee Break / Poster Session / Controversial Issues Session #1**		83-111

	Controversial Issues Session #1 -- "Is There a CB_3?" ➢*Chairs: Roger Pertwee / Patti Reggio*
2015	**Dinner** 

Day 2
Saturday, June 25th

Nervous Functions and Animal Models of Neuropsychiatric Disorders ➢*Chairs: Cecilia Hillard / Steve Varvel*			
0800	Hill, M.N., Carrier, E.J., Ho, W.-S.V., Meier, S., Hillard, C.J., and Gorzalka, B.B.	Pharmacologically Distinct Antidepressants Differentially Regulate the Endocannabinoid System	22
0815	Patel, S., Roelke, C.T., and Hillard, C.J.	The Anxiolytic Properties of Endocannabinoid Signaling: Implications for FAAH as a Therapeutic Target	23
0830	Musty, R.E., Gilbert, L., and Deyo, R.A.	Cannabichromene Reduces the Anxiogenic Effect of THC in Mice	24
0845	Abood, M.E., Kim, K., Moore, D.H., and Makriyannis, A.	AM1241 Slows Disease Progression in a Mouse Model of ALS	25
0900	Varvel, S.A., Wise, L.E., and Lichtman, A.H.	Facilitation of Extinction Learning Via FAAH Inhibition	26
0915-0945	**Coffee and Continental Breakfast** 		
0945	Lupica, C.R., Oz, M., and Hoffman, A.F.	Differential Effects of Repeated THC Exposure on Long- and Short-Term Synaptic Plasticity in the Hippocampus	27
1000	D'Souza, D.C., Braley, G., Perry, E., Mathalon, D., Ford, J., and Krystal, J.	Δ^9-THC Effects on Information Processing in Humans	28
The Endocannabinoid System in Non-Mammalian Organisms ➢*Chairs: Mauro Maccarrone / Ken Soderstrom*			
1015	Soderstrom, K., and Tian, Q.	CB_1 Receptor Activation Increases FOS Expression in a Subset of Brain Regions Important for Zebra Finch Vocal Learning and Production	29
1030	Johnson, F., and Soderstrom, K.	CB_1 Immunoreactivity in the Developing Songbird Telencephalon is Influenced by Food Availability	30
1045	Siafaka-Kapadai, A., Anagnostopoulos, D., Zafiriou, M.P., Farmaki, E., and Maccarrone, M.	The Endocannabinoid System in Unicellular Eukaryotes	31
Cannabinoids and Pain ➢*Chairs: Barbara Costa / John McPartland*			

1100	McPartland, J.M., Giuffrida, A., Scotter, J., and Musty, R.E.	Cannabimimetic Effects of Elevated Serum Anandamide Levels from Osteopathic Manipulation	32
1115	Costa, B., Colleoni, M., Trovato, A.E., Comelli, F., Franke, C., and Giognoni, G.	Anandamide Transport and Hydrolysis as Targets for the Treatment of Neuropathic Pain: Effects of AM404 and URB597 in Rats with Chronic Constriction Injury of the Sciatic Nerve	33
1130	Bisogno, T., Maione, S., Valenti, M., de Novellis, V., Palazzo, E., Petrosino, S., Rossi, F., and Di Marzo, V.	FAAH Inhibition Elevates Endocannabinoid Levels in the Periaqueductal Grey of the Rat: Dual Effect on Nociception via CB_1 and TRPV1 Receptors	34
1145	Stevens, D.L., Elmore, J.R., Sey, K., Williams, J., and Welch, S.P.	Enhancement of Morphine- and Tramadol-Induced Antinociception by THC in Diabetic Rodents	35
1200-1400	**Lunch / Free Time** 		
1400-1545	**Poster Session / Coffee Break**		112-135
Cannabinoids in the Immune System ➢*Chairs: Thomas Klein / Dana Selley*			
1545	Carrier, E.J., and Hillard, C.J.	Inhibition of Nucleoside Uptake in Microglia by the Plant-Derived Cannabinoids THC and CBD	36
1600	Kiertscher, S.M., Tashkin, D.P., and Roth, M.D.	THC Modulates the Ability of Human Dendritic Cells to Stimulate Primary and Recall T Cell Responses	37
1615	McHugh, D., and Ross, R.A.	Endocannabinoids and Phytocannabinoids Inhibit Human Neutrophil Migration	38
1630	Sugiura, T., Oka, S., Gokoh, M., Kishimoto, S., and Waku, K.	Physiological Roles of 2-Arachidonylglycerol as an Endogenous CB_2 Receptor Agonist	39
1645	Selley, D.E., Cassidy, M.P., Milstein, S., He, H-J., Sim-Selley, L., Spiegel, S., and Paugh, S.W.	Competitive Antagonism of CB1 Receptors by the Novel Immunosuppressant Drug FTY720	40
1700	Klein, T.K., Newton, C., Lu, L.T., Perkins, I., and Friedman, H.	THC Suppresses Dendritic Cell-T Cell TH1 Biasing by Inhibiting IL-12 Production and Increasing the Notch Pathway	41
1715-1830	**Kang Tsou Memorial Lecture: Mechanisms of Chronic Pain States** **by Frank Porreca** Professor of Pharmacology and Anesthesiology, University of Arizona, Tucson, AZ		
2015	**Beach Banquet / Dinner** 		

Day 3
Sunday, June 26th

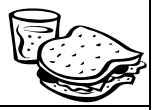
Human and Clinical Studies

➢*Chairs: Mary Lynch / Birgit Kraft*

0800	Lynch, M.E., and Young, J.	Report on a Case Series of Patients Using Medicinal Marijuana for Management of Chronic Pain Under the Canadian Medial Marijuana Access Regulations	42
0815	Kress, H.G., Nadulski, T., Frickey, N., Schnelle, M., Kaufmann, R., and Draft, B.	No Correlation of Plasma Kinetics and Clinical Effects After Standardized, Placebo-Controlled, Double-Blind Single Oral Administration of THC-Calibrated Cannabis Extract	43
0830	Kraft, B., Frickey, N., Kaufmann, R., Schnelle, M., Reif, M., Gustoff, B., and Kress, H.G.	No Analgesic and Antihyperalgesic Effects of Orally Administered Cannabis Extract in Two Different Human Pain Models	44
0845	Russo, E.B., and Robson, P.J.	Abrupt Interruption of Long-Term Treatment with Sativex was not Associated with a Withdrawal Syndrome or Serious Withdrawal Symptoms in a Sample of Patients with Multiple Sclerosis	45
0900	Ware, M., Collet, J-P., Shapiro, S., Wang, T., and the COMPASS Investigators	Cannabis for the Management of Pain – Assessment of Safety Study (COMPASS): Protocol Design	46
0915-0945	**Coffee and Continental Breakfast** 		
0945	Wright, S., and Johnson, J.R.	A Combination of Cannabidiol and Tetrahydrocannabinol is Superior to THC Alone in the Relief of Refractory Cancer Pain	47
1000	Leweke, F.M., Koehthe, D., Gerth, C.W., Nolden, B.M., Mauss, C., Schreiber, D., Hänsel, A., Neatby, M.A., Juelicher, A., and Klosterkötter, J.	Cannabidiol as an Antipsychotic. A Double-Blind, Controlled Clinical Trial on Cannabidiol vs Amisulpride in Acute Schizophrenia	48
1015	Morgenstern, H., Greenland, S., Zhang, Z-F., Cozen, W., Mack, T.M., and Tashkin, D.P.	Marijuana Use and Cancers of the Lung and Upper Aerodigestive Tract: Results of a Case-Control Study	49
1030	Teare, L.J., Zajicek, J.P., Wright, D., and Priston, M.J.	What is the Relationship Between Serum levels of Cannabinoids and Clinical Effect in Patients with Multiple Sclerosis and Does it Explain the Differences Between Pure Δ^9-Tetrahydrocannabinol and Whole Extract of Cannabis?	50
1045	Abrams, D.I., Jay, C., Shade, S., Vizoso, H., Petersen, K., and Rowbotham, M.	The Effects of Smoked Cannabis in HIV-Related Painful Peripheral Neuropathy: Results of a Randomized, Double-Blind, Placebo-Controlled Trial	51

Cardiovascular and Gastrointestinal Functions

➢*Chairs:George Kunos / Saoirse O'Sullivan*

1100	Maor, Y., Milman, G., Horowitz, M., Mo, F-M., Kunos, G., and Mechoulam, R.	Vasorelaxant Actions and Putative Vascular Protective Properties of Arachidonoyl Serine (ARA-S), A Novel Cannabinoid-Like Substance	52
1115	Ho, W.-S.V., and Hillard, C.J.	Endogenous Cannabinoids Modulate Physiologically Relevant Neuronal Activation in the Sensory Cortex	53
1130	Pazos, M.-R., Tolón, R.M., Benito, C., Gonzálex, Núñez, E., Almodóvar, F., Nevado, M., Alvarewz, M., Rodriguez, C.F., Santander, C., Aria, F., Gorgojo, J.J., and Romero, J.	Cannabinoid CB_1 Receptors are Present in Parietal Cells of the Human Gastric Mucosa	54
1145	O'Sullivan, S.E., Tarling, E., Bennett, A.J., Kendall, D.A., and Randall, M.D.	Vascular Actions of Δ^9-Tetrahydrocannabinol (THC) Mediated by Peroxisome Proliferator-Activated Receptor GAMMA	55
1200-1400	**Lunch / Free Time** 		
1315-1400	**NIDA Talk for Students: How to Write a Grant** ➢*Chairs: Allyn Howlett / Rao Rapaka*		
1400-1545	**Poster Session / Coffee Break**		136-159
Advanced Therapeutic Applications: Emesis, Obesity, and Smoking Cessation ➢*Chairs: Gérard Le Fur / Ester Fride*			
1545	Darmani, N.A., Gerdes, C., and Trinh, C.	Structurally Diverse Cannabinoids Prevent Substance P-Induced Emesis via Cannabinoid CB_1 Receptor in *Cryptotis Parva*	56
1600	Parker, L., Kwiatkowska, M., and Mechoulam, R.	Δ^9-Tetrahydrocannabinol and Cannabidiol Interfere with Conditioned Retching Elicited by a Lithium-Paired Context in the House Musk Shrew: A Model of Anticipatory Nausea	57
1615	Matias, I., Gonthier, M-P., Monteleone, P., and Di Marzo, V.	Peripheral Up-Regulation of the Endocannabinoid System in Obesity	58
1630	Gary-bobo, M., Bensaid, M., Gallas, J-F., Janiak, P., Marini, P., Trillou, C., Chabbert, M., Cruccioli, N., Pfersdorff, C., Elachouri, G., Roques, C., Arnone, M., Croci, T., Soubrié, P., Oury-Donat, F., Maffrand, J-P., Scatton, B., Lacheretz, F., Hebert, J-M., and Le Fur, G.	CB_1 Receptor Antagonist SR141716: Rimonabant, A New Promising Drug for the Treatment of Obesity-Associated Metabolic Syndrome Features	59
1645	Fride, E., Peretz-Ezra, D., Arshavsky, N., and Dahan, H.	CB_1 Receptor Blockade in Newborn Mice: An Etiologic Model for "Non-Organic Failure-To-Thrive" in Infants?	60
1700	Le Fur, G.	Clinical Results with Rimonabant in the Maintenance of Abstinence from Smoking	61
Control of Reproduction ➢*Chairs: Herebert Schuel / Mauro Maccarrone*			

1715	Maccarrone, M., Barboni, B., Paradisi, A., Bernabò, N., Gasperi, V., Pistilli, M., Fezza, F., Lucidi, P., and Mattioli, M.	The Endocannabinoid System in Boar Sperm and its Involvement in Male Fertility	62
1730	Schuel, H., and Burkman, L.J.	A Tale of Two Cells: Retrograde Endocannabinoid Signaling in Neurons and Sperm	63
1745	Burkman, L.J., Schuel., H., Makriyannis, A., Bodziak, M-L., Mroz, R., and Gurunatha, R.	In Human Spermatozoa, a Cannabinoid Agonist Can Inhibit the Effects of Nicotine on Hyperactivation (HA): Potent Interaction of Two Regulatory Systems	64
1800-1830	**Young Investigator Award Ceremony and Talk**		
2015	**Dinner** 		

Day 4
Monday, June 27th

Reward and Substances of Abuse ➢*Chairs: Jenny Wiley / Emmanuel Onaivi*			
0800	Cheer, J.F., Wassum, K.M., Wightman, M., and Wightman, R.	Endogenous Cannabinoid Tone Governs the Enhancing Effects of Cocaine on Sub-Second Dopamine Release	65
0815	Onaivi, E., Ishiguro, H., Gong, J-P., Patel, S., Meozzi, P., Myers, L., Tagliaferro, P., Leonard, C., Gardner, E., Brusco, A., Akinshola, B., Liu, Q-R., Hope, B., and Uhl, G.	Peripheral Cannabinoid CB_2 Receptors are Expressed in the Brain and Involved in Depression and Substance Abuse	66
0830	Wiley, J.L., O'Connell, M.M., Tokarz, M.E., and Wright, Jr., M.J.	Pharmacological Effects of Δ^9-Tetrahydrocannabinol in Adolescent Rats	67
0845	Ward, S.J., Gerald, T., Franklin, S.O., Howlett, A.C., and Dykstra L.A.	Disparate Role of Cannabinoid CB_1 Receptors in the Reinforcing and Conditioned Learning Effects of the Sweet Non-Drug Reinforcer Ensure® in Mice	68
0900	Solinas, M., Tanda, G., Justinova, Z., Makriyannis, A., Wertheim, C., and Goldberg, S.R.	Endogenous Cannabinoids Produce Discriminative-Stimulus Effects Similar to Those of Δ^9-Tetrahydrocannabinol	69
0915-0945	**Coffee and Continental Breakfast** 		
The Endocannabinoid System in the Eye ➢*Chairs: Alexandros Makriyannis / Steve Yazulla*			
0945	Woodward, D.F., Krauss, A.H-P., Nieves, A.L., Protzman, C.E., Wang, J-W., Donde, Y., Landsverk, K., and Struble, C.	First Report on a Prostaglandin-Ethanolamide (Prostamide) Receptor Antagonist AGN204396	70

1000	Yazulla, S., and Fan, S-F.	Retrograde Endocannabinoid Effects Exerted on IK(V) of Goldfish Retinal Cone Photoreceptors	71
1015	McLaughlin, P.J., Winston, K.M., Brown, C.M., Lu, D., Thakur, G., Makriyannis, A., and Salamone, J.D.	Biphasic Effect of the Novel CB_1 Agonist AM411 on Visual Signal Detection in Rats	72
Neuroprotection and Neurodegenerative Disorders ➢*Chairs: Vincenzo Di Marzo / Julien Romero*			
1030	Fernández-López, D., Martinez – Orgado, M., Nuñez, E., Romero, J., Bonet, B., Moro, M.A., and Lizasoain, I.	Neuroprotective Effect of WIN55212 in Newborn Rat Brain Slices Exposed to Oxygen-Glucose Deprivation	73
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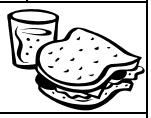
1200-1330	**Lunch / Free Time** 		
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Symposium XV
Clearwater Beach, FL
2005

Abstracts

3-PHENYLACETYLINDOLES, A NEW CLASS OF CANNABIMIMETIC INDOLES

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In a classical investigation of the structure-activity relationships of aminoalkylindoles the Sterling-Winthrop group found that a 3-(1-naphthoyl) substituent appended to the indole nucleus provided greater affinity for the cannabinoid CB_1 receptor than a substituted benzoyl group. Nearly simultaneously, we reported that an alkyl group could replace the *N*-aminoalkyl group without loss of cannabinoid activity. An *n*-pentyl group on the indole nitrogen provided maximum affinity for the CB_1 receptor, and *in vivo* potency typical of traditional cannabinoids.

In early work the Winthrop group also found that an aminoalkylindole with a 3-(1,2,3,4-tetrhaydro-1-naphthoyl) substituent had moderate affinity for the CB_1 receptor. This compound appeared to be a model for a 3-phenylacetylindole and we now report the synthesis and *in vitro* pharmacology of thirty 1-pentyl-3-phenylacetylindoles. In all cases the 2-methylindole analogs have from slightly to significantly lower affinity for the CB_1 receptor than the unsubstituted congener. The compounds with a *para*-substituent on the phenylacetyl moiety have uniformly poor affinity, while those with *meta*-substituents have somewhat to considerably greater affinity for the CB_1 receptor. Those phenylacetylindoles with *ortho*-substituents have from good to high affinity for the CB_1 receptor. 1-Pentyl-3-(2-chlorophenylacetyl) indole (JWH-203) and the 2-bromophenyl-acetyl compound (JWH-249) have the highest CB_1 receptor affinities with K_i = 8.0 ± 09 and 8.4 ± 1.8 nM, respectively. Molecular modeling and docking studies have been carried out in order to gain insight into these substituent effects.

The CB_2 receptor affinities of this class of indoles follow the same trend as their CB_1 affinities, however in contrast to many cannabimimimetic indoles, which show selectivity for the CB_2 receptor, two of these phenylacetylindoles show five-fold selectivity for the CB_1 receptor. 1-Pentyl-3-(2-methylphenylacetyl)indole (JWH-251) has good affinity for the CB_1 receptor (K_i = 29 ± 3 nM) with modest affinity for the CB_2 receptor (K_i = 146 ± 36 nM). 1-Pentyl-3-(3-methoxyphenylacetyl) indole (JWH-302) has K_i = 17 ± 2 nM for the CB_1 receptor, and K_i = 89 ± 15 nM at the CB_2 receptor. To evaluate the efficacy of these compounds their ability to stimulate GTPγS binding at CB_1 and CB_2 was determined. Both JWH-251 and JWH-302 stimulate GTPγS binding at CB_1 and are full agonists with E_{max} of greater than 90%. Although the affinities of these compounds at CB_2 are approximately one-fifth that of their affinities for the CB_1 receptor, both significantly stimulate GTPγS binding at the CB_2 receptor. At the CB_2 receptor both compounds are partial agonists with E_{max} values of less than 50%.

TETRAHYDROCANNABIVARIN IS MARKEDLY MORE POTENT AS AN ANTAGONIST OF *R*-(+)-WIN55212 AND ANANDAMIDE THAN OF Δ^9-TETRAHYDROCANNABINOL IN THE MOUSE ISOLATED VAS DEFERENS

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This investigation was directed primarily at establishing whether the phytocannabinoid tetrahydrocannabivarin (THCV), an analogue of Δ^9-tetrahydrocannabinol with a 3-propyl instead of a 3-pentyl side chain, behaves as a cannabinoid receptor agonist or antagonist. This objective was addressed by determining if THCV affects the amplitude of evoked contractions of the mouse isolated vas deferens when administered alone or blocks the ability of cannabinoid receptor agonists to inhibit electrically-evoked contractions of this tissue. Its ability to antagonize non-cannabinoids in the vas deferens and to displace [^{3}H]CP55940 from CB_1 binding sites was also investigated.

The methods used in this investigation have been detailed elsewhere (Thomas *et al.*, **2004**). Briefly, vasa deferentia obtained from adult MF1 mice were mounted in organ baths and contractions were evoked electrically or with beta,gamma-methylene-ATP or phenylephrine. THCV (GW Pharmaceuticals) was investigated by itself or added 30 min before *R*-(+)-WIN55212 (WIN), anandamide (AEA), clonidine, capsaicin beta,gamma-methylene-ATP or phenylephrine. THCV, THC, AEA and capsaicin were dissolved in DMSO, WIN in a 1:1 mixture of DMSO and saline, and other drugs in saline. Binding experiments were performed with mouse whole brain membranes.

THCV displaced [^{3}H]CP55940 from mouse brain membranes with a mean K_i value of 75 nM (n=4 to 8). At 3 to 1000 nM, THCV did not inhibit electrically-evoked contractions of the vas deferens. However, concentrations of THCV in this range did produce concentration-related parallel dextral shifts in the log concentration-response curves of WIN and AEA for inhibition of electrically-evoked contractions and these shifts were not accompanied by any significant decrease in the maximum effect of either agonist (n=6 to 9). Mean K_B values of THCV against WIN and AEA were 1.5 nM and 1.4 nM respectively. THC was also antagonized by THCV but was significantly less susceptible to THCV-induced antagonism (mean K_B = 97 nM) than WIN or AEA ($P<0.05$). THCV (100 nM) did not oppose the ability of clonidine or capsaicin to inhibit electrically-evoked contractions. At 1000 nM, neither THCV nor WIN altered the size of contractions induced by 10,000 nM beta, gamma-methylene-ATP or 3000 or 30,000 nM phenylephrine, suggesting that THCV interacts with WIN at prejunctional sites.

In conclusion, THCV is a potent antagonist of WIN and AEA that exhibits at least some degree of selectivity. As it appears to be more potent in antagonizing WIN and AEA than in antagonizing THC or in displacing [^{3}H]CP55940 from CB_1 receptors, further experiments are required to establish the basis of its antagonism of WIN and AEA.

Acknowledgements: Thomas, A. *et al.* (**2004**) *Eur. J. Pharmacol*., 487, 213-221.

TRIARYL BIS-SULFONES AS A NEW CLASS OF CANNABINOID CB_2 RECEPTOR INHIBITORS: IDENTIFICATION OF A LEAD AND INITIAL BIOLOGICAL CHARACTERIZATION

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The discovery of the cannabinoid receptor unique to immune cells, the CB_2 receptor, has stimulated interest in identifying receptor specific cannabinoids as novel immune modulators. We will discuss the identification and pharmacology of a novel class of cannabinoid CB_2-specific compounds, the triaryl bis-sulfones (1). We will show that selected members of this class of compounds, characterized as CB_2-selective inverse agonists using membrane binding studies, are capable of altering cellular chemotaxis mediated either by cannabinoids or by chemokines, both in vitro and in vivo. We also show that oral administration of triaryl bis-sulfones can decrease allergic eosinophilia in an animal model for asthma. We conclude that modulating cellular chemotaxis using triaryl bis-sulfones hold promise as therapeutics for modulating the immune system through the cannabinoid CB_2 receptor.

Acknowledgements: 1. Lavey BJ, Kozlowski JA, Hipkin RW, Gonsiorek W, Lundell DJ, Piwinski JJ, Narula S, Lunn CA. (**2005).** Bioorg Med Chem Lett. **2005** Feb 1;15(3):783-786

PHARMACOLOGICAL CHARACTERIZATION OF WATER-SOLUBLE CANNABINOIDS IN MICE

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Water-insolubility, a feature common to all currently available structural classes of cannabinoid agonists, results in the need for solubolization prior to experimentation. The purpose of this study was to investigate the pharmacological properties of a series of newly developed water-soluble tetrahydrocannabinols (THC). Two basic strategies for conversion to water-solubility were attempted. The first involved addition of substituents (R= carboxamido, imidazole, pyrazole, or triazole) to the terminal end of the pentyl side chain (Fig. 1).

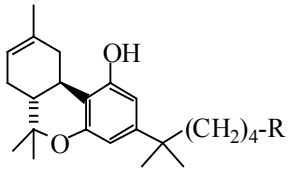

Fig. 1

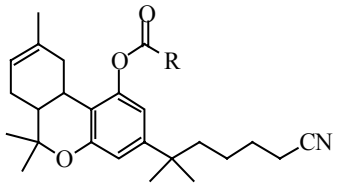

Fig. 2

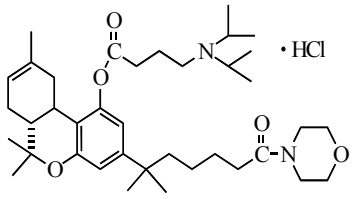

Fig. 3 0-2694

The second strategy was addition of a cyano group to the terminal end of the side chain and conversion of the phenolic hydroxyl to a morpholinobutyryloxy substituent (Fig. 2). Both strategies were used to synthesize O-2964 (Fig. 3). While the first strategy resulted in a series of potent THC analogs, none of the carboxamido, pyrazol-, or triazol-pentyl analogs were soluble in water. Water solubility was obtained with three hydrochloride salts of imidazol-pentyl side chain analogs of THC, with a range of affinities (K_i) for CB_1 and CB_2 receptors from 1.5 to 54 nM and 0.3 to 15 nM, respectively. Further, the imidazole-1-yl and 2-methyl-imidazole-1-yl analogs exhibited functional activity at the CB_1 receptor and were active *in vivo* when administered in saline. Similarly, all of the array of hydrochloride salts of substituted butyryloxy esters were water-soluble and highly potent. The substitutions include piperidine, piperazine, and alkyl substituted amino moieties. In addition, it was discovered that incorporation of a nitrogenous moiety in the alkyl side chain of tetrahydrocannabinol increased pharmacological potency. O-2694, which represents a synthesis of both strategies, was also water-soluble and active *in vivo*. It is now possible to conduct cannabinoid research with agonists that are water-soluble and thus obviating the need of solubilizing agents.

Acknowledgements: Research supported in part by NIDA grants DA-03672 and DA-05488.

2-THIOXOIMIDAZOLIDINONES AND IMIDAZOLIDINEDIONES DERIVATIVES: INHIBITION OF FAAH AND BINDING TO CB_1 CANNABINOID RECEPTOR

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Introduction: We are currently involved in the synthesis and pharmacological evaluation of 2-thioxo-imidazolidin-4-ones and imidazolidinedi-2,4-diones derivatives. First reports described the primary structure-affinity relationships of the 3-substituted-5,5'-diphenyl-imidazolidine-2,4-diones at the CB_1 cannabinoid receptor. These relationships were recently extended by bioisosteric replacement of the oxygen by a sulfur atom leading to the 3-substituted-5,5'-diphenyl-2-thioxo-imidazolidin-4-one derivatives. Among these compounds 3-allyl- and 3-butyl-5,5'-bis(4-iodophenyl)-2-thioxo-imidazolidin-4-one exhibited the highest affinity for the CB_1 cannabinoid receptor. Both the oxo- and thio- derivatives behave as inverse agonists of the human CB_1 cannabinoid receptor.

Here we report for the first time an inhibitory activity of these compounds on the fatty acid amide hydrolase (FAAH). Mode of inhibition as well as primary structure-activity relationships will be presented. Moreover, FAAH activity *vs* CB_1 cannabinoid receptor affinity will be discussed.

Methods and Results: Activity of the compounds on FAAH was evaluated using rat brain homogenates as enzyme source, and [^{3}H]-AEA as substrate. pI_{50} values were determined from dose-activity curves. Affinity for the cannabinoid receptors was assessed for each compound (10 μM) using CHO cells expressing selectively either the hCB_1 (against [^{3}H]-SR141716A) or the hCB_2 cannabinoid receptor (against [^{3}H]-CP55,940).

FAAH inhibition is the highest for compounds bearing no substituent on the phenyls and possessing a long alkyl chain on N_3. For instance, 3-heptyl-5,5'-diphenyl-imidazolidine-2,4-dione and 5,5'-diphenyl-3-tetradecyl-2-thioxo-imidazolidin-4-one exhibited pI_{50} values of 5.12 and 5.94, respectively. These two compounds showed no affinity for the cannabinoid receptors as radioligand displacements were less than 20% @ 10μM. Substitution of the phenyls, or shorter alkyl chains, resulted in less active compounds.

These structure-activity relationships are at the opposite of those described for CB_1 cannabinoid receptor activity. Indeed, introduction of a para substituent on the phenyl rings increases the affinity of the derivatives for the receptor.

Conclusions: We have reported here the inhibitory activity of 2-thioxo-imidazolidin-4-ones and imidazolidinedi-2,4-diones derivatives on the fatty acid amide hydrolase activity. Among the fifty compounds tested, 5,5'-diphenyl-3-tetradecyl-2-thioxo-imidazolidin-4-one was the most active in inhibiting FAAH and was devoid of cannabinoid receptor affinity.

5,5'-Diphenyl-2-thioxo-imidazolidin-4-one derivatives could constitute an attractive template for the synthesis of new FAAH inhibitors.

MOLECULAR CLONING AND CHARACTERIZATION OF *N*-ACYLETHANOLAMINE-HYDROLYZING ACID AMIDASE

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Bioactive *N*-acylethanolamines, including the endocannabinoid anandamide, are hydrolyzed to fatty acids and ethanolamine by fatty acid amide hydrolase (FAAH). In addition, we previously found another amidohydrolase "*N*-acylethanolamine-hydrolyzing acid amidase (NAAA)" which catalyzed the same reaction only at acidic pH. We purified NAAA from rat lung and determined its N-terminal sequence. By the use of database, we then identified complementary DNA of NAAA from human, rat, and mouse (Tsuboi *et al.* (January 17, **2005**) J. Biol. Chem. 10.1074/jbc.M413473200). The deduced primary structure did not show any homology to FAAH but had similarity to acid ceramidase, which hydrolyzes ceramide to sphingosine and fatty acid at acidic pH. At amino acid level, NAAA of human, rat, and mouse showed 33–34% homology to acid ceramidase of the same species over their entire length. When overexpressed in HEK293 cells, recombinant human NAAA hydrolyzed various *N*-acylethanolamines with *N*-palmitoylethanolamine as the most reactive substrate. The optimal pH was 4.5. Notably, NAAA also revealed a low ceramide-hydrolyzing activity whereas acid ceramidase showed an *N*-lauroylethanolamine-hydrolyzing activity. These results indicated structural and functional similarity of NAAA to acid ceramidase. With the aid of tunicamycin and endoglycosidase, NAAA was found to be a glycoprotein. Furthermore, a specific proteolytic cleavage of the enzyme was seen at pH 4.5, but not at pH 7.4. Expression analysis of a green fluorescent protein-NAAA fusion protein showed a lysosome-like distribution in HEK293 cells. The messenger RNA of NAAA was widely distributed in various rat organs with the highest expression in lung. These results strongly suggested that NAAA is a novel lysosomal enzyme acting as a second *N*-acylethanolamine hydrolase.

EXPRESSION ANALYSIS OF *N*-ACYLPHOSPHATIDYLETHANOLAMINE-HYDROLYZING PHOSPHOLIPASE D IN RAT BRAIN REGIONS

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It is widely accepted that long-chain *N*-acylethanolamines including the endocannabinoid anandamide are principally formed from their corresponding *N*-acylphosphatidylethanolamines (NAPEs) by a phosphodiesterase of the phospholipase D type (NAPE-PLD). Recently, we cloned cDNA of NAPE-PLD from rat, mouse, and human (Okamoto *et al.*, J. Biol. Chem. **2004**, 279, 5298). The enzyme was a novel member of the β-lactamase fold family, and was structurally and functionally distinguishable from the known PLDs. Although NAPE-PLD was reported to be present in the brain of various animal species, the distribution of the enzyme in brain areas has not been clarified yet. Here, we examined regional distribution of NAPE-PLD in rat brain using enzyme assay, Western blotting, and real-time PCR. The results revealed its wide distribution in different brain regions at the levels of enzyme activity, protein, and mRNA. The distribution pattern was not necessarily consistent with previously reported patterns of CB_1 receptor and fatty acid amide hydrolase (FAAH), and thalamus showed the highest expression level. Furthermore, NAPE-PLD activity in the brain markedly increased with advancing age as reported earlier, and the protein and mRNA levels of the enzyme were well correlated with the activity. *p*-Chloromercuribenzoic acid and cetyltrimethylammonium chloride inhibited both the recombinant NAPE-PLD and the native enzyme in all the tested brain regions. These results strongly suggested the central role of NAPE-PLD in the formation of anandamide and other *N*-acylethanolamines in the brain.

MICE LACKING FATTY ACID AMIDE HYDROLASE DEMONSTRATE INCREASED CROSS-TOLERANCE TO THC FOLLOWING SUBCHRONIC ANANDAMIDE

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Mice genetically altered to lack fatty acid amide hydrolase (FAAH(-/-)), the principle enzyme responsible for the degradation of anandamide (AEA) and other fatty acid amides (FAAs) possess significantly elevated levels of these endogenous lipid signaling molecules, show increased sensitivity to exogenously delivered AEA, but exhibit normal CB_1 receptor function compared to wild type mice. The aim of the present study was to examine tolerance and cross-tolerance to the cannabinoid-1 receptor (CB_1) agonists AEA and Δ^9-tetrahydrocannabinol (THC) in FAAH(-/-) mice following subchronic administration of either AEA or THC. Cumulative dose response curves were determined before and after subchronic administration of both drugs. Endpoints recorded were tail withdrawal latency, rectal temperature, and catalepsy. Subchronic administration of THC resulted in an equivalent degree of THC tolerance (as demonstrated by a rightward shift in the dose response curve for each endpoint) between FAAH(-/-) mice and wild type mice. Subchronic AEA administration led to tolerance to AEA, and cross-tolerance to THC. In contrast, FAAH(-/-) mice showed significantly greater cross-tolerance to THC than wild type littermates. These results indicate that subchronic AEA administration in mice lacking the ability to degrade fatty acid amides produces a greater magnitude of tolerance to cannabinoid agonists, which is likely due to a concomitant increase in AEA's half life in FAAH(-/-) mice. Taken together these data indicate that FAAH(-/-) exhibit normal CB_1 functioning, despite having constitutively increased levels of AEA, though they are more sensitive to AEA's effects after either acute or subchronic administration.

PHARMACOLOGICAL CHARACTERIZATION OF THE ANANDAMIDE TRANSPORTER – THE MISSING LINK IN ENDOCANNABINOID NEUROTRANSMISSION

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Eli Lilly and Company, Lilly Corporate Center,
Neuroscience Discovery Research, Indianapolis, IN

Endocannabinoids represent a novel class of lipid neurotransmitters with activity at specific metabotropic and ionotropic synaptic proteins. Anandamide (AEA), virodhamine, noladin ether, 2-arachidonylglycerol, oleamide and other related lipid signaling molecules modulate both pre- and post-synaptic GPCRs and ion channels including CB_1, CB_2, and VR1. In contrast to the well-characterized neurotransmission cycle of monoamine neurotransmitters, the release, disposition, and potential recycling of endocannabinoids is not well understood. In particular the mechanism of endocannabinoid reuptake has been elusive. Reports have implicated protein-independent diffusional pathways, FAAH-facilitated update and hydrolysis, as well as FAAH independent transmembrane transport. Here we provide pharmacological evidence that AEA transport is mediated by a protein independent of FAAH, but is significantly facilitated by FAAH activity. Using a novel radioligand and a variety of small molecule inhibitors of AEA uptake, we have identified a high affinity plasma membrane binding site with pharmacology correlated to AEA uptake. These novel inhibitors of AEA transport augment anandamide levels in vivo as well as demonstrate in vivo efficacy in animal models of nociception and convulsive behavior.

ANANDAMIDE UPTAKE AND IT'S INHIBITION IN RAT BASOPHILIC LEUKEMIA CELLS

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Arachidonoylethanolamide (anandamide, AEA) is a putative endocannabinoid and endovanilloid that is internalized by cells and subsequently inactivated by the enzyme fatty acid amide hydrolase (FAAH). The cellular uptake of AEA was originally characterized (Di Marzo et al., Nature. **1994** 372:686-91.) as a gradient-driven carrier-mediated process based upon its saturability and inhibition by selective compounds. Emerging data, however, suggest the existence of alternative pathways for the initial transport of AEA. Two such contrasting models have suggested the involvement of simple diffusion (Glaser et al., Proc Natl Acad Sci U S A. **2003** 100:4269-74) or caveolin-mediated endocytosis (McFarland et al., J Biol Chem. **2004** 279:41991-7). This study examined the mechanism(s) underlying the accumulation of AEA into the rat basophilic leukemia (RBL-2H3) cell line. This cell line, known to express FAAH, cannabinoid receptor 2, and the serotonin transporter, had unsaturatable AEA uptake when examined at short incubation times. In contrast, as a control, the uptake of 5-HT (serotonin) was found to be saturable under similar temporal conditions. As observed in other cell lines, (Ortega-Gutierrez et al., Biochemistry. **2004** 43:8184-90, Fowler et al., Eur J Pharmacol. **2004** 492:1-11) the concentration of BSA present in uptake media was found to influence the amount of AEA taken up into the cells but was nevertheless critical in keeping AEA in solution and preventing it from sticking to tissue culture plates and plastic ware. In addition, our attempts to examine AEA transport in the absence of BSA were likewise undermined by the instability of the uptake solution.

Recently, novel transport inhibitors have been reported to display negligible effects upon FAAH. Under our assay conditions, the inhibition of AEA uptake by the transport inhibitors UCM707, AM1172, OMDM2, VDM11 and the FAAH inhibitor CAY10400 were found to occur only under incubation times of >1 min and temporally correlated with the hydrolytic activity of FAAH. The ability of OMDM2 and AM1172 to reduce cellular AEA transport despite their reported lack of reactivity with FAAH *in vitro* suggested an alternative target for these compounds. To explore this possibility further, the uptake and hydrolysis of AEA following treatment with various inhibitors was examined under steady-state conditions (>1 min) in cell culture. Each compound was found to attenuate cellular AEA accumulation and to increase the relative levels of non-metabolized AEA while concurrently reducing AEA hydrolysis. These data suggest that 1) weak "transport"/FAAH inhibitors can significantly reduce the uptake of AEA in RBL-2H3 cells (via partial inhibition of FAAH), 2) the novel transport inhibitors have targets downstream of the plasma membrane, rather than a transmembrane transporter, and 3) FAAH seems to be selectively targeted by the "transport" inhibitors used in this study, although the involvement of other intracellular targets in this cell line cannot be excluded at the present time.

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ANANDAMIDE UPTAKE VIA ENDOCYTOSIS IN NEURONAL AND NON-NEURONAL CELL LINES

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CAD cells are neural cells, derived from the catecholaminergic Cath.a cell line, and undergo a reversible morphological differentiation that can be induced by the removal of serum from culture media. CAD cells are easily transfected and may present a new and useful model for studying the cellular uptake of anandamide and other cannabinoids. Differentiated and undifferentiated CAD cells displayed robust uptake and accumulation of radiolabeled anandamide. The accumulation of anandamide by CAD cells was inhibited by AM404 with an IC_{50} value in the high nanomolar range. Furthermore, cellular lysates from both differentiated and undifferentiated CAD cells were analyzed by Western blot and found to express high levels of the anandamide metabolizing enzyme fatty acid amide hydrolase (FAAH). Evidence from previous studies in our laboratory using pharmacological inhibitors of endocytosis has indicated that anandamide transport occurs via a caveolae-related endocytic process. Caveolae are a subset of membrane microdomains rich in cholesterol and sphingolipids called lipid rafts. Disruption of lipid rafts significantly inhibits the cellular uptake of anandamide in RBL-2H3 cells. We demonstrate that disruption of lipid rafts also decreased the cellular accumulation of anandamide in CAD cells confirming that endocytosis may play a role in anandamide uptake in neuronal cells. Proteomic analysis of lipid rafts has been performed seeking to identify putative proteins involved with anandamide endocytosis. These studies will reveal molecular information regarding anandamide uptake and provide new opportunities for pharmacologic manipulation of the endogenous cannabinoid system.

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IMAGING OF THE *IN VIVO* ACCUMULATION OF [^{3}H]ANANDAMIDE AND IT'S METABOLITES IN THE MOUSE BRAIN

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The putative endocannabinoid and endovanilloid anandamide (AEA) is inactivated by a process of uptake followed by hydrolysis by fatty acid amide hydrolase (FAAH). The accumulation of AEA in cultured cells has been proposed by some groups to be driven by a concentration gradient maintained by FAAH activity (Deutsch *et al. JBC* 276:10 6967-73; Day *et al. Mol Pharm* 59:6 1369-75). Few studies have examined the accumulation of [^{3}H]AEA in intact tissue. The first study observed the heterogeneous regional uptake of [^{3}H]AEA in rat brain slices, and blocked the pattern formation with the FAAH and putative transport inhibitor N-(4-hydroxyphenyl)arachidonylamide (AM404) (Giuffrida *et al. J PET* 298:7–14). The first study examining [^{3}H]AEA uptake on a cellular level linked FAAH immunoreactive cells with increased accumulation of [^{3}H]AEA and its metabolites in goldfish retina. This pattern was eliminated by AM404 and methyl arachidonyl fluorophosphonate (MAFP) (Glaser *et al. Visual Neurosci*., ***in press***).

We have examined the accumulation of [^{3}H]AEA and metabolites in wild-type (WT) C57BL/6 and FAAH knockout (KO) mouse brains following the *i.v.* administration of exogenous anandamide [arachidonyl 5,6,8,9,11,12,14,15-3 H] in ethanol/emulphor/saline vehicle (1:1:18 v/v). Following injection, blood and cerebellum samples were collected. Duplicate samples were counted in a scintillation counter, and processed for thin layer chromatography. The brain was fixed, serial cryosectioned, and imaged with a Betaimager. Surface radioactivity was determined using BetaVision+. Dose response curves from 1-10 mg/kg [^{3}H]AEA showed proportional increases of tritium in WT brains, with 2.5% injected dose/g 30 min after a 10mg/kg [^{3}H]AEA injection. Time course studies following injections of 10 mg/kg [^{3}H]AEA produced a sharp decline of available [^{3}H]AEA in WT blood from 30s to 5 min, and an increase of tritium in the brain. After 30 min, a heterogeneous tritium pattern was observed in WT mouse brains, with the highest levels in the cortex and thalamus, while FAAH KO brains had a uniform tritium distribution. 10 mg/kg [^{3}H]arachidonic acid accumulated significantly less ($p<0.005$) than ^{3}H-AEA in WT brains. Upon the co-administration of 10 mg/kg [^{3}H]AEA and increasing doses (1-8 mg/kg) of the irreversible FAAH inhibitor MAFP, brain [^{3}H]AEA levels significantly increased ($p<0.005$) at 8 mg/kg and the tritium distribution in the brain became uniform. While as potent as MAFP *in vitro* (Boger *et al. PNAS* 97:5044-5049), the reversible FAAH inhibitor CAY10435, was much less effective *in vivo*. At doses of 12 mg/kg, CAY10435 produced no significant increase ($p>0.05$) in [^{3}H]AEA brain levels. This study is the first to image the *in vivo* accumulation of exogenous anandamide and demonstrates the feasibility of imaging the inhibition of FAAH by pharmacological agents. In addition, these *in vivo* data support the conclusion of earlier *in vitro* studies suggesting that FAAH activity drives the intracellular accumulation of [^{3}H]AEA.

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NOVEL SELECTIVE INHIBITORS OF ANANDAMIDE RE-UPTAKE WITH ANTI-SPASTIC ACTIVITY IN A MOUSE MODEL OF MULTIPLE SCLEROSIS

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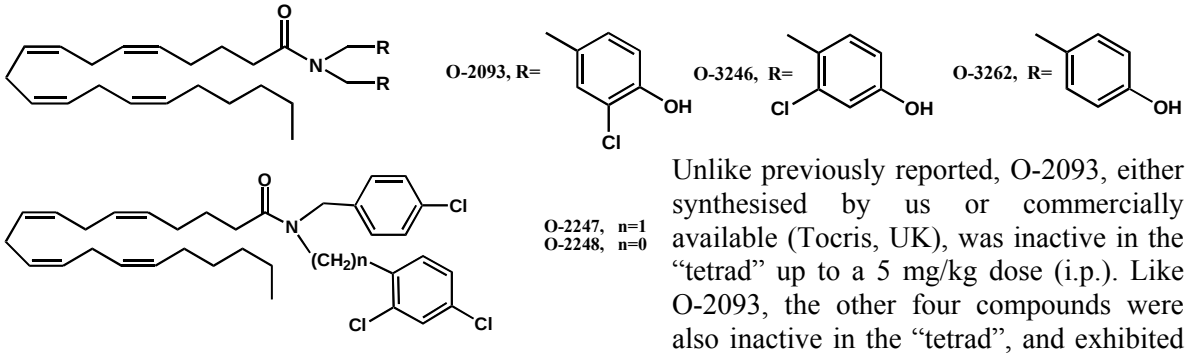
Inhibitors of the putative anandamide membrane transporter (AMT) were previously shown to counteract efficaciously spasticity in the chronic relapsing experimental allergic encephalomyelitis (CREAE) mouse model of multiple sclerosis (MS) (Baker et al., FASEB J., 2001; de Lago et al., Eur. J. Pharmacol., **2004**). We previously reported (Di Marzo et al., J. Pharmacol. Exp. Ther., **2001**) that the compound known as O-2093 is a selective AMT inhibitor (IC_{50}=11.5 μM), with no activity in the "tetrad" of *in vivo* tests diagnostic of cannabimimetic and, to some extent, vanilloid activity in mice. We have now reassessed the activity of O-2093 in vivo, synthesized four structural analogs (O-2247, O-2248, O-3246, O-3262), and assessed their activity in: 1) binding assays carried out with membranes from cells over-expressing the human CB_1 and CB_2 receptors; 2) functional ($[Ca^{2+}]_i$ fluorimetric) assays in cells over-expressing the human TRPV1 vanilloid receptor; 3) [^{14}C]anandamide cellular uptake and hydrolysis assays in RBL-2H3 cells; 4) the mouse "tetrad" tests (analgesia on a hot plate, immobility on a "ring", rectal hypothermia and hypolocomotion in an open field); and 5) the limb spasticity test in CREAE mice.



Unlike previously reported, O-2093, either synthesised by us or commercially available (Tocris, UK), was inactive in the "tetrad" up to a 5 mg/kg dose (i.p.). Like O-2093, the other four compounds were also inactive in the "tetrad", and exhibited low affinity in CB_1 and CB_2 binding assays (Ki> 5 μM), low efficacy in the TRPV1 functional assay (EC_{50}>10 μM), and low potency as inhibitors of rat FAAH activity (IC_{50}>25 μM). While O-2247 and O-2248 were also poor inhibitors of [^{14}C] anandamide cellular uptake (IC_{50}=50 and >50 μM, respectively), O-3246 and O-3262, like O-2093, were quite potent in this assay, with IC_{50} values of 2 and 4 μM, respectively. O-3246 is the most potent inhibitor of anandamide uptake ever found under our experimental conditions. O-3246 and O-3262, again like O-2093 and unlike O-2247 and O-2248, injected i.v. at the dose of 1 mg/kg, significantly inhibited limb spasticity in CREAE mice (30.4% and 32.7% inhibition of tension 30 min from administration, respectively *vs.* 30.3% inhibition observed with 0.5 mg/kg O-2093).

Given the chemical similarity of the compounds tested and the great differences in their potencies in AMT assays, our data further support the existence of a specific mechanism for endocannabinoid transport into the cell. In view of the strong correlation between inhibitory activity at the AMT and efficacy at reducing spasticity, these data confirm the potential utility of selective endocannabinoid uptake inhibitors as non-psychotropic anti-spasticity drugs in MS.

THE ROLE OF 2-ARACHIDONYLGLYCEROL IN RETROGRADE SIGNALING

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Activation of CB_1 cannabinoid receptors on axon terminals by exogenous agonists leads to presynaptic inhibition of neurotransmission at many synapses in the central nervous system. Recently it has been shown that these receptors are also activated by endogenous cannabinoids (endocannabinoids). Typically, endocannabinoids are synthesised by depolarised postsynaptic neurons. After synthesis, endocannabinoids diffuse to presynaptic axon terminals, where they inhibit transmitter release (retrograde signaling). If GABAergic neurotransmission is inhibited by this mechanism, the phenomenon is called depolarisation-induced suppression of inhibition (DSI; for review see Freund et al., Physiol Rev 83:1017-1066, **2003**). The aim of the present experiments was to clarify which of the two major endocannabinods, anandamide or 2-arachidonylglycerol, mediates DSI. We studied DSI at interneurone – Purkinje cell synapses in the cerebellar cortex; it has been shown previously that exogenous cannabinoids and endocannabinoids cause presynaptic inhibition at these synapses (Szabo et al., J Pharmacol Exp Ther 310: 915-925, **2004**).

Sagittal cerebellar slices were prepared from brains of young mice. GABAergic spontaneous inhibitory postsynaptic currents (sIPSCs) were recorded in cerebellar cortical Purkinje cells with patch-clamp techniques. Postsynaptic Purkinje cells were depolarised by 9 pulses at 1 Hz (from -70 mV to 0 mV for 100 ms) for inducing DSI. The cumulative amplitude of sIPSCs was inhibited by 35 ± 3 % (i.e., DSI was 35 ± 3 %). The CB_1 receptor antagonist rimonabant (10^{-6} M) abolished DSI, verifying involvement of endocannabinoids and CB_1 receptors. In order to determine whether 2-arachidonylglycerol is involved in DSI, we tested the effect of two inhibitors of diacylglycerol lipase on DSI (orlistat and RHC-80267). Diacylglycerol is a key enzyme in the synthesis of 2-AG. In the presence of orlistat (10^{-6} M) DSI was 17 ± 5 %. In the presence of a higher concentration of orlistat (10^{-5} M), DSI was 0 ± 7 %, i.e., it was eliminated. The other inhibitor of diacylglycerol lipase, RHC-80267 (10^{-4} M), also lowered DSI, to 18 ± 3 %. In order to determine whether anandamide is involved in DSI, we tested the effect of two inhibitors of fatty acid amide hydrolase on DSI (AA-5-HT and URB597). Fatty acid amide hydrolase is the main enzyme for anandamide hydrolysis. In the presence of AA-5-HT (1.5×10^{-5} M), DSI was 28 ± 12 %, i.e., unchanged. URB597 (5×10^{-7} M) also did not change DSI: DSI was 36 ± 4 % in the presence of this drug. In contrast, URB597 (5×10^{-7} M) potentiated the inhibitory effect of superfused exogenous anandamide (10^{-6} and 10^{-5} M) on sIPSCs.

These findings verify that CB_1 receptor-mediated retrograde signaling (DSI) is operating at interneurone – Purkinje cell synapses in the cerebellar cortex. A role of anandamide in DSI is unlikely, since blockade of anandamide metabolism did not enhance DSI. Inhibition of DSI by two inhibitors of the enzyme which synthesises 2-arachidonylglycerol - diacylglycerol lipase - suggests that DSI is mediated by 2-arachidonylglycerol.

ROLE OF THE RAS/ERK PATHWAY IN THE CB_1 RECEPTOR TRAFFICKING FOLLOWING CHRONIC CANNABINOID EXPOSURE

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We have recently demonstrated through genetical (Ras-GRF1 knock out mice) and pharmacological (SL327 pretreatment) approaches that ERK activation may play different roles in the events triggered by prolonged Δ^9-tetrahydrocannabinol (THC) exposure depending on the brain region considered. In the caudate putamen and cerebellum ERK activation could account for CB_1 receptor downregulation and desensitization, events likely to be involved in the development of tolerance to the THC hypolocomotor effect. In the hippocampus and prefrontal cortex other kinases might explain CB_1 receptor adaptation after chronic treatment. In these regions ERK activation could be involved in events not closely linked to CB_1 receptor activation but relevant for the synaptic plasticity associated with persistent drug exposure.

The aim of the present work was to investigate in different cerebral areas of mice chronically treated with THC the changes in G-protein coupled receptor kinases (GRKs) and beta-arrestins, that could represent the initial step in the complex molecular mechanims leading to cannabinoid CB_1 receptor desensitization and downregulation. Moreover we used mice lacking the THC-induced ERK activation (i.e. Ras-GRF1 knock out) to check the involvement of ERK in the occurrence of these adaptive events.

Five-days exposure to THC (10 mg/kg sc twice daily) leaded to different pictures depending on the brain areas considered and the genetic strain. In the caudate-putamen of wild type mice a significant increase in GRK2 (60%) and GRK4 (150%) paralleled the presence of CB_1 receptor downregulation and desensitization, together with beta- arrestin 2 alteration. All these changes were not observed in Ras-GRF1 -/- mice. In contrast in the cerebellum several GRKs play a role in the homologous regulation of CB_1 receptor, however the beta-arrestin 2 increases present in wild type animals was not observed in knockout mice. Finally, in the hippocampus and cerebellum, areas where CB_1 receptor adaptations occurred besides ERK inactivation, the significant increase observed in wild type mice of the transcription factor Fos B was completely inhibited in knockout mice.

Taken together, these data suggest that in the caudate-putamen and cerebellum the intracellular events leading to decreased CB_1 receptor functionality are under the modulation of the ERK pathway whereas in the hippocampus and prefrontal cortex this pathway seems to play a major role in the synaptic plasticity triggered by chronic THC exposure.

Acknowledgments: we thank GW Pharmaceuticals for THC supply and Greta Forlani for the maintenance of Ras-GRF1 knockout mice.

MOLECULAR IDENTIFICATION OF GPR55 AS A THIRD G PROTEIN-COUPLED RECEPTOR RESPONSIVE TO CANNABINOID LIGANDS

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The existence of sites of action for endocannabinoid ligands distinct from CB_1 and CB_2 has now been shown by multiple research groups. One site is present in brain and characterised by affinity for anandamide and WIN55212-2, which stimulate GTPγS binding both in brain sections and membranes. This site appears largely insensitive to the high-affinity CB_1 antagonist SR141716A. Another site has been described in vascular endothelium with affinity for anandamide and SR141716A but pharmacologically distinct from either CB_1 or CB_2. Both these sites are implicated as G protein-coupled receptors (GPCRs), from the observed stimulation of GTPγS binding and sensitivity to pertussis toxin, and both sites are present in CB_1 null or CB_1/CB_2 double-null mice. However, neither has yet been identified at a molecular level.

In an effort to identify GPCRs corresponding to these sites, we carried out ligand fishing. This approach used modified strains of the yeast *Saccharomyces cerevisiae,* in which receptor activation resulted in production of fluorescein from a conjugated substrate. Orphan GPCRs (i.e. GPCRs with unknown ligands) were identified through homology searches of the human genome, cloned into expression vectors, and introduced into cells. Using automated screening methods, panels of cannabinoid and other putative ligands were tested for activation of GPCR-expressing cells. This revealed the orphan receptor GPR55 to be activated by SR141716A, and its close analogues AM251 and AM281, at concentrations greater than 1μM. Subsequent screening identified CP55940 as an antagonist capable of blocking GPR55-mediated agonist effects (pA2 = 7.7). CP55940 also acts as an inverse agonist, inhibiting GPR55 constitutive activity in yeast. Agonist effects of anandamide at the rat orthologue of GPR55 were also shown. GPR55 is expressed in localised brain regions, particularly caudate and putamen, and possibly also in the periphery. In sequence, GPR55 is highly divergent to CB_1 and CB_2, and we propose has acquired affinity for anandamide by convergent evolution. We will discuss the possibility that GPR55 corresponds to one of the non-CB_1/non-CB_2 pharmacological sites described above.

AGONIST-STIMULATED SIGNALING, TRAFFICKING AND DESENSITIZATION OF CB_1 RECEPTORS IN A CULTURED CELL MODEL

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We have previously demonstrated that chronic treatment of mice with Δ^9-tetrahydrocannabinol (THC) or WIN 55,212-2 (WIN) produces brain region-dependent attenuation of CB_1 receptor-mediated G-protein activation and downregulation of CB_1 receptor binding sites. Recent studies have found that these adaptations persist for ≥ one week after cessation of THC treatment and do not involve a decrease in CB_1 receptor immunoreactive protein. To further examine potential mechanisms of CB_1 receptor adaptation, we have characterized a Chinese Hamster Ovary (CHO) cell culture model heterologously expressing the mouse CB_1 receptor. This cell line stably expresses CB_1 receptors at approximately 4 pmol receptor/mg membrane protein, as assessed by [^{3}H]SR141716A saturation analysis. Cannabinoid agonists stimulated G-protein activation in membranes prepared from these cells, with an order of efficacy of WIN = CP55,940 ≥ methanandamide > THC. Treatment of intact cells with WIN or THC caused internalization of CB_1 receptor within 30 min, but recruitment of green florescent protein-labeled β-arrestin was only modestly apparent. This same pretreatment, however, produced a 50% increase in optical co-localization of CB_1 receptors with caveolin-1 immunoreactivity. Concomitantly, there was a 30% decrease in co-localization of CB_1 receptors with the plasma membrane. In membranes prepared from cells pretreated for 18 hr with WIN or THC, significant desensitization of WIN-stimulated G-protein activation was observed. Interestingly, however, this treatment produced no significant downregulation of CB_1 receptor binding sites. These results demonstrate that mouse CB_1 receptors expressed in CHO cells exhibit similarities and differences in their responses to acute and chronic agonist exposure compared to CB_1 receptors in mouse brain. Further characterization of CB_1 receptor responses in this cell culture model should aid in elucidation of the mechanisms of CB_1 adaptation to chronic agonist treatment.

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THE ROLE OF DIFFERENT NEURONAL POPULATIONS IN THE PHARMACOLOGICAL ACTIONS OF Δ^9-TETRAHYDROCANNABINOL

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Δ^9-Tetrahydrocannabinol (THC) exerts profound effects on the brain through CB_1 receptors. As CB_1 is expressed in distinct neuronal populations in the central nervous system, we were interested in elucidating the exact roles of these neuronal populations in the well-known pharmacological actions of THC. We have generated a CB_1 knockout mouse line and a number of CB_1 conditional knockout mouse lines by the Cre / loxP system to dissect the involvement of different neuronal subpopulations in the physiological and pharmacological effects of agonists acting at the CB_1 receptor. We have tested mice lacking CB_1 expression in distinct neuronal populations in the so-called "tetrad" battery of pharmacological experiments. Here we present data showing that GABAergic forebrain interneurones are not required for the manifestation of the typical pharmacological / behavioural symptoms produced by THC treatment: hypolocomotion, hypothermia, catalepsy and increase of nociceptive threshold. Depolarisation induced suppression of inhibition (DSI) and LTD of inhibitory synapses (I-LTD) in the hippocampus were normally present in mice expressing CB_1 only on GABAergic interneurones and were abolished in mice lacking CB_1 expression from GABAergic neurones. This indicates that the physiological actions of endocannabinoids were conserved in mice irresponsive to THC. These results show that "classical" pharmacological actions of THC do not depend on functional expression of CB_1 on GABAergic interneurones, paving the way for a novel interpretation of cannabinoid pharmacology.

BETA-ARRESTIN2 AFFECTS CANNABINOID RECEPTOR/G-PROTEIN COUPLING

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Beta-arrestin2 has been implicated in desensitization of CB_1 receptors in cell culture models (W Jin, et al,**1999**; J Neurosci 19: 3773-80). Previously studies in my lab have shown that the effects of Δ^9-tetrahydrocannabinol (THC), but not other cannabinoid agonists, were greater in beta-arrestin2 -/- mice than in their wild type (+/+) counterparts.

Concentration-effect curves of THC and CP55940 were examined by [^{35}S]GTPγS binding at 30 μM GDP. There were no differences between beta-arrestin2-/- and +/+ cerebellar membranes in the effect of either agonist, but in cortex and hippocampal membranes, net THC-stimulated [^{35}S]GTPγS binding (in pmol/mg or %stimulation) in beta-arrestin2 -/- was only 50 or 70% (respectively) of that seen in +/+, there were no differences with CP55940.

Varying incubation time with 30 μM GDP showed that basal [^{35}S]GTPγS binding in -/- cortex membranes was half that in +/+ membranes at each time point. THC-stimulated [^{35}S]GTPγS binding in -/- cortex membranes was also ~50% of that in +/+. Using 60 min incubations, the difference between genotypes was reversed at low GDP concentrations; at 0 and 0.1 μM GDP, -/- cortex membranes showed more [^{35}S]GTPγS binding than +/+. At 1 and 10 μM GDP, binding was approximately the same, and at 30 and at 100 μM GDP, binding was lower in -/- than in +/+ membranes (as seen in the time course with 30 μM).

The binding of CP55940 at high and low affinity sites was assessed by displacement of [^{3}H]SR141716A binding. In cerebellar membranes, there was no difference between beta-arrestin2-/- and +/+ membranes. In cortex membranes, multi-component analysis indicated that CP55940 bound to 3 sites in +/+ but only 2 sites -/- membranes. The -/- membranes exhibited 3-4x as much binding to a high affinity site with a K_i of ~0.1 nM, but no binding to a low affinity site with K_i ~1200 nM. Both genotype bound a site with a K_i of ~50 nM.

These results indicate that among CB_1 agonists, THC alone was affected by beta-arrestin2. Results from whole animal agreed with the concept that beta-arrestin2 shut off CB_1 signaling, since THC's efficacy was increased in beta-arrestin2-/-. Deletion of beta-arrestin2 might permit greater spontaneous activity G-proteins by a variety of G-protein coupled receptors, resulting in higher levels of basal [^{35}S]GTPγS binding. While this was seen at low concentrations of GDP, the opposite was observed with high GDP. Also, activation by THC was reduced in the -/- membranes (the opposite effect from whole animals). One possibility is that GDP is more efficacious in reducing spontaneous G-protein activation or activation by THC in the absence of beta-arrestin2, but a mechanistic explanation will require further study. Beta-arrestin2 caused a fraction of CB_1 receptors to assume a low affinity state for agonist, and reduced the fraction of CB_1 in the high affinity state. This suggests that beta-arrestin2 displaced G-proteins from CB_1, since the high affinity agonist binding state is attributed to coupling of G-proteins to receptors. It also suggests that the low affinity state is attributable to the interaction of beta-arrestin2 with CB_1.

STRUCTURAL DETERMINATION OF THE THIRD INTRACELLULAR LOOP AND THE 8TH HELIX OF THE CB_1 CANNABINOID RECEPTOR

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In contrast to the 1st and 2nd intracellular loops (I1 and I2), I3 has a relatively long amino acid sequence and is important for the activation of the coupled G-protein, in particular Gαi1 or Gαi2 (Mukhopadhyay et al. *Mol. Pharmacol.* **2000**, *57*, 162). Thus, determining I3 conformation is crucial for examining the mechanism of G-protein activation. From recent NMR studies on the I3 peptide (Ulfers et al. *Biochemistry* **2002**, *41*, 11344; Ulfers et al. *Protein Sci.* 2002, *11*, 2526), a possible biological conformation of I3 has been reported. However, this NMR-determined 3D structure needs further validation. In the present study, we used a novel approach combining the following three sequential steps for assessing the structural suitability of this flexible loop region: 1) the consensus secondary structure was obtained by using various statistical programs in comparison with the reported NMR structure; 2) applying the secondary structure elements as the constraints, possible conformations were extensively sampled by simulated annealing (SA) simulations, some energetically plausible conformations were pre-screened; and 3) the best loop conformation was screened by the knowledge-based 3D-profiles (Lüthy et al. *Nature* **1992**, *356*, 83).

Our circular dichroism and NMR data have demonstrated that the 8th helix (H8) formed the helical structure only in SDS or phosphatidic acid micelles, with the formation of the 3_{10}helical structure being favored over the αhelical structure. An examination of the H8 sequence showed that the extent of the 3_{10}helical structural motif was associated with the positively charged amino acid residues. According to the x-ray structure of rhodopsin, the equivalent residues to these charged residues are pointing toward the intracellular side where G-proteins interact with the receptor, suggesting that the positively charged residues might be involved in charge-charge interactions with the polar solvents for the formation of the 3_{10}helical conformation. The pattern of the side chain conformation of the H8 in two distinct conformations (αand 3_{10}helical) was explored by MD simulations in aqueous solvent. It is likely that the inter-conversion between two structurally distinct α and 3_{10}helical conformations would be able to dictate different signals to the G-proteins or to turn on/off the signals.

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A CB_1 LIPID FACE V6.43A/I6.46A MUTATION COMPLETELY SEPARATES THE BINDING POCKETS OF SR141716A AND WIN55212-2 VS. AEA, CP55940 AND HU210: IMPLICATIONS FOR LIGAND ENTRY INTO CB_1

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We have identified two beta-branching residues (V6.43/I6.46) on the lipid face of transmembrane helix 6 (TMH6) in CB_1 that modeling studies suggest may be the initial interaction site for anandamide (AEA) with CB_1. To test if this interaction domain is specific for AEA or is a region that can interact with any alkyl chain, we undertook a series of MD lipid simulations in which AEA or its low affinity 20:2, n-6 congener (in combination with CB_1 TMH6) were simulated in a dioleoylphoshatidylcholine (DOPC) bilayer. A fully hydrated DOPC bilayer (28 waters/lipid) was built in order to generate a reasonable representation for the lipid bilayer. The calculations were run using an NPT (P = 1atm, T=310K) ensemble and the CHARMM27 set of all atom parameters designed for lipid simulations [Feller, S.E., et. al. *J. Am. Chem. Soc*., 74:2419 (**2002**)]. The ligand and TMH6 were placed in the equilibrated bilayer and each system was heated to 310K and equilibrated for 200ps using an NVT ensemble. The simulation then was switched to NPT ensemble conditions (P = 1atm, T=310K) and run for 6.0 ns. Simulations of AEA/TMH6 in DOPC revealed that the alkyl tail portion of AEA in its extended conformation can insert into the TMH6 V6.43 / I6.46 groove which is located near the bilayer center, but that the 20:2, n-6 congener of AEA (CB_1 Ki > 1500 nM) does not interact with this groove.

Based on these MD results, we then tested the importance of the V6.43/I6.46 groove to CB_1 recognition and activation via mutational analysis. A CB_1 V6.43A/I6.46A double mutant was stably transfected into HEK293 cells and the ligand binding and activation properties of the mutant were assessed. Using competition binding assays with [^{3}H]SR141716A, it was found that SR141716A and WIN55212-2 bound with near WT affinity to the mutant, but CP55940, HU210 and AEA were unable to compete for specific [^{3}H]SR141716A binding. In contrast, using competition binding assays with [^{3}H]CP55940, it was found that CP55940, HU210 and AEA bound with near WT affinity to the mutant, but SR141716A and WIN55212-2 were unable to compete for specific [^{3}H]CP55940 binding. These results suggest that the V6.43A/I6.46A mutation has separated the binding pocket for CP55940, HU210 and AEA from that of SR141716A and WIN55212-2. All agonists retained near WT efficacy at the V6.43A/I6.46A mutant. It is our hypothesis that the V6.43A/I6.46A mutation has "closed the door" for entry of AEA, CP55940 and HU210 into the CB_1 binding site crevice, but has left an "exosite" binding pocket for these ligands that stabilizes the CB_1 activated state. Modeling studies are currently underway to test this hypothesis.

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PHARMACOLOGICALLY DISTINCT ANTIDEPRESSANTS DIFFERENTIALLY REGULATE THE ENDOCANNABINOID SYSTEM

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While the mechanism of action of antidepressants is still not understood, it is becoming increasingly evident that their efficacy is related to more than just their actions on synaptic monoamine levels. Thus, research has accumulated implicating other systems, such as neuropeptides, signal transduction cascades, neurotrophins and transcription regulators, in the mechanism of action of antidepressants. The endocannabinoid (eCB) system has not been fully explored for a potential role in the pathophysiology of depression; however, given the very robust role this system appears to play in regulation of both emotionality and stress responsivity, investigation of how this system is modulated by antidepressants seems valid. In this study, we examined the effect of chronic treatment (21 days) with a variety of antidepressants on CB_1 receptor binding and endocannabinoid (eCB) content in the prefrontal cortex (PFC), hippocampus, hypothalamus and amygdala. Pharmacologically distinct antidepressants, fluoxetine (5 mg/kg; a selective serotonin reuptake inhibitor--SSRI), desipramine (10 mg/kg; a tricyclic antidepressant--TCA) and tranylcypromine (10 mg/kg; a monoamine oxidase inhibitor—MAOI), were employed to examine if common changes in the eCB system occurred following treatment with varying classes of drugs. It was found that fluoxetine had no effect on either CB_1 receptor binding or levels of the eCB's anandamide (AEA) or 2-arachidnoylglycerol (2-AG) in any brain regions measured, except for an upregulation of CB_1 receptor binding in the PFC. Desipramine on the other hand, had no effect on AEA or 2-AG in any brain region, but upregulated CB_1 receptor binding in the hypothalamus and induced a near-significant upregulation of CB_1 receptor binding in the hippocampus. Interestingly, tranylcypromine resulted in a significant downregulation of AEA, but not 2-AG, in the PFC, hippocampus and hypothalamus, with no effect in the amygdala. Additionally, tranylcypromine also induced an upregulation of CB_1 receptor binding in the PFC and hippocampus, but not the hypothalamus. This opposing effect of tranylcypromine and desipramine on the eCB system in the hypothalamus is of great interest given the role of eCB's in the regulation of the HPA axis, sleep cycles and feeding. The possible role of these differential effects of TCA's and MAOI's on the eCB system will be discussed with reference to specific depressive subtypes.

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EFFECTS OF ESTROUS CYCLE ON FAAH ACTIVITY IN MOUSE BRAIN

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The endogenous cannabinoid (eCB) system contributes to the modulation of anxiety and recent data suggest pharmacological potentiation of eCB signaling could represent a novel approach to the treatment of anxiety-related neuropsychiatric disorders. Several studies have explored the effects of cannabinoids on anxiety-like behaviors using the elevated plus-maze paradigm, however, differences in animal species and strain used, environmental test conditions, and the often limited pharmacological and dose-response relationships utilized by various laboratories, have made comparisons between studies difficult. As a result, incomplete and contradictory data exist regarding the effects of the eCB system on anxiety-like behaviors. In addition, there is little data available on the effects of indirect cannabinoid agonists, including eCB transport inhibitors, on anxiety-like behaviors. In the present study we explored the effects of wide dose ranges of direct and indirect cannabinoid agonists and cannabinoid antagonists on the anxiety-like behaviors using the elevated plus maze.

All experiments were carried out in male ICR mice (21-24g) under low light conditions. All animals were treated with drugs or corresponding vehicle 30 minutes prior to a 5 minute testing session. The time spent in the open arms (s), proportion of time spent in open arms over time spent in total arm exploration (% open time), and total number of arm entries (a measure of overall locomotor activity), were analyzed.

The direct CB_1 agonists CP55940, Win 55212-2, and Δ^9-tetrahydrocannabinol (THC), the fatty acid amide hydrolase (FAAH) inhibitor URB597, the mixed FAAH/eCB transport inhibitor AM404, and selective eCB transport inhibitor AM1172, and the CB_1 receptor antagonists SR141716 and AM251, were tested. CP55940 exhibited a bi-phasic effect; 0.001-0.03 mg/kg producing a significant anxiolytic effect, while 0.1 mg/kg was not significantly different from control. Win 55212-2 produced a significant, dose dependent anxiolytic effect between 0.3-3 mg/kg. In contrast, THC produced a significant, dose-dependent anxiogenic effect between 0.25-10 mg/kg. URB597 produced a significant, dose-dependent anxiolytic effect between 0.03-0.3 mg/kg. AM404 produced a significant, dose-dependent anxiolytic effect between 0.3-3 mg/kg. In contrast, AM1172 had no significant effect on anxiety-like behaviors. Lastly, both SR141716 and AM251 produced significant, dose-dependent anxiogenic effects between 1-10 mg/kg.

These data indicate that under our testing conditions, enhanced eCB signaling reduced anxiety-like behaviors and blockade of CB_1 receptors produces anxiogenic effects. Furthermore, these data indicate that inhibition of FAAH activity is critical to the anxiolytic profile of indirect cannabinoids. These data provide further evidence that pharmacological inhibition of FAAH activity, which is less likely to precipitate paradoxical anxiety than direct CB_1 agonists, could provide an effective approach to the treatment of anxiety-related neuropsychiatric disorders.

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CANNABICHROMENE REDUCES THE ANXIOGENIC EFFECT OF THC IN MICE

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The clinical use of THC has yielded significant benefits along with some challenges. Long-term marijuana clinical use is sometimes associated with anxiety and panic attacks in clinical populations as well as in the general community (Agosti & Levin, **2002**; Strike, Urbanoski & Rush, **2003**; Thomas, **1996**; Tunving, **1985**; Williamson & Evans, **2001**). Although in recent clinical trials using cannabis based medical extracts, very few anxiety or panic attacks were reported in patients with Multiple Sclerosis and neuropathic pain (Wade et al., **2003**).

Materials and Methods: The purpose of the first experiment was to verify the selection of an animal model that would be sensitive to the anxiogenic properties of THC. The rodent model of avoidance to light was selected because it relies on the rodent's natural anxiety response to light and does not use of aversive stimuli which might be confounded by the antinociceptive properties of the cannabinoids. In this model mice are placed in a two compartment box, one side is black (0 lux) and the other side is white and brightly lit (320 lux). Since rodents fear brightly lit areas, the latency to enter the white compartment is a measure of anxiety. Eighty C57BL6yj mice were randomly assigned to one of five treatment groups. These groups were vehicle, picrotoxin (a known anxiogenic, 2 mg/kg/0.01 cc/g body weight), THC (4 mg/kg/0.01 cc/g body weight), or the cannabinoid-cannabichromene (CBC) (40 mg/kg/0.01 cc/g body weight). In the second experiment we tested the effects CBC on THC induced anxiety. In the second experiment, fifty-four C57BL6yj mice were assigned to one of three treatment groups. These groups were vehicle, THC (4 mg/kg/0.01 cc/g body weight) and THC+CBC (4 mg/kg/0.01 cc/g body weight THC + 40 mg/kg/0.01 cc/g body weight CBC)

Results: For experiment 1: Both picrotoxin and THC were anxiogenic as indicated by increased latencies to enter the white compartment, while CBC had no effect.

Results: For experiment 2: Analysis indicated that THC significantly increased the emergence latencies. A 4 mg/kg dose of THC was found to increase anxiety without inhibiting movement. THC no longer produced a significant anxiogenic effect if 40 mg/kg of CBC was added to the THC extract.

Discussion: This is the first report that CBC has an anxiolytic effect. Our goal was to reduce the anxiogenic side effect of THC. This appears to have been accomplished in this study. It is unlikely that higher dosages of CBC would increase the effectiveness since we have found that as one approaches 80 mg/kg CBC inhibits movement which would appear to be an increase in anxiety on the test used here (unpublished observations). Thus, since CBC blocks or reduces THC's undesirable effects then it is also possible that CBC combined with THC might improve treatment outcomes in patients with various conditions, such as chronic pain and Multiple Sclerosis. These questions will need to be resolved in future studies.

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AM1241 SLOWS DISEASE PROGRESSION IN A MOUSE MODEL OF ALS

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Effective treatment for amyotrophic lateral sclerosis (ALS) remains elusive. Two of the primary hypotheses underlying motor neuron vulnerability are susceptibility to excitotoxicity and oxidative damage, including inflammatory damage due to microglial activation. We have previously found that Δ^9-tetrahydrocannabinol (THC) inhibits both excitotoxic and oxidative damage in spinal cord cultures and slows progression and improves survival in the ALS mouse model (*hSOD*^{G93A} transgenic mice) even when administered after onset of disease signs (Raman et al, Amyotroph Lateral Scler Other Motor Neuron Disord. 5: 33-39, **2004**). AM1241 is a CB_2 selective agonist that has been shown to be effective in models of inflammation and hyperalgesia. Thus, we evaluated the efficacy of AM1241 in the ALS mouse model.

Mice were treated daily beginning on day 75 (when tremors were first observed) with 1 mg/kg AM1241 or vehicle. Three conditions of ALS, the loss of motor function, paralysis scoring and survival time, were analyzed using a mathematical model. We can accurately estimate the age at which muscle endurance has declined by 50% and the predicted survival. Loss of motor function (as assessed by performance on a rotarod) was delayed by 12.5 days in male mice by AM1241. In female mice, AM1241 extended performance by 3 days. Paralysis was scored on a scale from 5 to 0; 5 is healthy, 1 is paralysis. AM1241 extended by 4.1 ± 1.7 days the time to reach a score of 2.5. AM1241 did not affect survival (129.8 ± 1.7 days, vehicle; 129.1 ± 7.0 days, AM1241, n =16). As AM1241 was well tolerated by the animals, CB_2-selective cannabinoids may be the basis for developing new drugs for the treatment of ALS.

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FACILITATION OF EXTINCTION LEARNING VIA FAAH INHIBITION

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A growing body of evidence suggests that the endocannabinoid system may play a facilitative modulatory role on extinction processes. Specifically, CB_1 knockout mice, or mice treated with the CB_1 antagonist SR141716 (SR), display attenuated extinction learning in several different paradigms (e.g. Marsicano et al., **2002**; Varvel et al., **2004**). We have recently investigated the ability of the FAAH inhibitor OL-135 to modulate performance in the Morris water maze (see Varvel et al., **2004** for general water maze methods). Mice pretreated with 30 mg/kg OL-135 (1 hour before the start of each session, i.p.) acquired the task faster than vehicle treated animals as assessed by path lengths ($F[1,133] = 4.9$, $p < 0.05$), while performance during a probe trial conducted the day after the last acquisition session showed no differences. Next, a series of weekly extinction probe trials were conducted. Importantly, mice treated with 30 mg/kg OL-135 extinguished significantly faster than did vehicle treated mice, as assessed by latency to target ($F[1,85] = 3.6$, $p < 0.05$). In parallel experiments, mice treated with 3 mg/kg SR (30 minutes before each session, i.p.) demonstrated the opposite effect and significantly attenuated extinction compared to vehicle treated mice, as assessed by latency to target ($F[1,65] = 8.6$, $p < 0.05$) and target entries ($F[1, 65] = 8.1$, $p < 0.05$). This experiment extends our previously published work with SR in the water maze by demonstrating that the same effect is seen when weekly extinction trials are employed (compared to biweekly trials – Varvel et al., **2004**). Interestingly, mice coadministered with 30 mg/kg OL-135 and 3 mg/kg SR acquired and extinguished this spatial task in a manner indistinguishable from vehicle controls. The OL-135 + SR group extinguished significantly slower than the OL-135 alone group as assessed by latency to target ($F[1,95] = 9.9$, $p < 0.01$) and target entries ($F[1,95] = 9.4$, $p < 0.01$), consistent with the hypothesis that the effects of OL-135 are mediated via increased endocannabinergic activity at CB_1 receptors. In order to separate effects of OL-135 on acquisition from those on extinction learning, mice were trained to locate the hidden platform without any drug treatments. The day after the last acquisition session mice received either a dose of 30 mg/kg OL-135 or vehicle and performed an extinction probe trial. No differences in latency to target were observed, indicating that that there were no effects on performance or retention of this task. However, when tested a week later (their second extinction trial) OL-135 treated mice had significantly higher latencies than did vehicle-treated mice ($t[11] = 2.3$, $p < 0.05$). Thus, under the influence of OL-135 a single extinction trial was sufficient to extinguish this learned response. Taken together, these experiments support the hypothesis that endocannabinoids acting via CB_1 receptors serve to positively modulate extinction learning, and that these processes may be enhanced by treatment with OL-135. Thus FAAH inhibition may represent a novel treatment strategy for a variety of behavioral disorders in which extinction of inappropriate behaviors is desirable.

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DIFFERENTIAL EFFECTS OF REPEATED THC EXPOSURE ON LONG- AND SHORT-TERM SYNAPTIC PLASTICITY IN THE HIPPOCAMPUS

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Memory deficits produced by marijuana are thought to arise, in part, via the interaction of the psychoactive component, Δ^9-tetrahydrocannabinol (Δ^9-THC), with cannabinoid receptors located in the hippocampus. Thus, acute cannabinoid exposure reduces glutamate release onto CA1 pyramidal neurons, and blocks hippocampal long-term potentiation (LTP), a putative synaptic correlate of memory. Despite this well-established effect of acute Δ^9-THC exposure the consequences of prolonged exposure to this drug on hippocampal physiology are poorly understood. Therefore, we examined effects of repeated cannabinoid exposure on a variety of physiological measures of hippocampal function in brain slices obtained from rats 24 hours following 7 consecutive daily i.p. injections of Δ^9-THC (10 mg/kg). Although baseline measures of glutamatergic and GABAergic synaptic transmission in CA1 pyramidal neurons were unchanged in slices from chronically treated animals, relative to vehicle-treated controls, chronic Δ^9-THC exposure produced tolerance to the ability of the cannabinoid agonist WIN55,212-2 to presynaptically inhibit GABAergic, but not glutamatergic synaptic transmission.

High-frequency (100 Hz, 1 sec x 3) electrical stimulation generated robust LTP of glutamatergic field EPSPs in control hippocampal brain slices, but LTP was absent following chronic exposure to Δ^9-THC. Furthermore, the Δ^9-THC-induced impairment of LTP was prevented by daily in vivo pretreatment with the cannabinoid antagonist AM251 (2 mg/kg, ip). Long-term depression (LTD), generated by low frequency (1Hz, 15 min) activation of glutamatergic synapses was significantly larger in hippocampal brain slices obtained from naive controls that in the chronic Δ^9-THC group. Unlike the impairment of these forms of long-term synaptic plasticity by chronic Δ^9-THC, the endocannabinoid-dependent short-term inhibition of GABAergic synaptic transmission caused by transient depolarization of CA1 pyramidal neurons (depolarization-induced suppression of inhibition, DSI) was unaffected by this treatment. These data suggest that repeated exposure to Δ^9-THC impairs long-term hippocampal plasticity (LTP and LTD), but does not affect DSI, a form of endocannabinoid-dependent short-term plasticity. These changes may contribute to memory impairments seen in humans following chronic marijuana use.

Δ^9-THC EFFECTS ON INFORMATION PROCESSING IN HUMANS

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Background: The mechanisms by which cannabinoids produce transient perceptual alterations, psychotomimetic effects, and impairments in memory and attention that resemble the aspects of schizophrenia, remain unclear. Perceptual, memory and attentional functions are based on distributed processes that are believed to be "bound" together by synchronous high frequency oscillatory activity. In humans, macroscopic neural synchrony can be evaluated noninvasively by entrainment of the EEG to sensory (auditory or visual) stimuli presented at various frequencies. The brain acts as a tuned oscillator, and the steady-state EEG waveform entrains to the frequency and phase of the presented stimulus, providing an indicator of the functioning state of the neural circuits supporting synchrony. Schizophrenia patients show evidence of impairments in neural synchrony and some studies suggest correlations between some clinical symptoms and alterations in neural synchrony. Preclinical studies demonstrate that cannabinoids via effects on GABA release disrupt neural synchrony in the gamma range. Further, cannabis users also show evidence of altered neural synchrony. Similarly, impairments in attention which are central to schizophrenia, are reflected in deficits in the P300 event related potential (ERP) associated with oddball task performance. Deficits in attention one of the most consistent effects of cannabis, can also be studied using the P300 oddball paradigm. However, the existing literature on cannabis effects in humans is mainly derived from comparisons of cannabis users/abusers with controls, which while informative, are confounded by several factors including premorbid differences, comorbidities, other substance use, etc. Some of these limitations can be addressed in laboratory studies of the acute effects of Δ^9-THC in carefully screened healthy volunteers.

Hypothesis: Δ^9-THC, will reduce neural synchrony, and amplitudes of target P3b and novel P3a in healthy individuals, in a dose related manner. The changes in neural synchrony induced by Δ^9-THC will correlate with changes in perception, memory and attention.

Methods: Carefully screened healthy individuals completed 3 test days during which they will receive (0, 0.015, 0.03 mg/kg) Δ^9-THC in a double-blind, randomized, counterbalanced design. Indices of neural synchrony were assessed by measurement of EEG spectral power evoked by presentation of auditory click trains at 20, 30 and 40 hz. The amplitude and latency of the ERP P300 components associated with the allocation target P3b and orientation novelty P3a to auditory and visual oddball tasks were measured. Finally, assessments of psychosis, perceptual alterations, short-term memory, working memory and sustained attention were also assessed.

Preliminary results: Δ^9-THC transiently 1) reduced spectral power evoked by entrainment to 20 and 30Hz stimulation on an auditory entrainment task, 2) reduced both target P3b and novelty P3a amplitude, and 3) reduced N100 amplitude. Δ^9-THC also reduced 1) immediate recall, 2) working memory, and 3) vigilance, while increasing scores on scales of psychosis and perceptual alterations.

CB_1 RECEPTOR ACTIVATION INCREASES FOS EXPRESSION IN A SUBSET OF BRAIN REGIONS IMPORTANT FOR ZEBRA FINCH VOCAL LEARNING AND PRODUCTION

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We have previously found distinct CB_1 cannabinoid receptor expression in brain regions important to zebra finch vocal learning and production [1,4]. These receptors are likely functionally important as exposure to the cannabinoid agonist WIN55212-2 (WIN, 1 mg/kg) during zebra finch vocal development alters song learning [2,3]. Because cannabinoid receptors are expressed in many relevant brain regions, we are interested to know which of these are involved in cannabinoid-altered song learning. To begin to address this question we have studied expression of the product of the immediate early gene, c-fos (FOS) as a function of WIN exposure. Immunohistochemistry reveals distinct expression in a subset of song-related brain regions that show distinct CB_1 expression. These regions include; the higher vocal center (HVC), the robust nucleus of archopallium (RA), and the caudal tip of hippocampus. Regions with distinctly high CB_1 expression that demonstrate little WIN-induced FOS expression include Area X, lMAN, the primary auditory field L2 and cerebellum. Our results demonstrate that distinct agonist responses occur across CB_1-expressing brain regions. These results also suggest that cannabinoid-altered vocal learning may involve changes in neuronal activity at the level of HVC and RA. Additional developmental experiments are planned to test this hypothesis.

[1] Soderstrom, K. and Johnson, F., Brain Research, 857 (**2000**) 151-157.
[2] Soderstrom, K. and Johnson, F., Brain Res Dev Brain Res, 142 (**2003**) 215-7.
[3] Soderstrom, K. and Tian, Q., Brain Res Dev Brain Res,, 153 (**2004**) 225-232.
[4] Soderstrom, K., Tian, Q., Valenti, M. and Di Marzo, V., J Neurosci, 24 (**2004**) 10013-21.

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CB_1 IMMUNOREACTIVITY IN THE DEVELOPING SONGBIRD TELENCEPHALON IS INFUENCED BY FOOD AVAILABILTY

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Rehearsal of learned song by male zebra finches shows acute regulation by the availability of food. Recent data suggest that endocannabinoids may mediate this environment/behavior interaction; food removal decreases singing and increases 2-arachidonyl glycerol levels in telencephalon whereas treatment with SR141716A rescues song production following food removal (Soderstrom et al., **2004**). Here, we report that manipulating the timing (but not the amount) of daily feeding in juvenile male zebra finches reduces vocal rehearsal and is associated with impairment of song learning as well as a striking decrease in CB_1 immunoreactivity in brain regions controlling song. Birds were raised by their parents up to 35 days of age when they were housed singly in visual but not auditory isolation. One group of juvenile males (n=5) was then placed on a daily regimen of Timed Access to Food (TAF), where food was only available during the last 6h of a 14h light phase; under these conditions birds maintained normal food intake and body mass, but vocal rehearsal decreased by more than two-thirds. Another group of free-feeding juveniles (n=5) served as control. All birds were recorded weekly to follow vocal development until they reached adulthood (>120 d/o). Examination of final recordings indicated that TAF was associated with profound impairment of note learning and note sequencing. Analysis of brain sections from TAF birds revealed reduced CB_1 immunoreactivity in all song regions of the telencephalon, whereas CB_1 labeling in the cerebellum was not different from free-feeding controls. Soderstrom et al. (**2004**) found that food removal specifically increased endocannabinoid levels in the telencephalon (with no effect in the cerebellum) so one possibility is that reduced CB_1 immunoreactivity in the telencephalon reflects desensitization due to chronic endocannabinoid release elicited by the daily feeding manipulation. Our data demonstrate that the development of brain CB_1 expression is sensitive to feeding state and we suggest that CB_1 may play an important role in mediating the well-known effects of the environment on cognitive and behavioral development.

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THE ENDOCANNABINOID SYSTEM IN UNICELLULAR EUKARYOTES

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The endocannabinoid system is a ubiquitous lipid signaling system with important regulatory functions throughout the body in vertebrates. Its multiple roles for some invertebrates have been also shown. Many of the actions of the most studied endocannabinoid, anandamide (AEA), are mediated by binding and activation of the cannabinoid receptors CB_1 and CB_2. AEA appears to be produced in a stimulus dependent manner by the consecutive action of two enzymes, a calcium dependent transacylase (for the production of N-acyl-phosphatidylethanolamine, NAPE) and a phospholipase D (NAPE-PLD) for the liberation of AEA. We have shown that the unicellular eukaryote *Tetrahymena pyriformis* is able to rapidly take up and metabolize [^{3}H]AEA and contains a FAAH-like activity with characteristics similar to the mammalian enzyme: anandamide hydrolysis followed Michaelis–Menten kinetics and western blot analysis, using an anti-FAAH polyclonal antibody, showed the presence of a 66kDa immunoreactive band. In the present study we demonstrate that *Tetrahymena* pyriformis contains endogenous anandamide and its basic levels were determined at 2.5 ± 0.5 pmol/mg lipid. Furthermore, *Tetrahymena* cell homogenate was found to contain NAPE-PLD activity (8.04 ± 1.60 pmol/min*mg protein). In addition, preliminary experiments suggest the presence of cannabinoid receptors (western blot analysis, using CB_1 and CB_2 receptor polyclonal antibodies), as well as the presence of MAG lipase activity. The physiological significance of the endocannabinoid system in *Tetrahymena* is not known. Nevertheless, this study provide further evidence for the existence of the endocannabinoid signaling system in lower eukaryotes and suggests the importance of this signaling system throughout the evolution.

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CANNABIMIMETIC EFFECTS OF ELEVATED SERUM ANANDAMIDE LEVELS FROM OSTEOPATHIC MANIPULATION

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Cognitive and mood-altering effects may be elicited by endogenous cannabinoids when they activate cannabinoid receptors in the brain. Analogous mood-altering effects may be elicited by osteopathic manipulative treatment (OMT), including anxiolysis, analgesia, sedation, even euphoria. The founder of osteopathy, Andrew Taylor Still, MD, originally intended the system to be a drugless school of medicine, "Man should study and use the drugs compounded in his own body." Still hypothesized in **1899** that manipulative treatment stimulated the production of endogenous compounds that promoted homeostasis and healing. Previous research in the **1970**s and **1980**s indicated the endorphin system is not responsible for OMT's mood-altering effects.

A randomized, double-blind, controlled clinical trial measured pre- and post-treatment serum levels of anandamide (AEA), 2-arachidonoylglycerol (2-AG), and oleylethanolamide (OEA) in 31 healthy subjects that received OMT or control (light physical contact). Serum levels were determined by chemical ionization gas chromatography/mass spectrometry. Subjects were also measured with a neuropsychological questionnaire, the Drug Reaction Scale (DRS). The DRS has been used previously to measure the cannabimimetic effects of marijuana and THC.

In subjects receiving OMT, post-treatment AEA levels (mean 8.01 pmol/ml) increased 168% over pre-treatment levels (2.99 pmol/ml), with no changes in control subjects. OEA levels decreased 27% in post-OMT subjects, with no changes in control subjects. No changes occurred in 2-AG levels. In the DRS questionnaire, subjects receiving OMT recorded highly significant increases in DRS cannabimimetic descriptors *good, high,* and *light-headed,* and significant increases in *hungry* and *stoned*, with significant decreases in *sober, inhibited,* and *uncomfortable.*

When changes in serum AEA were correlated with changes in DRS scores, increased AEA significantly correlated with increased feelings of *rational* and *cold,* and decreased sensations of *paranoid, bad* and *warm.*

We propose that healing modalities popularly associated with changes in the endorphin system, such as OMT, chiropractic, acupuncture, massage, and meditation, may actually be mediated by the endocannabinoid system – a widespread but heretofore unrecognized therapeutic phenomenon.

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ANANDAMIDE TRANSPORT AND HYDROLYSIS AS TARGETS FOR THE TREATMENT OF NEUROPATHIC PAIN: EFFECT OF AM404 AND URB597 IN RATS WITH CHRONIC CONSTRICTION INJURY OF THE SCIATIC NERVE

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Many studies have suggested that the endocannabinoid system may operate at multiple levels, both central and peripheral, to mitigate responses to a variety of acute and chronic nociceptive stimuli. Although CB_1 agonists have long been considered as potential drugs for the treatment of pain, these compounds also produce substantial psychotropic effects that limit their clinical utility. The main aim of this work has been to target components of the endocannabinoid system to obtain relief of pain without adverse psychotropic effects. We adopted two different powerful strategies to upregulate endocannabinoid tone: the pharmacological inactivation of FAAH, by employing URB597, and the inhibition of anandamide (AEA) intracellular uptake, by using AM404. The antihyperalgesic effects of these compounds have been tested in a widely used animal model of neuropathic pain: the chronic constriction injury of the sciatic nerve in the rat (CCI). Both drugs have been daily administered to rats starting from the day after the surgical procedure. AM404 was subcutaneously injected at the dose of 10mg/kg, while URB 597 was intraperitoneally given at the doses of 3 and 10 mg/kg. Repeated administration with AM404 resulted in a relief of both thermal hyperalgesia (assessed by plantar test) and mechanical allodynia (assessed by Randall-Selitto test) which reached the maximum after 7 days. The dose employed did not affect the pain response of the contralateral paw and it was ineffective when acutely given to CCI rats. We also evaluated whether this AM404-induced antihyperalgesic effect was accompanied with alteration in the production of some mediators known to be increased during the development and maintenance of neuropathic pain. AM404 abolished the increase in nitric oxide production and brought the expression of iNOS and nNOS down to the level of control animals. Furthermore, it induced the restore of the physiological level of the proinflammatory and pronociceptive cytokine TNFα together with a significant enhancement of the anti-inflammatory interleukin-10. All these effects are associated to the inhibition of the activation of the transcription factor NF-kB which induces the transcription of genes encoding for both NOS enzymes and cytokines. Since AM404, as many other AEA reuptake inhibitors, has also been shown to interact with either FAAH or the TRPV1 receptor, studies are in progress to characterize whether the antihyperalgesic effect of this compound can be mediated via CB_1, CB_2 and/or TRPV1 receptors. The repeated administration of FAAH inhibitor, URB597, led to a dose-dependent antihyperalgesic effect which was obtained only after 14 days of treatment. Furthermore, URB597 administration seemed to increase the nociceptive thresholds of the contralateral paw.

All together these findings suggested that the increase in the endocannabinoid tone, by modulating AEA uptake and metabolism can induce pain relief and highlighted these processes as drug targets for compounds useful in the treatment of chronic pain states, such as the neuropathic one.

FAAH INHIBITION ELEVATES ENDOCANNABINOID LEVELS IN THE PERIAQUEDUCTAL GREY OF THE RAT: DUAL EFFECT ON NOCICEPTION VIA CB_1 AND TRPV1 RECEPTORS

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Systemic administration of fatty acid amide hydrolase (FAAH) inhibitors elevates the brain levels of anandamide and results in cannabinoid CB_1 receptor-mediated analgesia (Lichtman et al., J. Pharmacol. Exp. Ther. **2004**). Anandamide also affects pain transmission by acting at vanilloid TRPV1 receptors (Di Marzo et al., Curr. Opin. Neurobiol., **2002**). Periaqueductal grey (PAG) neurons projecting to the rostral ventromedial medulla (RVM) play an important role in setting the threshold for pain sensitivity. Intra-PAG glutamate release may cause both anti-and pro-nociceptive responses by activating – via PAG output neurons – "OFF" and "ON" neurons in the RVM respectively, and is under the negative and positive control by CB_1 and TRPV1 receptors, respectively. We studied in healthy rats the effect of intra-PAG injections of the selective FAAH inhibitor, URB-597, on: 1) the nocifensive response in the "plantar test"; 2) PAG levels of anandamide and 2-arachidonoylglycerol (2-AG); and 3) the firing activity of "OFF" and "ON" neurons in the RVM of anaesthetized rats.

URB-597 was injected by stereotaxis into the dorsal lateral PAG at different doses, alone or together with the TRPV1 and CB_1 receptor antagonists, capsazepine and AM251. The two agonists of TRPV1 and CB_1 receptors, capsaicin and WIN55,212-2, were also studied. PAG endocannabinoid levels were quantified by isotope dilution LC-MS. Extracellular recordings were made from single neurons in the RVM with glass insulated tungsten filament electrodes (3-5 MΩ) using the following stereotaxic coordinates (Paxinos and Watson, 1997): 2.8-3.3 mm caudal to lambda, 0.4-0.9 mm lateral and 8.9-10.7 mm depth, and were amplified and displayed on analogic and digital storage oscilloscopes.

We found that URB-597 either suppresses or enhances thermal nociception in the "plantar test" depending on the dose and the time from administration. A low dose (0.25 μg) of URB-597 caused immediate nociception, which, on co-administration with AM251, was transformed into a capsazepine-sensitive analgesic effect. An intermediate dose of URB-597 (1.25 μg) caused a bi-phasic response, with immediate analgesia followed by delayed nociception. The former effect was erased by capsazepine, whereas the nociceptive phase was again transformed into an analgesic effect by AM251. Accordingly, capsaicin (6 nmol) and a low dose of WIN55,212-2 also suppressed or enhanced nociception, respectively. The bi-phasic effects of URB-597 correlated with the dose-and time-dependent enhancement of PAG anandamide or 2-AG levels, which likely results in either TRPV1 or CB_1 activation, respectively. The TRPV1-mediated antinociception of URB-597 correlated with enhanced activity of RVM "OFF" neurons, suggesting that it occurred via the stimulation of glutamate release from PAG neurons. Finally, at the highest dose tested, URB-597 (2.0 μg) and WIN55,212-2 (40 nmol) exerted a typical CB_1-mediated analgesia.

This study demonstrates for the first time that endocannabinoids affect the descending pathways of pain control by acting at both CB_1 and TRPV1 receptors under physiological conditions

ENHANCEMENT OF MORPHINE- AND TRAMADOL-INDUCED ANTINOCICEPTION BY THC IN DIABETIC RODENTS

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We have previously demonstrated that synergy is observed between morphine and THC in the expression of antinociception in acute pain models such as the tailflick test and in arthritic models of chronic pain. Our data have been extended to include both acute and neuropathic pain in diabetic rats and mice. Mice were rendered diabetic (blood glucose levels greater than 450 mg/dl) 7 to 14 days following the i.p. injection of 75 mg/kg streptozocin which selectively destroys pancreatic beta cells. Δ^9-THC p.o. was more potent, but equally efficacious, in the tailflick test in the diabetic mouse than in the non-diabetic mouse (ED50's + 95% CLs of 55.3 [32 - 96] versus 210 [105 – 421] mg/kg, respectively). Conversely, morphine s.c. was less potent in diabetic mice than in non-diabetic mice (6.1 [5.1 – 7.2] versus 3.2 [2.4 – 4.1] mg/kg, respectively), an effect previous extensively documented in preclinical and clinical testing. Tramadol, considered to be a drug of choice for the treatment of diabetic neuropathic pain, was more potent in diabetic mice than non-diabetic mice in the tailflick test (46 versus 86 mg/kg, p.o.), respectively. An inactive dose of THC (20 mg/kg, p.o.) produced a 2-fold enhancement of morphine-induced antinociception in non-diabetic mice in the tail-flick test, but produced a 4-fold enhancement of morphine-induced antinociception in diabetic mice in the tailflick test. In addition, THC (20 mg/kg, p.o.) significantly enhanced the antinociceptive effects of tramadol by nearly 4-fold in non-diabetic mice. In the streptozocin-induced rat model of diabetic allodynia, maximal pressure (using von Frey filaments to induce paw withdrawal) in the non-diabetic rat was 67.9 gms of force versus 3.7 gms of force in the diabetic rats. A combination of inactive doses of THC and morphine raised the threshold for withdrawal in the diabetic rats to 55.7 gms of force. Subsequent work determined that the effects of THC and morphine were synergistic in reducing allodynia in the diabetic rats. These studies indicate that pain in diabetic animals responds well to treatment with THC alone and further, that the combination of THC with morphine produces a synergistic relief of acute pain and allodynia in mice and rats, respectively, which is greater in magnitude than in normal mice. Extrapolation of such studies to the clinical setting may indicate the potential for use of THC-like drugs in the treatment of diabetic neuropathic pain, alone, or in combination with very low doses of opioids.

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INHIBITION OF NUCLEOSIDE UPTAKE IN MICROGLIA BY THE PLANT-DERIVED CANNABINOIDS THC AND CBD

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Microglia are the resident immune cells of the central nervous system, and respond to neuronal damage or infection by proliferating and migrating to the site of injury. We examined the effects of cannabinoids on the microglial proliferation by measuring incorporation of [^{3}H]thymidine into a murine microglial cell line. We found that the plant-derived cannabinoids Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) potently inhibit [^{3}H]thymidine incorporation, independent of cannabinoid receptors. Derivatives of these plant-derived cannabinoids were also tested for their ability to inhibit [^{3}H]thymidine incorporation, and a distinct structure-activity profile was determined. While this profile implies a specificity of action, the decrease in [^{3}H]thymidine incorporation did not correspond with a decrease in reduction of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrasodium bromide tetrazolium (MTT) or a decrease in the number of cells in S, G_2, or M phase, suggesting that microglial proliferation remains normal while the amount of [^{3}H]thymidine taken up is reduced.

Given these data, we examined the effects of THC and CBD on [^{3}H]thymidine uptake into microglial cells. Treatment with 500 nM THC decreased [^{3}H]thymidine uptake into whole cells, at time periods from 1 minute to 4 hours. THC, CBD, and cannabinoid analogues all inhibited [^{3}H]thymidine transport, and did so with almost identical potency as measured in [^{3}H]thymidine incorporation "proliferation" experiments. As a result, we conclude that, rather than inhibiting microglial proliferation, THC and other plant-derived cannabinoids in fact inhibit thymidine uptake. As similar transporters mediate thymidine and adenosine transport into cells, we also examined effects of THC on adenosine transport into EOC-20 microglia. THC decreased uptake of [^{3}H]adenosine to a similar extent as [^{3}H]thymidine, suggesting that it acts upon a common transporter. There are two basic types of nucleoside transporters: concentrative (CNT), which are sodium-dependent but insensitive to the drug 6-(4-nitrobenzyl)-thio-9-ß-*d*-ribofuranosylpurine (NBMPR), and equilibrative (ENT), which are sodium-independent but blocked by NBMPR. As inhibitory effects of THC are additive with sodium-free buffer but not with nanomolar concentrations of NBMPR, this suggests that THC acts at the ENT1 transporter to decrease uptake of nucleotides into microglia. Because uptake of adenosine is a primary mechanism of terminating adenosine signaling and adenosine transporter inhibitors can have agonist-like effects, this raises the possibility that THC and CBD can enhance adenosine signaling by decreasing its uptake into cells.

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THC MODULATES THE ABILITY OF HUMAN DENDRITIC CELLS TO STIMULATE PRIMARY AND RECALL T CELL RESPONSES

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We previously reported that human monocytes express CB_2 and that exposure to THC altered their capacity to differentiate into dendritic cells (DC) when cultured for 7 days *in vitro* with GM-CSF and IL-4. THC produced a dose-dependent decrease in the expression of MHC molecules (HLA-DR) and co-stimulatory molecules including CD40, CD80, and CD86. DC prepared in the presence of THC produced normal amounts of IL-10, a cytokine associated with activation of T-helper type 2 (Th2) cells, but were impaired in their capacity to secrete IL-12, a cytokine associated with activation of T-helper type 1 (Th1) cells. We hypothesized that these changes in DC differentiation produced by THC would impact on their ability to stimulate antigen-specific T cell responses. DC generated in the presence of THC (THC-DC) were compared to control DC for their ability to take up antigen in a receptor-mediated fashion. THC-DC showed little to no uptake of FITC-dextran, while 100% of the control DC demonstrated robust uptake. THC-DC were tested for their ability to stimulate recall antigen T cell responses to Hepatitis B surface antigen (HBsAg). DC generated in the presence or absence of 500 ng/ml THC were pulsed with 40 μg/ml HBsAg and used to stimulate autologous T cells from HBV-immunized donors in a tritiated thymidine incorporation assay. Control DC pulsed with HBsAg stimulated the proliferation of antigen-specific T cells in a dose-dependent manner, which was enhanced by the addition of 2 ng/ml IL-7. Depletion of $CD45RO^+$ T cells prior to DC stimulation ablated the proliferative response, indicating that memory T cell populations were required for the antigen-specific T cell response to HBsAg. In contrast, THC-DC stimulated a poor antigen-specific T cell response, which was only minimally improved by the addition of IL-7. As a measure of the effect of THC exposure on primary T cell responses, DC generated in the presence or absence of 500 ng/ml THC were used to stimulate allogeneic naïve T cells in a mixed leukocyte reaction (MLR). $CD45RA^+$ T cells were labeled with CFSE, cocultured with DC for 5 days, and collected for analysis by FACS. In contrast to control DC, THC-DC were deficient in their ability to induce naïve T cell proliferation, and failed to promote the normal shift from CD45RA (naïve) to CD45RO (memory) T cell populations. The addition of SAC (as a DC maturation stimulus) and IL-7 (to promote T cell responses) partially corrected the deficiencies of THC-DC in the MLR. We conclude that exposure to exogenous THC impairs human monocyte differentiation into functional antigen-presenting DC. The results of these studies suggest that the cannabinoid receptor system plays an important role in regulating antigen presentation and T cell immunity.

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ENDOCANNABINOIDS AND PHYTOCANNABINOIDS INHIBIT HUMAN NEUTROPHIL MIGRATION

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The endocannabinoid anandamide binds to cannabinoid CB_1 and CB_2 receptors and has both analgesic and anti-inflammatory actions (Rice *et al*, 2002). The non-psychoactive cannabis constituent cannabidiol has low affinity for cannabinoid receptors yet it is an oral anti-arthritic therapeutic in murine collagen-induced arthritis (Malfait *et al*, **2000**). Neutrophils play a critical role in the development of inflammation. The aim of this study was to investigate the effect of cannabinoids on the migration of human peripheral polymorphonuclear neutrophils (PMNs).

Peripheral PMNs were isolated from normal whole blood by centrifugation over Polymorphprep™ and re-suspended at a concentration of $1x10^6$ $cellsml^{-1}$ in PBS containing $CaCl_2$ and $MgCl_2$. *In vitro* cell migration was investigated using a modified 48-well Boyden Chamber. PMNs were pre-incubated with vehicle (0.01% DMSO) or test compound for 30 min at 37ºC before loading into the upper wells. The lower wells contained the corresponding concentration of test compound and N-formyl-methione-leucine-phenylalanine (fMLP). After incubation for an additional 30 min in a 5% CO_2 atmosphere at 37°C, each well on the underside of the 3μm pore filter was stained using Diff-Quik and the cells counted in ten non-overlapping fields (x40) with a light microscope.

Anandamide significantly (one-way ANOVA; n = 8) inhibited neutrophil migration induced by fMLP (1μM). The fMLP-induced migration was -3.87 ± 14.5% ($P<0.001$), 56.5 ± 10.2% ($P<0.01$), 44.2 ± 7.4 % ($P<0.001$), 56.4 ± 10.2% ($P<0.01$), and 75.2 ± 10.1% of vehicle control in the presence of 100nM, 10nM, 1nM, 0.1nM and 0.01nM anandamide respectively. N-arachidonoyl dopamine (NADA), another endocannabinoid, and the phytocannabinoid cannabidiol also significantly (one-way ANOVA; n = 6) inhibited migration. The fMLP-induced migration was 28.7 ± 12.4% ($P<0.01$), 49.1 ± 17.8% ($P<0.05$), 56.8 ± 15.7% ($P>0.05$), 59.9 ± 15.3% ($P>0.05$), 61.3 ± 14.9% ($P>0.05$) of vehicle control in the presence of 100nM, 10nM, 1nM, 0.1nM and 0.01nM NADA respectively. The fMLP-induced migration was 18.1 ± 2.9% ($P<0.001$), 58.1 ± 4.5 % ($P<0.05$), 21.0 ± 3.7 % ($P<0.001$), 49.9 ± 2.4 ($P<0.05$) and 110 ± 13.9% ($P>0.05$) of vehicle control in the presence of 100nM, 10nM, 1nM, 0.1nM and 0.01nM cannabidiol respectively. The inhibitory effect produced by anandamide (100nM) was significantly (one-way ANOVA; n = 3) different in the presence of SR141716A (1μM) ($P<0.001$) and SR144528 (100nM) ($P<0.01$); the TRPV1 receptor antagonist, capsazepine (1μM) did not affect the compound ($P>0.05$). The inhibition produced by anandamide (100nM) was 57.6 ± 0.49%, 85.2 ± 4.93%, 32.8 ± 0.42%, 64.73 ± 0.86% of vehicle control in the presence of vehicle, SR141716A, SR144528, and capsazepine respectively. These data demonstrate that anandamide, NADA and cannabidiol inhibit migration of PMNs.

Malfait, A.M., Gallily, R., Sumariwalla, P.F., Malik, A.S., Andreakos, E., Mechoulam, R., & Feldmann. M. (**2000**) *Proc Natl Acad Sci* U S A. 97, 9561.
Rice, A.S.C., Farquhar-Smith, W.P. & Nagy, I. (**2002**). *Prostaglandins Leukotrienes and Essential Fatty Acids*, 66, 243.

PHYSIOLOGICAL ROLES OF 2-ARACHIDONOYLGLYCEROL AS AN ENDOGENOUS CB_2 RECEPTOR AGONIST

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2-Arachidonoylglycerol (2-AG) is an endogenous ligand for the cannabinoid receptors (Sugiura *et al*., (**1995**) *Biochem. Biophys. Res. Commun*. 215, 89; Mechoulam *et al*., (**1995**) *Biochem. Pharmacol*. 50, 83). Since 1995, we have focused on 2-AG and studied its physiological and pathophysiological significance in various mammalian tissues and cells. Recently, we found that 2-AG induces enhanced formation of chemokines such as IL-8 and MCP-1 in HL-60 cells and the migration of HL-60 cells differentiated into macrophage-like cells, human peripheral blood monocytes, natural killer cells and eosinophils. These results strongly suggest that 2-AG plays important stimulative roles in inflammatory reactions and immune responses. In the present study, we first examined the effect of 2-AG on morphology as well as the actin filament system in HL-60 cells differentiated into macrophage-like cells. We found that 2-AG induces rapid morphological changes such as the extension of pseudopods. We also found that 2-AG provokes a rapid actin polymerization in these cells. The actin polymerization induced by 2-AG was abolished when cells were treated with SR144528, a CB_2 receptor antagonist, and PTX, suggesting that the response was mediated by the CB_2 receptor and Gi/o. We also found that 2-AG induces augmented adhesion of macrophage-like cells via a CB_2 receptor- and Gi/o-dependent manner. It is possible that 2-AG plays physiologically essential roles in various inflammatory cells and immune-competent cells by inducing a rapid actin rearrangement. We then examined the effects of the topical application of 2-AG to mouse ear. We found that the application of 2-AG induced ear swelling. Importantly, 2-AG-induced ear swelling was abolished by treatment of the ear with SR144528, whereas AM251 exerted only a slight effect. Similar results were obtained for 2-AG ether, a non-hydrolyzable analog of 2-AG. In contrast to 2-AG and 2-AG ether, anandamide did not induce ear swelling. We then examined the effects of 2-AG on several intracellular signaling molecules. We found that the topical application of 2-AG to mouse ear induces augmented phosphorylation of p42/44 MAP kinase and p38 MAP kinase. We confirmed that the phosphorylation of p42/44 MAP kinase and p38 MAP kinase was markedly reduced when SR144528 was applied together with 2-AG. Finally, we examined the effects of L-NMMA and 7-nitroindazole, NO synthase inhibitors, on 2-AG-induced ear swelling. The application of L-NMMA and 7-nitroindazole markedly reduced ear swelling induced by 2-AG, suggesting that NO is closely involved in 2-AG-induced ear swelling. These results support our hypothesis that 2-AG plays important stimulative roles in inflammatory reactions and immune responses.

COMPETITIVE ANTAGONISM OF CB_1 RECEPTORS BY THE NOVEL IMMUNOSUPPRESSANT DRUG FTY720

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The immunosuppressant drug FTY720 (2-amino-2-(2-[4-octylphenyl]ethyl)-1,3-propanediol) prevents human transplant graft rejections and effectively suppresses a variety of autoimmune disorders in animals. In contrast to other immunosuppressive agents, FTY720 induces sequestration of lymphocytes in secondary lymphoid tissues without cytotoxicity or inhibiting growth potential. FTY720 is a sphingosine analogue, which when phosphorylated in vivo, can interact with four of the five sphingosine-1-phosphate (S1P) receptors. It is unclear, however, whether S1P-like activity can account for all of the in vivo function of FTY720. Because S1P receptors have homology with CB_1 and CB_2 cannabinoid receptors and because cannabinoid receptors are important in regulating immune function, we investigated the hypothesis that FTY720 interacts with cannabinoid receptors. The ability of FTY720 to compete for radioligand binding to CB_1 or CB_2 receptors was examined in cells heterologously expressing either receptor. To determine efficacy for activation of cannabinoid receptors by FTY720, its ability to stimulate or antagonize G-protein or effector activity was also examined. Results showed that FTY720 inhibited binding of [^{3}H]SR141716A and [^{3}H]CP55,940 to CB_1 receptors with modest affinity, but did not significantly inhibit [^{3}H]CP55,940 binding to CB_2 receptors. Interestingly, sphingosine, but not S1P, also inhibited radioligand binding to CB_1 receptors. Both FTY720 and sphingosine, but not S1P, antagonized WIN55,212-2 (WIN)-stimulated [^{35}S]GTPγS binding in CB_1 receptor-expressing cells in a concentration-dependent manner. Low μM concentrations of FTY720 or sphingosine also inhibited WIN-stimulated phosphorylation of extracellular signal-regulated kinases (ERK 1/2) and protein kinase B (PKB/Akt). Preincubation of cells with FTY720 or sphingosine also inhibited WIN-stimulated internalization of CB_1 receptors. The antagonism of WIN-stimulated G-protein activation by FTY720 or sphingosine was shown to be competitive, as indicated by their ability to produce rightward shifts in the WIN concentration-effect curve. These results indicate that FTY720 and sphingosine act as competitive CB_1 antagonists with moderate affinity. These results imply that treatment with FTY720 could antagonize CB_1 receptors in the immune system and in the CNS, because FTY720 can cross the blood-brain barrier. Furthermore, sphingosine is an endogenous lysolipid that might act as an endogenous CB_1 antagonist, providing cross-talk between endocannabinoid and sphingolipid signaling cascades in the CNS and immune system.

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THC SUPPRESSES DENDRITIC CELL-T CELL TH1 BIASING BY INHIBITING IL-12 PRODUCTION AND INCREASING THE NOTCH PATHWAY

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Legionella pneumophila infection causes in mice increased blood levels of IL-12 and IFNg followed by antigen-specific production of IFNg and specific anti-*L. pneumophila* IgG_{2a} antibodies (i.e., Th1 response). Injection with THC prior to *L. pneumophila* infection results in a biasing from this Th1 response to a Th2 response characterized by decreased IFNg and IL-12 production and increased IL-4 and IgG_1 antibodies. Injection with cannabinoid receptor (CBR) antagonists prior to THC and utilizing CBR gene deficient mice showed an attenuation of the THC effect on T helper biasing, indicating a receptor-mediated response. Previously we showed that THC suppressed IL-12p40 production in murine bone marrow-derived dendritic cells (DCs) infected with *L. pneumophila* and that cannabinoid receptors were involved in this suppression. DCs are major antigen presenting cells and regulate the differentiation of either Th1 or Th2 CD4+ lymphocytes by varying the production of different biasing factors. One of these factors is Jagged1, a member of the Notch receptor ligand family, which is expressed on DCs and induces Th2 responses by increasing IL-4 and GATA3 in CD4+ cells. We hypothesized that THC was biasing toward Th2 by decreasing IL-12 production and increasing Jagged1 expression on DCs. Bone marrow-derived DCs were isolated from mice and THC treatment of the cultures was shown to suppress IL-12p40 production and increase Jagged1 mRNA. Regarding lymphocyte activation in this system, Notch pathway associated factors such as IL-4 protein and GATA3 mRNA were shown to be increased in spleen cells from *L. pneumophila*-infected and THC-treated mice. Furthermore, THC treatment of *L. pneumophila*-infected DCs followed by co-culture with *Legionella*-primed splenic $CD3^+$ T cells was observed to suppress IFNg production by the T cells. These studies suggest that THC treatment biases toward Th2 immunity by suppressing IL-12 production and increasing the expression of the Jagged1 ligand of the Notch pathway in DCs making these cells a major target of drug action.

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REPORT ON A CASE SERIES OF PATIENTS USING MEDICINAL MARIJUANA FOR MANAGEMENT OF CHRONIC PAIN UNDER THE CANADIAN MEDICAL MARIJUANA ACCESS REGULATIONS

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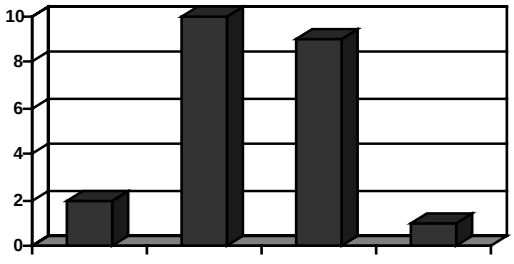
The Canadian Medical Marijuana Access Regulations (MMAR) were formally launched July 30, 2001, allowing patients to apply for a license to possess marijuana for medicinal purposes where traditional therapies have been tried or considered, and have been found to be inadequate in relieving patient suffering. Previous surveys of patients presenting to our clinics (Ware, Doyle et al. 2003) have identified that 15% of patients presenting to a tertiary care pain clinic have used and 10% continue to use cannabis for pain control and 14% of patients presenting to the multiple sclerosis clinic (Clark, Ware et al. **2004**) use cannabis for symptom control. The current paper presents a case series of 22 patients followed at a tertiary care pain clinic who have used cannabis for a year or more under the MMAR program. All subjects completed a structured follow-up questionnaire containing demographic and dosing information, a series of 11 point numerical symptom relief rating scales, a side effect checklist and a subjective measure of improvement in function.

Mean age was 46 with a range from 35-61 (11 men and 11 women). Diagnosis of pain included: post-traumatic pain (5), arthritis (4), MS (2), spinal cord injury (2), HIV related pain (1), neuropathic pain other than spinal cord injury (3), chronic low back pain (2), fibromyalgia (1), congenital multiple exostosis (1) and chronic visceral pain (1). Results revealed that 100% of patients reported relief in pain, the majority 91% reported pain relief greater than or equal to 6 (figure 1). Eighty two percent of patients reported relief in other symptoms as well (figure 2). Doses ranged from less than 1 to 5 grams per day, (average dose 2.5 grams per day). The majority of patients used the smoked route, most patients used a dose of 6 puffs per dose or less four times a day or less. Side effects included: increased appetite (14), a sense of well being (8), weight gain (7, 5 of whom needed to gain weight), slowed thought (7), fatigue or tired (4), decreased energy (3), rapid heart rate (4), confusion (1), poor concentration (1), anxiety (1), paranoia (2). Benefits outweighed the side effects in every case. 68% (15) were able to decrease other medications that had been causing side effects. 95% of patients reported subjective improvement in function.

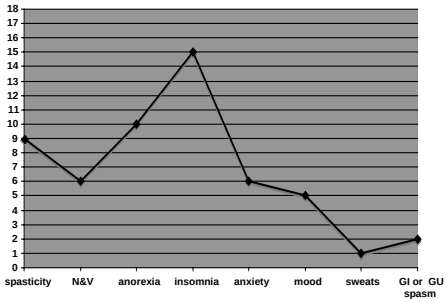
Magnitude of pain relief according to numeric pain rating scale



<5 6-7 8-9 10

0=no relief 10=complete relief

Number of patients reporting moderate to complete relief of other symptoms



Clark, A. J., M. A. Ware, et al. (**2004**). "Patterns of cannabis use among patients with multiple sclerosis." Neurology 62: 2098-2100.
Ware, M. A., C. R. Doyle, et al. (**2003**). "Cannabis use for chronic non-cancer pain: results of a prospective survey." Pain 102(1-2): 211-6.

NO CORRELATION OF PLASMA KINETICS AND CLINICAL EFFECTS AFTER STANDARDIZED, PLACEBO-CONTROLLED, DOUBLE-BLIND SINGLE ORAL ADMINISTRATION OF THC-CALIBRATED CANNABIS EXTRACT

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Introduction: The use of oral cannabinoids is hampered by first-pass-effect and variable gastrointestinal absorption. In our study, cannabinoid plasma levels and their correlation with clinical effects were investigated after a single oral dose of THC-calibrated cannabis extract in healthy, cannabis-naïve probands

Methods: After IRB approval, capsules containing THC-calibrated cannabis extract (20mg THC per capsule; THC:CBD = 2:1) or active placebo (5mg diazepam) were orally administered together with a standardized breakfast to healthy female volunteers (n = 16) in a randomized, double-blind, placebo-controlled crossover design. Plasma levels of THC, cannabidiol (CBD) and the two active metabolites THC-11OH and THC-COOH were measured before, 2, 4 and 8h after administration. The typical THC effects (sedation, "feeling high", vertigo, dry mouth) were determined every 60min during an 8h period by both the proband and a blinded observer using 11-point visual analogue scales (self-rating VAS and observer VAS). Blood pressure (BP), SaO_2, heart rate, and body temperature were recorded every 30 min.

Results: In 12 individuals (75%), peak plasma levels of THC and CBD were reached within 2h, in 25% (n = 4) between 2 and 4h after intake, with THC values between 1.29 ng/ml and 7.91 ng/ml. Peak values of CBD were found after 2h in 10 (63%) and after 4h in 6 (37%) subjects with a range between 0.40 and 3.58 ng/ml. Levels of CBD, THC and metabolites showed a similar variance and time dependence, but individuals with slow absorption rate or delayed metabolism could be observed. The maximum VAS for all drug effects was seen after 2.6h and differed significantly from baseline and placebo. Only one subject presented extensive adverse effects such as vomiting, tachycardia, anxiety and a psychotic episode, but all symptoms disappeared within 8h. No correlation was found between the magnitude of the individual plasma level and the occurrence and intensity of the observed effects. Heart rates were significantly elevated and median systolic BP was lower in the cannabis group, whereas median diastolic BP, body temperature and SaO_2 remained unaltered.

Conclusion: Even after a well controlled, strictly standardized single oral administration of a defined THC/CBD dose, the bioavailabilty and the effects of cannabis extract vary considerably and are not closely correlated. Since our study demonstrates that an oral dose containing 20 mg of THC does not allow to predict the plasma level or the occurrence and intensity of clinical effects, the individual titration of the cannabinoid dose in each subject should be an essential part of future study protocols for pain patients.

Acknowledgements: Supported by "Fonds Soziales Wien".

NO ANALGESIC AND ANTIHYPERALGESIC EFFECTS OF ORALLY ADMINISTERED CANNABIS EXTRACT IN TWO DIFFERENT HUMAN PAIN MODELS

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Introduction: The objective of our study was to investigate the effects of orally administered cannabis extract on pain response and hyperalgesia in probands using two different human pain models, sunburn and intradermally administered capsaicin.

Methods: Healthy female volunteers (n=16, median age 23y, median BMI 22) were randomized for this double-blind, cross-over study. Twenty hrs before the respective test session, a standardized circular sunburn was induced by UV-B irradiation on the skin of one upper leg. After baseline measurements of thresholds for heat pain (HPT), heat pain tolerance, (HPTT), electrical pain (EPT) and electrical pain tolerance (EPTT) in the sunburn spot and the contralateral site, capsules containing THC-calibrated cannabis extract (20mg THC, THC:CBD = 1:2) or placebo (5mg diazepam) were administered orally together with a standardized breakfast. After 120min, HPT, HPTT, EPT and EPTT measurements were repeated, and 150 min after medication, 20µl of 0.1% capsaicin were intradermally injected into one forearm. Using an 11- point visual analogue scale (VAS), initial pain intensity and its decrease were determined at 15s intervals during the first 2min after injection, followed by measurements at 2.5, 9 and 15min. The flare area was assessed by tracing on an acetate sheet 10min after injection. The hyperalgesic area was determined by pinprick and brush.

Results: In the cannabis and in the placebo group, HPT, HPTT, EPT and EPTT were not significantly altered. Under cannabis, surprisingly, pain thresholds were slightly lower compared to baseline or placebo. Immediately after capsaicin injection, maximum pain intensity was measured and disappeared almost completely within 15min. There was no significant difference in spontaneous pain intensity between both groups, but pain decreased more rapidly under cannabis compared to placebo. Cannabinoid medication had no influence on flare response or hyperalgesic area.

Discussion: In the two pain models used in our study, cannabis extract did not significantly reduce hyperalgesia and inflammatory pain. Our results obtained with an inflammatory and hyperalgesic pain model were surprisingly similar to those reported by Naef et al.using an acute pain model in normal skin. Although, in our study, pain was induced 20 hrs before the measurements, the sunburn model, too, does not represent actual chronic pain, and therefore the effect of cannabis extract may differ in patients with fully developed chronic pain.

References: Naef M et al., Pain **2003**; 105(1-2):79-88

Acknowledgements: Supported by "Fonds Soziales Wien".

ABRUPT INTERRUPTION OF LONG-TERM TREATMENT WITH SATIVEX WAS NOT ASSOCIATED WITH A CONSISTENT WITHDRAWAL SYNDROME OR SERIOUS WITHDRAWAL SYMPTOMS IN A SAMPLE OF PATIENTS WITH MULTIPLE SCLEROSIS

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The issue of withdrawal with respect to recreational cannabis usage has been extensively studied with claims of associated symptoms (Budney AJ et al. *Journal of Abnormal Psychology* **2003**; 112:393-402). As clinical cannabis returns to the pharmacopoeia the question of withdrawal in the context of therapeutic usage of prescription cannabis is an important one that heretofore has been little investigated. A cohort of 160 multiple sclerosis patients with varied associated symptoms was previously examined in a double-blind randomised placebo-controlled Phase III clinical trial of Sativex standardised oromucosal cannabis based medicine (Wade, DT et al. *Multiple Sclerosis* **2004**; 10(4):434-41). From this group, 137 patients then continued in a long-term safety-extension (SAFEX) trial (Wade DT et al. *Clinical Rehabilitation* **2005** (in press)), in which it was demonstrated that MS symptoms of spasticity, spasm, pain, bladder dysfunction and sleep disturbance continued to improve with Sativex administration over the course of more than one year with no evidence of tolerance and with slight decreases in mean required daily dosages. Twenty-five patients maintained for at least one year on Sativex from this cohort then elected to abruptly withdraw from this medication for a two-week period. None of the patients subsequently met the criteria recently proposed for a cannabis withdrawal syndrome. Approximately half the patients experienced symptoms previously reported in connection with abrupt withdrawal from regular use of recreational cannabis, but these did not occur with a consistent pattern or time-profile. No patients withdrew prematurely from the study as a result. Abstinence from Sativex was associated with re-emergence of MS-related symptoms over a period of 7–10 days, and in a minority of patients these became severe enough to result in premature termination of the abstinence period. The psychoactivity profile of Sativex in patients bears little resemblance to that of smoked cannabis in recreational users because of differences in composition, dose and route of administration, pharmacokinetic profile, and motivation of the user. Intoxication scores in both acute and chronic trials have been indistinguishable from placebo. In more than 400 patient-years of treatment with Sativex there have been no instances of excess personal use or diversion. In long-term treatment there is no evidence of dose escalation or tolerance. The results of this study indicate that if abrupt withdrawal from Sativex in clinical practice is medically indicated, the likely consequence will be limited to transient disturbances of sleep, emotion or appetite in some patients.

CANNABIS FOR THE MANAGEMENT OF PAIN – ASSESSMENT OF SAFETY STUDY (COMPASS): PROTOCOL DESIGN

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Introduction: An increasing number of patients in Canada are legally using cannabis for medical purposes under new federal access regulations. Research-grade cannabis is currently cultivated under contract to Health Canada, and a quality–controlled product has been available for research purposes since early 2003.

Objectives: To collect standardized safety data on the use of cannabis when used in the treatment of chronic pain. The secondary objectives are to describe dosage patterns for the various pain disorders, collect data on satisfaction with the Health Canada cannabis product, explore predisposing factors for adverse events and examine the feasibility of web-based adverse event reporting.

Methodology: We are conducting an open-label cohort study with one-year of follow-up, to be conducted using a phase-IV type surveillance design. 350 subjects will be recruited from eight clinical sites across Canada. While these subjects will all use cannabis for pain management, we will also follow 1050 control subjects with chronic pain who will continue routine treatment without cannabis. Patients who are 18-year-old or above, with chronic non-cancer pain for 6 months or longer, and a diagnosis of moderate to severe pain in whom conventional treatments have been considered medically inappropriate or inadequate will be eligible. Patients who are pregnant or breast-feeding, or who have a history of psychosis, or with significant and unstable ischaemic heart disease or arrhythmia, or with significant and unstable bronchopulmonary disease will not be eligible for enrolment. Standardized quality-controlled herbal cannabis will be supplied by Prairie Plant Systems Inc. to the site pharmacies for dispensing. Subjects will use the cannabis in the manner with which they are familiar. Dosing will be established at onset with study physicians will be titrated gradually over a one month period to the desired drug effect or until intolerable side effects develop. All adverse events will be recorded for each enrolled subject over one-year of follow-up. Four clinical visits and 3 telephone interviews are required for the subjects, and 3 clinical visits and 2 telephone interviews are required for controls. A range of data on physiological, cognitive and drug use patterns, as well as laboratory and clinical examination findings will be collected according to the study schedule.

Analysis: Descriptive analyses, subgroup analyses and multivariate analysis will be used to present the results. Incidence rates of adverse events will be calculated, with 95% confidence intervals. Conditional logistic regression models will be fitted to find the incidence rate ratio of adverse event of subjects compared with controls.

Implications: This study will provide 350 patient-years of safety data on medical cannabis use. The information will assist in policy decisions and inform discussions of cannabis use between patients and physicians. Further data collection using this methodology may be expanded to include physicians located in rural sites, and longer follow-up may be envisaged for long term effects. This type of monitoring may also be applied to post-marketing safety studies of other medications.

A COMBINATION OF CANNABIDIOL AND Δ^9-TETRAHYDROCANNABINOL (THC) IS SUPERIOR TO THC ALONE IN THE RELIEF OF REFRACTORY CANCER PAIN

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Methods: All patients had a clinical diagnosis of cancer related pain, not adequately relieved by regular strong opioid treatment (i.e. NRS pain score ≥4 on a 0-10 NRS pain scale on at least one occasion per day, during a 2 day run-in assessment period) were enrolled. Study medication was administered for a period of at least 2 weeks in addition to strong opioid analgesic maintenance medication. The use of strong opioids as breakthrough pain medication was permitted during the study period. Patients self-titrated their study medication: each 100µl oro-mucosal spray of Sativex® delivered 2.7mg Δ^9-tetrahydrocannabinol (THC) and 2.5mg of cannabidiol (CBD). Each 100µl spray of Tetranabinex® (a high THC content extract) delivered 2.7mg THC.

The primary outcome of the study was the comparison of each active treatment with placebo in the change from baseline in NRS pain score at the end of 2 weeks' treatment, along with the consumption of breakthrough analgesia. Secondary endpoints were Brief Pain Inventory Short Form (BPI-SF), quality of life (EORTC-QLQC30) and 0-10 NRS scores for sleep disturbance, nausea, memory, appetite and concentration.

Results: 177 patients were randomised to one of 3 treatments (n= 60 Sativex®, n= 58 Tetranabinex®, n= 59 Placebo) for a period of 2-3 weeks. The NRS pain score results and dosing details are tabulated below:

Dosing Details				
Mean No. of sprays per day (SD)	9.3 (5.5)	8.5 (5.4)	10.9 (5.8)	
NRS Pain Scores	Change from Baseline			p
	Sativex®	Tetranabinex®	Placebo	
NRS Pain Score (ITT, Baseline)	5.68	5.77	6.05	
Change in NRS Pain Score (ITT, ANCOVA)	-1.37	-1.01	-0.69	0.0142
NRS Pain Score (Per protocol, ANCOVA)	-1.41	-0.94	-0.59	0.0047

There were no significant differences in the usage of escape medication (number of days on which escape was used (ITT): Sativex® v placebo, p=0.91; Tetranabinex® v placebo, p=0.41; number of doses of escape medication taken per day (ITT): Sativex® v placebo, p=0.69; Tetranabinex® v placebo, p=0.90). Tolerability of both active treatments was good.

Conclusion: The combination of CBD and THC provides significant advantages in the relief of refractory cancer pain compared with THC alone.

CANNABIDIOL AS AN ANTIPSYCHOTIC: A DOUBLE-BLIND, CONTROLLED CLINICAL TRIAL ON CANNABIDIOL VS. AMISULPRIDE IN ACUTE SCHIZOPHRENIA

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The endogenous cannabinoid system has recently been shown of particular importance in the pathophysiology of acute schizophrenia. It interacts with various neurotransmitter systems in the central nervous system including the dopaminergic, glutamatergic and GABAergic system. While the psychedelic properties of the natural cannabis compound Δ^9-tetrahydrocannabinol are widely known, there is some experimental and clinical evidence that other herbal cannabinoid compounds may have antipsychotic properties.

Based on these confounders we designed a four week, double-blind, controlled clinical trial on the effects of purified cannabidiol, a major compound of herbal cannabis, in acute schizophrenia and schizophreniform psychosis compared to the antipsychotic amisulprid. The antipsychotic properties of both drugs were the primary target of the study. Furthermore, side-effects and anxiolytic capabilities of both treatment strategies were investigated.

Cannabidiol significantly reduced psychopathological symptoms of acute psychosis after both, week two and four, when compared to the initial status. There was no statistical difference of this effect to the control condition. In contrast, Cannabidiol revealed significantly less side effects when compared to Amisulpride.

This phase II clinical trial on the effects of Cannabidiol in acute schizophrenia and schizophreniform psychosis raises evidence for its antipsychotic properties that exceeds by far the evidence from open observations available up to now. Furthermore, it raises evidence that the endogenous cannabinoid system may provide a valid target in the search for new treatments for schizophrenia.

Acknowledgements: Funded by the Stanley Medical Research Institute (00-093 to FML) and the Koeln Fortune Program (107/**2000** + 101/**2001** to FML).

MARIJUANA USE AND CANCERS OF THE LUNG AND UPPER AERODIGESTIVE TRACT: RESULTS OF A CASE-CONTROL STUDY

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Marijuana smoke contains several known carcinogens, and heavy habitual use can produce both accelerated malignant change in lung explants and pre-malignant histopathologic and molecular changes in bronchial biopsies. Nonetheless, experimental animal studies using different tumor models have shown both pro- and anti-tumor effects of THC, and the results of epidemiologic studies have been mixed. Most epidemiologic studies, however, have been limited by small numbers of heavy longterm marijuana users and by sources of possible bias. The purpose of this study is to assess the possible effects of marijuana–including heavy longterm use–on the risks of cancers of the lung and upper aerodigestive tract (UAT) among middle-aged adults living in Los Angeles County.

Methods: A population-based case-control study was conducted by identifying cancer cases, ages 18-59, through rapid ascertainment by the Los Angeles County Cancer Surveillance Program. Controls were identified by canvassing the residential neighbor-hoods of cases and were matched to cases on age, gender, and neighborhood. Personal interviews were completed on 1,209 cancer cases (611 lung, 403 oral/pharyngeal, 90 laryngeal, 108 esophageal) and 1,040 controls. Data were collected on lifetime histories of marijuana, tobacco, alcohol and other drug use, sociodemographic factors, diet, occupational exposures, and family history of cancer. Cumulative marijuana use was measured in joint-years (j-yr), where 1 j-yr = 365 joints. Logistic regression was used to estimate the effect of marijuana use on each cancer type, adjusting for age, gender, race/ethnicity, education, cumulative tobacco smoking, and cumulative alcohol use.

Results**:** Among controls, 46% had never used marijuana, 31% had used ≤1 j-yr, 12% had used 1-10 j-yrs, 5% had used 10-30 j-yrs, 2% had used 30-60 j-yrs, and about 3% had used >60 j-yrs. Compared with subjects who had used ≤1 j-yr, the estimated odds ratios (OR; and 95% CI) for lung cancer were 0.78 (0.53, 1.2) for 1-10 j-yrs, 0.74 (0.43, 1.3) for 10-30 j-yrs, 0.85 (0.41, 1.8) for 30-60 j-yrs, and 0.81 (0.44, 1.5) for >60 j-yrs. The estimated ORs for oral/pharyngeal cancers were 0.92 (0.62, 1.4) for 1-10 j-yrs, 0.89 (0.52, 1.5) for 10-30 j-yrs, 0.81 (0.40, 1.6) for 30-60 j-yrs, and 1.0 (0.57, 1.9) for >60 j-yrs. Similar, though less precise, results were obtained for the other cancer sites.

Conclusion**:** In this preliminary analysis, we did not observe a positive association of marijuana use–even heavy longterm use–with lung or UAT cancers, controlling for cigarette smoking and other potential confounders.

Acknowledgments: Supported by NIDA grants DA11386 and DA03018.

WHAT IS THE RELATIONSHIP BETWEEN SERUM LEVELS OF CANNABINOIDS AND CLINICAL EFFECT IN PATIENTS WITH MULTIPLE SCLEROSIS AND DOES IT EXPLAIN THE DIFFERENCES BETWEEN PURE Δ^9-TETRAHYDROCANNABINOL AND WHOLE EXTRACT OF CANNABIS?

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The CAMS study investigated the treatment of spasticity and other MS-related symptoms with cannabinoids, in a large multicentre randomised placebo controlled trial. Over 52 weeks there was a significant improvement in spasticity in patients treated with Δ^9-THC compared to either cannabis extract (mainly containing both Δ^9-THC and the non-psychoactive cannabidiol) or placebo. There were also significant improvements in measures of disability, namely the Rivermead Mobility Index and the Expanded Disability Status Scale.

We went on to investigate whether the clinical effects of Δ^9-THC are related to serum level and at what level the difference between cannabis extract and Δ^9-THC occurred We wanted to determine if the lack of clinical effect with cannabis extract was due to interaction between cannabidiol and Δ^9-THC, either due to cannabidiol affecting the pharmacokinetics of Δ^9-THC, interacting at the receptor level or whether there was an effect on absorption.

Analysis of plasma cannabinoid levels when patients were in steady state showed a weak correlation between clinical effect and increasing concentration of Δ^9-THC for the Δ^9-THC group but in the cannabis extract group, increasing Δ^9-THC was associated with a decrease in clinical effect. Increasing cannabidiol concentration is also associated with less clinical effect.Analysis of log concentrations shows a significant difference between the two treatment arms and this reflects the differences found in the CAMS trial. Analysis of levels of 11-hydroxytetrahydrocannabinol show no significant difference between the two different formulations implying that the difference is unlikely to be at a metabolic level. Further work is now underway to try and determine at what level the CBD-THC interactions may be operating.

THE EFFECTS OF SMOKED CANNABIS IN HIV-RELATED PAINFUL PERIPHERAL NEUROPATHY: RESULTS OF A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

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Introduction: There is significant evidence that cannabinoids may be involved in the modulation of pain, especially of neuropathic origin. An open-label pilot study of smoked cannabis in patients with HIV-related painful peripheral neuropathy demonstrated enough possible effectiveness that a follow-on randomized, double-blind, placebo-controlled trial was designed.

Methods: Fifty patients with HIV-related painful peripheral neuropathy were randomized to smoke either three 3.56% THC cigarettes daily for 5 days or three identical placebo cigarettes from which the cannabinoids had been extracted. The study was conducted in the inpatient General Clinical Research Center at San Francisco General Hospital. An experimental pain model with heat/capsaicin was performed on all participants to allow an assessment of the effect of smoked cannabis on the experimental pain as well as their chronic neuropathic pain.

Results: The last patient will complete the trial in mid-April. The study remains blinded at this time. Results will be available to present in June. This abstract will be updated and re-submitted prior to the meeting.

Conclusion: As above.

Acknowledgements: Funded by the University of California Center for Medicinal Cannabis Research

VASORELAXANT ACTIONS AND PUTATIVE VASCULAR PROTECTIVE PROPERTIES OF ARACHIDONOYL SERINE (ARA-S), A NOVEL CANNABINOID-LIKE SUBSTANCE

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Arachidonoyl-Serine (ARA-S) is a cannabinoid-like substance recently identified in rat brain, which exerts strong vascular activity *in vivo*. We have conducted a series of experiments to determine the mechanism underlying the vasodilatory properties of ARA-S.

Intact and endothelium-denuded rat abdominal aortic rings (2-3 mm length) were placed in a 10 ml organ bath containing Krebs-Henseleit buffer gassed with 95% O_2/5% CO_2 and maintained at 37°C. The rings were stretched to an optimal basal tension of 1.0 g and allowed to equilibrate for 60 minutes. The segment was then stimulated with phenylephrine (1 μM) leading to force generation and in turn vasoconstriction. Addition of ARA-S in different concentrations led to relaxation of the pre-contracted segments. The levels of relaxation were then measured, and dose response curves were established.

The vasorelaxation by ARA-S is inhibited by pertussis toxin, but not by the nitric-oxide synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (300 μM), SR 141716A (CB_1 receptor antagonist; 1 μM), SR 144528 (CB_2 receptor antagonist; 1 μM) or by the vanilloid VR1 receptor antagonist capsazepine(10 μM).

ARA-S relaxes the rat abdominal aorta by endothelium-dependent activation of a target coupled to Gi/Go protein in a cannabidiol-sensitive manner, which does not involve CB_1 and CB_2 receptors. Its effects may be related to the Abn-CBD sensitive receptor.

Incubation of cultured human umbilical vein endothelial cells (HUVEC) with 1 μM ARA-S or 30 μM Abn-CBD for 30 min caused a significant increase in the phosphorylated forms of akt and p44/42 MAP kinase, and the effects of both compounds were significantly attenuated in cells pre-incubated overnight with 400 ng/ml pertussis toxin. These findings suggest that a receptor similar to that in rat aorta is present in HUVEC and is involved in mediating the effect of ARA-S on MAP kinase and Akt.

The pertussis toxin sensitivity of the response suggests that this receptor is coupled to Gi/Go and that this endothelial receptor is involved not only in vasomotor activities but also in processes involving the homeostasis of the vascular system. The results point towards the possible importance of this compound as a vascular protective agent.

ENDOGENOUS CANNABINOIDS MODULATE PHYSIOLOGICALLY RELEVANT NEURONAL ACTIVATION IN THE SENSORY CORTEX

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Increases in local neuronal activity are accompanied by increases in blood flow (hyperemic response) within the active region. For example, stimulation of whiskers, a physiological stimulus, increases blood flow to the whisker-barrel cortex of the rat. Using this model, we have recently shown that CB_1 receptor activation enhances the hyperemic responses. Given that application of the CB_1 receptor antagonists, SR141716 or AM251 alone inhibits hyperemic responses, we have further explored the role of endogenous cannabinoids in neurovascular coupling.

Over an open cranial window (with intact dura), local cerebral blood flow of male Sprague-Dawley rats (250-350g) was measured by Laser Doppler Flowmetry (LDF). Contralateral whiskers were stimulated (lateral movement of approx. 5mm at 10Hz, on for 13s and off for 27s) for 8-15 times at each data point. Drugs are applied locally by injection underneath the dura. The magnitude of hyperemic responses was expressed as the area under curve of average increases in blood flow during whisker stimulation (i.e. % change from baseline X unit time). Data are reported as mean±s.e.m.; $n \geq 4$ rats unless otherwise stated.

Inhibition of catabolism by FAAH or cellular uptake is known to enhance the effects of the endocannabinoid anandamide, including activation of CB_1 receptors. Subdural application of the covalent fatty acid amide hydrolase (FAAH) inhibitor, URB597 (1μM, 60min incubation) significantly potentiates hyperemic responses to whisker stimulation (control, 125±19s; at 60 min, 308±40s, $P<0.05$; 75min, 349±39s, $P<0.05$; 90min, 389±43s, $P<0.01$; 105min: 395±68s, $P<0.01$). Membrane extracts from the whisker-barrel cortex exposed to URB597 (1μM) displayed reduced FAAH activity, which was measured by percent conversion of [^{3}H]-AEA to [^{3}H]-ethanolamine (n=2). Neither 100nM URB597 nor the vehicle increased the hyperemic responses. AM404 (100μM, 30min incubation), which inhibit cellular uptake of anandamide and FAAH, had no significant effect (control, 208±72s; 30min, 278±105s; 45min, 254±54s; 60min, 273±70s; 75min: 329±73s). Since AM404 is itself a substrate of FAAH and rapidly metabolized by brain membranes, the experiment was repeated with 15 min incubation. No significant effect on hyperemic response was observed.

This study shows that prolonged inhibition of FAAH enhances the hyperemic response elicited by whisker stimulation. Previously, we found that CB_1 receptor agonists similarly potentiate the hyperemic response whereas CB_1 receptor antagonists alone cause inhibition. Taken together, these findings suggest that activation of CB_1 receptors by endogenous cannabinoids promotes the recruitment of blood flow during neuronal stimulation in the somatosensory cortex of the rat.

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CANNABINOID CB_1 RECEPTORS ARE PRESENT IN PARIETAL CELLS OF THE HUMAN GASTRIC MUCOSA

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Experimental data suggest that cannabinoid CB_1 receptors are involved in digestive functions in different animal species. Among these, inhibition of acid secretion, decrease in motility and regulation of feeding are some of the most prominent. So far, it is thought that these effects are mediated by CB_1 receptors located on neuronal terminals of the vagal nerve. However, few data exist on the presence and possible functions of different components of the endocannabinoid system in the human digestive tract. We aimed to study the distribution of these membrane receptors in the human gastric mucosa. To this end, we performed immunohistochemical analysis of CB_1 protein distribution in biopsy samples of healthy individuals. Our results show that CB_1 receptors are expressed by a specific population of mucosal cells in which the signal was limited to the cytoplasmic compartment, leaving the round-shaped, big-sized cell nuclei devoid of any staining. By double immunohistochemical staining with a specific anti-H^+/K^+ ATPase antibody we conclude that these cells are acid-secreting parietal cells. In addition, western blots from human biopsies of gastric mucosa showed a single band of approximately 60 KDa, consistent with the expected molecular weight of the CB_1 protein. Finally, CB_1 presence in human mucosa was further corroborated by CB_1-mRNA detection by RT-PCR. In summary, we report on the presence of cannabinoid CB_1 receptors in parietal cells of the human gastric mucosa. The functional consequences of these observations are currently under study in our laboratory.

Acknowledgements: Supported by Pfizer.

VASCULAR ACTIONS OF Δ^9-TETRAHYDROCANNABINOL (THC) MEDIATED BY PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA (PPARγ)

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Cannabinoids have widespread effects on the cardiovascular system, only some of which are mediated via G protein-coupled cell surface receptors (Randall *et al*., **2004**). The principal active ingredient of cannabis, Δ^9-tetrahydrocannabinol (THC), causes acute vasorelaxation in a number of blood vessels (O'Sullivan et al., **2005**). We have now shown that THC also causes a more slowly developing and sustained vasorelaxation through activation of the peroxisome proliferator-activated receptor gamma (PPARγ). *In vitro*, THC was found to cause time-dependent vasorelaxation of rat isolated arteries (the aorta and superior mesenteric artery). This response was found to require the presence of nitric oxide, an intact endothelium and protein synthesis. The time-dependent vasorelaxation was mimicked by the PPARγ agonist rosiglitazone and inhibited by the PPARγ antagonist GW9962, but not by the cannabinoid CB_1 receptor antagonist AM251. In transactivation assays in cultured HEK293 cells, THC activated PPARγ, transiently expressed in combination with retinoid X receptor gamma (RXRγ) and a luciferase reporter gene. THC also stimulated adipocyte differentiation in cultured 3T3 L1 cells, a well-accepted property of PPARγ ligands (Mueller et al., **2002**). The present results provide strong evidence that THC is a PPARγ ligand, stimulation of which causes time-dependent vasorelaxation, implying that some of the pleiotropic effects of cannabis are mediated by nuclear receptors.

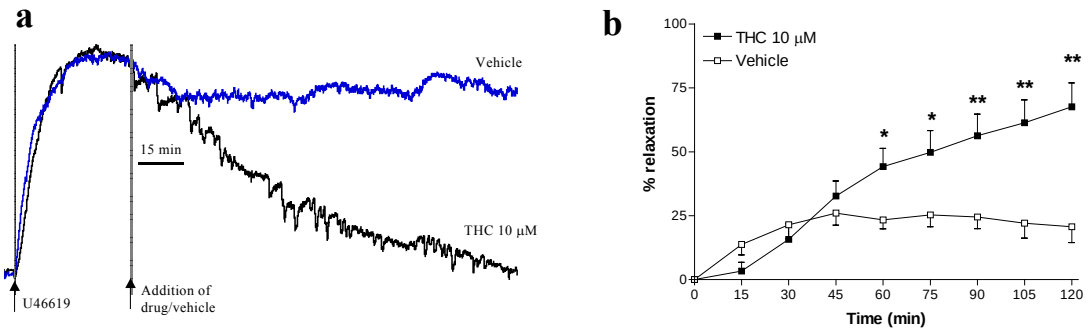

Figure 1. **a**, An original tracing showing the vasorelaxant effects of THC over time compared with a vehicle treated segment of the same aorta. **b**, Mean vasorelaxant response of THC (10 μM) versus vehicle over time in the rat isolated aorta.

Randall, M.D., O'Sullivan, S.E. & Kendall, D.A (**2004**). *Br. J. Pharmacol.* 142: 20-26.
O'Sullivan, S.E., Kendall, D.A. & Randall, M.D. (**2005**). *Br. J. Pharmacol (in press).*
Mueller, E., Drori, S., Aiyer, A. *et al.*, (**2002**). *J. Biol. Chem.* 277: 41925-30.

STRUCTURALLY DIVERSE CANNABINOIDS PREVENT SUBSTANCE P-INDUCED EMESIS VIA CANNABINOID CB_1 RECEPTOR IN CRYPTOTIS PARVA

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Chemotherapeutic agents such as cisplatin cause emesis via a number of emetic neurotransmitters including substance P (SP). SP is a member of the tachykinin family of bioactive peptides which produces emesis via activation of neurokinin NK_1 receptors. The goals of this study were to investigate whether: 1) SP possesses emetic activity in the least shrew (Cryptotis parva); 2) structurally diverse cannabinoids can prevent SP-induced emesis; 3) the antiemtic action of cannabinoids is $CB_{1/2}$-receptor-mediated, and 4) combinations of a noneffective antiemetic dose of the NK_1 receptor antagonist, CP99,994, with varying doses of Δ^9-THC can produce additive or synergistic antiemetic activity. SP (0, 10, 25, 50 and 100 mg/kg, i.p., n = 7-11 per group) caused significant increases both in the mean frequency of emesis ($P<0.01$ at 50 mg/kg and $P<0.05$ at 100 mg/kg) and the number of shrews vomiting ($P<0.0002$ 50 mg/kg and $P<0.007$ 100 mg/kg) in the 30 min postinjection observation period. A 30 min pretreatment with CP99,994 (0, 5 and 10 mg/kg, n = 9-10 per group), dose-dependently decreased both the mean vomit frequency ($P<0.01$ at 10 mg/kg) and the number of shrews vomiting ($P<0.003$ at 10 mg/kg) produced by SP (50 mg/kg, i.p.). Likewise, a 30 min prior exposure to different doses of structurally diverse cannabinoids [Δ^9-THC (0, 0.5, 1, 2.5 and 5 mg/kg, i.p., n = 8-9 per group); Δ^9-THC (0, 2.5, 5, 10 and 20 mg/kg, s.c., n = 7-9 per group); WIN55,212-2 (0, 1, 2.5 and 5 mg/kg, i.p., n = 8-11 per group); and CP55,940 (0, 0.025, 0.05 and 0.1 mg/kg, n = 8 per group)] to different groups of shrews, attenuated both the mean emesis frequency and the number of animals vomiting in a dose- (and route-) dependent manner with an ID_{50} order of CP55,940<WIN55,212-2<Δ^9-THC.

A 30 min prior injection of either vehicle or a non-effective dose of the NK_1 antagonist CP99,940 (i.e. 0 and 5 mg/kg) with different doses of Δ^9-THC (0, 0.5, 1, 2.5 and 5 mg/kg, i.p., n = 8-12 per group) did not significantly increase the antiemetic efficacy of Δ^9-THC against SP (50 mg/kg, i.p.)-induced emesis. Moreover, a 30 min prior exposure to the CB_1 receptor antagonist SR 141716A (0, 1, 2.5, 5, 10 and 20 mg/kg, s.c.,n = 6-9 per group) had no significant effect on SP-induced emesis. However, 30 min pretreatment with varying doses of SR 141716A (0, 5 and 10 mg/kg, s.c., n = 8-10 per group) dose-dependently reversed the antiemetic activity of a fully effective dose of Δ^9-THC (20 mg/kg, s.c.) against SP-induced emesis. These results suggest: 1) substance P produces emesis via activation of NK_1 receptors; 2) structurally diverse cannabinoids prevent substance P-induced emesis via cannabinoid CB_1 receptors, and 3) no additive antiemetic activity occurs when an infective dose of an NK_1 antagonist is administered with varying doses of Δ^9-THC.

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Δ^9-TETRAHYDROCANNABINOL AND CANNABIDIOL INTERFERE WITH CONDITIONED RETCHING ELICITED BY A LITHIUM-PAIRED CONTEXT IN THE HOUSE MUST SHREW: A MODEL OF ANTICIPATORY NAUSEA

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Chemotherapy patients report not only acute nausea and vomiting during the treatment itself, but also report anticipatory nausea and vomiting upon re-exposure to the cues associated with the treatment. We present a model of anticipatory nausea based on the emetic reactions of the *Suncus murinus* (musk shrew). Following three pairings of a novel distinctive contextual cue with the emetic effects of an injection of lithium chloride, the context acquired the potential to elicit conditioned retching in the absence of the toxin. The expression of this conditioned retching reaction was completely suppressed by pretreatment with Δ^9-tetrahydrocannabinol or cannabidiol at a dose that did not suppress general activity. On the other hand, pretreatment with a dose of ondansetron (a 5-HT_3 antagonist) that interferes with acute vomiting in this species, did not suppress the expression of conditioned retching during re-exposure to the lithium-paired context. These results support anecdotal claims that marijuana, but not ondansetron, may prevent the expression of anticipatory nausea.

PERIPHERAL UP-REGULATION OF THE ENDOCANNABINOID SYSTEM IN OBESITY

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There is growing interest in the outcome of phase III clinical trials with the CB_1 receptor antagonist rimonabant against obesity and metabolic syndrome. CB_1 receptors are found not only in CNS but also in peripheral organs involved in the control of energy balance. Several studies have also shown that the endocannabinoid (EC) system controls food-intake via both central and peripheral mechanisms (Bensaid et al., **2002**; Cota et al., **2003**; Ravinet-Trillou et al., **2003**). Since rimonabant ameliorates the metabolic complications (high triglyceride levels, low HDL levels, leptin and insulin resistance) of abdominal obesity, we have been interested in testing the hypothesis of an overactivated EC system being responsible for a part of these dysfunctions. We investigated the mechanisms through which EC regulate, and are regulated by, a number of mediators involved in energy balance in adipocytes and pancreatic β-cells. In this context, we examined for the first time by isotope-dilution LC-MS the presence of ECs in: 1) human visceral fat and blood from normoweight and overweight patients, 2) a mouse pre-adipocyte cell line (3T3F44A) before and after differentiation; and 3) rat RINm5F pancreatic β-cells following stimulation with insulin, glucose and peroxisome proliferator activated receptor (PPAR)-α and γ agonists.

In human visceral fat we detected anandamide (AEA, 0-76 pmol/g weight tissue) and 2-arachidonoylglycerol (2-AG, 12-304 pmol/g weight tissue). The levels of 2-AG were significantly (1.8-fold) elevated in overweight/obese patients (BMI>25), whereas those of AEA were enhanced in the plasma of obese women (BMI>30). Up-regulation of ECs was also observed at the cellular level, in the 3T3F44A mouse pre-adipocytes, where both AEA and, particularly, 2-AG levels dramatically increased (2-15-fold) concomitantly with adipocyte differentiation induced by insulin alone or insulin + dexamethasone + IBMX. We also found that RINm5F pancreatic β-cells produce AEA and 2-AG, and express both CB_1 and CB_2 receptors and EC metabolic enzymes. Preliminary data suggest that the levels of two ECs in these cells are modulated by high glucose, insulin, PPARα and PPARγ.

Our data indicate that during obesity ECs become up-regulated in the adipose tissue and, possibly, in pancreatic β cells, where they might contribute to excessive fat accumulation and the development of leptin and insulin resistance.

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CB_1 RECEPTOR ANTAGONIST SR141716: RIMONABANT, A NEW PROMISING DRUG FOR THE TREATMENT OF OBESITY-ASSOCIATED METABOLIC SYNDROME FEATURES

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Rimonabant (SR141716) a CB_1 receptor antagonist has been shown to possess potent anti-obesity effects and to improve several pathological features of obesity-associated chronic and progressive diseases. In various rodent models of obesity and diabetes, rimonabant reduces food intake, body weight, body fat mass, hyperinsulinemia and improves insulin sensitivity and plasma lipid parameters. In addition, rimonabant inhibits cell proliferation of cultured mouse preadipocytes. Importantly, rimonabant increases the mRNA expression in adipose tissue as well as plasma levels of the adipocytokine: adiponectin. This adipo-hormone has been shown to be involved in obesity and in obesity-associated metabolic diseases including diabetes, insulin resistance, dyslipidemia, cardiovascular risk factors, inflammation and cancer. Adiponectin is now considered as a biomarker of the metabolic syndrome.

Here we report the results of studies performed in order to evaluate the effect of rimonabant on inflammation, dyslipidemia and hepatic diseases associated with obesity and diabetes in a congenital model of obesity: Zucker (fa/fa) rats. Our results support the fact that rimonabant is a promising candidate for the treatment of obesity-associated features of the metabolic syndrome.

1) * These authors contributed equally to this work.

CB_1 RECEPTOR BLOCKADE IN NEWBORN MCE: AN ETIOLOGIC MODEL FOR "NON-ORGANIC FAILURE-TO THIVE" IN INFANTS?

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"Non-organic failure-to-thrive" (NOFTT) afflicts 2-4% of infants and is characterized by low body weight and height for age, without any known organic cause. Until some years ago, impaired mother-infant relationships were held responsible for the development of NOFTT. More recently, several researchers have hypothesized a "biological vulnerability" leading to hypotonia, deficient oral-motor function and impaired suckling.

Previously, we have reported that administration of the CB_1 receptor antagonist rimonabant to newborn mice, caused severe growth failure and death in up to 80% of the neonates, which could be prevented by co-administration of Δ^9-tetrahydrocannabinol (*Fride et al., Eur J. Pharmacol. 419:207-14* ***2001****, 461:27-34* ***2003***). In the present study we sought to determine psychobiological mechanisms underlying the dramatic inhibition of milk ingestion which develops after rimonabant administration on the first day of life.

General Methods: One day old mouse pups (ICR strain) were injected with rimonabant (10 or 20 mg/kg). Nursing-related behaviors were recorded on an anesthetized dam.

Experiment 1. On days 2, 3, 4, 6 and 9 of age, parameters of a. motivation to suckle (opening mouth to maternal or artificial nipple) b. oral-motor performance (holding onto the nipple, rate of sucking) and c. general motor behavior (locomotion, righting reflex, motor strength and stability) were recorded. The results indicated that motivation was normal, while general as well as oral-motor behavior were significantly impaired.

Experiment 2. In order to find out whether we could improve feeding by circumventing the generalized motor weakness of the rimonabant-treated pups, we positioned part of the experimental pups adjacent to the nipple ("helped" pups). Compared to rimonabant-treated pups which received no "help", the "helped" pups displayed more sucking behavior, but similar growth failure. This suggests that general hypotonia contributes, but does not decisively determine to the failure to nurse after neonatal CB_1 receptor blockade.

Experiment 3. We investigated whether CB_1 receptor blockade specifically impairs the sucking mechanism, or other parameters associated with milk ingestion: licking and swallowing. After 3 h of separation from the mother, neonates were placed in a dish containing 16% fat milk/cream which induced the ingestion of significant amounts of milk. Growth and survival were significantly improved in rimonabant-treated pups exposed to the cream/milk mixture.

Conclusions: We have shown that the CB_1 receptor antagonist rimonabant injected into mice within 24h after birth, results in a dramatic impairment of milk ingestion and severe growth failure frequently followed by death. These pups displayed motor weakness and oral-motor deficiencies but no decrease in motivation to suckle. Moreover, the detrimental effects of CB_1 receptor blockade could be counteracted by aiding the pups to approach the maternal nipple as well as by allowing the pups to ingest milk by licking.

We conclude that the syndrome observed in newborn pups with blocked CB_1 receptors, may serve as the first animal model for NOFTT. Furthermore, we suggest that a deficient Endogenous Cannabinoid-CB_1 Receptor System represents the presumed "biological vulnerability" underlying the etiology of NOFTT.

CLINICAL RESULTS WITH RIMONABANT IN THE MAINTENANCE OF ABSTINENCE FROM SMOKING

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Rimonabant (SR 141716) is a cannabinoid CB_1 receptor antagonist showing high selectivity for the central CB_1 receptor compared to the peripheral cannabinoid receptor CB_2 in rat tissues and in CHO cells expressing human CB_1 and CB_2 receptors.

It is now generally accepted that endocannabinoid systems are involved with brain reward function. Consistent with the expression of CB_1 receptors in limbic areas, Δ^9- tetrahydrocannabinol (Δ^9-THC) and synthetic CB_1 agonists (like WIN 55212-2) activate
the mesolimbic dopaminergic system, which is a recognised pathway in mediating the addictive properties of drugs. Δ^9-THC enhances, and rimonabant reduces, the rewarding effects of electrical stimulation of brain reward circuits.

A great of interest has been centred on the role of CB_1 receptors and eating behavior as they appear to be largely distributed in brain areas involved in the control of feeding behavior (i.e. lateral hypothalamus, limbic system) and additionally seem to be implicated in food intake control.

Results show that endocannabinoids in the hypothalamus may tonically activate CB_1 receptors to maintain food intake, and may increase the incentive value of food. Further evidence shows that the CB_1 receptors may be involved in the motivational aspects of eating by enhancing the satisfaction derived from eating through activation of the meso-limbic dopaminergic system. All this evidence would seem to indicate that specific CB_1 antagonists like rimonabant should have some effect in body weight control and this has been confirmed in numerous pharmacological studies in different species.
Nicotine itself and environmental cues associated with nicotine delivery are critically important for sustaining smoking in humans and nicotine self-administration in animals. Recent studies have shown that pre-treatment with rimonabant decreases nicotine self-administration and decreases compulsive behavior maintained by conditioned stimuli in rats. Rimonabant has also been shown to reduce ethanol intake and ethanol-induced dopamine release in rodents.

These results suggest that the activity of drugs of abuse, like nicotine and ethanol, to facilitate the meso-limbic dopaminergic transmission also involves activation of CB_1 receptors.

Initial clinical studies with rimonabant have shown that it reduces hunger, caloric intake and body weight in obese patients. In smoking cessation studies in man, the compound produced an increased abstinence in patients as well as preventing the secondary weight gain often seen in this situation. In addition, the compound has shown a very good safety profile.

Results from the Phase III STRATUS (STudies with Rimonabant And Tobacco USe) clinical studies in smoking cessation include a WorldWide, double-blind, placebo-controlled, randomized, parallel-group, fixed dose, 1-year treatment, 1-year follow-up study in more than 1,000 patients. The recent results of this study confirm that both the 5mg and 20mg doses continued to show efficacy in the maintenance of abstinence from smoking. The 20mg dose also demonstrated efficacy in the reduction of weight gain as well as significantly increasing the HDL-Cholesterol levels. The overall safety profile after 12 months was good and consistent with the safety profile observed with similar exposure in the RIO programme for rimonabant in obesity.

The results of these studies confirm the dual role of the compound against risk factors, essentially cardiovascular, associated with obesity and smoking, is likely to make rimonabant a cornerstone therapy in the future management of patients with cardiovascular risks.

THE ENDOCANNABINOID SYSTEM IN BOAR SPERM AND IT'S INVOLVEMENT IN MALE FERTILITY

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Anandamide (AEA) is the endogenous ligand of cannabinoid (CB) receptors, and as such it plays several central and peripheral activities. Among the latter effects, regulation of female fertility has attracted growing interest. Yet, a role for AEA in controlling sperm function and male fertility in mammals remains unknown. In this investigation we report unprecedented evidence that boar sperm cells have the biochemical machinery to bind and degrade AEA, i.e. type-1 cannabinoid receptors (CB_1R), vanilloid receptors (TRPV1), AEA-synthesizing phospholipase D (NAPE-PLD), AEA transporter (AMT) and AEA hydrolase (FAAH). We also show that the non-hydrolyzable AEA analogue methanandamide reduces sperm capacitation and zona pellucida-induced acrosome reaction, according to a cyclic AMP-dependent pathway triggered by binding to CB_1R. Furthermore, activation of TRPV1 receptors blocks spontaneous acrosome reaction of sperm cells, which is not affected by CB_1R, and inhibition of AMT fully prevents this effect. Taken together, we show that sperm cells have a complete and efficient endocannabinoid system, and that activation of cannabinoid or vanilloid receptors controls at different time-points sperm functions required for fertilization. These observations open new perspectives to the understanding and treatment of male fertility problems.

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A TALE OF TWO CELLS: RETROGRADE ENDOCANNABINOID SIGNALING IN NEURONS AND SPERM

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Chemical-signaling is the universal language for communication between cells from unicellular organisms (bacteria and protozoa) to somatic and reproductive cells in mammals. Sperm and neurons are highly polarized excitable cells which contain voltage- and ligand-gated ion channels for rapid electrical signaling. Sea urchin and human sperm express functional receptors for numerous neurotransmitters and psychoactive drugs, including cannabinoid receptors [CBRs]. Anandamide, arachidonoylethanolamide [AEA], is an endogenous agonist for CBRs in neurons and non-neural cells in the brain and peripheral organs, including the reproductive system. AEA is rapidly released from membrane phospholipids when neurons are stimulated. Retrograde AEA signals from depolarized postsynaptic neurons inhibit neurotransmitter release at excitatory synapses in mammalian brain by activating CB_1 receptors on presynaptic terminals. Analogous processes operate during fertilization in sea urchins. AEA and (-)Δ9tetrahydrocannabinol [THC], the major psychoactive constituent of *Cannabis*, reversibly inhibit fertilization in sea urchins by blocking the egg-jelly-stimulated acrosome reaction [AR]. Acrosomal exocytosis enables sperm to penetrate and activate the egg. The acrosome is a Golgi-derived secretory granule in sperm analogous to synaptic vesicles in neurons. Consistent with observations that AEA and THC inhibit release of neurotransmitters at synaptic endings, ultrastructural studies on sea urchin sperm show that THC prevents the membrane fusion step in acrosomal exocytosis. AEA and THC do not block ionophore-induced-AR. These data suggest that cannabinoids inhibit AR by modulating signal transduction event(s) prior to the opening of ion channels, findings consistent with results obtained with neurons. Unfertilized sea urchin eggs have enzymes required to release AEA from membrane phospholipids, and to hydrolyze AEA. These results indicate that sea urchin eggs may release AEA after activation by the fertilizing sperm. Released AEA may then activate sperm CBR to prevent other sperm in the vicinity from undergoing AR, thereby helping to prevent polyspermic fertilization. With respect to sea urchin gametes, the sperm is functionally equivalent to a presynaptic neuron in the brain, while the egg is equivalent to a postsynaptic neuron. AEA-signaling via CB_1 modulates capacitation and fertilizing potential of human sperm. These findings suggest that: 1) AEA-signaling via CBRs directly affects sperm functions required for fertilization in sea urchins and humans; 2) these processes are critically important for normal sperm function since they have been conserved for over 600 million years of evolutionary history; 3) exogenous cannbinoids derived from marijuana smoke might directly affect sperm functions in humans; and 4) also provide additional evidence for common signaling processes in neurons and sperm.

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IN HUMAN SPERMATOZOA, A CANNABINOID AGONIST CAN INHIBIT THE EFFECTS OF NICOTINE ON HYPERACTIVATION (HA): POTENT INTERACTION OF TWO REGULATORY SYSTEMS

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We have previously shown that human sperm carry a cannabinoid (CB) receptor and that THC from marijuana affects several sperm functions. Sperm hyperactivated swimming (HA) and zona binding are altered by other endogenous and synthetic cannabinoids. A nicotinic cholinergic receptor is also believed to have a role in sperm flagellar activity. Linked with a study of tobacco smokers, we found that both nicotine and cotinine (metabolite) can significantly affect sperm functions in vitro. We hypothesized that the two receptor systems (CB and cholinergic) may interact in regulating normal sperm functions. In Study I, sperm from non-smoking donors were capacitated with a synthetic CB_1 agonist (AM-1346, 10 pM) in the presence of nicotine plus cotinine (100 pM or 10 nM; Control had no drug treatment). Data were normalized as % of the Control value. HA motility was evaluated after 2, 4, 6 and 24 hr using computerized image analysis (IVOS). In Study II, sperm were washed in culture medium containing nicotine+cotinine (NC, 100 pM) at the outset. After 6 hr or 20 hr of capacitation, AM-1346 was added to these same treatments (delayed addition). HA was analyzed at multiple time points up to 30 hr. In Study I, the cholinergic and CB systems appeared to have a significant interaction in regulating human sperm HA. Relative to the Control, drug treatment produced no effect until 24 hr. NC at both concentrations showed stimulation of HA after 24 hr that was 250% of the Control. AM-1346 alone produced some stimulation of HA (163% ± 56). However, AM-1346 present with NC significantly lowered the stimulation of HA that was induced by NC alone (146% ± 39; $p < 0.04$). Furthermore, in Study II, even delayed addition of AM-1346 produced inhibition of the cholinergic effect, indicating that the CB agonist has an effect on sperm function even late into the "capacitation" process. At the 24 hr and 30 hr readings, hyperactivation with NC alone was 158% and 132% of the Control, compared to 72% and 68% of the Control when AM-1346 was present simultaneously with NC (ANOVA, $p = 0.039$). It was striking that the combination suppressed HA below Control levels. Timely hyperactivation is required for fertilization, yet its endogenous regulation is not well understood. Based on these findings, the endogenous interaction of different receptor systems which impact sperm function may be quite complex. Practically speaking, the imbalance in sperm regulation that is created by exogenous marijuana and/or tobacco use can be significant.

ENDOGENOUS CANNABINOID TONE GOVERNS THE ENHANCING EFFECTS OF COCAINE ON SUB-SECOND DOPAMINE RELEASE

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Several drugs of abuse, act through dopamine release in the nucleus accumbens to create an intensely pleasurable effect driving the abuser towards subsequent use and abuse. Spontaneous dopamine release can occur on a tonic (minute to minute) or a phasic (sub-second) time scale and may be involved in the effects of abused drugs. Behavioral evidence has alluded to the possible involvement of the endogenous cannabinoid system in the rewarding effects of drugs of abuse. Therefore, we have investigated the involvement of the endogenous cannabinoids in the effects of cocaine on phasic dopamine release measured with fast-scan cyclic voltammetry. We found that cocaine increases phasic dopamine activity, manifested as an increase in the number of concentration transients which occur over a slower change in concentration in the nucleus accumbens of freely moving rats. This increase was diminished when the drug was administered in the presence of SR141716A, a selective CB_1 receptor antagonist. Therefore, the effects of cocaine on phasic dopamine release are mediated through the endogenous cannabinoid system. These results provide preliminary evidence of SR141716A's potential as a treatment for cocaine abuse, because drug seeking behavior, which creates abuse potential, has previously been shown to be mediated by sub second dopaminergic neuronal activity.

PERIPHERAL CANNABINOID CB_2 RECEPTORS ARE EXPRESSED IN THE BRAIN AND INVOLVED IN DEPRESSION AND SUBSTANCE ABUSE

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Depression and substance abuse are mental health problems associated with stress. The presence and expression of the peripheral type cannabinoid CB_2 receptors in the brain and its regulation in depression and substance abuse is unknown. Therefore, mice were subjected daily for four weeks to chronic mild stress (CMS) and anhedonia was measured by the consumption of 2% sucrose solution. Behavioral and rewarding effects of abused substances were determined in the CMS and control animals. The expression of CB_2 receptors and their gene transcripts were compared in the brains of CMS and control animals by Western blotting using CB_2 receptor antibody and RT-PCR. We have also mapped the distribution of cannabinoid CB_2 receptor immunoreactivity in the rat brain. CMS induced gender specific aversions, which were blocked by WIN55212-2, a non-specific CB_1 and CB_2 cannabinoid receptor agonist. Direct CB_2 antisense oligonucleotide microinjection into the mouse brain induced anxiolysis indicating that CB_2 receptors are present in the brain and may influence behavior. The expression of CB_2 gene, which was influenced by CMS, was dependent on the brain region examined and the strain of the mice used. Furthermore, mice treated chronically, but not acutely with 20 mg/kg morphine showed enhanced expression of CB_2 gene in the midbrain, but not in striatum. Abundant CB_2 immunostaining was found in neuronal and glial processes in a number of brain areas examined. In the cerebellum for example, the CB_2 immunoreactivity was greater in the Purkinje cells than molecular layer and low immunostaining was detected in the inner granular cell layer. The CB_2 immunostaining in the cerebellum was abolished when the CB_2 antibody was preadsorbed with CB_2 receptor blocking peptide. A major finding from these studies was the expression of peripheral cannabinoid CB_2 receptors and its gene transcript in the mouse brain that was enhanced by CMS. These preliminary results suggest that CB_2 receptors are expressed in the mammalian brain and may play a role in depression and substance abuse.

PHARMACOLOGICAL EFFECTS OF Δ^9-TETRAHYDROCANNABINOL IN ADOLESCENT RATS

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Marijuana is the most commonly used illicit drug during adolescence. Despite this high prevalence of marijuana use, however, little is known about short- or long-term effects of its use during development in humans. One of the few studies in which age of onset was examined found that early-onset users (< 16 years), but not late-onset users, showed impaired reaction times in a visual scanning task (Ehrenreich et al., **1999**, Psychopharmacology, 142: 295-301). Besides the paucity of controlled studies in this area, however, any long-term marijuana effects are potentially confounded with the fact that human users are self-selected. Hence, much of what we know about the acute and long-term effects of marijuana and other cannabinoids on the developing brain derives from preclinical research with immature animals.

Like humans, rodents and other mammals undergo physical and behavioral changes around the time of puberty, albeit the duration of these changes is much shorter for rats than for humans. Importantly, characteristic patterns of adolescent behavior such as increased risk taking and increased social interaction with peers that have been observed cross-species occur in the rat from approximately postnatal day 28 to 42 (PN28-PN42) {Spear LP, **2000**, Neurosci. Biobehav. Rev., 24: 417-463}. Concomitant with these behavioral changes are substantial alterations in the central nervous system.

In this study, we evaluated the effects of Δ^9-THC in adolescent (PN29) and adult (> PN 60) in four tests: spontaneous activity, tail flick, rectal temperature, and catalespy. Following initial testing, we dosed the rats twice daily with 10 mg/kg Δ^9-THC. Subsequently, rats in each group were again injected with Δ^9-THC and tested in the four assays. Δ^9-THC suppressed activity and produced antinociception, hypothermia, and catalepsy to approximately the same degree and with similar potency in adolescent and adult female rats. Further, tolerance to these effects developed with repeated dosing. In contrast, male adolescents were more sensitive to the activity-decreasing and hypothermic effects of Δ^9-THC than were male adults, although potencies and maximal effects for producing antinociception and catalepsy were similar. Despite their increased sensitivity to the activity-decreasing and hypothermic effects of Δ^9-THC, however, male adolescents developed full tolerance to these effects (i.e., return to vehicle levels) by the end of the repeated dosing period. Male adults, who were less affected initially, also showed less tolerance to these two effects. These results suggest that adolescent males show greater initial response to Δ^9-THC than adult males in some tests of cannabinoid activity, but that they also may develop tolerance to its effects quickly. These age differences were not noted for female rats.

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DISPARATE ROLE OF CANNABINOID CB_1 RECEPTORS IN THE REINFORCING AND CONDITIONED LEARNING EFFECTS OF THE SWEET NON-DRUG REINFORCER *ENSURE*® IN MICE

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Addiction resulting from either the uncontrollable intake of drugs of abuse or sweet foods shares many underlying factors. Among these, both the reinforcing effects and conditioned learning properties of the abused substance have been implicated in both drug addiction and compulsive overeating. Recently, the cannabinoid CB_1 receptor system has been demonstrated to be involved in the reinforcing effects of several drugs, such as heroin, nicotine, and alcohol. Also, CB_1 receptors appear to be involved in the appetitive conditioning effects of these drugs as well, as both CB_1 knockout mice and mice treated with the CB_1 antagonist SR141716 show attenuated conditioned place preference for heroin, nicotine, and alcohol. The reinforcing effects of sweets such as sucrose are also sensitive to CB_1 receptor blockade; however, the role of this system in the appetitive conditioning effects of sweet reinforcers is not as well characterized. We assessed the role of CB_1 receptors in both reinforcement and appetitive conditioned learning using CB_1 knockout mice as well as the CB_1 antagonist SR141716 (3.0 mg/kg). Operant responding for the sweet liquid nutritional drink *Ensure*® was evaluated under a progressive ratio (PR) schedule of reinforcement, and the acquisition and expression of a place preference for *Ensure*® was assessed using the conditioned place preference procedure. We found that both mature adult CB_1 knockout mice and mature adult mice pretreated with 3.0 mg/kg SR141716 showed attenuated responding for several concentrations of *Ensure*® under the PR schedule, indicating that *Ensure*® was less reinforcing in these groups compared to vehicle treated mice. However, both CB_1 knockout mice and mice pretreated with SR141716 showed an enhanced place preference to *Ensure*® in the conditioned place preference procedure. These results are contrary to what has been reported by others regarding the drug reinforcers heroin, nicotine and alcohol, in that both the reinforcing properties and conditioning effects of these drugs were decreased with CB_1 receptor blockade/deletion. In conclusion, blockade of CB_1 receptors can both attenuate the reinforcing effects of *Ensure*® while enhancing appetitive conditioned place preference for *Ensure*®. This implies that the neural mechanisms underlying reinforcing effects versus conditioned effects are not identical, at least as they pertain to sweet food reward. Interestingly, there is a well established literature revealing a facilitory role of CB_1 receptor blockade/deletion on learning and memory, and emerging evidence that this blockade can improve memory performance in appetitively motivated learning specifically. This suggests that although CB_1 knockout mice and mice pretreated with SR141716 show reduced sensitivity to the reinforcing effects of *Ensure*®, a concomitant enhancement of conditioned learning may be driving the behavior in the conditioned place preference procedure. Additionally, the role of CB_1 receptors in extinction to appetitive conditioning may be implicated.

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ENDOGENOUS CANNABINOIDS PRODUCE DISCRIMINATIVE-STIMULUS EFFECTS SIMILAR TO THOSE OF Δ^9-TETRAHYDROCANNABINOL

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Anandamide is an endogenous ligand for cannabinoid receptors and produces many effects qualitatively similar to those of Δ^9-tetrahydrocannabinol (THC), but behavioral effects of anandamide have been difficult to demonstrate, probably due to its rapid inactivation by transport into neurons and inactivation by fatty acid amide hydrolase (FAAH). In this study, we used a two-lever choice drug-discrimination procedure to investigate whether anandamide and its metabolically stable analog methanandamide produce discriminative-stimulus (subjective) effects similar to those of THC in rats. When injected intravenously (i.v.), methandandamide, but not anandamide, completely substituted for the training dose of THC. We then used pharmacological tools that inhibit transport and hydrolysis of anandamide to investigate whether rapid inactivation of anandamide was responsible for the lack of THC-like discriminative effects. When the anandamide transport inhibitor AM404 (10 mg/kg) was given before anandamide, anandamide produced small but not significant THC-like discriminative effects. In contrast, when URB-597 (0.3 mg/kg), an inhibitor of fatty acid amide hydrolase (FAAH), was administered before anandamide, anandamide then produced significant and dose-related THC-like discriminative effects, with 3mg/kg anandamide completely substituting for the training dose of THC. However, URB597 produced no THC-like effects when give alone. The THC-like discriminative-stimulus effects of anandamide and methanandamide appeared to be mediated by cannabinoid CB_1 receptors since they were significantly reduced by the CB_1 receptor antagonist rimonabant (SR-141718A). In contrast, the vanilloid VR1 antagonist capsazeine (10 mg/kg) did not reduce these THC-like discriminative effects of anandamide, suggesting that vanilloid VR1 receptors were not involved. Thus, anandamide can produce discriminative effects similar to THC when its duration of action is increased by inhibition of its metabolism.

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FIRST REPORT ON A PROSTAGLANDIN–ETHANOLAMIDE (PROSTAMIDE) RECEPTOR ANTAGONIST AGN 204396

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Prostaglandin (PG) –ethanolamides (prostamides) and PG-glyceryl esters are biosynthesized anandamide and 2-arachidonyl glycerol, respectively. Early studies on the agonist effects of prostamide $F_{2\alpha}$ and PGE_2-glyceryl ester suggested that they are pharmacologically distinct from PGs. In these present studies, the prostamide receptor concept was further explored by using a selective antagonist. The isolated feline iris sphincter was used to provide an exacting test of the prostamide receptor hypothesis, since it is not only prostamide sensitive but contains functional prostanoid FP receptors

In the isolated feline iris sphincter, the prostamide antagonist AGN204396 blocked the effect of prostamide $F_{2\alpha}$ and its congener bimatoprost but did not antagonize the effects of $PGF_{2\alpha}$ and prostanoid FP receptor selective analogs. In an attempt to perform the FP receptor counterpart experiment, the FP receptor antagonist AL-8810 was employed. AL-8810 behaved as a weak, full agonist in the feline iris and was unsuitable for antagonist studies. The prostamide antagonist AGN204396 did not antagonize responses to AL-8810, thereby providing additional evidence that prostamide and prostanoid FP activity exist as distinct entities in the feline iris. AGN204396 was not an antagonist at recombinant human prostanoid DP, $EP_{1\text{-}4}$, FP, and IP receptors, AGN204396 did not antagonize the iridial effects of PGE_2-glyceryl ester.

Studies in the feline iris demonstrated that AGN204396 selectively blocks the effects of prostamide $F_{2\alpha}$ and bimatoprost but not $PGF_{2\alpha}$ and PGE_2-glyceryl ester. The identification of an antagonist that selectively blocks prostamide effects provides further support for a novel prostamide-sensitive receptor.

RETROGRADE ENDOCANNABINOID EFFECTS EXERTED ON $I_{K(V)}$ OF GOLDFISH RETINAL CONE PHOTORECEPTORS

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Retrograde release of endocannabinoids is a common feature of numerous synapses in the CNS. We previously showed that voltage-dependent outward K-current elicited by depolarizing pulses ($I_{K(V)}$) of goldfish cones is modulated biphasically by cannabinoid CB_1 receptor agonist WIN55212-2: enhanced at < 1 μM via G_s and suppressed at > 1μM via $G_{i/o}$ (Fan & Yazulla, Vis. Neurosci. **2003**, 20:177). Here, we determined if retrograde effects of endocannabinoids could be measured on $I_{K(V)}$ of cones by K^+ depolarization of cells in the inner nuclear layer (INL) that are postsynaptic to the cones.

Experiments were performed on goldfish retinal slices, from which whole-cell recordings were obtained from the inner segment of long single cones under voltage clamp. A single-short puff (50 msec, 15 psi) of physiological saline with 70 mM KCl was directed through a pipette (tip diameter ~2 μm) to depolarize bipolar cell bodies in the middle of the INL. The rationale was that, if present, such stimulation would trigger the release of an endocannabinoid that could retrogradely modulate $I_{K(V)}$ of cones.

Following a single K^+ puff in the INL, effects on $I_{K(V)}$ of cones were observed after ~200 msec; the change in $I_{K(V)}$ reached the maximum at ~400 msec and gradually returned to control in minutes. $I_{K(V)}$, recorded ~400 msec after the K^+ puff, either decreased to 77 ± 5% (mean ± S.E., n = 10) or increased to 139 ± 15% (n = 5) relative to control. The direction of the effect reflected the biphasic action of WIN55212-2 and presumably was determined by the local concentration of endocannbinoid around the cone terminal. The effect of the K^+ puff on $I_{K(V)}$ was blocked by the CB_1 antagonist SR141716A (3 μM, n = 5) but not by combined 300 μM picrotoxin and 3 μM CNQX (n = 7). Anandamide and 2-arachidonylglycerol may be degraded *in vitro* by cyclooxygenase-2 (COX-2). Application of COX-2 inhibitor nimesulide had no effect on the amplitude of $I_{K(V)}$ but it prolonged the effects of the K^+ puff on $I_{K(V)}$ at least by a factor of five. In control conditions, $I_{K(V)}$ returned to baseline in 6.7 ± 2.7 min (n =10). However, in 30 μM nimesulide, full recovery to baseline was not observed, rather the half-recovery of $I_{K(V)}$ increased to 36.0 ± 14.6 min (n = 5).

Depolarization of cells in the INL releases an endocannabinoid that exerts a retrograde effect on $I_{K(V)}$ of long single cones. COX-2 appears to one limiting factor of endocannabinoid action in the retina. Based on the position of the puff pipette in the middle of the INL, we suggest that bipolar cells are a source of endocannabinoids.

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BIPHASIC EFFECT OF THE NOVEL CB_1 AGONIST AM 411 ON VISUAL SIGNAL DETECTION IN RATS

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Research in humans and animals suggests that CB_1 receptor agonists impair detection of brief salient stimuli. However, some studies have failed to find impairments in selection of attended targets in human subjects administered marijuana. Moreover, CB_1 agonists have been shown to influence acetylcholine release in a biphasic manner across different doses. In the current study, Sprague-Dawley rats were trained on a two-choice operant detection task, which required subjects to respond to the brief illumination of one of two cue lights by pressing the lever nearest the illuminated lamp. Stimuli varied in duration from 100-1000 ms. Subjects (n=9) were administered systemic AM 411 (doses of 0.25, 0.5, 1.0, 2.0 mg/kg and vehicle) once per week in a counterbalanced design. Another group of subjects (n=12) was administered the CB_1 inverse agonist AM 251 (doses: 0.5, 1.0, 2.0, 4.0 mg/kg and vehicle). For comparison, an additional group of subjects (n=8) was administered the muscarinic antagonist scopolamine (doses: 0.0625, 0.125, 0.25 mg/kg and vehicle). AM 411 significantly altered overall performance. Choice accuracy did not interact with stimulus duration; however, planned comparisons revealed that performance under the 0.5 mg/kg dose was significantly enhanced relative to vehicle. No other doses were significantly different from vehicle. Furthermore, analysis of response times suggest that the enhanced performance at the 0.5 mg/kg dose did not result from an altered speed/accuracy tradeoff. AM 251 produced no effects on any measure. As expected, scopolamine impaired performance in a dose- and duration-dependent fashion. No alterations in performance bias were found in any group, however, response strategy was altered slightly at the highest doses of AM 411 and scopolamine. While an enhancement in accuracy in visual stimulus detection is puzzling in the face of contradictory reports in the literature, the present results suggest that low doses of a cannabinoid receptor agonist may enhance performance in situations in which it is necessary to monitor a visual field for expected but very brief salient stimuli.

NEUROPROTECTIVE EFFECT OF WIN-55212 IN NEWBORN RAT BRAIN SLICES EXPOSED TO OXYGEN-GLUCOSE DEPRIVATION

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AIM: to characterize the neuroprotective effect of the cannabinoid agonist WIN55212 (WIN) in ex-vivo model of neonatal hypoxic-ischemic encephalopathy (Oxygen-Glucose Deprivation (OGD) of forebrain slices).

Methods: 500 μm-thick forebrain slices obtained from 7-day-old Wistar rats were incubated in oxygenated physiological solution, and then exposed to OGD for 30 min, (OGD group, n=30), whereas in control group the medium composition remained unchanged (CG, n=28). The effect of incubation of OGD slices with WIN (50 μM) alone (WIN, n= 14) or together with the CB_1 or CB_2 receptor antagonists SR 141716 (50 μM; SR1, n=7) or SR 144528 (50 μM; SR2, n=8) was studied. Neuronal damage was assessed by histological analysis of Nissl stained slices and by determination of LDH efflux to incubation medium by spectrophotometry. Additionally, medium glutamate (Glu) levels were determined by HPLC and those of TNFα by ELISA. Finally, inducible nitric oxide synthase (iNOS) and CB_1 and CB_2 receptor expression were determined in slices homogenate by Western blot.

Results: OGD led to brain damage, reversed by WIN. This effect was histologically apparent and significant in terms of LDH efflux (area under the curve (AUC) of LDH efflux: 73.7±2.4, 148,3±6.8 and 76.3±5.5 a.u. for CG, OGD and WIN, respectively, p<001), Glu release (51.3±5.9, 1115±53.1, and 526.4±46.9 ng/mL for CG, OGD and WIN, respectively, p<0.01), TNFα release (49.2±3.1, 84.4±4.2, and 20.1±0.7 pg/mL for CG, OGD and WIN, respect., p<0.01) and iNOS expression (densitometric analysis of bands signal, expressed as percentage of CG band signal: 300±35 and 163±21% for OGD and WIN, respect., p<0.01). WIN effect on LDH efflux, TNFα release and iNOS expression was abolished by co-administration of either SR1 or SR2 (AUC of LDH efflux: 136.8±6.5 and 151.5±6.8 a.u.; TNFα release: 60.1±3.5 and 95.2±6.5 pg/mL; and iNOS expression: 281±49 and 433±57 %, for SR1 and SR2, respectively, all p<0.01 vs. WIN), whereas that on Glu release was abolished by co-administration of SR1 (Glu release: 1607.3±38.5 ng/mL, p<0.01 vs. WIN). The expression of CB_1 and CB_2 was observed in slices; OGD led to an increase of CB_1 expression.

Conclusions: WIN afforded robust neuroprotection in newborn forebrain slices exposed to OGD, by modulating glutamatergic excitotoxicity, TNFα release and iNOS expression; this neuroprotective effect was mediated by CB_1 and CB_2 receptors.

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ARVANIL, A HYBRID ENDOCANNABINOID AND VANILLOID COMPOUND, BEHAVES AS AN ANTIHYPERKINETIC AGENT IN A RAT MODEL OF HUNTINGTON'S DISEASE

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Arvanil, *N*-arachidonoyl-vanillyl-amide, is an endocannabinoid/vanilloid structural "hybrid" that blocks the endocannabinoid transporter but also possesses direct activity at the cannabinoid CB_1 and vanilloid TRPV1 receptors. Due to these hybrid characteristics, arvanil seems an interesting compound to provide alleviation of symptoms in the rat model of Huntington's disease (HD) generated by bilateral intrastriatal application of 3-nitropropionic acid (3-NP), where hybrid cannabinoid/vanilloid compounds have been previously found to be effective as antihyperkinetic compounds. As expected, arvanil did reduce ambulation and stereotypic and exploratory activities, and increased the inactivity, in control rats. It was also active in 3-NP-lesioned rats, where, despite its lowering effects on stereotypic and exploratory activities, arvanil reduced the hyperkinesia (increased ambulation) typical of these rats, and also increased the inactivity, being these two effects more moderate than those found in control rats. These antihyperkinetic effects of arvanil were not due to correction of the characteristic neurochemical anomalies observed in the caudate-putamen in this rat model of HD and that involve GABA, glutamate and dopamine deficits. Arvanil caused its antihyperkinetic effects presumably by enhancing excitatory transmission at the globus pallidus, since it increased glutamate content in this region. This contrasts with its effects in control rats where arvanil enhanced GABA transmission at the globus pallidus. In summary, arvanil might be a promising compound for the alleviation of hyperkinesia typical of HD, which represents an important goal considering the lack of efficacious pharmacological treatments in this basal ganglia disorder.

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DUAL EFFECT OF INHIBITORS OF ENDOCANNABINOID INACTIVATON ON MEMORY RETENTION IN EXPERIMENTAL MODELS OF ALZHEIMER'S DISEASE

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Endocannabinoids exert neuroprotective actions but also inhibit memory processes in rodents. We investigated if endocannabinoids are involved in controlling neuronal damage and memory retention in an experimental model of Alzheimer's disease (AD). We analysed by isotope dilution LC-MS the brain endocannabinoid levels in rats and mice treated with the β-amyloid peptide-(1-42) (BAP). Furthermore, we administered selective inhibitors of endocannabinoid inactivation to BAP-treated rodents, with different time schedules, in order to assess their effect on: 1) brain endocannabinoid levels, and 2) memory retention in a passive avoidance task.

In rats, 12 days after stereotaxic injection of BAP into the brain cortex, we observed an enhancement of 2-arachidonoylglycerol (2-AG), but not anandamide, levels in the hippocampus ipsilateral to the injection *vs.* the contralateral one. RT-PCR analyses revealed that enzymes catalyzing 2-AG biosynthesis or hydrolysis were also over-expressed following BAP injection, with no effect on anandamide biosynthetic and degradative enzymes. Chronic treatment with a selective inhibitor of endocannabinoid cellular uptake, VDM-11 (5 mg/kg, i.p.), starting from either 3 or 7 days after BAP injection, enhanced anandamide, but not 2-AG, hippocampal levels.

In mice, no enhancement of whole brain endocannabinoid levels was observed 14 days after i.c.v. injection of BAP. However, chronic treatment with VDM-11 (5 mg/kg, i.p.), starting 3 days after BAP injection, reversed BAP-induced loss of memory retention in a passive avoidance test, and strongly enhanced both 2-AG (~3-fold) and anandamide (~6-fold) brain levels. Instead, administration with VDM-11 from the 7th day after BAP injection reduced memory retention in this test, while still potently enhancing the levels of the two endocannabinoids. *N*-arachidonoylserotonin (5 mg/kg, i.p.), a selective inhibitor of FAAH, irrespective of the time of administration, caused a significantly less strong enhancement of endocannabinoid levels (~1.2-2-fold), and reduced memory retention when administered from the 7th day. The amnesic effects of the two inhibitors were also observed in normal mice only when they were administered from the 7th day.

These data suggest that the enhancement of endocannabinoid levels might help controlling the memory loss typical of AD only if sufficiently strong and long-lasting, possibly because of concurrent inhibitory actions of endocannabinoids on neuronal damage and memory consolidation. Of these two actions, only the latter would undergo tolerance, thus possibly explaining why inhibitors of endocannabinoid degradation, while effective at enhancing endocannabinoid levels irrespective of the time of administration, reduce memory loss only when administered starting 3 days from injection of BAP. These findings confirm the hypothesis that endocannabinoids, although produced to counteract neuronal damage and neurochemical imbalances during neurodegenerative disorders, may also contribute to some of their symptoms.

PROTECTIVE ACTIVATION OF ENDOGENOUS CANNABINOIDS DURING ISCHEMIA IN DOPAMINE NEURONS

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We previously demonstrated that dopamine (DA) neurons in the ventral tegmental area (VTA) release the endocannabinoid 2-arachidonoyl-glycerol to modulate impinging excitatory afferents. *In vivo*, stimulation of the prefrontal cortex, one of the major excitatory afferent to the VTA, evokes endocannabinoid release to depress stimulation-evoked firing and bursting activity of DA neurons. Consistently, depolarization of DA neurons *in vitro,* or stimulation of glutamatergic afferents, induces an endocannabinoid-mediated suppression of excitation. Although conditions triggering endocannabinoid release may occur physiologically, for example in response to behaviourally relevant salient stimuli, membrane depolarization and excessive glutamatergic activity leading to excitotoxicity are also expression of neuronal suffering and may lead to protective activation of the endocannabinoid system. To test this hypothesis, we carried out single unit extracellular recordings from DA neurons in urethane anesthetized rats. Rats were prepared with the four-vessel occlusion protocol to induce transient bilateral general brain ischemia. During ischemia (7 min. artery occlusion) in control conditions, activity of DA neurons displayed a progressive but reversible decline (minimal reduction to 58.3 ± 12.9% of baseline firing rate). Conversely, when the CB_1 receptor antagonist SR141716A (1 mg/kg, i.v.) was administered 4 min. prior ischemia, DA neurons were strongly excited (peaking at 187.8 ± 41% of baseline firing rate) and displayed an enhanced bursting pattern of firing activity. Whole-cell patch-clamp experiments carried out on VTA DA neurons corroborated our *in vivo* results. Under these experimental conditions, these neurons underwent a membrane depolarization ranging from 10 to 29 mV (mean 19.8 ± 3 mV) that caused, after an initial increase, an irreversible block of firing. Blockade of CB_1 receptors (AM281, 500 nM) robustly prolonged ischemia-induced membrane depolarization and cessation of spontaneous firing, thus suggesting that endocannabinoids were released during ischemia and accelerated DA neurons recovery following reoxigenation. Interestingly, the effect of the CB_1 agonist WIN55212 was beneficial only at the lowest concentrations tested (3-30 nM), whereas was detrimental at the highest (1 μM). Thus, in the VTA, a low to moderate stimulation of CB_1 receptors during ischemia may improve neuronal survival, while a strong stimulation by high doses of the agonist is unfavourable.

Our results corroborate the notion that endocannabinoids regulate afferent activity to DA neurons and protect them when excessively depolarized. As a consequence, an unbalanced endocannabinoid signal might be correlated to both altered DA-dependent processes (stress, drug abuse and other psychiatric disorders like schizophrenia), and to specific vulnerability during stroke or neurodegenerative diseases such as Parkinson's.

ARE ENDOCANNABINOIDS INVOLVED IN CHRONIC HEPATIC ENCEPALOPATHY?

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Introduction: Cirrhosis is the end stage of many forms of liver injury. It causes numerous complications, one of which is hepatic encephalopathy (HE), a reversible impairment of neurological functions. Its pathogenesis involves raised levels of ammonia and γ-aminobutyric acid as well as functional changes associated with neurotransmitter systems within the central nervous system.

The endocannabinoid system has been found to be involved with the effects due to cirrhosis, however its possible role in HE has not been explored.

Recently we showed that the endocannabinoid system may play an important role in the pathogenesis of an experimental model of fulminant hepatic failure in mice induced by intraperitoneal injection of the hepatotoxin thioacetamide (TAA), a model of acute HE. Modulation of the endocannabinoid system, either by specific antagonists to the CB_1 cannabinoid receptor, or by endocannabinoid agonists for the CB_2 receptor caused improvement in neurological score, cognitive function and activity and indicated that endocannabinoids may have therapeutic potential in acute HE.

Our study aimes at exploring the possible role for ECs in the pathogenesis of chronic HE in a model of secondary biliary cirrhosis in the rat.

Methods: Male, Sprague-Dawley rats were subjected to a bilateral ligation of the bile duct (BDL), under ketamine hydrochloride anesthesia and were administered SR141716, a CB_1 receptor antagonist. Sham operated animals were used as controls. Two and four weeks after surgery, animals which had received either vehicle or SR141716 were evaluated for cognitive and neurological function in the Morris Water Maze and in the Neurological Severity Score (NSS) test, respectively. The animals were sacrificed and their hippocampi were taken to determine 2-AG levels by GC-MS analysis.

Results: The CNS levels of 2-AG were elevated in the BDL group. Cognitive function in the Morris Water Maze was significantly impaired in the BDL rats 2 and 4 weeks post surgery. 5mg/kg SR141716 improved these deficits in the BDL animals compared to controls. NSS was significantly higher in the BDL group compared to the sham group and SR141716 returned this score to normal values.

Conclusion: The results presented now, together with those previously reported indicate an involvement of the endocannabinoid system in the pathogenesis of both acute and chronic HE. Modulation of this system, either by specific antagonists to the CB_1 cannabinoid receptor (in both models), or by exogenous endocannabinoid agonists for the CB_2 receptor (in the acute model) leads to improvement in neurological score, cognitive function and activity and that endocannabinoids may have therapeutic potential in acute hepatic encephalopathy.

MODULATION OF THE ENDOCANNABINOID CB_2 RECEPTOR IN MIGROLGLIAL CELLS IN RESPONSE TO INFLAMMATORY STIMULI

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Evidence exists that the cannabinoid system, which includes the endocannabinoids, 2-AG and their receptors CB_1 and CB_2 can modulate immune function. Microglial cells are thought to play a role in the progression of CNS inflammation and express CB_2 during certain pathological conditions. To examine the expression of the cannabinoid system during CNS autoimmunity, we used the mouse model of multiple sclerosis, EAE, which can be induced by adaptive transfer of MBP specific T cells. Using real-time RT-PCR, we determined that CB_2 was upregulated ~100x in the CNS at EAE onset. Next, we determined whether microglial cells express CB_2 in the CNS during EAE. To distinguish between microglial cells and blood-derived macrophages that migrate into the CNS during EAE, we generated MHC class I mismatched bone marrow chimeras by transplanting H-$2^{u/u}$ B10.PL mice with H-$2^{u/b}$ donor bone marrow. Ten days after EAE induction, we sorted four cell populations from the CNS: 1) peripheral macrophages ($CD11b^+$, $CD45^{high}$, K^b), 2) resting microglial cells ($CD11b^+$, $CD45^{low}$), 3) activated microglial cells ($CD11b^+$, $CD45^{high}$), and 4) $CD4^+$ lymphocytes. By real-time RT-PCR, we found that activated microglial cells and macrophages expressed 10-fold more CB_2 than resting microglial cells and CD4 T cells. We did not detect changes in the expression of 2-AG during EAE using Liquid Chromatography–Mass Spectrometry. These data show that CB_2 and the cannabinoid system play an important role in early microglial cell functions in the CNS during EAE.

HU331 AND OTHER CANNABINOIDS AS ANTI-ANGIOGENIC DRUGS

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Introduction: Angiogenesis, the process of new blood vessel formation, is crucial for tumor growth. Much effort has been put into the development of compounds with anti-angiogenic activity. Recent findings on the ability of anthracycline antibiotics, a family of standard chemotherapy drugs, which inhibit angiogenesis and vascular endothelial cell proliferation make this field very promising in cancer research/therapy. The development of quinonoid compounds that display antineoplastic and/or anti-angiogenic activity, but are less toxic than the standard anthracyclines is a major therapeutic goal. A new anticancer quinone, HU331, was synthesized from cannabidiol. It is highly effective against human cancer cell lines *in-vitro* and also against *in-vivo* tumor grafts in *nude* mice, due to its anticancer and probably also to its anti-angiogenic effects.

Methods: For evaluation of the anti-angiogenic action of HU331 and some other cannabinoids, collagen-embedded aortic rings were incubated for 5-7 days with these compounds in the presence of FGF or VEGF (or with FGF/VEGF alone as positive controls). Four parameters were chosen to quantify the anti-angiogenic effects of HU compounds: 1-the area of endothelial cells that proliferate from aorta, 2-the number of new vessels, 3-average vessel length, and 4-maximal vessel length. The ability of cannabinoids to inhibit endothelial cell proliferation was assayed as well.

Results: HU331is strongly anti-angiogenic. It partially inhibited aortic ring angiogenesis in concentrations as low as 0.1 μg/ml (300nM). All the parameters (area of endothelial cells that proliferate from aorta, number of new vessels formed, average vessel length, maximal vessel length) were affected by HU331. The number of new vessels formed was not only lower, but even those that were formed were shorter. HU331 also lowered endothelial cell proliferation. The principle plant cannabinoids, cannabidiol (CBD) and tetrahydrocannabinol (THC), which are not toxic, also possess some anti-angiogenic activity. In a MTT proliferation assay on bovine aortic endothelial cells CBD was almost as potent as HU331; however in the aortic ring assay it showed much lower activity. Thus at 0.78 μg/ml there was no inhibition of angiogenesis. Both CBD and THC have some biphasic effects. At very low concentrations (0.01μg/ml) they induced some aortic ring angiogenesis, with CBD causing proliferation, but not new vessel formation, while THC induces new vessel formation, but no proliferation. Other cannabinoid quinones, such as those formed from CBN and THC also inhibit aortic ring angiogenesis more potently than their parent compounds, but are less active than HU331, which is the most potent compound in this series. We are currently studying in greater detail the mechanism by which HU331 inhibits angiogenesis.

Summary: HU331 shows potent anti-angiogenic activity. As this compound has anti-cancer properties and is more selective and potent in our assays than standard chemotherapy drugs, it has a high potential as a new drug. The anti-angiogenic effects of other cannabinoids, especially CBD, are also of interest, as CBD is non-toxic and non-psychotropic and can be administered in high doses.

THE COMPARABLE EFFECT OF EXO- AND ENDOGENOUS CANNABINOIDS ON APOPTOSIS IN HUMAN MEDULLOBLASTOMA AND RAT GLIOMA CELLS

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Synthetic cannabinoids act by mimicking endocannabinoids, which activate specific cannabinoid receptors. CB_1 receptors are primarily distributed in the CNS with CB_2 receptors mainly expressed in cells of the immune system. Cannabinoids have been shown to induce apoptosis, decrease tumour growth and inhibit tumour angiogenesis in a number of primary cells and transformed cells. They are selective in their targets and kill tumour cells without affecting non-transformed cells, thus have favourable drug safety profiles and do not lead to the general toxic effects of conventional chemotherapy. The aim of this study was to investigate any role of cannabinoids and endocannabinoids on human medulloblastoma (DAOY) and rat glioma (C6) cells.

Cells were seeded in 6-well plates at a density of 50,000cells/ml. The apoptotic effect of cannabinoids and endocannabinoids was studied by replacing the media after 24 hours with WIN55,212-2 mesylate (CB_1 and CB_2 agonist), Methanandamide (CB_1 agonist), JWH133 (CB_2 agonist), Anandamide (AEA), 2-Arachidonylglycerol (2-AG) (endogenous cannabinoid agonists), or AM281 (CB_1 antagonist). Drug concentrations used were 100µM, 50µM, 25µM, 10µM, 1µM and 100nM. Positive controls were incubated with 10µM MG132, a known apoptotic inducer. Cells incubated in the presence of DMSO or ethanol (carriers) provided the baseline level of apoptosis. Cells were incubated for 3 and 7 days. Cells were harvested and incubated with Annexin V and propidium iodide. The number of live, apoptotic and necrotic cells were counted using flow cytometry.

The results showed drug dependent apoptosis after 3 days in DAOY cells incubated with WIN55,212-2 mesylate (100µM, 50µM and 25µM), Methanandamide (100µM), JWH133 (100µM) and AEA (100µM). Incubation of C6 cells for 3 days with 100µM Methanandamide resulted in drug dependent apoptosis. Drug dependent apoptosis was observed in DAOY cells after 7 days incubation with WIN55,212-2 mesylate (100µM and 50µM), Methanandamide (100µM and 50µM), JWH133 (100µM), AEA (100µM), and 2-AG (100µM and 50µM). Trends show drug dependent apoptosis after 7 days in C6 cells incubated with WIN55,212-2 mesylate (100µM), JWH133 (50µM, 25µM and 100nM), and all concentrations of Methanandamide, AEA and 2-AG. Treatment with the antagonist AM281 alone did not result in apoptosis at all concentrations and time points. Control experiments showed the carriers had no significant effect on apoptosis at all concentrations and time points.

These results provide strong evidence to justify further investigation of exo- and endogenous cannabinoids as therapeutic agents in the treatment of medulloblastoma and glioma brain tumours. Cannabinoid-based therapies which do not result in unwanted psychoactive side effects would be preferable, thus CB_2 agonists may offer major potential benefits.

EFFECT OF CANNABINOIDS ON THE CELL CYCLE: POTENTIAL THERAPEUTIC IMPLICATIONS IN BREAST CANCER

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It is well known that cannabinoids are involved in the control of cell fate. Thus, these compounds can induce cell death, survival or differentiation depending on the cell type and its physio-pathological context. However, little is known about the role of cannabinoids in the cell cycle, the main control of cell fate.

We studied the effects of cannabinoids on several breast cancer cell lines. Our observations show that THC reduces cell viability by interfering with the cell cycle at two different levels depending on the dose. Lower doses of the cannabinoid induce an arrest in G0/G1 phase, which is not accompanied by cell death and seems to be independent of CB_1 and CB_2 receptors. In contrast, higher doses of cannabinoids produce an arrest in a different phase of the cell cycle (G2/M). In this case, the effect is accompanied by CB_2-dependent apoptosis, and involves the down-regulation of cycline dependent kinase-1, the CDK controlling the transition from G2 phase to mitosis. Of interest, viability of normal human mammary epithelial cells is not affected by any of the doses of THC tested.

We have also analyzed by quantitative PCR the expression of cannabinoid receptors in a wide range of human breast tumors. Our results show that CB_1 expression is low and very similar in all the tumors analyzed, whereas CB_2 expression is higher than CB_1 expression and, of interest, seems to correlate with the grade of malignancy of the tumor.

Taken together, these data show that cannabinoids can alter the cell cycle of tumor cells, inducing them to stop proliferation or to die depending on the dose. These results confirm the antiproliferative potential of cannabinoids and might set the bases for a cannabinoid-based therapy for the management of breast cancer.

ENDOCANNABINOID METABOLISM IN HUMAN GLIOBASTOMAS AND MENINGIOMAS COMPARED TO HUMAN NON-TUMOR BRAIN TISSUE

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The endogenous levels of the two cannabinoid receptor ligands 2-arachidonoyl glycerol and anandamide and their respective congeners, monoacyl glycerols and *N*-acylethanolamines as well as the phospholipid precursors of *N*-acylethanolamines were measured by gas chromatography-mass spectrometry in glioblastoma (WHO grade IV) tissue and meningioma (WHO grade I) tissue, and compared to human non-tumor brain tissue. Furthermore, the metabolic turnover of *N*-acylethanolamines was compared by measurements of the enzymatic activity of *N*-acyltransferase, *N*-acylphosphatidylethanolamine-hydrolyzing phospholipase D and fatty acid amide hydrolase in the same three types of tissue. Glioblastomas were characterized by enhanced levels of *N*-acylethanolamines (8-fold, 128 ± 59 pmol/μmol lipid phosphorus) including anandamide (17-fold, 4.6 ± 3.1 pmol/μmol lipid phosphorus) and several species of *N*-acylphosphatidylethanolamines (3- to 8-fold). This was accompanied by a more than 60% reduction of the enzyme activities of *N*-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D and fatty acid amide hydrolase. By contrast, meningiomas were characterized by massively enhanced level of 2-monoacyl-glycerols (20-fold, 2293 ± 361 pmol/μmol lipid phosphorus) including 2-arachidonoyl glycerol (20-fold, 1524 ± 361 pmol/μmol lipid phosphorus). This was accompanied by an enhanced *in vitro* conversion of phosphatidylcholine to monoacylglycerol (5-fold). The enhanced level of the endocannabinoids 2-arachidonoyl glycerol and anandamide and their congeners in the two types of tumor tissue may possibly act as endogenous antitumor compounds by stimulation of both cannabinoid and non-cannabinoid receptor-mediated mechanisms.

STRUCTURE ACTIVITY RELATIONSHIP STUDY AROUND A NON-CLASSICAL CANNABINOID

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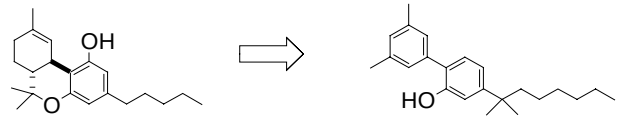
Classical cannabinoids display a wide range of physiological effects including analgesic, anti-inflammatory, anti-convulsive and immunosuppressive activities. Previous SAR studies have shown that the tricyclic moiety in 1 is not essential for high cannabinoid receptor affinity as demonstrated by the biaryl phenol 2, a cannabinoid mimetic originally described by researchers at Merck Frosst.

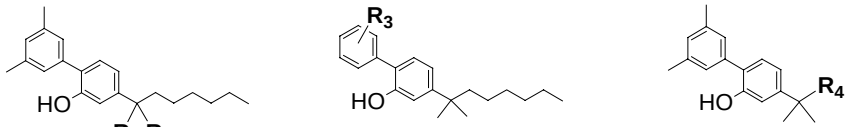


1 **2**

Previously 2 was synthesized in 9 steps starting from 3,5-dimethoxybenzoic acid. This synthesis included an extremely low yielding monophosphonation step (16%) resulting in a 4% yield overall. We have shortened the synthesis to 6 steps with 42% yield overall by adapting a procedure recently described by Papahatjis et al.

It was also shown that substitution of the *n*-pentyl chain of Δ^9-tetrahydro-cannabinol **1** with a 1′**,**1′-dimethylheptyl or 1′**,**1′-cyclopropylheptyl chain led to enhanced affinity for both cannabinoid receptors. Therefore we substituted the 1′**,**1′-dimethyl group in **2** with carbocycles of various ring sizes **3** to probe the stereochemical limits of this system.

A solid phase synthesis approach and the commercial availability of a diverse set of aryl boronic acids allowed access to a large number of analogs **4**. Analogs **5** with modifications of the hydrocarbon tail of varying lengths and substitution patterns were investigated to complete the SAR.



3 **4** **5**

Substitutions up to a 6-membered ring in 3 were well tolerated. The bulkier substituents also seemed to enhance selectivity for the CB_1 receptor about threefold.

Analogs **4** did not show significant selectivity for either receptor. Activity was retained when the top aryl ring was substituted in the 2 and/or 3 positions. Substitution in the 4-position generally led to receptor affinities that were reduced by about 2 orders of magnitude. The CB_2 receptor seemed more tolerant towards R_4 substitution in **5**, accepting alcohol, ester and amide functionalities. Introduction of carboxylic acid groups resulted in loss of affinity to both receptors (K_i > 1000 nM). All of the compounds behaved as full agonists in the [^{35}S]GTPγS assay with potencies consistent with their binding affinities. Data for over 50 compounds will be presented.

NOVEL CB_2 RECEPTOR INVERSE AGONISTS

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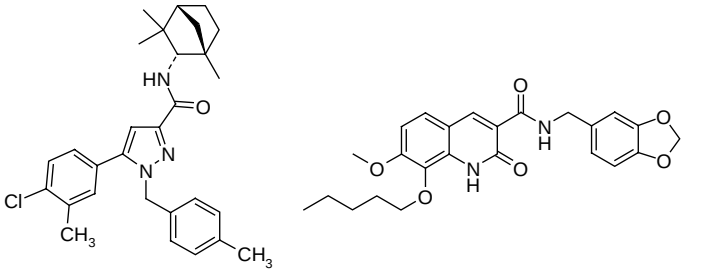
The CB_2 receptor has been suggested to have a significant role in mediating the immunologic effects of cannabinoid receptor ligands. This is supported by the periphery-limited expression of CB_2, concentrated in cells and tissues of the immune system such as spleen, tonsils, thymus, natural killer cells, T cells and B cells. The level of CB_1 receptors, widely expressed in the CNS, only reaches 1-10% of the corresponding CB_2 content in the immune tissues [1,2]. Besides immunological disorders, such as inflammation [3] and multiple sclerosis [4], the medical conditions possibly treatable with CB_2 receptor ligands include pain [5], osteoporosis [6], and growth of malignant gliomas [7] and tumors of immune origin [8].

Novel inverse agonists for the CB_2 receptor have been designed and synthesized. Their CB_2 activities have been compared to those determined for JTE-907 and SR144528 (Figure 1), both of which have been reported to possess anti-inflammatory properties *in vivo* [9]. The *in vitro* evaluation of the CB_2 activity of all the compounds has been performed in Chinese hamster ovary (CHO) cell membranes stably expressing the human CB_2 receptor using a method based on the CB_2 receptor activation-induced [^{35}S]GTPγS binding to the G protein. Efficacies and potencies comparable to those of SR144528 were achieved in our CB_2 ligand development.



SR144528

CB2 activity 59.8+-3.7 %basal (10^{-6} M)
log IC_{50} -8.59+-0.08

JTE-907

CB2 activity 76.3+-3.9 %basal (10^{-6} M)
log IC_{50} -7.65+-0.38

Figure 1. The chemical structures and CB_2 activity data of SR144528 and JTE-907.

[1] Galiègue, S *et al*. *Eur. J. Biochem.*, **1995**, 232, 54.
[2] Ameri, A. *Prog. Neurobiol.*, **1999**, *58*, 315.
[3] Iwamura, H. *et al. Pharmacol. Exp. Ther.*, **2001**, *296*, 420.
[4] Pertwee, R. G. *Pharmacol. Ther.*, **2002**, *95*, 165.
[5] Malan, T. P. *et al. Curr. Opin. Pharmacol.*, **2003**, *3*, 62.
[6] Bab, I. *et al*. *Symposium on the Cannabinoids*; **2004**, 74.
[7] Sanchez, C.; *et al*. *Cancer Res.*, **2001**, *61*, 5784.
[8] McKallip, R. J. *et al*. *Blood*, **2002**, *100*, 627.
[9] Iwamura, H. *et al*. *J. Pharmacol. Exp. Ther.*, **2001**, *296*, 420.

COMPARISON OF THE EFFECTS OF TWO NOVEL CANNABINOID RECEPTOR LIGANDS (O-2050 AND O-2654) WITH SR141716 ON FOUR NERVE-SMOOTH MUSCLE PREPARATIONS *IN VITRO*

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The selective CB_1 cannabinoid receptor antagonist SR141716 has been shown to potentiate electrical field stimulation (EFS) evoked contractions of the mouse vas deferens (MVD) and the guinea pig ileal myenteric plexus longitudinal muscle (MPLM) preparations (Pertwee *et al*. **1992**). Whether this is a consequence of inverse agonism or blockade of the inhibitory effect of endogenously released cannabinoids is not clear. O-2050 a recently introduced cannabinoid ligand, is claimed to be a "silent" CB_1 receptor antagonist. The present study compares the effects of SR141716 and O-2050 with those of a cannabidiol derivative O-2654 on EFS evoked contractions of the MVD, rat vas deferens (RVD) and guinea pig and rat MPLM preparations *in vitro*. Vasa deferentia were obtained from TWA mice (30-50 g) and male Wistar rats (350-550 g) whereas strips of MPLM were dissected from the small intestine of male Dunkin-Hartley guinea pigs (400-650 g) and Wistar rats. All preparations were mounted in organ baths containing Krebs solution for recording of isometric contractions to EFS. MVD, RVD and guinea pig MPLM preparations were stimulated as described by Pertwee *et al*. (**1992**), whereas rat MPLM strips were stimulated as described by Makwana *et al*. (**2004**). Values are expressed as mean potentiation or inhibition of maximal response ± s.e.m. All cannabinoid drugs were dissolved in absolute ethanol. EFS contractile responses of all preparations were confirmed to be neurogenic (TTX 10^{-6} M sensitive). Both SR141716 (10^{-9} M to 10^{-6} M) and O-2050 (10^{-9} M to 10^{-6} M) dose dependently enhanced EFS contractile responses of the MVD (maximum percentage potentiation at 10^{-6} M and EC_{50}: 183.4%, 3.9 x 10^{-8} M (n=5) and 215.4%, 1.4 x10^{-7} M (n=5) respectively), but O-2654 (10^{-9} M to 3x10^{-6} M (n=3)) had no effect. On the guinea pig MPLM both SR141716 (10^{-9} M to 10^{-6} M) and O-2050 (10^{-9} M to 10^{-6} M) produced a concentration related enhancement of EFS contractions (maximum percentage potentiation at 10^{-6} M and EC_{50}: 287.6 %, 1.1 x 10^{-7} M (n=5) and 215.4 %, 1.1 x 10^{-7} M (n=5) respectively). However O-2654 (10^{-8} M to 3x10^{-6}M (n=5) induced a dose dependent inhibitory effect (maximum percentage inhibition at 3x10^{-6} M and EC_{50}: 56.1 %, EC_{50}: 1.3 x 10^{-7} M (n=5). In contrast to the MVD and guinea pig MPLM, on the rat MPLM all three ligands i.e. SR141716 (10^{-7} M-10^{-4} M), O-2050 (10^{-7} M-10^{-4} M) and O-2654 (10^{-7} M-10^{-5} M) dose dependently inhibited contractile responses (maximum percentage inhibition at the highest concentrations and EC_{50}: 98.3 %, 3.6x10^{-6} M (n=9); 76.92 %, 3.5x10^{-6} M (n=4) and 65.18 %, 1.3 x10^{-6} M (n=7) respectively. In contrast, none of the cannabinoid ligands (10^{-9}-10^{-4} M) had any effect on the RVD. Our findings indicate that the effects of cannabinoid receptor antagonists on EFS-evoked contractions are dependent on the species studied and the tissues used and the term silent should be used with caution.

Makwana *et al*., (**2004**) *Br.J. Pharmacol* proceedings (in press)
Pertwee RG *et al*., (**1992**) *Br.J. Pharmacol* 105, 980-984

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DEOXY ANALOGS OF CP47,497 AND CP55,940 AS POTENTIAL CB_2 SELECTIVE LIGANDS

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Although the benzopyran ring system of THC was considered essential for cannabinoid activity, non-traditional cannabinoids lacking the benzopyran ring system, such as CP47,497 and CP55,940, were developed by Pfizer. These compounds have high affinity for the CB_1 and CB_2 receptors and are potent cannabinoids *in vivo*.

1-Deoxy-3-(1',1'-dimethylbutyl)-Δ^8-THC (JWH-133) is a highly selective CB_2 agonist with K_i = 3.4 nM at the CB_2 receptor and K_i = 677 nM at CB_1 (Huffman, J.W.; *et. al. Bioorg. Chem.* **1999**, *7*, 2905). In an effort to develop new CB_2 selective ligands, the synthesis of a series of deoxy analogs of the Pfizer bicyclic non-traditional cannabinoids was initiated.

3-[4-(1,1-Dimethylalkyl)phenyl]-1-cyclohexanols, deoxy analogs of CP47,497, have been synthesized. These compounds were prepared by coupling an aryllithium with 3-ethoxy-2-cyclohexen-1-one followed by a dissolving metal reduction of the cyclohexenone. Stereoselective reduction of the carbonyl group provided the CP47,497 analogs. In all cases both epimeric alcohols were prepared. With the exception of commercially available 4-*tert*-butylbromobenzene the aryl bromide starting materials were prepared from the corresponding phenol by a new procedure developed in our laboratory (Thompson, A.L.S.; *et. al. Synthesis* **2005**, *4*, 547).

3-[4-(1,1-Dimethylalkyl)phenyl]-4-(3-hydroxypropyl)-1-cyclohexanols, deoxy analogs of CP55,940, have been synthesized. These compounds were prepared via conjugate addition of an aryl Grignard reagent to an enone, followed by stereoselective reduction of the ketone, and hydroboration-oxidation to form the propanol chain (Johnson, M.R.; *et. al. U.S. Patent No.: US 4,371,720*, **1981**). The structure and conformation of *cis*-3-[4-(1,1-dimethylethyl)phenyl]-*trans*-4-(3-hydroxypropyl) cyclohexanol has been confirmed by X-ray crystallography.

The lower members of both homologous series have little affinity for either the CB_1 or CB_2 receptor. Increased chain length (up to 1,1-dimethylheptyl) has increased the affinity for both receptors with the CB_2 receptor affinities being slightly higher than those at CB_1 but are still very weak. The syntheses and receptor affinities of these CP47,497 and CP59,940 analogs will be discussed.

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EFFECTS OF HALOGEN SUBSTITUENTS IN THE 1-ALKYL-3-(1-NAPTHOYL) INDOLE SERIES ON CB_1 AND CB_2 RECEPTOR AFFINITIES

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The structure of THC, as determined in **1964** by Gaoni and Mechoulam, has a dibenzopyran nucleus. This finding led to the development of structure-activity relationships (SAR) based upon this skeleton. Pfizer extended these sets to their non-traditional cannabinoids, which do not contain the dibenzopyran nucleus. In the late **1980**'s it was found by a group at Sterling-Winthrop that pravadoline, an indole based non-steroidal anti-inflammatory agent inhibited contractions of the mouse vas deferens. It was subsequently determined that this was due to effects caused by interaction with the CB_1 receptor. This compound and other structurally related aminoalkylindoles have since been shown to exhibit typical cannabinoid pharmacology *in vivo*.

1-Pentyl-2-methyl-3-(1-naphthoyl)indole (JWH-007) has high affinity for the CB_1 and CB_2 receptors with $K_i = 9.5 \pm 4.5$ at CB_1 and $K_i = 2.9 \pm 2.6$ at CB_2. The propyl analog (JWH-015) has good affinity for the CB_2 receptor with $K_i = 13.8 \pm 4.6$ while it has weak affinity for CB_1 with $K_i = 164 \pm 22$. (Huffman, J.W.; *et.al. Bioorg. Med. Chem.* **2005**, *13*, 89). In further studies to develop improved SAR for these indole derivatives it was discovered that an *n*-pentyl nitrogen substituent has optimal affinity for CB_1. As the carbon chain decreases or increases in length the affinity for the CB_1 receptor decreases.

Addition of substituents to the naphthyl ring of these derivatives causes variation in the affinities for both the CB_1 and CB_2 receptors. The synthesis of a series of indole derivatives with halogens as substituents in the C-4 position of the naphthyl ring has been initiated. These compounds were prepared from the corresponding 4-bromo or 4-chloro naphthoic acids. The synthesis of these acids was performed by Friedel-Crafts acylation of 1-bromo or 1-chloronaphthalene followed by the King modification of the haloform reaction. This yielded a pyridinium salt which upon basic hydrolysis gave the crude acid. Purification of this acid through the methyl ester followed by another basic hydrolysis yields pure 4-bromo or 4-chloro-1-naphthoic acid. The acid is subsequently converted to the naphthoyl chloride. Indole or 2-methylindole is stirred with methylmagnesium bromide and then combined with the naphthoyl chloride to form 3-(4-bromo or 4-chloro-1-naphthoyl)indole or the 2-methylindole analogs. Alkylation using either 1-propyl or 1-pentyl bromide yields the final compounds 1-alkyl-3-(4-halo-1-napthoyl)indoles or their 2-methylindole analogs. The *in vitro* pharmacology of these compounds will be discussed.

OXY-HOMOLOGUES OF ANANDAMIDE AND RELATED ENDOLIPIDS: SYNTHESIS AND BIOLOGICAL EVALUATION

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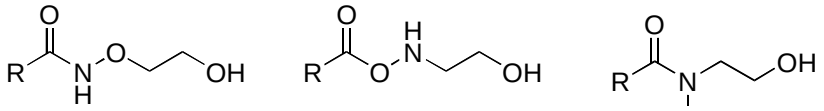
N-Acylethanolamines are an important class of endolipids that function as chemical messengers in the central nervous system. These compounds are exemplified by the endocannabinoid anandamide (arachidonylethanolamide, AEA), the PPAR-γ activator oleylethanolamide (OEA), and the neuroprotective agent palmitylethanolamide (PEA). Despite their chemical similarity, these compounds can show antagonistic activity at a functional level [AEA and PEA on neuroprotection (Leon et al., WO-03006007, **2003**), AEA and OEA on appetite (Rodriguez de Fonseca et al., Nature **2001**, 414,209)], and interference with their production and/or degradation has therapeutic potential for the management of pathological conditions that are top priorities in biomedical research (pain, anxiety, sleep and eating disorders). These compounds are rapidly inactivated by hydrolysis from a serine hydrolase widespread in the central nervous system (FAAH). S/A relationships within *N*-acylethanolamines have mainly been investigated in AEA, and have highlighted the critical role of the amide bond for bioactivity. Isosteric modification of this group was pursued with the aim of increasing metabolic stability, but was disappointing in term of activity. We have reasoned that oxygen homologation of the amide bond, while maintaining the basic topology of this group, has marked effect on its electronic properties. This could in principle provide the opportunity to dissect receptor interaction, a non-covalent event depending essentially on the spatial relationship between the heteroatoms of the polar head of ethanolamides, and the capacity of these compounds to act as substrates for FAAH inactivation, a dynamic event depending on the electronic properties of the substrate.

In principle, three distinct types of oxygen homologation of an amide bond are possible, resulting in *N*- or *O*-acylhydroxylamides and *N*-hydroxyamides, respectively (**A**-**C**). The chemistry of these functional groups has been so far poorly investigated. From a synthetic standpoint, while hydroxylamine would be a suitable and common starting material, the lability of the polyunsaturated olefin system of arachidonic acid greatly restricts the conditions available for its *N*-or *O*-alkylation. The full implementation of this project, will be presented.



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INCENSOLE ACETATE: A PSYCHO-ACTIVE COMPOUND DERIVED FROM FRANKINCENSE, WITH A PARTIAL CANNABIMIMETIC PROFILE

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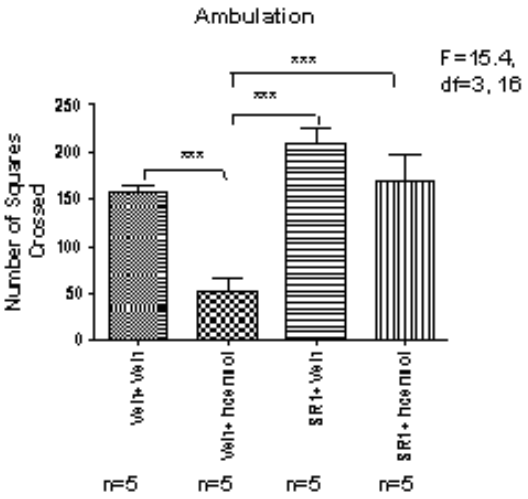
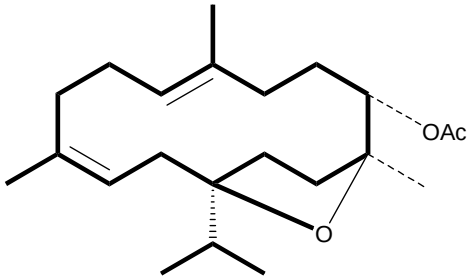
The resin of Boswellia species (Frankincense, Olibanum) has been used since ancient times as incense in religious ceremonies. It is mentioned in Assyrian clay tablets, in the Old Testament, and in Ayurveda texts as well as in modern books on incense. We assumed that this resin may contain psychoactive constituents. Indeed, we isolated a compound from Boswellia carterii resin, incensole acetate (IA), a macrocyclic diterpenoid, which was tested for anandamide-like activity.





Incensole acetate

Methods: IA (dissolved in ethanol:cremophore: saline = 1:1:18) was administered *i.p.* to mice (Sabra strain, aged 21-22 weeks) at a dose of 50 mg/kg. They were assayed in all 4 tests of the cannabinoid tetrad (Fride and Mechoulam, Eur. J. Pharm. 231:313, **1993**). In additional groups of mice, we administered the CB_1 receptor antagonist SR141716 (5 mg/kg). The CB_1 binding assay was performed as previously reported (Devane et al., Science 258:1946, **1992**).

Results: IA significantly reduced the activity of mice in the open field and ring tests and lowered temperature. It also reduced the pain threshold in the hot plate test, but this effect was not significant. The CB_1 receptor antagonist SR141716 significantly blocked the IA-induced effects on locomotion and rearing, but did not reverse the effects of IA on body temperature and pain. IA did not bind to the CB_1 receptor.

Summary: Incensole acetate, a constituent of Boswellia carterii, partially mimics anandamide in its pharmacological activity. Its effects are blocked in part by a CB_1 receptor antagonist. These effects may perhaps explain its wide use as an incense.

VIRTUAL SCREENING OF NOVEL CANNABINERGIC LIGANDS USING A COMPARISON MODEL OF THE CB_2 RECEPTOR

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The CB_2 receptor is a peripheral G-protein coupled receptor (GPCR) found mainly in the tissues of immune system. It is an attractive target for drug development that aims, among others, to alleviate pain [1] and inflammation [2]. In the absence of an experimental structure of a particular GPCR, rhodopsin crystal structure-based comparison models have been shown to be useful for structure-based virtual screening and lead optimization [3]. In order to find novel selective CB_2 lead compounds, a comparison model of the CB_2 receptor was constructed using the recently determined high-resolution bovine rhodopsin x-ray structure [4] as a template. The model was subjected to a 500-picosecond molecular dynamics (MD) simulation and thereafter, new conformers of the receptor binding site were produced in a simulated annealing procedure. Known CB_2 ligands were then docked into five chosen conformers of the receptor model and the docking results were ranked by scoring algorithms. The best-ranked docking conformations of the ligands were used to build rough 3D-QSAR models utilizing the CB_2 affinity data collected from literature. The receptor conformer that produced the best QSAR models was chosen to be used for virtual database screening. Three different search queries were built: the first based on the known CB_2 agonists in their docking conformation, the second based on the structure of the receptor binding site and the third based on the ligand-receptor complex. Maybridge and LeadQuest® databases were searched through and the hit molecules were ranked by docking and scoring. Finally, about 120 best-ranked hit compounds were purchased from the commercial databases and tested for CB_2-mediated G-protein activation. One of the hits was shown to act as an agonist at CB_2. In the future, this low-potency lead compound will be further optimized to develop more potent CB_2 agonists that could be used for therapeutic purposes.

[1] Malan, T. P., Jr.; Ibrahim, M. M.; Lai, J.; Vanderah, T. W.; Makriyannis, A.; Porreca, F. Curr. Opin. Pharmacol., **2003**, 3, 62.
[2] Iwamura, H.; Suzuki, H.; Ueda, Y.; Kaya, T.; Inaba, T.J. Pharmacol. Exp.Ther., **2001**, 296, 420.
[3] Evers, A.; Klabunde, T. J. Med. Chem., in press.
[4] Palczewski, K.; Kumasaka, T.; Hori, T.; Behnke, C. A.; Motoshima, H.; Fox, B. A.; Le Trong, I.; Teller, D. C.; Okada, T.; Stenkamp, R. E.; Yamamoto, M.; Miyano, M. Science, **2000**, 289, 739.

CANNABINOID CB_2 RECEPTORS: IMMUNOHISTOCHEMICAL LOCALIZATION IN RAT BRAIN

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Brain expression of CB_2 cannabanoid receptors has been much less well established and characterized in comparison to the expression of the more abundant brain CB_1 receptors. Since CB_2 receptors are intensely expressed in peripheral and immune tissues, expression in brain microglia has been anticipated. Nevertheless, we now describe expression of CB_2 receptor like immunoreactivity in brain in neuronal patterns that support broader CNS roles for this receptor. Two anti-CB_2 affinity purified polyclonal antibodies were raised in rabbits immunized with peptide conjugates that corresponded to amino acids 1-33 and 20-33. Western blot analyses revealed specific 60 kDa bands that were identified using these sera and were absent when the sera were preadsorbed with 8.3 μg/ml of the immunizing peptides. These studies, and initial RT-PCR analyses of brain CB_1 and CB_2 mRNAs, also supported brain CB_2 expression at levels much lower than those of CB_1 receptors. Immunohistochemical analyses revealed abundant CB_2 immunostaining in apparent neuronal and glial processes in a number of brain areas. Cerebellar Purkinje cells and hippocampal pyramidal cells revealed substantial immunoreactivity that was absent when sections were stained with preadsorbed sera. CB_2 immunoreactivity was also observed in striatum, thalamic nuclei, hippocampus, substantia nigra, pontine nuclei, inferior colliculus and the parvicellular portion of the medial vestibular nucleus. The multifocal expression of CB_2 immunoreactivity in neuronal patterns in a number of brain regions suggests reevaluation of the possible roles that CB_2 receptors may play in the brain.

LOCALIZATION OF THE CB_1 CANNABINOID RECEPTOR IN THE CHICK BRAIN AND ITS IMPLICATIONS IN PASSIVE AVOIDANCE LEARNING

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The type 1 cannabinoid receptor (CB_1) has recently been in the focus of interest due to its several possible roles in behavior and memory. However, distribution and function of this receptor in the avian brain has not yet been studied. We investigated the immunohistochemical localization of the CB_1 receptor in the brain of the domestic chick using polyclonal antibody against CB_1 receptor in coronal vibratome sections. To study the role of the cannabinoid system on avian learning, the effect of the CB_1 antagonist SR141716A was assessed in a passive avoidance learning task. Intensely labelled CB_1 immunoreactive (CB_1+) neurons were present in the ventral tegmental area and the hippocampus, showing an even cytoplasmic staining. In the latter area CB_1+ axon fibers were also observed. We found labelled cells also in the lateral septum, where the immunostaining occurred as a cup-like mass surrounding the cell body. Many intensely stained fibers were detected in the arcopallium, a region homologous to the mammalian amygdala. We also found several CB_1+ fibers located in the medial striatum and nucleus accumbens. Overall, the distribution of CB_1 receptor appeared similar to previous findings on mammalian brains. Intraperitoneal treatment with SR141716A 30 minutes before passive avoidance training had no effect on the retention of the learning task. Conversely, when the SR141716A was administered after the passive avoidance training trial, 30 minutes before the recall, the chicks showed strongly impaired memory retention as compared with the control animals. The neuroanatomical observations indicate that the CB_1 receptor is abundant in the avian brain areas with a known relevance to learning, i.e. limbic structures and the basal ganglia. The results of the behavioral study suggest that CB_1 receptors may be active at the time of the second wave of protein synthesis associated with the consolidation of memory.

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ELEVATION OF INTRACELLULAR CALCIUM BY TRICYCLIC CANNABINOIDS IN T CELLS INVOLVES THE TRPC1 CHANNELS

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Cannabinoid compounds have been widely reported to alter immune function. Previous studies from this laboratory have shown T cells to be a sensitive target to cannabinoid treatment. The objective of the present studies was to examine the effect of the classical tricyclic cannabinoids, Δ^9-THC, CBN and HU210, on intracellular calcium ($[Ca^{2+}]_i$) elevation in HPB-ALL cells, a human CB_2 expressing T cell line. All three compounds elevated $[Ca^{2+}]_i$ in a concentration-responsive manner in resting HPB-ALL cells with rank order potency: Δ^9-THC > HU210 > CBN. The cannabinoid-mediated elevation of $[Ca^{2+}]_i$ was attenuated upon pretreatment with SR144528 and SR141716A, the CB_2 and CB_1 receptor antagonists, respectively. Moreover, the elevation in $[Ca^{2+}]_i$ elicited by Δ^9-THC, HU210 and CBN was attenuated in the absence of extracellular calcium. Further mechanistic studies performed with Δ^9-THC revealed that neither pretreatment with thapsigargin nor the ryanodine receptor antagonist, 8-Br-cADP-ribose, abrogated the Δ^9-THC-mediated elevation in $[Ca^{2+}]_i$, indicating that Δ^9-THC-mediated rise in $[Ca^{2+}]_i$ was independent of calcium stores. Pretreatment of cells with inhibitors of calcium channels showed that the Δ^9-THC-mediated elevation in $[Ca^{2+}]_i$ was attenuated strongly by the receptor-operated calcium channel blocker, SK&F96365, but weakly by the store-operated calcium channel blockers, 2-APB and $LaCl_3$. RT-PCR analysis for members of the transient receptor potential canonical (TRPC) channel subfamily, several of which have been implicated in the formation of receptor-operated cation channels, demonstrated that HPB-ALL cells express TRPC1, a channel gated by diacylglycerol (DAG). Treatment of HPB-ALL cells with 1-oleoyl-2-acetyl-*sn*-glycerol (OAG), a cell-permeant diacylglycerol analog, led to a rise in $[Ca^{2+}]_i$. In addition, when cells were sequentially treated with OAG followed by Δ^9-THC, Δ^9-THC failed to elicit a further rise in $[Ca^{2+}]_i$. Finally, siRNA knockdown of TRPC1 in HPB-ALL cells attenuated both the mRNA expression of TRPC1, as well as the Δ^9-THC-mediated elevation of $[Ca^{2+}]_i$. Collectively, the present investigation demonstrates that the mechanism of $[Ca^{2+}]_i$ elevation by tricyclic cannabinoids in T cells is independent of intracellular calcium store-release, is attributable entirely to extracellular calcium influx, and involves diacylglycerol-sensitive TRPC1 channels.

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G-PROTEIN ACTIVATION BY CANNABINOID AND OPIOID LIGANDS

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Although the existence of the functional links between the endogenous cannabinoid and opioid systems has already been demonstrated, extensive research is still needed to elucidate the biochemical mechanisms involved in this interaction Both cannabinoid and opioid receptors are members of the superfamily of G-protein-coupled receptors, so the signal transduction pathways of these receptors are mediated by the process of G-protein activation and can be measured by agonist stimulated [^{35}S]GTPgammaS binding.

In the recent study G-protein activation was measured with 2AG and 2AGE and with mu(DAMGO) and delta(DeltorphinII) specific opioid ligands in homozygote $CB_1^{+/+}$(wild type) and $CB_1^{-/-}$(knockout) adult, male mice brain.

Synergistic interaction was observed with the combination of opioid and cannabinoid agonist ligands in preparations from the wild type brain. The effect was more pronounced (approximately 35%) with the mu specific opioid ligand DAMGO. There was no significant change in GTPgammaS binding in the preparations in the CB_1 knockout animals and in CHO cells expressing mu or delta receptors. The interaction was inhibited by antagonists.

These investigations prove the interactions of these two systems at the level of G-protein coupling.

Acknowledgements: This work was supported by OTKA(T046434) research grant.

IN VITRO PHARMACOLOGICAL CHARACTERIZATION OF AM1241 IN RECOMBINANT CELL LINES EXPRESSING THE RAT AND HUMAN CB_1 AND CB_2 RECEPTORS

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There is an increasing body of evidence to support the potential utility of selective cannabinoid CB_2 receptor agonists for the treatment of pain in the absence of psychoactive side effects associated with the activation of the CB_1 receptor subtype (Malan *et al.,* **2001**, Pain, 93, 239). Strong supporting evidence for this hypothesis is provided from studies with the CB_2-selective agonist AM1241, which has demonstrated efficacy in preclinical models of inflammatory and neuropathic pain (Ibrahim *et al.*, **2003**, PNAS, 100, 10259). The aim of the current study was to evaluate the *in vitro* profile of AM1241 at both the rat and human cannabinoid CB_1 and CB_2 receptor subtypes.

AM1241 was evaluated and compared to the non-selective cannabinoid agonist CP55,940 and the selective CB_2 antagonist SR144528 in cell lines that express recombinant CB_1 or CB_2 receptors using radioligand binding assays, calcium flux (FLIPR) assays, cyclase assays measuring the inhibition of forskolin-stimulated adenylyl cyclase activity, and mitogen-activated protein kinase (p42/p44 MAPK) phosphorylation assays. Radioligand binding assays confirmed that AM1241 exhibited high selectivity for the CB_2 receptor subtype in both human and rat. However, discrepancies were encountered across these functional assays. AM1241 exhibited antagonist activity in the FLIPR assay, neutral antagonist-like effect in cyclase assay and partial agonist efficacy in the MAPK phosphorylation assay. In contrast, the prototypical CB_2 antagonist SR144528 exhibited highly efficacious inverse agonist activity in the adenylyl cyclase and MAPK phosphorylation assays, and it behaved as an antagonist in the FLIPR assay, whereas CP55,940 appeared to be a full agonist in all these functional assays.

This apparent inconsistency between the *in vivo* agonist efficacy of AM1241 as an analgesic agent whose effects are fully antagonized by selective CB_2 antagonists, and the lack of robust agonist efficacy in recombinant systems may suggest an elevated level of constitutive activity in the recombinant cell line assay systems that results in the apparent antagonist behavior of AM1241 in FLIPR and cyclase assays. The finding that AM1241 exhibited agonist, antagonist and inverse agonist properties depending upon the assay systems used suggests that AM1241 is a protean agonist, and physiologically relevant *in vitro* assay systems are important for accurate prediction of compound efficacies *in vivo*.

CB_1 MEMBRANE TRAFFICKING: A ROLE FOR CRIP1b?

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G protein-coupled receptors (GPCRs) are one of the largest protein families encoded in the human genome and provide a vital function by conveying cellular information. As GPCRs must be properly trafficked to the cell membrane to be available for ligand binding and thus signal transduction, mechanisms of membrane localization are integral to receptor function. Indeed, adjusting surface expression levels is a robust means to control receptor function as the number of receptors at the membrane is influenced by rates of delivery, retention and turnover, which can be individually altered in response to specific signaling mechanisms. Some GPCR-interacting proteins, such as RAMP1 or Homer1a, have been shown to operate as molecular chaperones or to stabilize cell surface expression of GPCRs.

We have previously identified a CB_1 cannabinoid receptor interacting protein, CRIP1b, which interacts with CB_1 at the distal C-terminal tail. CRIP1b has thus far been identified only in human and chimpanzee genome databases and may be unique to primates. Electrophysiology results indicate that CRIP1b does not affect CB_1-mediated modulation of Ca^{2+} channels; however, CRIP1b may function instead in membrane localization of CB_1 receptors. The ability of CRIP1b to alter CB_1 surface expression is currently under investigation using biotin labeling experiments. We hypothesize that CRIP1b could regulate the function of CB_1 through adjustment of CB_1 membrane expression.

CANNABINOID-INDUCED NITRIC OXIDE (NO) PRODUCTION IN N18TG2 NEURONAL CELLS

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Nitric oxide is a highly reactive molecule involved in numerous physiologic and pathophysiological signaling pathways. In the central nervous system, NO has been shown to be important in long term potentiation, neurotransmitter release, and synaptic plasticity. NO production is catalyzed by one of three enzymes namely endothelial (e), inducible (i), or neuronal (n) isoforms of nitric oxide synthase (NOS). N18TG2 neuroblastoma cells expressed two nNOS protein bands detected using Western analysis, the nature of which are currently under investigation. We have not found conditions in which iNOS or eNOS proteins were detected in these cells. NO production in N18TG2 neuroblastoma cells was detected intracellularly using fluorescence microscopy of a complex of diaminofluorescein and NO. Fluorescence in cells could be attenuated by L-nitroso-N-arginine, demonstrating that the NO was derived from enzymatic production. CP55940, WIN55212-2, and (R)-methanandamide stimulated a 10-fold increase of NO accumulation in N18TG2 cells. Pre-treatment with SR141716 significantly lowered agonist-stimulated NO production by N18TG2 cells, suggesting that CB_1 receptor stimulation led to NO synthesis. When cells were pretreated with pertussis toxin to block Gi interaction with receptors, cannabinoid-stimulated NO production was attenuated, indicating that signaling through $G\alpha i$ proteins appears to be important in the regulation of levels of NO in N18TG2 cells. The pathways and/or scaffolding mechanisms leading to the CB_1-receptor regulation of nNOS are currently under investigation.

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REGULATION OF CB_1 CANNABINOID RECEPTOR POLARITY IN CULTURED HIPPOCAMPAL NEURONS

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The CB_1 receptor is the main subtype of cannabinoid receptor expressed in the central nervous system (CNS). At the cellular level, the surface distribution of CB_1 receptors is polarised, with high levels of expression on axons and at presynaptic terminals, where they are well positioned to modulate neurotransmitter release (Irving et al., Neuroscience. **2000**;98(2):253-62.). Two distinct mechanisms have been proposed for targeting proteins to the axonal surface, involving selective retention or selective delivery (Sampo et al., Neuron. **2003** Feb 20;37(4):611-24.). In this study we have investigated the role of endocytosis in regulating CB_1 receptor surface expression, using a previously characterized N-terminally tagged CB_1-GFP chimera (McDonald et al, ICRS Symposium, Paustum, **2004**). When transfected into primary cultures of hippocampal neurons and cerebellar granule cells, CB_1-GFP fluorescence is observed throughout the cell in living neurons. However, when the cells are fixed and surface staining for GFP is carried out, surface CB_1-GFP is expressed predominantly on the axon. This suggests that retention, rather than specific targeting to the axon might be responsible for CB_1 receptor polarity in these neurons. In order to test whether preferential endocytosis in the somato-dendritic compartment could account for this we co-transfected with a dominant-negative dynamin (K44A) construct to prevent clathirin-dependent endocytosis. In co-transfected neurons, polarization of CB_1 was lost, suggesting that selective endocytosis may underlie CB_1 receptor targeting to the axon.

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THE CRITICAL DOMAIN OF THE CB_1 RECEPTOR THAT INTERACTS WITH CRIP1A

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A number of proteins interact with the C-termini of the GPCRs and play a very important role in signal transduction. Two novel alternatively spliced proteins CRIP1b and CRIP1a (Cannabinoid Receptor Interacting Proteins), which interact with CB_1 receptors were discovered. The CB_1 receptor C-terminal last 9 amino acids (aa) was the minimal domain tested that gave a strong interaction with CRIP1b in a yeast two-hybrid assay. We investigated whether the same domain is required for the interaction between CB_1 receptors and CRIP1a by deleting the last 9 aa of the CB_1 receptor.

The C-terminal last 9 aa were deleted from the HA-tagged rat CB_1 receptor, and the mutant rCB_1-464 receptor was subcloned into pcDNA 3 vector. Immunocytochemistry showed that rCB_1-464 receptors successfully trafficked to the membrane in HEK293 cells. The signal transduction through rCB_1-464 was investigated by using a superior cervical ganglion (SCG) neuronal expression system. In SCG neurons expressing rCB_1-464 receptors, the cannabinoid receptor inverse agonist SR141716 (SR) increased the Ca^{2+} current 45.3±5.7% (n=11) versus 56.7±9.4% (n=8) in SCG neurons expressing wild type rCB_1 receptors. The cannabinoid receptor agonist WIN55,212-2 (WIN) inhibited Ca^{2+} current 56.9±3.0% (n=11) versus 40.3±7.7% (n=8) in SCG neurons expressing rCB_1-464 and rCB_1 receptors respectively. The effects of SR and WIN between the mutant rCB_1-464 receptor and the wild type rCB_1 receptor are not significantly different.

We next investigated whether deletion of the C-terminal last 9 aa of the CB_1 receptor could block the effect of CRIP1a. In SCG neurons expressing rCB_1-464 and CRIP1a, SR increased Ca^{2+} current 43.2±8.2% (n=6), which is not significantly different from rCB_1-464 alone; while WIN inhibited Ca^{2+} current 64.2±1.7% (n=6), which is significantly increased compared to rCB_1-464 alone. In the presence of CRIP1a, SR increased the Ca^{2+} current 43.2±8.2% through rCB_1-464, but only 23.9±2.3% through rCB_1; while WIN inhibited Ca^{2+}current 64.2±1.7% through rCB_1-464, but only 45.2±4.0% through rCB_1. The differences were statistically significant.

	WIN	SR
rCB_1	40.3±7.7	56.7±9.4
rCB_1-464	56.9±3.0	45.3±5.7
rCB_1-464+CRIP1a	64.2±1.7	43.2±8.2
rCB_1+CRIP1a	45.2±4.0	23.9±2.3

In summary, deletion of the C-terminal last 9 aa of the CB_1 receptor did not alter its ability to inhibit voltage-gated Ca^{2+} channels. However the mutant rCB_1-464 receptor blocked the ability of CRIP1a to reduce the effect of SR. Ca^{2+} current inhibition by the WIN was increased in SCG neurons expressing rCB_1-464 with CRIP1a compared to either rCB_1-464 alone or rCB_1 with CRIP1a. The data demonstrated that the C-terminal last 9 aa of the CB_1 receptor were critical for the interaction between the CB_1 receptor and CRIP1a.

NMR STRUCTURAL REFINEMENT OF THE HOMOLOGY-BUILT 3D CB_2 RECEPTOR MODEL

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The CB_2 receptor, a subtype of cannabinoid (CB) receptors, was identified as a member of the rhodopsin-like family of seven-transmembrane G protein-coupled receptors (GPCRs). A potential for therapeutic specificity in regulating diseases has made CB receptors one of the important GPCR targets for new drug discovery. The CB_2 receptor is likely to be involved in signal transduction in the immune system, and can be a potential target for immuno-treatments. However, our understanding of its action mode and rational drug design are limited because of the absence of a 3D receptor structure. Its intrinsic membrane protein property makes the CB_2 receptor difficult to crystallize for X-ray study. Direct NMR study is also restricted due to the large protein size and slow correlation time in membrane-mimicking environments. The Comparative structural modeling has been widely used to predict the structural characters of many structure-unknown proteins including CB receptors on the basis of the crystal structure of bovine rhodopsin. Conversely, the current CB_2 structural model is lacking experimental support. Without experimentally based structural data, the existing theoretical model may mis-predict the helix ends, orientations, and the binding site conformation. We are developing a combined NMR and computational approach to refine the 3D structural model of the CB_2 receptor. Based on the Homology-constructed CB_2 3D structural model, the CB_2 polypeptide fragments were designed to contain the extracellular or intracellular loop and about three helical turns in each of two adjacent transmembrane helices. Then, we used the 2D NMR techniques to resolve the secondary structure of these chemically synthetic polypeptides in DMSO, which has been verified to be suitable an alternative to detergent micelle for the secondary structure forming in polypeptide. The NMR-determined helical conformations at the N-terminal and C-terminal regions of the CB_2 polypeptide fragments may reveal the amino acid ends of CB_2 seven transmembrane domains. Such studies also provided valuable experimental data integrated into the comparative predicting 3D structure model to refine the computational 3D structural model of the CB_2 receptor

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IDENTIFICATION OF POSSIBLE CB_1/DOPAMINE D2 HETERODIMER INTERFACES USING CORRELATED MUTATION ANALYSIS

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Kearn and Glass have recently reported evidence that CB_1/Dopamine D2 receptor complexes exist, are dynamic, and are agonist regulated with highest complex levels detected when both receptors are stimulated with sub-saturating concentrations of agonist (C. S. Kearn, K. Blake-Palmer, E. Daniel, K. Mackie and M. Glass Mol. Pharmacol., in press: E.Pub Feb 14, **2005**). We have taken a BioInformatics approach to identify the most likely sites for formation of these CB_1/D2 heterodimers. This approach uses an algorithim to calculate correlated mutations (Correlated Mutation Analysis, CMA) (O. Olmea and A. Valencia Fold Des 2: S25-32, **1997**). First, multiple sequence alignments are performed with the CLUSTALW program (J.D. Thompson, D.G. Higgins and T.J. Gibson Nucleic Acids Research 22, 4673-4680, **1994**), using the human sequence for each receptor as reference. Each of these multiple sequence alignments is then used as an input to the CMA computational procedure that automatically calculates correlated mutations on the lipid-exposed faces of the transmembrane (TM) helices. Solvent accessibility values are calculated from the atomic coordinates of our current 3D model of the CB_1 receptor and a 3D model of the D2 receptor built using the rhodopsin crystal structure as a template (K. Palczewski et al. Science 289, 739-745, **2000**). Possible heterodimer interfaces will be identified by considering only residues that form "interaction neighborhoods". These are regions of the receptor sequence where at least three residues identified by CMA appear close to each other, i.e., within i+7 on the same alpha helix. The results of this analysis will be presented.

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THE HIGH CONSTITUTIVE ACTIVITY EXHIBITED BY CB_1 IS DUE IN PART TO THE LACK OF AROMATIC RESIDUES I-4 and I+3 FROM W6.48

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The CB_1 receptor exhibits high levels of constitutive activity. In contrast, rhodopsin exhibits an exquisite lack of constitutive activity. Based upon spectroscopic evidence, W6.48 has been proposed to undergo a rotamer switch upon activation of rhodopsin. In rhodopsin, W6.48(265) on TMH6 is flanked by aromatic residues at positions I-4 (F6.44) and I+3 (Y6.51), while in CB_1 the residues I-4 and I+3 to W6.48 are leucines (L6.44 and L6.51). A recent conformational memories (CM) calculation on wildtype CB_1 TMH6 revealed that the presence of leucines at 6.44 and 6.51 provides W6.48 with greater conformational mobility. In contrast, an aromatic residue at 6.44 and 6.51 tends to disfavor activation (Singh et al. J. Pep. Res. 60:357-370 (**2002**)). In the current study, aromatic mutations at positions 6.44, 6.51, and 6.52 (L6.44F, L6.51F, L6.52F, L6.44F/L6.51F, and L6.44F/L6.52F) were made in CB_1 to test the hypothesis that aromatic stacking interactions between W6.48 and aromatic residues at position 6.44, 6.51 or 6.52 may influence the rotamer population leading to a helix that favors an inactive receptor state. The L6.44F, L6.51F, L6.52F, L6.44F/L6.51F, and L6.44F/L6.52F mutant receptors were stably transfected into HEK293 cells. Ligand binding and cAMP accumulation assays were performed on these mutant receptors.

The L6.44F mutant receptor retained its ability to bind CP55940 and SR141716A, but the ability of CP55940 to inhibit forskolin-stimulated cAMP accumulation in cells expressing L6.44F mutant receptors was greatly reduced. In addition, this mutation caused a loss of CB_1 receptor constitutive activity. The L6.51F mutant receptor lost its ability to bind SR141716A, but retained its ability to bind CP55940. In cells expressing L6.51F mutant, the ability of CP55940 to inhibit forskolin-stimulated cAMP accumulation was markedly reduced. Furthermore, the L6.51F mutation caused a loss of constitutive activity of CB_1. In contrast, the L6.52F mutation had no effect on ligand binding, agonist-induced inhibition of cAMP accumulation and constitutive activity of CB_1. The double mutation L6.44F/L6.51F caused a complete loss of ligand binding, agonist-induced inhibition of cAMP accumulation and constitutive activity. The double mutation L6.44F/L6.52F retained its ability to bind both CP55940 and SR141716A, but in cells expressing this mutant receptor, the ability of CP55940 to inhibit forskolin-stimulated cAMP accumulation, and the constitutive activity of the transfected receptor, were markedly reduced.

These data demonstrate that L6.44F, L6.51F, L6.44F/L6.51F, and L6.44F/L6.52F mutations caused either a complete loss or a marked reduction of CB_1 receptor constitutive activity. Therefore, the high constitutive activity exhibited by CB_1 is due, at least in part, to the lack of aromatic residues at positions 6.44 and 6.51.

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LACK OF AROMATIC RESIDUE AT POSITION 6.44 OF HUMAN CB_2 CANNABINOID RECEPTOR CONTRIBUTES TO CONSTITUTIVE ACTIVITY

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The activation of G protein-coupled receptors (GPCRs) is thought to be associated with the movement of transmembrane helices (TMH) 3 and 6. P6.50 in the highly conserved CWXP motif in TMH6 may act as a flexible hinge, permitting TMH6 to straighten upon activation. In contract to the well-studied GPCR models rhodopsin and β2-adrenergic receptor, which are natively inactive and in which the CWXP motif in TMH6 are flanked with aromatic residues, wild-type CB_2 cannabinoid receptor is constitutively active and with no aromatic residues flanking this motif. We hypothesized that lack of aromatic residues around the CWXP motif may contribute to the constitutive activity of CB_2.

In the present study, we mutated L6.44, L6.45, V6.51 and L6.52 of CB_2 receptor to Phe, one at a time, or in combination, to test this hypothesis. These mutant CB_2 receptors were stably transfected into the HEK293 cells and the effects of these mutations were examined with Western blot analysis, ligand binding, and cAMP accumulation assays. Western blot analysis demonstrated that all the CB_2 mutants expressed at levels similar to that of the wild type CB_2. In the ligand binding studies, while single mutant V6.51F and all the combined mutants containing V6.51F completely lost binding to [^{3}H]CP55940, all the other CB_2 mutants had ligand binding affinities similar to that of wild-type CB_2 for both CP55940 and SR144528. SR144528, an inverse agonist of CB_2, could prevent constitutively active wild-type CB_2 from decreasing forskolin-stimulated cAMP accumulation. It had the same effect on the mutants L6.45F, L6.52F, and L6.45F/L6.52F. However, SR144528 had no such effect on the single mutants L6.44F and V6.51F or on any of the combined mutants containing L6.44F or V6.51F. Furthermore, in cells transfected with the L6.44F or V6.51F single mutants or any of the combined mutants containing L6.44F or V6.51F, the ability of cannabinoid agonist CP55940 to lower cAMP levels was remarkably reduced. In contrast, in cells expressing other mutants, the ability of CP55940 to inhibit cAMP accumulation was not changed.

These data demonstrate that the L6.44F mutation caused a loss of constitutive activity as well as agonist-induced activation of CB_2 cannabinoid receptor. The V6.51F mutation caused a complete loss of ligand binding as well as the functions of CB_2. The mutations at the other sites, such as 6.45 and 6.52, had no evident effects. Therefore, the constitutive activity of CB_2 receptor may due to the lack of an aromatic residue at position 6.44.

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MOUSE NEURO2A CELLS REQUIRE FUNCTIONAL ERK-MAPK PATHWAY FOR CB_1 MEDIATED KROX 24 INDUCTION

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Considerable diversity has been reported for the signal transduction pathways activated by the CB_1 receptor and the consequence of activation on growth, proliferation, neurite modeling, ability to induce apoptosis or promote cell survival. We have identified endogenous CB_1 receptor expression in the neuroblastoma Neuro2a cell line. We have used a combination of immunocytochemical imaging with high throughput quantification using the Molecular Devices Discovery 1 system to measure CB_1 receptor expression and signaling responses in these cells.

The localization of CB_1 receptors is intriguing in Neuro2a cells as a considerable portion of the total receptor expression is present in the cytoplasm with only a small percentage on the cell membrane. Stimulation of the CB_1 receptors with the agonist HU210 leads to rapid induction of Krox 24 mRNA levels as measured by Northern blot analysis within 20 minutes and these remain elevated for approximately 2 hours post stimulation. By immunocytochemistry the number of Krox 24 positive cells increases from 60 minutes post stimulation and peaks between 90-120 minutes, with a maximum of 15-25% of cells responding to the CB_1 agonist in this time period. Thereafter the number of Krox 24 positive cells remains significantly elevated for at least 24 hours. The CB_1 mediated Krox 24 induction is not associated with a decrease in the rate of cells proliferation, nor are enzymes involved with apoptosis (caspase-3, JNK-MAPK) induced within this 24h time frame. The incrase in Krox 24 protein is dependent upon a functional ERK-MAPK pathway as treatment with the inhibitor UO126 abolishes the induction of Krox 24 by HU210 and also reduces Krox 24 levels in unstimulated cells. These data suggest that a functional MEK/ERK pathway is required for CB_1 to induce expression of the Egr-1 transcription factor, however, in unstimulated Neuro2a cells pERK levels are readily detectable in most cells, whereas strong Egr-1 staining is only present in 3-5% of the cells under the same conditions, therefore activation of ERK is probably not sufficient to generate a Krox 24 response on its own.

CHANGE OF A CONSERVED PHENYLALANINE RESIDUE TO ALANINE IN THE THIRD TRANSMEMBRANE HELIX OF HUMAN CANNABINOID 1 RECEPTOR CONVERTS AM2233 FROM AGONIST TO INVERSE AGONIST

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The cannabinoid 1 receptor (CB1R) is a member of the G-protein coupled receptor family. We changed a conserved phenylalanine in the third transmembrane helix of the human CB1R (hCB1R) to alanine (F200A). CHO cell lines stably expressing wild type (WT) and the F200A mutant hCB1R were examined for modulation of forskolin-stimulated cAMP accumulation and 3H-CP55940 radioligand binding. AM2233 functions as a full agonist to decrease forskolin-stimulated cAMP accumulation at hCB1R WT (EC50=0.93 nM). However at the F200A mutant, AM2233 further augments the forskolin-stimulated cAMP accumulation in a dose dependant manner (EC50=18nM). F200 does not contribute substantially to the high affinity binding of AM2233 at hCB1R, since the F200A mutation had a minor effect on AM2233 binding affinity (hCB1R WT IC50=5.7 nM; F200A mutant IC50=18 nM). The F200A mutation had a more significant effect on the binding of AM251 to WT receptor (IC50=3.5, and 30 nM, WT and F200A respectively). CP55940, HU-210 and Win55212-2, which are agonists with different structures, still function as agonists with similar efficacy and potency and have the same binding affinity between hCB1R WT and F200A mutant. We conclude that phenylalanine 200 of hCB1R is critical to the high affinity binding to AM251 and the functional property of AM2233.

A NEW RECEPTOR FOR CANNABINOID LIGANDS

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Endocannabinoids and extracts of the cannabis plant C. sativa affect a plethora of biological processes via CB_1 and CB_2 receptors. However, much evidence has emerged to suggest that additional receptors exist that mediate certain cannabinoid functions.

Here we show, that mammalian and plant derived cannabinoid ligands bind to and affect the activity of the orphan G-protein coupled receptor (GPCR) GPR55. In turn this results in activation of the intracellular signaling mediator RhoA via activation of G_{13} G-proteins. RT-PCR expression analysis, immunohistochemistry and in situ hybridisation using human material revealed that GPR55 is exressed in a number of tissues including certain vascular beds. In vivo, a GPR55 selective agonist, O1602, induces a reduction in blood pressure in spontaneously hypertensive rats. This effect is significantly enhanced by concomitant selective CB_1 receptor blockade, and is sensitive to a GPR55 selective antagonist. These data suggest that GPR55 is a novel cannabinoid receptor that may play a role in blood pressure regulation.

VANILLOID RECEPTOR LIGANDS MODULATE EXCITATORY SYNAPTIC TRANSMISSION IN THE RAT HIPPOCAMPAL SLICE

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We have investigated the possibility (Hajos & Freund, **2002**, Neuropharmacology, 43:503) that vanilloid receptor ligands inhibit excitatory synaptic transmission in the CA1 region of the rat hippocampus. Transverse hippocampal slices prepared from young adult Sprague-Dawley rats were maintained in an interface-type recording chamber perfused with artificial cerebrospinal fluid maintained at 28-30^{o}C. Schaffer collateral-commissural fibres were stimulated every 30 sec using a stimulus intensity that evoked a half-maximal population spike recorded from stratum pyramidale of the CA1 region. Drugs were made up in stock solutions of DMSO and applied by addition to the perfusing medium at dilutions of at least 1000-fold. The drug vehicle had no effect at these concentrations.

The TRPV1 agonists capsaicin (10 microM) and resiniferatoxin (50 nM) perfused onto the slice for 20 minutes caused a slowly developing inhibition of the amplitude of the population spike to 54 $\pm$ 8 and 56 $\pm$ 4% (mean $\pm$ standard error) of control respectively. Prior perfusion of the TRPV1 antagonist capsazepine (10 microM) had no effect by itself, but blocked the effects of both capsaicin and resiniferatoxin (population spike amplitudes were 100 $\pm$ 4 and 90 $\pm$ 3% of control respectively).

The results suggest that capsaicin and resiniferatoxin can indeed inhibit evoked excitatory transmission in the CA1 region, and that this effect is blocked by capsazepine. Whether this is via a TRPV1 receptor, or a different receptor with similar properties is unclear. Note that the effective concentrations of capsaicin and resiniferatoxin used to inhibit excitatory transmission in these experiments are 10-fold higher than those which we have previoulsy reported to selectively increase the paired-pulse depression of population spikes (Al-Hayani et al, **2001**, Neuropharmacology, 41:1000). Vanilloid receptor ligands may therefore have multiple effects on synaptic transmission in the hippocampus mediated by different receptors.

EFFECTS OF ENDOCANNABINOID UPTAKE INHIBITOR UCM707 ON CAPSAICIN AND *N*-ARACHIDONOYL-DOPAMINE-EVOKED RESPONSES OF RAT DRG NEURONES

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The endocannabinoid / endovanilloid *N*-arachidonoyl-dopamine (NADA) is an agonist at both anti-nociceptive CB_1 receptors and pro-nociceptive TRPV1 receptors, both of which are co-expressed by sensory primary afferent fibres. Previously we, and others, have demonstrated NADA-evoked increases in intracellular calcium ($[Ca^{2+}_i]$) in small diameter dorsal root ganglion (DRG) neurones (Sagar *et al.,* **2004**, E.J.N., 20, 175). It has been suggested that the anandamide membrane transporter may determine whether anandamide acts at extracellular CB_1 receptors, or intracellular sites on the TRPV1 receptor (De Petrocellis *et al.,* **2001**, J.B.C. 276, 12856). UCM707 is a potent and selective inhibitor of endocannabinoid uptake, with low affinity for the CB_1 receptor and TRPV1 receptor (Lopez-Rodriguez *et al.,* **2003**, E.J. Med. Chem. 38, 403). The aim of this study was to determine whether NADA-evoked activation of primary afferent fibres requires the uptake of NADA by the AMT. Here the effects of UCM707 on NADA-evoked increases in $[Ca^{2+}_i]$ in DRG neurones are reported.

Single cell calcium imaging studies were performed in adult rat DRG neurones. Neurones were cultured for 24 hours and loaded with Fura-2AM. Drugs were applied for 60 sec. with a 45 min. washout period between drug treatments. DRG neurones (n=36 neurones) were superfused with capsaicin (100 nM), followed by NADA (1μM), NADA (1μM) and UCM707 (1 μM) and finally KCl (60mM, 60 sec). In separate experiments, the effects of UCM707 (1 μM) on capsaicin (100nM)-evoked $[Ca^{2+}_i]$ were also studied on DRG neurones (n=65 neurones). Effects of UCM707 alone on $[Ca^{2+}_i]$ of DRG neurones (n= 65) were determined. Data are expressed as % KCl response. Statistical analysis used a Wilcoxon signed rank test with Gaussian approximation.

Both capsaicin and NADA increased $[Ca^{2+}_i]$ in DRG neurones (79±7 % of KCl and 57±9 % of KCl, respectively). In contrast, UCM707 alone produced no significant response (7±3 % of KCl). In the presence of UCM707, NADA-evoked increases in $[Ca^{2+}_i]$ were significantly smaller (29±5 % KCl, $p<0.001$) than NADA alone. Similarly, in the presence of UCM707, capsaicin-evoked increases in $[Ca^{2+}_i]$ were significantly smaller (35±6 % KCl, $p<0.0001$) than capsaicin alone. UCM707 did not alter KCl-evoked increases in $[Ca^{2+}_i]$ in DRG neurones.

Effects of both capsaicin and NADA were significantly attenuated by inhibition of the anandamide transporter. UCM707 has a low affinity for the TRPV1 receptor and, therefore, effects of UCM707 are unlikely to be due to TRPV1 antagonism. Our data suggest that the full expression of capsaicin and NADA-evoked increases in $[Ca^{2+}_i]$ in DRG neurones requires facilitated uptake of the drugs into DRG neurons and implies that at least part of their action is mediated by interaction with an intracellular receptor site.

CO-LOCALIZATION OF TRPV1 AND CB_1 RECEPTORS IN THE MOUSE BRAIN: AN IMMUNOHISTOCHEMICAL STUDY

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Cannabinoid CB_1 and transient receptor potential vanilloid type 1 (TRPV1) receptors have been proposed to act as metabotropic and ionotropic receptors, respectively, for the same class of endogenous polyunsaturated fatty acid amides, the acylethanolamides and the acyldopamides (Di Marzo et al., Curr. Opin. Neurobiol. **2002**). Furthermore, we and others have shown that functional cross-talks occur between these two receptors when they are expressed in the same cell (Hermann et al., Cell. Mol. Life Sci., **2003**; Kim et al., J. Neurosci. **2005**). Although demonstrated in sensory neurons of the dorsal root ganglia (Bridges et al., Neuroscience, **2003**), co-expression of CB_1 and TRPV1 has not yet been studied in the brain. In the present study, we addressed this issue by using double immunofluorescence and ABC immunohistochemistry techniques.

We used commercially available specific anti-TRPV1 or anti-CB_1 receptors antibodies and exploited the availability of the TRPV1 or CB_1 receptor null mice (-/-) as a control of the specificity of the antibodies. Eight anesthetized male mice (4 wild-types, 2 $TRPV^{-/-}$ and 2 $CB_1^{-/-}$) were transcardially perfused with 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). The brains were removed, cryoprotected in sucrose and cut on a cryostat into 20 μm-thick frozen coronal sections. Double immunofluorescence was carried out on free floating sections incubated for 2 days in a mixture of the following antibodies: goat anti-TRPV1 N-terminus (1:100; Santa Cruz) and rabbit anti-CB_1 N-terminus (1:400; Calbiochem). Subsequently, the sections were incubated for 4 hours at room temperature in a mixture of secondary IgG antibodies including goat anti-rabbit Alexa 488 and rabbit anti-goat Alexa 546 (1:100; Molecular Probes). For single ABC immunohistochemistry the same primary antibodies as for immunofluorescence were used (anti-TRPV1, 1:200; anti-CB_1, 1:800) followed by biotin-conjugated IgG secondary antibodies and avidin-biotin-peroxidase solution (ABC, Vector).

Both methods used point to the same results, the specificity of TRPV1 and CB_1 antibodies being confirmed by data obtained with brains from knock-out mice. TRPV1/CB_1 co-expression was detected in the hippocampus, substantia nigra compacta and cerebellum of the mouse brain. In particular in the cerebellum, high co-expression of TRPV1/CB_1 receptors was found as fine dots surrounding the Purkinje cell bodies, in their growth cone and axon. At high magnification the cytoplasm appeared CB_1-unstained and lightly TRPV1-stained. More intensely stained TRPV1/CB_1 immunoreactive bundle fibers were found in the cerebellum white matter. In the hippocampus TRPV1/CB_1 co-expression was detected in the principal neurons of the Ammon's horn and dentate gyrus, whereas in the stratum oriens many CB_1 immunoreactive cells were found that were negative for TRPV1. These data support the hypothesis of a functional relationship between the two receptor types also in the CNS.

IDENTIFICATION OF $TRPV_1$ RECEPTORS IN DIFFERENT NERVE TERMINALS OF THE RODENT HIPPOCAMPUS

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The presence of $TRPV_1$ (vanilloid) receptor immunoreactivity, mRNA, and radioligand binding in the rodent hippocampus has been indicated by previous studies. However, the exact cellular and subcellular distribution of the vanilloid receptor, and its function to presynaptically modulate the release of different transmitters are not well defined. We performed Western blot analysis of the subsynaptic fractions, and immunochemistry, release experiments with radiolabelled preloaded transmitters, and microfluorimetrical studies, all in hippocampal nerve terminals, as described in our previous publications [Katona et al. (**1999**) *J Neurosci* 19:4544; Díaz-Hernández et al. (**2002**) *J Pharmacol Exp Ther* 301:441; Köfalvi et al. (**2003**) *Eur J Neurosci* 18:1973; Köfalvi et al. (**2005**) *J Neurosci in press*]. With the highly sensitive immunohystochemical technics, we show now that $TRPV_1$ receptors are present in the active zone of the synapse, and co-localize with selective markers of glutamatergic, GABAergic, and cholinergic nerve terminals, and with CB_1 receptors. This latter finding suggests an interesting dual modulatory role in the same nerve terminals for anandamide and N-arachydonoyl-dopamine (NADA), the endogenous agonists for the inhibitory CB_1 receptor and the excitatory $TRPV_1$ receptor. NADA and the selective $TRPV_1$ agonist capsaicin inhibited the potassium (20 mM) evoked Ca^{2+}-entry into synaptosomes, but the underlying mechanisms were likely different. NADA, but not capsaicin induced Ca^{2+}-entry in the synaptosomes, comparable to the effect of epibatidine and kainate. Antagonists of the $TRPV_1$ receptor rather exacerbated than prevented the NADA-induced Ca^{2+}-entry. Capsaicin, only at 100 microM concentration, and only in the presence of 2-aminoethoxydiphenyl borate (100 microM), was able to evoke Ca^{2+}-entry. Finally, capsaicin and NADA differently modulated the resting and potassium-evoked releases of GABA and glutamate from hippocampal synaptosomes. Together, the immunochemical approaches confirmed the presence of the $TRPV_1$ receptor at sites crucial to modulate neural transmission, in agreement with previous electrophysiological findings. However, the conventional pharmacological assays failed to serve clear-cut evidence for the functionality of the $TRPV_1$ receptors in the hippocampus. These data also indicate that the non-specific effect of conventional vanilloid ligands must be carefully taken into account.

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ANTAGONISM OF THE CANNABIMIMETIC EFFECTS OF THC, ANANDAMIDE, CAPSAICIN, AND THEIR ANALOGS

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The endogenous cannabinoid (CB) receptor ligand anandamide has a similar pharmacological profile as Δ^9-tetrahydrocannabinol (THC), the main psychoactive constituent of marijuana. In addition, anandamide has been found to elicit similar pharmacological effects as capsaicin, the pungent component of chili peppers, by also activating the heat activated vanilloid receptor (VR1). THC, anandamide, capsaicin and other CB_1 and VR1 receptor agonists exhibit similar responses in the tetrad mouse model. Research has demonstrated that a number of anandamide and vanilloid analogs induce cannabimimetic behavioral effects regardless of whether they have moderate or high affinity at both CB_1 and VR1 receptors (CB_1/VR1 hybrids) or were more selective for just one of these receptors (Di Marzo, Bisogno et al. **2001**, Biochem Biophys Res Commun, 281(2): 444-51).

The present study sought to determine if inhibition of CB_1 or CB_2 receptors, with the selective antagonists SR141716A and SR144528, respectively, would block the pharmacological effects of CB_1 and VR1 receptor agonists in the mouse tetrad test. These effects include 1) antinociception, determined by tail-flick latency, 2) catalepsy, by the ring stand test, 3) hypothermia, by rectal temperature, and 4) hypomobility, by spontaneous activity in the open-field test. THC, anandamide, capsaicin, three anandamide and four vanilloid analogs were tested.

As expected, SR141716A attenuated THC and anandamide-induced hypomobility, antinociception, hypothermia and catalepsy whereas SR144258 did not. Similar results were observed with the anandamide analogs 0-1860, 0-1812, and 0-1811 such that cannabimimetic effects were blocked with SR141716A but not with SR144528. These results support previous research that has demonstrated that cannabimimetic effects are induced by activation of CB_1 receptors and that peripheral CB_2 receptors do not play a role in these effects. In contrast, capsaicin-induced hypomobility, antinociception, hypothermia and catalepsy were not attenuated by either SR141716A or SR144258. Likewise, none of these pharmacological effects exhibited by the vanilloid analogs, 0-1856, 0-1839, 0-1895, and 0-1861, were antagonized by pre-administration of SR141716A or SR144258, suggesting that these effects of capsaicin and vanilloid analogs were not mediated by CB_1 or CB_2 receptor activation. Current studies are investigating the inhibition of cannabimimetic effects with vanilloid receptor antagonists.

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Di Marzo, V., T. Bisogno, et al. (**2001**). "Highly selective CB(1) cannabinoid receptor ligands and novel CB(1)/VR(1) vanilloid receptor "hybrid" ligands." Biochem Biophys Res Commun 281(2): 444-51.

CONTROL OF NEURONAL CB_1 RECEPTORS BY LIPID RAFTS, AND MODULATION OF ANANDAMIDE-INDUCED APOPTOSIS

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Several G protein-coupled receptors function within lipid rafts, plasma membrane microdomains which may be important in limiting signal transduction. Here we show that treatment of rat C6 glioma cells with the raft disruptor methyl-β-cyclodextrin (MCD) doubles the binding efficiency (i.e., the ratio between maximum binding and dissociation constant) of type-1 cannabinoid receptors (CB_1R), which belong to the rhodopsin family of G protein-coupled receptors. In parallel, activation of CB_1R by the endogenous agonist anandamide (AEA) leads to ~3-fold higher [^{35}S]GTPγS binding in MCD-treated cells than in controls, and CB_1R-dependent signaling via adenylate cyclase and p42/p44 mitogen-activated protein kinase is almost doubled by MCD. Unlike CB_1R, the other AEA-binding receptor TRPV1, the AEA synthetase NAPE-PLD and the AEA hydrolase FAAH are not modulated by MCD, whereas the activity of the AEA membrane transporter AMT is reduced to ~50% of the controls. We also show that MCD reduces dose-dependently AEA-induced apoptosis in C6 cells but not in human CHP100 neuroblastoma cells, which mirror the endocannabinoid system of C6 cells but are devoid of CB_1R. MCD reduces also cytochrome c release from mitochondria of C6 cells, and this effect is CB_1R-dependent and partly mediated by activation of p42/p44 mitogen-activated protein kinase. Altogether, the present data suggest that lipid rafts control CB_1R binding and signaling, and that CB_1R activation underlies the protective effect of MCD against apoptosis.

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SPATIAL AND FUNCTIONAL SEPARATION BETWEEN ANANDAMIDE UPTAKE AND HYDROLYSIS IN HUMAN KERATINOCYTES

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The signaling activity of anandamide (AEA) is terminated by its uptake across the cellular membrane and subsequent intracellular hydrolysis by the fatty acid amide hydrolase (FAAH). To date, the existence of an AEA membrane transporter (AMT) independent of FAAH activity remains questionable, though it has been recently corroborated by pharmacological and genetic data. We performed confocal microscopy and biochemical analysis in human HaCaT keratinocytes, in order to study the cellular distribution of AMT and FAAH. We found that FAAH is intracellularly localized as a punctate staining partially overlapping with the endoplasmic reticulum. Consistently, subcellular fractionation and reconstitution of vesicles from membranes of different compartments demonstrated that FAAH activity was localized mainly in microsomal fractions, whereas AMT activity was almost exclusively in plasma membranes. These results provide the first morphological and biochemical evidence to support the view that transport and hydrolysis are two spatially and functionally distinct processes in AEA degradation.

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DIFFERENTIAL ACTIVITY OF CANNABIDIOL AND TETRAHYDROCANNABINOL AT $5HT_{1A}$ RECEPTORS

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One of the prominent biologically active components of cannabis is cannabidiol (CBD). Unlike tetrahydrocannabinol (THC), which is psychoactive, CBD is not. The human $5HT_{1a}$ receptor is a member of the seven transmembrane (7TM) superfamily, and is implicated in a variety of processes, including anxiety, depression, headache, pain, and thermoregulation. In this project, using filtration-based drug-receptor binding techniques, CBD (4-32 μM) displaced the highly specific agonist, [^{3}H]8-OH-DPAT from the human $5HT_{1a}$ receptor. The apparent IC_{50} was 8 μM in this concentration-dependent relationship. On the other hand, in these cell culture-based experiments, THC was unable to displace agonist from the receptor in the same micromolar range. The intrinsic activity of CBD was determined in two signal transduction formats. First, CBD (16 μM) increased GTP binding in this G protein coupled receptor (GPCR) system as do the agonists, serotonin and 8-OH-DPAT. Second, in this negatively coupled system, both CBD (16 μM) and other known agonists decrease cAMP concentration at similar levels of receptor occupancy. In preliminary comparative studies with the cloned rat $5HT_{2a}$ receptor (also a 7TM/GPCR) in culture, CBD was active but less so (apparent IC_{50} of 32 μM) than at the human $5HT_{1a}$ receptor. In all experiments, the methanol that accompanied CBD was shown to be inactive in the assays utilized here. We conclude that CBD is a modest affinity agonist at the human $5HT_{1a}$ receptor in cell culture. Further experiments are needed to detail CBD's properties at the $5HT_{2a}$ receptor to compare its potential in other species and at other serotonin receptors, and to explore signal transduction characteristics in depth. The results reported here suggest that cannabidiol may have interesting and useful potential beyond the realm of cannabinoid receptors.

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HETEROLOGOUS EXPRESSION OF FATTY ACID AMIDE HYDROLASE AS A FUSION TO MALTOSE-BINDING PROTEIN

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Introduction: Fusion proteins have been widely used as strategy for the recombinant expression of a target protein. In this context, maltose-binding protein (MBP) as the carrier protein was reported to improve the solubility and expression level of passenger proteins as well as to facilitate their subsequent purification.

Fatty acid amide hydrolase is an eukaryotic membrane protein and its heterologous expression in *E.coli* has therefore proven to be very difficult to handle, with low yields of production.

Here we present the results of the overexpression of FAAH in *E.coli* as a C-term fusion to maltose-binding protein (MBP-FAAH).

Methods and Results: For this purpose, cDNA encoding rat FAAH was cloned into pMALcRI plasmid and expression carried out in *E.coli* with IPTG (0.3mM) induction for 2h at 37°C. Kinetics experiments, done in order to validate the fusion protein, and efforts towards its purification will be presented.

Briefly, fusing FAAH to MBP will be very helpful for an efficacious overexpression of the enzyme in *E.coli*. This strategy can be used in order to obtain large quantities of the enzyme, suitable for kinetics and inhibitors screening experiments.

At this point however, efforts in order to completely purify the fusion protein turned out to be very difficult. Chromatography exploiting affinity of the maltose-binding protein moeity for amylose as well as either ion metal affinity chromatography or ion exchange chromatography were undertaken, leading to an only partial purification of the recombinant protein. Reasons explaining such difficulties seem to come from the fact that overexpression of the interest protein is accompagnied by the expression of a serie of by-products of MBP-FAAH.

Conclusion: In conclusion, fusing fatty acid amide hydrolase to maltose-binding protein is a strategy suitable for the production of large quantities of enzyme for kinetics or inhibitors screening experiments. Purification of the fusion protein has until now proven to be exceedently difficult and further highlights the problems encompassing heterologous expression of eukaryotic proteins.

EFFECT OF INHIBITORS OF ENDOCANNABINOID UPTAKE AND FAAH ON BRAIN ENDOCANNABINOID LEVELS

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Several inhibitors of endocannabinoid inactivation via either the fatty acid amide hydrolase (FAAH) or the putative endocannabinoid transporter have been developed so far. Although these compounds have been shown to enhance the pharmacological actions of endocannabinoids in vitro and in vivo, and to exert beneficial actions in several animal models of neurodegenerative disorders, their effects on brain endocannabinoid levels has never been reported. Here we have studied the effect in rats of three recently developed selective inhibitors of endocannabinoid uptake (VDM-11, UCM-707 and OMDM-2) and of a selective FAAH inhibitor (*N*-arachidonoyl-serotonin) on the brain concentrations of anandamide and 2-arachidonoylglycerol.

The compounds were administered intraperitoneally at a dose of 5 mg/kg to male Wistar rats. The treatment included 5 injections done every 12 hours over a total period of 48 hours. The animals were sacrificed after 1, 5 and 12 hours from the last administration and brains were immediately removed and stored at –80° until lipid extraction. Brain concentrations of anandamide and 2-AG, expressed as pmol or nmol/g wet tissue weight, respectively, were then measured by means of isotope dilution LC-MS.

Of the three uptake inhibitors tested, OMDM-2 was the most efficacious since it significantly enhanced the levels of anandamide at all time points, with a maximal effect (1.9-fold enhancement) after 5 h. This compound also enhanced 2-AG levels by ~1.3-fold, but only 5 and 12 h from administration. VDM-11 slightly, albeit significantly, enhanced anandamide levels (1.25-fold) only at 1 h from administration, and 2-AG levels (1.3-fold) only after 5 h. Finally, UCM-707 only affected 2-AG levels (by 2-fold) at 1 h from administration. FAAH inhibition by *N*-arachidonoyl-serotonin significantly enhanced the levels of both anandamide (between 1.25- and 1.5-fold, maximal effect after 1 h) and 2-AG (between 1.3- and 1.6-fold, maximal effect after 12 h) at all time points. In this last group, we also measured serotonin contents by HPLC-ED to control the metabolic stability of this FAAH inhibitor.

These data indicate that: 1) the several pharmacological effects reported so far for the four compounds under study in animal models of diseases are indeed due, at least in part, to enhancement of endocannabinoid levels; 2) OMDM-2 and *N*-arachidonoyl-serotonin should be regarded as enhancers of endocannabinoid levels particularly suitable for use *in vivo*; and 3) 2-AG levels seem to need a longer time after the last administration in order to be augmented, at least in the case of *N*-arachidonoyl-serotonin, OMDM-2 and VDM-11.

N-ARACHIDONYLMALEIMIDE IS A POTENT INHIBITOR OF MGL-LIKE ENZYMATIC ACTIVITY IN RAT CEREBELLAR MEMBRANES

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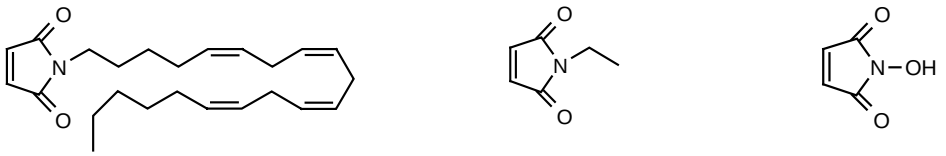
Introduction: The endogenous cannabinoid 2-arachidonoylglycerol (2-AG) activates CB_1 and CB_2 cannabinoid receptors, inducing cannabimimetic effects. However, *in vivo* effects of 2-AG are weak due to its rapid enzymatic hydrolysis.

Monoglyceride lipase (MGL), the main enzyme responsible for 2-AG hydrolysis, is sensitive to inhibition by *p*-chloromercuribenzoic acid (*p*CMB), mercury chloride ($HgCl_2$) and *N*-ethylmaleimide (NEM), suggesting the presence of essential sulfhydryl group(s). The aim of this study was to characterize a putative *N*-ethylmaleimide-sensitive site on the MGL-like enzyme, which was previously found in rat cerebellar membranes (Saario et al., *Biochem Pharmacol* **2004**;67**:**1381-87).

Methods: Nine *N*-alkyl/aryl maleimides of diverse hydrophobicity were examined for their ability to inhibit MGL-like enzymatic activity in rat cerebellar membranes. Rat cerebellar membranes were preincubated with each maleimide derivative prior to their incubation with 2-AG. Inhibition activities of the maleimides were determined by the production of arachidonic acid, the hydrolysis product of 2-AG, as measured by HPLC. Additionally, the positions of the cysteine residues in 3D model of the rat and human MGL enzyme were visualized.

Results**:** *N*-arachidonylmaleimide, NEM and *N*-hydroxymaleimide (Figure 1) inhibited enzyme activity with IC_{50}-values (mean ± SEM, n=3) of 180 ± 10 nM, 55 ± 14 μM and 500 ± 27 μM, respectively. A comparison of these maleimides revealed a correlation between increasing potency of enzyme inhibition and increasing hydrophobic character. According to the 3D enzyme structure, the NEM-sensitive amino acid residue is either Cys208 or Cys242, both of which are located at the hydrophobic 2-AG binding site.

Conclusion**:** The relatively high potency of *N*-arachidonylmaleimide is most probably due to its structural similarity with the natural MGL substrate 2-AG. These findings provide further support to our previous conclusion that the MGL-like enzymatic activity in rat cerebellar membranes corresponds to MGL. Furthermore, our data suggest that the NEM-sensitive cysteine residue on MGL is located in the nonpolar 2-AG binding region of this enzyme.



1 **2** **3**

Figure 1. Chemical structures of *N*-arachidonylmaleimide (1), NEM (2) and *N*-hydroxymaleimide (3).

THE ANANDAMIDE HYDROLASE INHIBITOR PHENYLMETHYLSULPHONYLFLUORIDE DEMONSTRATES ANTICHOLINESTERASE ACTIVITY IN TWO ISOLATED NERVE-SMOOTH MUSCLE PREPAPRATIONS

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The endogenous cannabinoid anandamide (AEA) is highly susceptible to metabolic degradation by the enzyme fatty acid amide hydrolase (FAAH) (Cravatt *et al.,* **1996**). Inhibition of FAAH may serve as an attractive therapeutic strategy for selectively augmenting local endocannabinoid levels without the side effects that may be elicited by exogenously applied cannabinoid receptor agonists. The compound phenylmethylsulphonylfluoride (PMSF) is used as a FAAH inhibitor to potentiate the effects of endogenously released AEA (Pertwee *et al*., **1995**), but it can also inhibit the enzyme acetylcholinesterase (Skau *et al*., **1999**). Exogenously applied AEA inhibits contractions of the ileal myenteric plexus longitudinal muscle (MPLM) preparation of the rat (unpublished observations) and guinea pig (Pertwee *et al*., **1995**) evoked by electrical field stimulation (EFS). Therefore if the endogenous cannabinoid system is tonically active in these preparations, PMSF would be expected to exert an inhibitory effect. The aim of the present study was to examine the effects of PMSF on electrically evoked contractions of the guinea pig and rat isolated ileal MPLM preparation. Strips of ileal MPLM were dissected from male Dunkin-Hartley guinea pigs (400-650g) and male Wistar rats (350-550 g) and mounted in organ baths for recording of isometric contractions to EFS. Guinea pig MPLM strips were stimulated at 0.1 Hz frequency, 0.5 ms pulse duration at supramaximal voltage, whereas rat MPLM strips were stimulated at 30 Hz frequency, 0.5 ms pulse duration for 2 sec every min at 30 V intensity. Values are expressed as mean potentiation or inhibition of maximal response ± s.e.m. All drugs were dissolved in distilled water with the exception of PMSF, which was dissolved in absolute ethanol. PMSF (10^{-6} to 3 x 10^{-5} M) had no effect on EFS contractile responses of both guinea pig and rat MPLM, but dose dependently potentiated EFS responses between 10^{-4} to 10^{-3} M, (mean maximal potentiation at 10^{-3} M: 428.40 ± 40.57 % (n=6) and 143.60 ± 19.96 % (n=12) respectively). These EFS contractile responses were confirmed to be cholinergic and nerve mediated by their abolition with either tetrodotoxin (10^{-6}M) or atropine (10^{-6}M). The possibility that PMSF was demonstrating anticholinesterase activity in the gut was confirmed by its ability to produce a statistically significant leftward shift of the concentration response curve to exogenously administered acetylcholine (10^{-10} to 10^{-5} M) and an increase in tissue maximal response (42% and 30% increase by PMSF (10^{-3} M)) on the rat and guinea pig MPLM respectively. $P<0.0001$ two-way ANOVA). PMSF had no effect on the carbachol (10^{-10} to 10^{-5} M) cumulative dose response curves on either rat or guinea pig ileal MPLM preparations. These findings suggest a possible absence of an endogenous cannabinoid tone in the gut and also indicate that care must be taken in the use of PMSF as a FAAH inhibitor with consideration being given to the tissue under study and the concentration of PMSF being used.

Cravatt BF *et al* (**1996**) *Nature* 384:83-7
Pertwee RG *et al*., (**1995**) *Eur. J. Pharmacol* 272: 73-8
Skau KA *et al*., (**1999**) *Neuropharmacology* 38(5): 691-8

NOVEL ANANDAMIDE BIOSYNTHETIC PATHWAY IN RAW264.7 MACROPHAGES

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We previously found that bacterial endotoxin (lipopolysaccharide [LPS], 10 ng/ml) increases anandamide (AEA) levels in RAW264.7 cells >10-fold by increasing both the generation of the AEA precursor NAPE and its subsequent metabolism to yield AEA, presumably via *N*-acyltransferase and a phospholipase D (NAPE-PLD), respectively. LPS also induces the AEA-degrading enzyme fatty acid amidohydrolase (FAAH). (J Biol Chem. 278:45034-9, **2003**). We now report that LPS actually decreases NAPE-PLD gene expression by ~50% in RAW264.7 cells, while it increases the gene expression of a protein tyrosine phosphatase (non-receptor type 8) 3.2-fold. The PLC inhibitor neomycin (3 mM) or the non-selective tyrosine phosphatase inhibitor sodium orthovanadate (SOV, 100 μM) greatly reduced LPS induction of AEA. TLC analysis of cellular lipids revealed the presence of phospho-AEA in extracts from SOV-pretreated but not from control cells. Synthetic phospho-AEA incubated with cell homogenates is time-dependently converted to AEA, which is blocked in the presence of SOV or when the cell homogenates is boiled before the assay. These data indicate the existence in RAW264.7 cells of a novel AEA biosynthetic pathway where NAPE generated via *N*-acyltransferase is hydrolyzed by PLC to yield phospho-AEA, which is then dephosphorylated by a tyrosine phosphatase. In LPS-stimulated cells this pathway becomes predominant, whereas NAPE-PLD is suppressed.

IDENTIFICATION OF NOVEL BRAIN-DERIVED FATTY ACID AMIDES IN EXTRACTS OF THE RAT BRAIN

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The discovery and study of putative lipid neurotransmitters like the endogenous cannabinoids arachidonoyl ethanolamide (anandamide) and 2-arachidonoyl glycerol has lead to an explosion of work in many fields of cannabinoid-related neuroscience. Our lab has been involved in the further discovery and study of the cannabimimetic lipid amides N-arachidonoyldopamine, oleoyldopamine, stearoyldopamine, palmitoyldopamine, N-arachidonoylGABA, and N-arachidonoylglycine that may add to our understanding of signaling pathways in pain and inflammation. In this study we continued the search for novel brain fatty acid amides similar to those listed above. To extract the compounds, homogenates of 6 male Sprage-Dawley rats were subjected to a modified liquid-liquid Folch extraction with further purification on diethylamino, C18, and silica separation columns. Effluent was concentrated and subjected to gradient capillary HPLC on a 75μm C18 column flowing at 400nL/min, ionized by an Applied Biosystems nanospray ion source. Parent and fragment ions were monitored using an Applied Biosystems/MDS Sciex QStar quadrupole/time-of-flight mass spectrometer. Preliminary data suggest the existence of 11 novel brain-derived lipid amides: N-palmitoylglycine, N-oleoylglycine, N- stearoylglycine, N-palmitoylGABA, N-oleolylGABA, N-palmitoylmethionine, N-palmitoylglutamic acid, N-palmitoylglutamine, N-arachidonoylglutamine, N-arachidonoylserine, and N-arachidonoyltyrosine. Each of these compounds may have unique neurophysiological effects that have yet to be determined.

PHARMACOLOGICAL CHARACTERIZATION OF THE ENDOCANNABINOID TRANSPORTER USING A NOVEL HIGH AFFINITY LIGAND

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Anandamide (arachidonylethanolamide; AEA) is an endogenous lipid agonist for the CB_1 and CB_2 cannabinoid receptors. Because of its lipid structure, AEA's characteristics are quite distinct from classical monoamine neurotransmitters. The mechanism of AEA synthesis and release has been described; however the mechanism of removal of AEA from the synapse remains quite controversial. Evidence exists to support diffusional, protein-mediated, and endocytic processes. To further evaluate the mechanism of AEA uptake, a novel small molecule probe was developed from a group of compounds exhibiting high potency inhibition of AEA uptake in RBL-2H3 cells. An analogue of LY2183240 was iodinated for use in radioligand binding assays to characterize the specific protein involved in AEA uptake. This novel radioligand ([^{125}I]-LY2318912) maintained high potency as an inhibitor of AEA uptake in RBL-2H3 cells. Radioligand binding assays performed in membranes prepared from RBL-2H3 cells revealed a specific, high affinity, saturable binding site with pharmacology correlating to affinities observed in functional AEA uptake assays. To verify this protein was distinct from fatty acid amide hydrolase (FAAH), radioligand binding was performed in membranes prepared from wild-type HeLa cells lacking detectable expression or activity of the FAAH enzyme (Day et al., **2001**). Binding constants measured were identical in membranes prepared from both HeLa (FAAH-/-) and RBL-2H3 cells, indicating the presence of a specific binding site distinct from FAAH.

The role of FAAH in AEA uptake was assessed through pharmacological evaluation of uptake inhibitor compounds in both HeLa (FAAH-/-) and RBL-2H3 cells. In HeLa (FAAH-/-) cells there was a reduction in total AEA uptake compared to RBL-2H3 cells, as well as a right shift of the dose response curves for these inhibitors. However, transfection and expression of FAAH in the wild-type FAAH (-/-) HeLa cells restored the potency of these compounds to near that observed in RBL-2H3 cells. These data indicate that AEA uptake occurs via a specific process distinct from FAAH, but requires FAAH for facilitation.

ENHANCED PERIPHERAL CANNABINOID (CB_2) RECEPTOR EXPRESSION IN THE BRAIN AND REDUCED ALCOHOL CONSUMPTION IN MOUSE CHRONIC MILD STRESS (CMS) MODEL OF DEPRESSION

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Depression is characterized by a lack of interest in pleasurable things of life (termed anhedonia) and depressed mood but the involvement and expression of CB_2 cannabinoid receptors in the mouse brain or in depression is unknown. The aim of these studies is to determine whether the link between depression and stress factors that are known to be involved in problem drug and alcohol dependency, may be associated with the endocannabinoid hypothesis of substance abuse. The endocannabinoid physiological control system (EPCS) exerts a powerful modulatory action on synaptic transmission and in most biological systems (for a review see Onaivi et al., **2002**). We have set up the chronic mild stress (CMS) model using mice in order to simulate the symptoms of anhedonia, a major feature of depression. This was achieved by subjecting mice to mild stressors every day for four weeks and anhedonia was measured by the consumption of 2% sucrose solution. Once anhedonia was established the behavioral and rewarding effects of abused substances were determined in the CMS and control animals. The expression of CB_2 cannabinoid receptors was determined and compared in the brains of the CMS and control animals by Western blotting immunoreactivity using CB_2 cannabinoid receptor antibody. The reduction in locomotor activity induced in mice by CMS was further enhanced by treatment with WIN55212-2, a cannabinoid receptor agonist. The influence of CMS on the performance of mice in the plus-maze test of emotionality was gender specific with the female mice being more susceptible to open arm aversions in the plus-maze. Mice that were prenatally exposed to capsaicin, a vanilloid receptor agonist, also demonstrated this gender dependent response. However, the treatment with WIN55212-2 reduced the mouse aversions induced by CMS. These reduced aversions by WIN55212-2 were also enhanced in animals prenatally exposed to capsaicin. There was cannabinoid CB_2 receptor immunoreactivity with enhanced expression in the brains of CMS animals in comparison to controls and no detectable immunostaining post incubation with CB_2 receptor blocking peptide. Two major and striking findings from these studies were the decrease in alcohol intake induced by the CMS and the expression of CB_2 cannabinoid receptor in the mouse brain that was enhanced in the brains of the CMS animals. Therefore the present results demonstrated that CMS model of depression alters the behavioral and rewarding effects of abused substances in a cannabinoid and vanilloid receptor sensitive manner. The findings suggest that the CB_2 cannabinoid receptors that are expressed in the mouse brain may be involved in depression and substance abuse.

ENDOCANNABINOID REGULATION OF ANHEDONIA: ROLE OF STRESS-INDUCED CHANGES OF ENDOCANNABIONOID SIGNALING IN THE AMYGDALA

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Depression is a complex, heterogeneous disorder and several neurotransmitter and neurohormone pathways have been implicated in its pathophysiology. An emerging hypothesis suggests that depression may arise when neural systems do not exhibit appropriate, adaptive plasticity in response to an external stimulus such as stress. As endocannabinoids (eCBs) play roles in the processing of reward, stress, and are involved in responses to subserve synaptic plasticity including depolarization-induced suppression of inhibition (DSI) and excitation (DSE), we hypothesized that eCBs play a role in the pathophysiology of depression. Anhedonia, a core symptom of depression, was modeled in mice using repeated restraint stress. Anhedonia is operationally defined as a decrease in sensitivity to reward. Sensitivity to natural reward was determined by measuring sucrose consumption and preference in a two-bottle choice protocol. A dose of the CB_1 receptor agonist, CP55940 (30 μg/kg), which had no effect on sucrose consumption and preference, blocked stress-induced decreases in sucrose and saccharin consumption and preference. A dose of the CB_1 receptor antagonist/inverse agonist, SR141716 (1 mg/kg), which had no effect on sucrose consumption and preference, accentuated stressed induced decreases in sucrose and saccharin consumption and preference. Thus, restraint stress decreased sensitivity to natural reward regardless of caloric value and CB_1 receptor activation blocked while CB_1 receptor blockade exacerbated stress-induced anhedonia. These findings suggest that eCBs are part of a homeostatic system that protects animals from stress-induced anhedonia. Pharmacological agents that increase eCB tone could block the development of anhedonia and may be useful in the treatment of depression.

Stress-induced changes in sensitivity to reward require that stimulus-reward value associations be updated. The basolateral amygdala (BLA) contributes to updating stimulus-reward value associations. We hypothesized that stress-induced anhedonia is accompanied by changes in eCB signaling in the amygdala. Fatty acid amide hydrolase (FAAH) activity was increased in the amygdala after 10 days of restraint stress. Ten days of restraint stress had no effect on either monoacylglycerol lipase (MGL) activity or CB_1 receptor binding in the amygdala. Amygdalar content of the *N*-acylethanolamines (NAEs), anandamide (AEA) and palmitoylethanolamide (PEA), were decreased on restraint days 1, 7, and 10. Restraint stress increased 2 arachidonylglycerol (2-AG) content in the amygdala on restraint day 10 only. We speculate that the eCBs, AEA and 2-AG, may act in concert to increase excitatory drive in the amygdala, which could be a component of a homeostatic system that protects animals from stress-induced anhedonia.

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REGION SPECIFIC MODULATION OF THE ENDOCANNABINOID SYSTEM FOLLOWING PROLONGED GLUCOCORTICOID EXPOSURE

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Substantial evidence has accumulated that suggests that the endocannabinoid system could, in part, be regulated by glucocorticoid activity. Previously we have demonstrated that chronic, unpredictable stress, which results in both hypersecretion of glucocorticoids and adrenal hypertrophy, down-regulates both CB_1 receptor binding and protein and decreases endocannabinoid content in the hippocampus. However, whether these changes are due to the experiential nature of stress and subsequent neurochemical responses, or the hormonal substrate of the stress response are still unknown. Removal of endogenous glucocorticoids has been shown to increase CB_1 receptor mRNA expression, implying that glucocorticoids negatively regulate CB_1 receptor expression. To further examine the role of glucocorticoids in regulation of the endocannabinoid system, we administered male Long-Evans rats 20 mg/kg corticosterone-21-acetate for 21 days and examined CB_1 receptor binding and protein expression, endocannabinoid content and metabolic enzyme expression or activity. Chronic corticosterone administration resulted in a non-statistically significant decrease in both CB_1 receptor density (B_{max}) and protein expression. Interestingly, the binding affinity (K_d) of the CB_1 receptor was reduced, an effect not seen following chronic stress. Also unlike chronic unpredictable stress, long-term glucocorticoid exposure did not affect levels of any endocannabinoid (anandamide, 2-AG, PEA and OEA) in the hippocampus. No changes were seen in expression of FAAH or cytosolic MAG lipase activity. In the amygdala, chronic corticosterone treatment did not affect CB_1 receptor binding parameters (B_{max} or K_d), but did lead to a significant increase in 2-AG content (without effecting PEA or OEA levels). These findings lead to two conclusions regarding the role of glucocorticoids and the endocannabinoid system: 1) some of the effects of chronic stress on the endocannabinoid system are likely due to alterations independent of glucocorticoid hypersecretion; 2) the effects of glucocorticoids on the endocannabinoid system are brain-region-specific. Further research is required to fully understand the mechanisms mediating stress-induced alterations of the endocannabinoid system.

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ENDOCANNABINOIDS MODULATE GLUTAMATERGIC BUT NOT GABAERGIC NEUROTRANSMISSION ONTO LAYER V PYRAMIDAL NEURONS IN MOUSE SENSORY CORTEX

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Depolarization-induced suppression of inhibition (DSI) or excitation (DSE) are forms of short-term synaptic plasticity mediated by the brains endocannabinoid system that are known to occur at GABAergic or glutamatergic synapses, respectively. DSI and DSE have been shown to co-exist in many brain regions including the hippocampus, cerebellum, and ventral tegmental area. In the present study, using whole-cell patch clamp recordings from layer V pyramidal neurons in slices of mouse sensory cortex, we show that depolarization of layer V pyramidal neurons triggers DSE but not DSI of evoked intralaminar postsynaptic currents. Consistent with this finding, evoked EPSCs but not IPSCs recorded from layer V pyramidal neurons were suppressed by bath application of 5 μM WIN55,212-2, a cannabinoid receptor agonist. The reduction in evoked EPSC amplitude by WIN55,212-2 was reversed by co-application of 2 μM SR141716, a cannabinoid receptor antagonist. The degree of WIN55,212-2-mediated reduction in evoked EPSCs was highly correlated with the magnitude of suppression observed during DSE. Furthermore, the reduction in evoked EPSC amplitude by WIN55,212-2 was accompanied by an increase in the paired pulse ratio indicating that it was most likely due to a reduction in the probability of presynaptic neurotransmitter release. These results demonstrate that layer V pyramidal neurons are capable of modulating glutamatergic but not GABAergic inputs via presynaptic cannabinoid receptors.

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GETTING LOST: THE EFFECTS OF CANNABINOIDS ON SPATIAL REPRESENTATION IN THE RODENT HIPPOCAMPUS

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The present study investigates the role of exogenous cannabinoids in the formation and regulation of hippocampal place fields using multiple single unit recording technology. The hippocampus is a primary brain area affected by exogenous cannabinoid administration. Modulation of hippocampal network dynamics via exogenous cannabinoids has been shown to result in deficits in learning and memory. Presently, we have examined the effects of Δ^9-THC, and the reverse agonist SR171416A (both in combination and separately), on the quality and stability of spatial representation in the hippocampus, levels of ensemble reactivation, and intrinsic network oscillations. Preliminary results indicate a reduction in the theta band oscillations (between 7-10 Hz), as well as an increase in the information content of place fields, at low-doses. At higher doses, there appears to be an increase of place field size from baseline. The present study lends insight not only into the nature of the deleterious effects of exogenous cannabinoids, but also the significance of basal endocannabinoid signaling on information processing in the hippocampus. Further studies may serve to elucidate how retrograde endocannabinoid synaptic signals influence neural computation.

EFFECT OF THE ANANDAMIDE TRANSPORT INHIBITOR, AM 404, ON ANXIETY RESPONSE IN RATS

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There are many and contradictory reports on the interaction between cannabinoids and anxiety. Both cannabinoid agonists and antagonists have been shown to have anxiolytic- and anxiogenic-like behavioural reactions in rodents depending on the dose and the context (Onaivi et al. **1990**; Crawley et al. **1993**; Onaivi et al. **1995**; Rodriguez de Fonseca, **1996**; Navarro et al., **1997**; Akinshola et al. **1999**; Haller et al., **2002**; Berrendero and Maldonado **2002**;Valjent et al.. **2002**; Haller et al. **2004**).

The aim of the present work was to further elucidate the role of endocannabinoid system in anxiety response. For this purpose, the anandamide transport inhibitor, AM 404 (2.5-10 mg/kg), and Δ^9–THC (0.015-1.5 mg/kg), previously evaluated in our laboratory for its reinforcing properties in a Conditioned Place Preference test (Braida et al., **2005**), were studied in a plus-maze apparatus according to Pellow et al. (**1985**) The test length was 5 min and the total time spent in each arm and the number of arm entries were scored by trained observers in male Sprague-Dawley rats, 30 min after treatment. The role of the CB_1 cannabinoid and opioid receptor was investigated pre-treating rats with SR141716 (0.25-1 mg/kg) and naloxone (0.5-2 mg/kg), 10 min before Δ^9–THC or AM 404.

Both Δ^9–THC (0.75 mg/kg) and AM 404 (10 mg/kg) significantly elevated the percentage of open arm entries and the time spent in the open arms, showing an anxiolytic activity. This effect was reversed by pre-treatment with SR141716. An interaction with opioid system was also found.

These findings further support a key role of endocannabinoid system in the regulation of emotional states.

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ARE THE EFFECTS OF WIN55,212-2 ON SPATIAL REFERENCE MEMORY MEDIATED BY CB_1 RECEPTORS IN THE HIPPOCAMPUS?

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Previous studies have reported that the cannabinoid agonist WIN55,212-2 (WIN-2) induces spatial learning and memory impairments in both the radial arm maze (Lichtman et al. **1995**) and DMTS/DNMTS tasks (Hampson and Deadwyler, **1999**; **2000**; Han et al. **2000**). The majority of research to date suggests that the effects of cannabinoids on spatial memory are mediated via hippocampal CB_1 receptors, since intrahippocampal (ihc) infusion of the cannabinoid agonists CP55,940 and Δ^9-tetrahydrocannabinol (Δ^9-THC) impaired spatial working memory (Egashira et al. **2002**; Lichtman et al. **1995**). However, ihc effects of the effects of WIN-2 have not been studied. The main objective of this study was to determine whether WIN-2 induced effects on spatial reference memory using the water maze are mediated by the hippocampus and more specifically if they are mediated by hippocampal CB_1 receptors.

Two experiments employed naïve Lister hooded rats:

In Exp. 1 animals were assigned to drug groups of WIN-2 (1mg/kg); WIN-2 (3mg/kg); WIN-2 (1mg/kg) + AM281 (0.5mg/kg); Tween 80 was used as the vehicle (N=9 per group). All drugs were injected intraperitoneally (ip) 30 minutes prior to testing.

In Exp. 2 animals were assigned to the drug groups of WIN-2 (0.125μg), WIN-2 + AM281 (0.0625μg) or WIN-3 (0.125μg) (N=8 per group). WIN-3 the inactive isomer of WIN-2 was used as the vehicle. The animals were surgically implanted with bilateral ihc cannulae attached to minipumps, and drugs were infused chronically for 14 days. In both experiments animals performed a standard reference memory task using 4 trials a day for 4 days (max. trial time 90s and all ITI's 30s). Probe trials (max. trial time 60s) were performed 24hr and 4days post-acquisition.

Systemic infusion of WIN-2 (1mg/kg and 3mg/kg) significantly impaired acquisition performance compared to Tween controls with the animals requiring longer path lengths to locate the platform. Treatment with the CB_1 antagonist AM281 failed to reverse the WIN-2 induced deficit. In probe trial 1, 24 hrs post-acquisition all groups displayed a spatial bias for the target quadrant whereas, in probe trial 2, 4 days post-acquisition only the Tween and WIN-2 + AM281 treated animals continued to present with a spatial bias for the target quadrant. A significant impairment in the performance of WIN-2 treated animals compared to WIN-3 controls was also evident following ihc infusion. However, unlike systemic infusion ihc co-administration of AM281 withWIN-2 reversed the deficit. Similar to Exp. 1 on probe trial 1 all groups displayed a spatial bias for the target quadrant and on probe trial 2 only the control and WIN-2 + AM281 groups continued to show retention for the platform location.

These results corroborate previous work that reported WIN-2 induced spatial learning and memory impairments and suggests that the effects of WIN-2 on spatial reference memory using the water maze may be mediated by CB_1 receptors located in the hippocampus.

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CB_1 RECEPTOR ANTAGONISTS INCREASE HIPPOCAMPAL ACETYLCHOLINE RELEASE: SITE AND MECHANISM OF ACTION

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Evidence suggests that cannabinoid receptor antagonists increase acetylcholine (ACh) release in the hippocampus. Although it is assumed that this type of effect is mediated through CB_1 receptor (CB_1R) blockade, recently an array of functional studies has suggested non-CB_1R involvement in actions mediated by CB_1R antagonists. In addition, it is possible that the effect is mediated through cannabinoid receptors at a neuroanatomical site other than the hippocampus or indirectly through the dopaminergic system. We thoroughly examined these issues using a combination of systemic and local administration of CB_1R antagonists, different methods of microdialysis, CB_1R knockout (KO) mice, tissue measurements of ACh, and immunohistochemistry. First, we showed that systemic injections of the CB_1R antagonists SR141716A and AM251 dose-dependently increased hippocampal ACh efflux in rats. Similarly, local hippocampal, but not septal, perfusions of SR141716A or AM251 increased hippocampal ACh release. Importantly, these stimulatory effects were completely abolished in CB_1R KO mice. CB_1R KO mice had similar basal, but higher stress-induced hippocampal ACh levels, compared to wild-type controls. Interestingly, pretreatment with a D_1 receptor antagonist counteracted the stimulatory effect of SR147161A on hippocampal ACh release. Finally, immunohistochemical methods confirmed that the hippocampus contained a high proportion of CB_1R positive nerve terminals and illustrated colocalization of CB_1 receptors with cholinergic and dopaminergic nerve terminals. In conclusion, CB_1R antagonists increase hippocampal ACh release probably through a dual CB_1R mediated action that involves disinhibition of both cholinergic and dopaminergic function.

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ANTICONFLICT EFFECT OF CANNABIDIOL IN THE RAT VOGEL PUNISHED LICKING TEST

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Cannabidiol (CBD) is a compound present in *Cannabis sativa.* It may induce anxiolytic-like effects in rats exposed to the elevated plus maze model. The Vogel test is a conflict model in which water-deprived rats are punished with electric shocks when they lick the spout of a bottle containing water. Anxiolytic drugs inhibit the conflict and increase the number of punished licks. The aim of this work was to test the hypothesis that CBD would induce an anxiolytic-like effect in the Vogel test. Male Wistar rats (180-220 g; n = 7/group) were water deprived for 24 hours. Afterwards, they were pre-exposed to the apparatus and allowed to drink during 3 min. After another 24-hours of water deprivation, the rats were exposed to the test. The contact of the animal with the spout of a drinking bottle and the grid floor closed an electrical circuitry producing 7 pulses each seconds. Each pulse was considered as a lick. The rats received a 0.5 mA shock for 2 seconds every twenty licks. The total number of licks and the number of punished licks were recorded for 3 minutes and are presented as median (+/- interquartil range). Vehicle (VEH), diazepam (DZP, 3.0 mg/kg) or CBD (2.5, 5.0 and 10.0 mg/kg) were injected i.p. 30 min before the test. The data were analysed by Kruskal-Wallis followed by the Mann-Whitney test. Statistical significance was set at $p<0.05$. DZP and CBD 10 mg/kg significantly increased the total number of licks [VEH: 80 (60-238); DZP 3.0: 216 (100-376); CBD 2.5: 100 (60-408); CBD 5.0: 125 (80-200); CBD 10.0: 320 (180-419)] and the number of punished licks [VEH: 4 (3-4); DZP 3.0: 10 (5-18); CBD 2.5: 5 (3-20); CBD 5.0: 6 (4-10); CBD 10.0: 14 (9-20)]. These results extend the data suggesting that CBD has anxiolytic properties. The mechanisms of action of these properties, however, remain unclear.

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Δ^9-THC EFFECTS ON VIRTUAL WATER MAZE PERFORMANCE IN HUMANS

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Background: Δ^9-tetrahydrocannabinol (THC), the principal psychoactive ingredient of cannabis, has been shown to result in spatial memory impairments in rodents similar to impairments seen following hippocampal lesions. Moreover, these impairments are blocked by cannabinoid receptor antagonists. This suggests that cannabinoid receptors may have a role in spatial memory. The Morris watermaze task has been used in animal studies to assess spatial working memory. We have developed a virtual watermaze task for use in humans that requires and engages the hippocampus.

Hypothesis: We hypothesized that THC would performance on a watermaze task in humans.

Methods: 6 healthy individuals without any psychiatric illness and 4 cannabis abusers completed a two day double-blind study where they received pretreatment with naltrexone or placebo, followed by placebo or intravenous 1.75 mg/kg THC (over 20min) in a fixed order. Subjects were tested on a version of the Morris water task in which they had to use distal cues in a virtual room to navigate to a hidden platform. Each subject was tested 4 times over 2 days using a within-subjects design. In each test session, participants were placed in a unique virtual room with a pool that had a hidden platform located in one area of the pool. Participants were to navigate to the hidden platform as quickly as possible by manipulating a joystick. There were 16 hidden platform trials, 1 30 s probe trial, and 4 visible platform trials. The order of the pools was counterbalanced across participants.

Results: Subjectively, participants report feeling stoned / high, euphoric, and had perceptional alterations. THC impaired performance on a verbal recall task.

All subjects learned the task well, and they displayed normal learning curves. However, THC did not impair escape Latency ($p > 0.05$) and there were no group differences in escape latencies between cannabis abusers and controls following THC.

Conclusions: THC does not impair spatial memory as measured by the Morris water task despite that fact that participants reported being stoned and displayed other cognitive impairments.

CHARACTERIZATION OF THE CANNABINOID CB_1 RECEPTOR ANTAGONIST SURINABANT (SR147778) IN MODELS OF COGNITION IN RODENTS

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A growing body of pharmacological evidence suggests that the central endocannabinoid system modulate different aspects of cognitive processes. Disruptive effects of cannabinoids on short-term memory were shown in tests based either on conditioned or on spontaneous behavior. All these effects were blocked by CB_1 receptor antagonists, indicating that the cognitive-modulating action of cannabinoids are mediated by this receptor subtype. In line with this idea are findings with CB_1 receptor knockout mice that showed improved memory performance in these animals compared to their wild-type counterparts, in several tasks, including the object recognition and Morris water maze tests. The present series of experiments aimed at examining the effects of the new selective CB_1 receptor antagonist Surinabant (SR147778) in animal models measuring different aspects of cognitive processes. They include the social recognition paradigm in aged mice confronted with a conspecific juvenile mouse and the visual object recognition task in adult rats. Results from the social recognition test showed that Surinabant (3 mg/kg, i.p.) improved significantly short-term juvenile recognition in aged mice, an effect indicative of increased memory retention. In the visual object recognition test in rats, Surinabant (1-3 mg/kg, i.p.) significantly and fully reversed short-term episodic memory deficit induced by scopolamine (with a 1 h intertrial interval). It is unlikely that this effect involves other behavioral processes that are not related to cognition, since spontaneous exploratory activity during learning and retention was not modified. In the same model, Surinabant (1 mg/kg, p.o.) also improved long-term memory (with a 24 h intertrial interval). Taken together, these results indicate that Surinabant is able to improve different aspects of cognitive processes, including social and episodic memory. Since both are particularly affected in Alzheimer's patients, our data suggest that Surinabant may represent a promising drug candidate for the treatment of the cognitive symptoms related to Alzheimer's disease.

Δ^9-THC IMPAIRS MEMORY RETRIEVAL THROUGH A CB_1 RECEPTOR MECHANISM IN A REPEATED ACQUISITION MORRIS WATER MAZE TASK

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The primary psychoactive component of marijuana, Δ^9-Tetrahydrocannabinol (Δ^9-THC), has been shown to disrupt performance in a wide range of rodent short-term memory assays, including a working memory version of the Morris water maze (MWM) task. However, Δ^9-THC is generally given prior to learning, which makes it difficult to distinguish among the various processes necessary for performance (e.g., attention, consolidation, and retrieval) that may be influenced by the drug. The aim of the present study was to investigate whether Δ^9-THC specifically impairs memory retrieval in the MWM. On each test day, mice were given a single retrieval tests following repeated acquisition training in which a hidden platform, also called the target, was placed in a random location. Mice were given five two-min trials to locate the platform in the MWM and were required to locate the platform in less than 30 s on at least two of the last three trials. After achieving these criteria, the platform was removed from the MWM and the mice were returned to the tank for a 60 s probe trial following various delays (60 s, 1 h, 2 h, 6, h, or 18 h) to assess memory duration. During the probe trial, the time spent in the target zone (i.e., an area approximately six times larger than the platform area) was recorded and used to assess the recall of the target location. Additionally, the time spent in an area opposite to the target zone was scored for a comparison. In another set of experiments, we examined whether Δ^9-THC (1, 3, and 10 mg/kg) administered 30 min after the five training trials would impair memory retrieval when the probe trial was given at 1 h. Mice exhibited significant decreases in latency to find the platform across the five acquisition trials ($p < 0.001$), indicating that the subjects learned the location of the platform. The mean latency $\pm$ SEM to locate the platform was 65 $\pm$ 6, 43 $\pm$ 5, 19 $\pm$ 3, 21 $\pm$ 2, and 28 $\pm$ 4 s for each of the five respective trials. The time spent in the target zone during the probe trial significantly decreased as a function of time delay ($p < 0.01$). In particular, the time spent in the target zone following the 18 h delay was significantly reduced compared with both 1 min and 1 h delays ($p<0.05$). The mean % time spent in the target zone $\pm$ SEM for the 60 s, 1 h, 2 h, 6, h, and 18 h delays was 21.6 $\pm$ 1.9, 20.6 $\pm$ 4.5, 16 $\pm$ 3.1, 12.7 $\pm$ 2.1, and 11.6 $\pm$ 1.4%, respectively. Δ^9-THC dose-dependently disrupted the memory retrieval of the target zone location ($p<0.01$; ED_{50} (95% CI) = 2.5 (1.5 to 4.1) mg/kg). The mean % time spent in the target zone $\pm$ SEM for each group treated with vehicle, and 1, 3, and 10 mg/kg of Δ^9-THC was 18.7 $\pm$ 2.5, 18.0 $\pm$ 1.7, 14.3 $\pm$ 1.5, and 10.3 $\pm$ 1.1%, respectively. The 10 mg/kg dose of Δ^9-THC led to a significant decrease in the amount of time spent in the target zone compared with the vehicle and 1 mg/kg Δ^9-THC conditions ($p<0.05$). The disruptive effects of 10 mg/kg Δ^9-THC on memory retrieval were completely blocked by 3 mg/kg SR141716 ($p<0.05$). The mean % time spent in the target zone $\pm$ SEM for the vehicle-Δ^9-THC and Δ^9-THC-SR 141716 groups was 10.1 $\pm$ 1.5 and 18.4 $\pm$ 2.4%, respectively. In contrast, both the time delay and Δ^9-THC failed to affect the amount of time in the control zone. Collectively, the results of the present study indicate that Δ^9-THC dose-dependently disrupts memory retrieval through a CB_1 receptor mechanism of action.

CANNABINOID RECEPTORS IN INVERTEBRATES: THE ECDYSOZOA HYPOTHESIS REVISITED

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Studies of invertebrates have revealed strong evidence for cannabinoid receptors in some species (eg, sea squirt, *Ciona Intestinalis*) and lack of cannabinoid receptors in other species (fruit fly, *Drosophila Melanogaster*; nematode, *Caerorhabditis Elegans*). The presence or absence of specific neuroreceptors may serve as taxonomic markers; restriction of cannabinoid receptors from one major clade of invertebrates, the Ecdysozoa, may support phylogenetic relationships originally deduced by 18S rDNA studies. The Ecdysozoa hypothesis (proposed in **1997**) divides invertebrates into organisms that molt (eg, arthropods and nematodes) versus organisms that do not molt (eg. Annelids and mollusks). One accepted characteristic of molting is the loss of β-thymosin, which is absent in ecdysozoans. Cannabinoid receptors also modulate locomotion, thus it has been proposed that cannabinoid receptors may be absent in ecdysozoans.

We performed tritiated ligand binding assays with [^{3}H]CP55,940, displaced with the cannabinoid receptor 1 (CB_1) receptor selective antagonist SR141716A, which provide robust evidence, albeit indirect, of CB_1 gene expression. Seven invertebrates were tested: *C. intestinalis* (sea squirt; Deuterstoma), *Lumbricus terrestris* (nga noke o te whenua, earthworm; Lophotrochozoa), *Actinothoe albocincta* (kotare moana, sea anemone; Cnidaria), *Tethya aurantium* (hautai, golf ball sponge; Porifera), and three ecdysozoans: *Peripitus novae-zealandiae* (ngoakeoke, velvet worm; Onychophora), *Jasus edwardi* (koura waitai, rock lobster; Crustacea) and *Panagrellus redivivus* (beer mat nematode; Nematoda).

Specific bining of [^{3}H]CB55940 was found in all organisms except *A. albocincta* and *T. aurantium*. The *C. intestinalis* cannabinoid receptor was previously characterized *in silico* as the descendant of a receptor that predated the CB_1-CB_2 duplication event. Our *in vitro* results suggest the ancestral sequence may have functioned more like present-day CB_1 than CB_2.

Taken together with previous work, our findings suggest the presence of CB_1 receptors in all major subdivisions of bilaterians (deuterostomes, lophotrochozoans, ecdysozoans). The presence of CB_1 in onychophorans, crustaceans, and some nematodes does not contradict the Ecdysozoa hypothesis, but gives it no support.

NEUROANATOMICAL DISTRIBUTION OF THE CANNABINOID SYSTEM IN THE GOLDFISH CNS: POSSIBLE FUNCTIONAL IMPLICATIONS

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Although the presence of a cannabinoid system has been demonstrated in the CNS of almost all the vertebrate classes, neuroanatomical reports of its distribution in non-mammals are still few. In our work, we analyzed CB_1 cannabinoid receptor and FAAH distribution in the CNS of the goldfish *Carassius auratus*. CB_1- and FAAH-immunopositive neurons and fibres were found throughout the CNS, being particularly abundant in specific brain areas.

CB_1-LI-immunoreactivity (CB_1-LI-IR) was detected in the basal telencephalon and preoptic area. Since in these same regions co-distributions of CB_1-immunolabellings with salmon GnRH, seabream GnRH-associated peptide and tyrosine hydroxilase (TH, the catecholamine biosynthetic enzyme assumed as a marker for dopaminergic innervation) immunoreactivities were found, possible roles of the cannabinoid system in the regulation of the reproduction were postulated. Moreover, CB_1 expression was also demonstrated in the goldfish gonads, indicating a cannabinergic control of teleost reproduction at both central and gonadal levels.

CB_1-LI-IR was also observed in both Dorsal and Ventral Areas of the telencephalon and hypothalamic lateral lobes, neural centres which control bony fish feeding response. Such finding, together with the evidence of CB_1-immunopositivities in prosencephalic regions where a well developed NPY (an orexigenic signal) neural system was described (Pontet et al., **1989**, *Cell Tissue Res., 255: 529-538*), could indicate for cannabinoid system a possible role in the regulation of goldfish feeding response.

In conclusion our data might support the possibility that cannabinoids participate, also in bony fish, to the regulation of fundamental biological functions, such as reproduction and food intake, as described in mammals.

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SUPPRESSION OF SYNOVIAL CELL METALLOPROTEINASE PRODUCTION BY A NONPSYCHOACTIVE CANNABINOID ACID

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Production of matrix metalloproteinases (MMP) in joint tissue of patients with inflammatory arthritis facilitates cartilage degradation and bone erosion and leads to joint deformities and crippling. Thus, MMPs are important targets for agents designed to treat inflammatory arthritis. Oral administration of 1′, 1′ dimethylheptyl-THC-11-oic acid (ajulemic acid; AJA), a synthetic, nonpsychoactive cannabinoid acid, prevents joint tissue injury in rats with adjuvant arthritis. AJA binds to and activates PPARγ directly, and increases production of the PPARγ agonist prostaglandin J_2 (PGJ_2) that is probably the result of stimulating the release of free arachidonic acid and increasing the expression of COX-2. Therefore, we investigated the influence of AJA on MMP production in human fibroblast-like synovial cells (FLS), and examined the role of PPARγ in the mechanism of action of AjA.

Methods: FLS were isolated from synovial tissue of patients with inflammatory arthritis. Cells were treated for 60 min with AJA (1-30 μM), then stimulated with 1 ng/ml TNFα. After 4 hr, release of MMP-1, 3, and 9 were measured by ELISA after 24 hr stimulation. $PPAR\gamma^{+/-}$ and $PPAR\gamma^{-/-}$ mouse embryonic fibroblasts (gift from Dr. Bruce Spiegelman) were treated in a similar manner.

Results: Addition of AJA to FLS suppressed production of MMP-1, 3, and 9 in a dose-dependent manner. Secretion of MMP-3 was also suppressed completely by 20 μM AJA in TNFα- and IL-1α-stimulated $PPAR\gamma^{+/-}$ and $PPAR\gamma^{-/-}$ fibroblasts. Moreover, treatment of human FLS cells with the PPARγ antagonist GW9662 did not alter the effect of AJA on MMP-3 secretion. Thus, the influence of AJA on MMP-3 secretion appears to be PPARγ independent. It is of interest that PPARγ was necessary for optimal production of MMP-3.

Conclusions: Treatment of synovial fibroblasts in vitro with AJA suppressed MMP production in a PPAR γ-independent manner. Although rats with adjuvant arthritis continue to exhibit some joint inflammation when treated with AJA, cartilage and bone damage is prevented and normal joint architecture is preserved. Thus, prevention by AJA of joint tissue injury and crippling in this animal model may be explained in large part by inhibition of MMPs. These results suggest that AJA may be useful for treatment of patients with inflammatory arthritis.

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IN VIVO CHARACTERIZATION OF A NON-CLASSICAL BIARYL CANNABINOID, ADC00007609

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Cannabinoids relieve nausea and vomiting, stimulate appetite in patients with severe wasting disease associated with cancer and AIDS, and reduce hyperalgesia and allodynia in rodent models of inflammatory, neuropathic and visceral pain (Martin, WJ, *IASP Newsletter*, **1999**). The therapeutic utility of cannabinoids has been limited due to psychotropic effects. A previously reported biaryl cannabinoid ADC00007609 (Gareau, Y., et. al., *Bioorganic & Medicinal Chemistry Letters*, **1996**, 6), was resynthesized at Adolor and was shown to have antihyperalgesic activity in rats treated with Freund's complete adjuvant (Stabley, G., et. al. Society for Neuroscience National Meeting, **2003**). The objective of the present study was to evaluate further the pharmacological activity of ADC00007609. The L5 spinal nerve injury model in the rat is a validated neuropathic pain model that involves a tight ligation of the left L5 spinal nerve. ADC00007609 produced potent antiallodynic activity at 1 and 3 mg/kg (i.p.), with paw withdrawal thresholds of 262% and 465% of vehicle-treated animals, respectively. WIN55,212-2 was less efficacious, with responses of 171% and 270% of vehicle response at 1 mg/kg and 2.5 mg/kg i.p., respectively.

A comparison of the potential side effects of ADC00007609 and WIN55,212-2 was determined. ADC00007609 and WIN55,212-2 produced significant and dose-dependent catalepsy in the mouse ring test with almost identical ED_{50} values of 6.6 and 6.5 mg/kg i.p., respectively. In rats, ADC00007609 and WIN55,212-2 significantly potentiated haloperidol-induced catalepsy at a dose of 1 mg/kg i.p. The catalepsy data indicate that ADC00007609 and WIN55,212-2 produce activation of central CB_1 receptors at very similar doses. Cannabinoids have been shown to affect gastrointestinal function (Pertwee, *Gut*. **2001**). ADC00007609 and WIN55,212-2 were equipotent at inhibiting gastrointestinal transit in the mouse, with ED_{50} values of 1.8 mg/kg i.p.

In conclusion, ADC00007609 and WIN55,212-2 have very similar *in vivo* profiles, and ADC00007609 may represent a useful starting point to explore the structure activity relationships of novel biaryl cannabinoids.

NOXIOUS-EVOKED CHANGES IN ENDOCANNABINOID LEVELS IN RAT CENTRAL NERVOUS SYSTEM AND PERIPHERAL TISSUE

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The endocannabinoid (EC) receptor system plays an important role in modulating nociceptive processing (Walker et al. (**1999**) PNAS, 96: 12198-12203). The role of the peripheral EC system during nociceptive processing is unclear (Calignano et al. (**1998**) Nature, 394: 277-280; Beaulieu et al. (**2000**) Eur. J. Pharmacology, 396: 85-92). The aim of this study was to measure levels of endocannabinoids in the rat spinal cord and peripheral hindpaw tissue, following noxious stimulation, using a liquid chromatography-mass spectrometry method.

Male Sprague-Dawley rats (150-180g) received an intraplantar injection of formalin (2.5%, 50µl) or saline (n=4 per group). Behavioural responses (licking and flinching) of rats were measured for 60 minutes. Rats were killed and spinal cord and paw tissue dissected rapidly. A control group of rats (n=4) received no injection, with tissue collected as above. A lipid extraction method (Kingsley et al. (**2003**) Anal. Biochem, 314: 8-15) with liquid chromatograpy-mass spectrometry was used to quantify levels of anandamide (AEA), 2-arachidonyl glycerol (2-AG), noladin ether, virodhamine, N-arachidonyl dopamine (NADA) and oleoyl ethanolamine (OEA).

AEA, 2-AG and OEA were detected in all tissues analysed. In spinal cord samples, no significant changes between endocannabinoid levels of untreated and saline-treated animals were seen. AEA levels in the ipsilateral spinal cord of formalin-treated animals were significantly decreased, compared to control tissue (Table 1). AEA and OEA levels were significantly decreased in the ipsilateral spinal cord in formalin-treated rats, compared to saline-treated rats. In formalin-treated rats, AEA, 2-AG and OEA in the ipsilateral paw were significantly decreased compared to saline-treated rats.

Table 1: Levels of endocannabinoids (means ± s.e.mean) in control, saline and formalin treated rats (wet weight). Statistical comparisons made with Students' t-test
◆ different from untreated animals ($p<0.05$)
* different from saline-treated animals ($p<0.05$)

		AEA (pmol/g)	2-AG (nmol/g)	OEA (nmol/g)
Ipsilateral Spinal Cord	Control	35.7±6.3	22.1±2.6	2.2±0.63
	Saline	38.8±3.0	35.9±11	1.8±0.14
	Formalin	17.5±3.1*◆	14.4±1.9	1.0±0.14*
Contralateral Spinal Cord	Control	22.3±2.2	22.7±3.9	1.4±0.15
	Saline	27.0±4.8	13.8±0.3	1.6±0.3
	Formalin	22.3±4.2	17.4±2.1	1.2±0.1
Ipsilateral Paw Tissue	Control	55.2±30.0	8.0±4.0	0.8±0.4
	Saline	217.2±68.0	12.8±1.8	1.5±0.4
	Formalin	34.5±16.3*	3.4±0.7*	0.4±0.2*
Contralateral Paw Tissue	Control	11.6±4.4	7.6±4.0	0.06±0.002
	Saline	14.6±2.1	4.7±0.2	0.2±0.03
	Formalin	8.1±1.3*	4.9±1.2	0.09±0.02

This study demonstrates that AEA, 2-AG and OEA are present in the spinal cord and paw tissue of naïve rats. Intraplantar injection of formalin reduced levels of some endocannabinoids in the hindpaw tissue and spinal cord, which may reflect an increased metabolism of endocannabinoids during hindpaw inflammation.

EFFECTS OF THE FAAH INHIBITOR URB597 ON MECHANICALLY-EVOKED RESPONSES OF SPINAL NEURONES IN A RAT MODEL OF NEUROPATHIC PAIN

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Activation of cannabinoid receptors by the endocannabinoids produces anti-nociceptive effects in models of pain. Endocannabinoids are metabolised by fatty acid amide hydrolase (FAAH). Inhibition of FAAH, or deletion of the FAAH gene, increases levels of endogenous cannabinoids and produces anti-nociception (Kathuria *et al*., **2003**, Nature Med, 9, 76; Lichtman *et al*., **2004**, Pain, 109, 319). The aim of this study was to determine whether inhibition of FAAH is anti-nociceptive in a model of neuropathic pain. Here, effects of peripheral and spinal administration of the FAAH inhibitor, URB597, on mechanically-evoked responses of spinal neurones in the spinal nerve ligation (SNL) model of neuropathic pain are described.

Tight ligation of L5-L6 spinal nerves was performed in male Sprague-Dawley rats. 14-18 days post-surgery, extracellular single-unit recordings of ipsilateral deep dorsal horn neurones were made in anaesthetised SNL and sham-operated rats. Innocuous (10, 15 g) and noxious (26 g) mechanical stimuli were applied to the hindpaw and responses of spinal neurones (spikes/s) were recorded. URB597 was injected peripherally into the receptive field (25 μg in 50 μl of 3% Tween 80 in saline) or spinally (10, 25 and 50 μg in 50 μl). Effects of URB597 on evoked responses of spinal neurones were recorded and analysed.

Mechanical stimulation of the hindpaw receptive field produced graded stimulus-intensity-dependent increases in firing of spinal neurones in SNL and sham rats. In sham rats, intraplantar injection of URB597 (25 μg) inhibited mechanically-evoked responses of spinal neurones (Table 1). In marked contrast, in SNL rats, URB597 produced a modest facilitation of evoked responses of spinal neurones (Table 1).

Table 1. Effects of intraplantar injection of URB597 (25 μg) on mechanically-evoked responses of WDR neurones in sham-operated and SNL rats. Data are means ± SEM of % control mechanically-evoked responses(*n*=5-6).

	von Frey weight (g)		
	10 g	15 g	26 g
Sham	33 ± 5	49 ± 17	63 ± 10
SNL	124 ± 22	131 ± 15	127 ± 16

Spinal administration of URB597 reduced most mechanically-evoked responses of spinal neurones in sham rats (Table 2) and had similar effects in SNL rats (data not shown).

Table 2. Effects of spinal administration of URB597 on mechanically-evoked responses of WDR neurones in sham-operated rats. Data are means ± SEM of % control mechanically-evoked responses

Dose of URB597	von Frey weight (g)		
	10 g	15 g	26 g
10 μg	59 ± 36	35 ± 7	99 ± 21
25 μg	19 ± 2	39 ± 11	40 ± 10
50 μg	19 ± 8	22 ± 6	39 ± 6

These data suggest that there is a reduced peripheral role of FAAH in neuropathic rats. By contrast the role of spinal FAAH appears to be unaltered in neuropathic rats.

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THE EFFECTS OF THE FATTY-ACID AMIDES, ANANDAMIDE, *N*-PALMITOYL ETHANOLAMINE, *N*-OLEOYL ETHANOLAMINE, AND OLEAMIDE ON CARRAGEENAN-INDUCED PAW EDEMA

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Fatty-acid amide hydrolase (FAAH) is an integral membrane enzyme that catalyzes the hydrolysis of fatty-acid amides (FAAs). Mice in which FAAH has been genetically deleted (FAAH -/- mice) and transgenic mice that express FAAH in the nervous system but not the periphery (FAAH –NS mice) exhibit reduced inflammation in carrageenan-induced paw edema (Cravatt et al., **2001**, Proc Natl Acad Sci 98: 9372-9376; Cravatt et al., **2004**, Proc Natl Acad Sci 101: 10821-10826; Lichtman et al., **2004**, Pain 109:319-327). The observation that this anti-inflammatory phenotype was blocked by separate or combined administration of the CB_1 receptor antagonist SR141716 or the CB_2 receptor antagonist SR144528 (Cravatt et al., **2004**, Proc Natl Acad Sci 101: 10821-10826) indicates the involvement of FAAs acting at a non-cannabinoid site of action. The present study sought to determine which particular members of the FAA family may be involved in this phenotype. The anti-inflammatory effects of the FAAs anandamide (AEA – 50 mg/kg i.p.), *N*-palmitoyl ethanolamine (PEA – 6.26, 12.5, 25.0, and 50.0 mg/kg i.p.), *N*-oleoyl ethanolamine (OEA - 12.5, 25.0, and 50.0 mg/kg i.p.), and oleamide (50 mg/kg i.p.), as well as the non-steroidal anti-inflammatory drug (NSAID) diclofenac (DIC – 5.0 mg/kg i.p.) as a positive control, were investigated. Drugs were administered 30 min prior to an intraplantar injection of λ-carrageenan (0.3%, 20 μl). Paw diameter (PD) was measured using digital calipers pre-injection and at 1, 3, 5, and 24 h post-carrageenan injection. FAAs producing anti-inflammatory effects were then tested in FAAH -/- and FAAH +/+ mice. As previously reported, FAAH -/- mice exhibited a 30 percent decrease in edema (mean = 0.69 ± 0 .01 mm) compared with wild type mice (mean = 1.02 ± 0.03 mm). In wild type mice, PEA (approximately 35 % at the 50 mg/kg dose, $p < .01$), OEA (approximately 15 % at the 50 mg/kg dose, $p < .01$), and DIC (approximately 35 % at the 5 mg/kg dose, $p < .01$) decreased inflammation at the 5-h time point, but AEA and oleamide failed to affect paw diameter. Interestingly, PEA failed to have further anti-inflammatory effects in FAAH -/- mice. These studies confirm that FAAH -/- mice have an anti-inflammatory phenotype in carrageenan-induced paw edema. Moreover, these findings suggest that PEA and OEA, but not AEA or oleamide, may contribute to the anti-inflammatory phenotype of FAAH (-/-) mice.

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THE EFFECT OF SDF-1 ALPHA AND FRACTALKINE ON THE ANTINOCICEPTION INDUCED BY THE CANNABINOID AGONIST WIN55212-2 IN RATS

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We have previously reported cross-desensitization between opiod receptors (mu, kappa, and delta) and receptors for the chemokines RANTES/CCL5 or the SDF-1 alpha/CXCL12 in the regulation of antinociception in rats. Similarly, we also reported cross-desensitization between RANTES receptors and cannabinoid receptors. In the present experiments, th chemokines SDF-1-alpha and fractalkine/CX3CL1 were tested for their effect on antinociception induced by WIN55212-2 (WIN: a non-selective cannabinoid agonist). Male Sprague-Dawley rats, weighing 175-200 g, wer housed in groups of 3-4 for at least 1 week in an animal room maintained at 22 $\pm$ 2^{o}C and approximately 50% relative humidity. Lighting was on a 12/12 h light/dark cycle. The animals were allowed free access to food and water. They were anesthetized with a mixture of ketamine hydrochloride (100-150 mg/kg) and acepromazine maleate (0.2 mg/kg). A cannula was implanted into the preiaqueductal grey (PAG) according to standard procedures inour laboratory and fixed with dental cement according to Pellegrine and Cushman, system A (**1967**). The rats were kept in individual plastic cages for about 1 week after surgery. Each rat was used only once. At the end of the experiment, sites of injection were verified using microinjection of bromobenzene blue. The latency to flick the tail in cold water (-3^{o}C) was used as the antinociceptive index, according to a standard procedure in our laboratory (Pizziketti, et al., **1985**). WIN55212-2 was dissolved in 10% Cremophor SDF-1 and fractalkine were dissolved in artifical cerebrospinal fluid (aCSF). The data are expressed as the mean and standard error. Statistical analysis of difference between groups was assessed with a two-way analysis of variance (ANOVA) following by Duncan's test and with a gropued t-test. $P \leq 0.05$ was taken as the significant level of difference. Rats were given a PAG injection of SDF-1alpha or aCSF 30 min before sc injection of 1 mg WIN55212-2 or vehicle alone (10% Cremophor). Pretreatment with SDF-1 alpha (100 ng) 30 min before WIN significantly reduced the antinociceptive effective induced by WIN. In the next experiment, the design was similar to that in the previous one, except for substitution of the SDF-1 alpha by fractalkine. Fractalkine (100 ng, PAG) 30 min before sc injection of WIN had no effect on the antinociceptive effect induced by WIN. These results demonstrate that activation of CXCL12 (and CCR5, as shown previously) caused cross-desensitization of the cannabinoid receptor in rats, whereas fractalkine/CX3CL1 failed to alter the analgesic activity of the cannabinoid agonist WIN55212-2.

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PARTICIPATION OF THE ENDOCANNABINOID SYSTEM IN THE EFFECT OF TNF-ALPHA TO DECREASE HYPOTHALAMIC cAMP CONTENT

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It is well known that cannabinoids alter many reproductive parameters in animals and humans. Our group reported previously that delta-9-tetrahydrocannabinol (THC) injected into the third cerebral ventricle (icv) reduced plasma luteinizing hormone (LH) levels, and *in vitro* studies indicated that this suppressive effect was mediated by a blockade of LH releasing hormone (LHRH) release. More recent studies indicated that the endocannabinoid anandamide (AEA) injected icv decreased plasma LH. Also, we reported previously that AEA decreased cAMP content in medial basal hypothalamus (MBH) incubated *in vitro* inducing a decrease in LHRH release. On the other hand, we have shown previously that the intraperitoneal (ip) administration of lipopolysaccharide (LPS) decreases plasma LH as well as icv injection of TNF-alpha. Since endocannabinoids have been shown to affect a variety of immune cell functions, we hypothesized that TNF-alpha could decrease LHRH release by increasing AEA in the hypothalamus. It was reported that LPS down regulates fatty acid amide hydrolase (FAAH) expression and therefore increases AEA plasma levels. The present studies show that an acute ip injection of LPS to male Wistar adult rats significantly ($p<0.05$) increased hypothalamic AEA synthesis after 3 hs. It is well known that the activation of CB receptors (CB-r) negatively regulates adenylyl cyclase (AC) activity. The present experiments demonstrated that the incubation of MBH in the presence of forskolin (an activator of AC) increased highly significantly ($p<0.001$) cAMP that was almost completely abolished ($p<0.001$) by the presence of TNF-alpha. Moreover this effect of TNF-alpha was significantly ($p<0.05$) but not completely blocked by the addition of AM251 (a CB_1-r antagonist) indicating that it is mediated at least in part by endocannabinoids. In summary, these results showed that AEA may play a pathophysiological role in mediating the action of proinflamatory cytokines, such as TNF alpha by decreasing cAMP, resulting in decreased LHRH followed by decreased LH release.

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CANNABINOIDS HAVE A POTENTIAL THERAPEUTIC USE IN THE TREATMENT OF PSORIASIS

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Psoriasis is a common inflammatory skin condition notable for the manifestation of unsightly lesion ('scale') that develop within the epidermis. Whilst the understanding of the aetiology of psoriasis is incomplete, it is always characterized by epidermal keratinocyte hyper-proliferation and accompanied by the infiltration and increased expression of pro-inflammatory mediators into the skin. Affecting between 2 and 4% of the population, patient morbidity is significant, and causes distress in many patients.

Cannabis, extracts of cannabis and isolated cannabinoids, are known to have anti-inflammatory properties and are reported to have an inhibitory effect on rapidly proliferating cell lines *in-vitro* as well as the ab ility to modulate the cytokine network.

Initially in this study we examined, in addition to extracts of cannabis, the ability of the isolated natural cannabinoids, Δ^9-tetrahydrocannabinol, (THC), cannabinol (CBN), cannabidiol (CBD) and cannabigerol (CBG) to inhibit proliferation of an HPV immortalized human keratinocyte (ATTC #CCD1106 KERTr) cell line that is acknowledged as representative of hyper-proliferating psoriatic keratinocytes. Additionally, the effect of the synthetic cannabinoid receptor ligands, WIN55212-2 HU210, JWH015 and BML190 and the Endocannabinoid anadamide were also examined.

Furthermore, major cytokines associated with the causative factors of psoriasis such as IL-6 and IL-10, which are significantly overexpressed and down-regulated respectively in psoriatic lesions, the ability of cannabinoids to alter the expression of these cytokines by the cell line was also examined. In addition, expression of both cannabinoid receptors, CB_1 and CB_2, by the same cell line, were assessed by RT-PCR and Western blot analysis.

Cannabinoids inhibit hyper-proliferation and modulate cytokine expression through a non-CB_1/CB_2-mediated mechanism. Although both WIN55 and HU210 significantly inhibited proliferation, the absence of CB_1 receptors, as demonstrated by RT-PCR and western blot analysis, rules out a CB1-mediated mechanism. The poor activity of JWHO15 and BML190 indicates that while CB2 may elicit a circumstantial mediatory role in keratinocyte proliferation, it does not support a significant CB2 contribution. Our evidence demonstrates a potential role for cannabinoids in the treatment of psoriasis.

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ROLE OF CB_2 RECEPTOR IN MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) ACTIVATION IN PRIMARY IMMUNE CELLS

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Two G-protein coupled cannabinoid receptors, the central cannabinoid (CB_1) receptor and the peripheral cannabinoid (CB_2) receptor, have been identified to date. The signaling cascades associated with the CB_1 receptor in cell lines and primary immune cells have been extensively studied. However, the role of the CB_2 receptor in the activation of signaling cascades in primary immune cells has not been elucidated. To study whether the CB_2 receptor is involved in the mitogen-activated protein kinase (MAPK) pathway in primary cells, we have developed a quantitative sandwich-ELISA based colorimetric assay. Myelin Basic Protein (MBP), an ERK1/2 substrate, is the key component of the assay to detect levels of phosphorylation in lipopolysaccharide-induced (LPS) and cannabinoid-treated primary immune cells. LPS is known to activate the MAPK pathway in B cells. Cell lysates of primary spleenocytes were treated with 10μg/ml LPS in the absence or presence of WIN55,212-2. We found that WIN55,212-2 increased phosphorylation of MBP in LPS-induced spleenocytes. Next, we will test this assay in macrophages and will also investigate the effects of Δ^9-tetrahydrocannabinol (THC) and 2-arachidonoylglycerol (2-AG). Our preliminary study suggests that the CB_2 receptor is involved in MAPK activation in primary immune cells.

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ECHINACEA PURPUREA'S IMMUNOMODULATING EFFECTS ON SPLENOCYTE PROLIFERATION AND CYTOKINE SECRETION IN THE PRESENCE OF WIN55,212-2

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Echinacea purpurea (*E.p.)* is an herbal remedy used as a therapeutic agent for ailments such as chronic arthritis, cancer, and skin diseases. *E.p.* has been suggested to reduce both the severity and duration of cold and flu. As an immunostimulant, *E.p.* has been shown to enhance the activity of certain immune cells such as natural killer cells and macrophages, cells of the non-specific immune system. However, the effect of *E.p.* on B and T-lymphocyte activity, cells of the specific immune system, is conflicting. Recently, in our lab, water extracted roots of *E.p.* (*wE.p.*), and WIN55,212-2, an agonist of cannabinoid receptors, were observed to increase splenocyte proliferation. Using cells extracted from the spleens of c57BL/6 mice 8-12 week old, the effects of *E.p.* and WIN55,212-2 on splenocyte proliferation were further investigated. Splenocytes were incubated *in vitro* with different concentrations of *wE.p.* (0 mg/ml, 0.75 mg/ml, 1.5 mg/ml, and 3.0 mg/ml) in the absence or presence of Concanavalin A (Con A) or plate bound anti-CD3 antibody (anti-CD3), T cell mitogens. In addition, splenocytes were treated with *wE.p.* and WIN55,212-2 in the absence or presence of Con A or anti-CD3. A splenocyte proliferation assay was conducted at the 72-h post incubation time-point using a BrdU kit and ELISA. Previous data demonstrates that both *wE.p.* and WIN55,212-2 individually induces splenocyte proliferation. Yet, splenocytes stimulated with *wE.p.* and either Con A (2.5 μg/ml) or anti-CD3 (5 μg/ml) had reduced cell proliferation in comparison to *wE.p.* alone. Further analysis showed an even greater inhibitory effect on splenocyte proliferation when *wE.p.* was added to the splenocytes treated with 10nM or 100nM WIN55,212-2 in the presence of either Con A or anti-CD3. Because the components of *E.p.* have yet to be isolated and identified, the receptor and mechanism of cell stimulation by *E.p.* remain uncertain. Future investigation includes more in-depth analysis of the effects of *E.p.* and WIN55,212-2 on T cell proliferation and cytokine secretion. In conclusion, examining the interaction between *E.p.* and WIN55,212-2 provides additional insight into their roles in the innate and adaptive immune systems.

WIN55,212-2 ALTERS T-CELL PROLIFERATION AND CYTOKINE SECRETION IN A CB_2-DEPENDENT MECHANISM

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Cannabinoids have been shown to exhibit immunomodulatory effects. Because CB_2 receptors are found primarily in the periphery, especially in immune cells, these effects are thought to be mediated via the CB_2 receptor. More specifically, it has been demonstrated that cannabinoids can alter T-cell differentiation, proliferation, cytokine production, and gene expression. At the nanomolar concentrations utilized in our lab, cannabinoids have been shown to actually increase cell proliferation and cytokine secretion in a dose dependent manner in splenocytes activated with Concanavalin A (Con A). It is believed that the mechanism involves the CB_2 receptor, as the same treatment in CB_2 knockout splenocytes does not display any increase in proliferation or cytokine secretion. Although Con A is believed to specifically activate T-cells, it is not known whether other immune cells of the spleen (B-cells, dendritic cells etc.) are somehow contributing to these events. This leads us to our present study, where we investigated the immunomodulatory effects of WIN55,212-2 in wildtype and CB_2 knockout mice derived T cells. Spleens were extracted from c57BL/6 mice aged 8-12 weeks and placed into single cell suspension. T-cells were separated using a MACS immunomagnetic separation column and treated with WIN55,212-2 (0 – 1000 nm) in the presence of Con A (2.5 μg/mL). T-cell proliferation was assayed with a BrdU ELISA at 72 hours. IL-2 and IL-4 secretions were quantified using an ELISA. Treatment of T-cells with WIN55,212-2 showed an increase in cellular proliferation and IL-2 and IL-4 secretion in wildtype only. Knockout T-cells showed no such effect. Interestingly, Con A induced IL-2 and IL-4 secretions were substantially increased in knockout as compared to wildtype T cells in the absence of WIN55,212-2. Our findings suggest that cannabinoids do in fact modulate T-cell proliferation and cytokine production through a CB_2-dependent mechanism, as modulation does not occur in T-cells lacking the receptor. Thus, we have found that the T cell population within the spleen is affected by cannabinoids via the CB_2 receptor. However, we can not exclude that cannabinoids may also affect other cell populations in the spleen. The mechanism of T-cell activation by Con A is not entirely clear. Thus, future studies will incorporate anti-CD3 and anti-CD28, which are specific T-cell activators.

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CANNABINOID MODULATION OF MIGRATION BY MURINE MACROPHAGES AND MACROPHAGE-LIKE CELLS

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Exogenous and endogenous cannabinoids have been reported to modulate functional activities of macrophages and microglia, the resident macrophages of the central nervous system. These cells undergo a multi-step process to "full activation" in response to multiple signals in which they progress successively from "resting" to "responsive", "responsive" to "primed", and "primed" to "fully activated" states. It is recognized that macrophages express primarily the CB_2 cannabinoid receptor, but recent studies indicate that its expression is differential in relation to activation state with maximal levels occurring when cells are in "responsive" and "primed" states. These results suggest that the functional activities of macrophages when in these states of activation are the most susceptible to the action of cannabinoids, at least in terms of a functional linkage to the CB_2. Consistent with these observations, we have observed that cannabinoids inhibit migration, a signature activity attributed to "responsive" macrophage-like cells. The partial agonist delta-9-tetrahydrocannabinol (THC), when administered *in vitro* (10^{-5}M – 10^{-8}M), inhibited in a dose-related fashion the migratory response of thioglycollate-elicited $(B_6C_3)F_1$ murine peritoneal macrophages to the chemokine RANTES (Regulated upon Activation Normal T-cell Expressed and Secreted). *In vivo* treatment with THC (25 and 50 mg/kg) also resulted in inhibition of the *in vitro* migratory response of murine peritoneal macrophages to RANTES. A comparable inhibition *in vitro* of this response was obtained for murine EOC-20 microglial-like cells that were treated (10^{-5}M – 10^{-9}M) with THC. A more robust inhibitory response was observed when the potent cannabinoid agonist CP55940 (10^{-6}- 10^{-12}M) was employed. THC treatment *in vitro* had no effect on the migratory response when peritoneal macrophages from CB_2 receptor knockout mice were used. Collectively, these results are consistent with a role for the CB_2 in this cannabinoid-mediated inhibition of the migratory response to RANTES.

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MICROGLIAL CANNABINOID RECEPTOR EXPRESSION IN MURINE BRAIN TISSUE

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Microglia are resident macrophages within the brain and respond to sites of brain injury. It has been demonstrated that these cells express CB_1 and CB_2 receptors *in vitro* and that the CB_2 is expressed differentially in relation to cell activation state. Activated and inflammatory microglia have been implicated as contributors to neurodegenerative diseases such as AIDS dementia, Parkinson's disease, Alzheimer's disease, and multiple sclerosis. The definition of whether microglia co-express CB_1 and CB_2 *in vivo* is significant since these receptors could be targeted selectively using appropriately designed cannabinoid analogs to ablate untoward inflammatory responses. Immunocytochemical studies at the light and electron microscopy levels have demonstrated the presence of CB_1, but not CB_2, in glial cells in intact "normal" brain tissue. It is our hypothesis, however, that microglia express CB_2 *in vivo* when these cells are in "responsive" and/or "primed" state(s). We have employed an *in vitro* murine brain slice organotypic model in order to monitor CB_2 expression, as well as that of CB_1, with respect to different inflammatory activation states in intact brain tissue. The use of brain slices in culture obviates potential confounding receptor expression that could be introduced by immunocytes from peripheral tissues. Murine brain slices were maintained (18h) in medium or in medium supplemented with murine interferon gamma (IFNγ; 100 U/ml) to allow for generation of "resting" and "primed" microglia, respectively. Assessment of CB_1 and CB_2 mRNA expression was performed on murine brain slice homogenates using Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) in concert with Southern Blotting. Preliminary data demonstrate that IFNγ-treated brain slices constitutively express CB_1 mRNA at levels comparable to those for slices maintained only in medium. In contrast, signal suggestive of CB_2 mRNA was obtained for murine brain slices treated with IFNγ. Studies are in progress to confirm these observations and to define expression of the CB_1 and CB_2 at the protein level, both in whole tissue homogenates as well as immunocytochemically using phenotypic markers to identify the specific cell types that may express CB_2.

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CP55,940 INCREASES ANTIBODY CLASS SWITCHING FROM IgM TO IgE IN CULTURES OF MOUSE PURIFIED B LYMPHOCYTES

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We reported that THC injection prior to antigen challenge in mice caused an increase in antibodies of the IgG_1 class and an increase in Th2 activity. B lymphocytes (B cells) express Ig receptors and differentiate into antibody producing cells under the influence of antigen stimulation and cytokines produced by Th cells, and as they mature they undergo the process of antibody class switching from IgM and IgD isotypes to IgG and IgE isotypes. Because B cells express relatively high levels of CB_2 and proliferate in response to stimulation with CP55,940, we hypothesized that a portion of the cannabinoid-induced increase in IgG_1 production involves a direct effect of the drugs on B cell class switching. Previous studies have shown that the addition of both IL-4 and anti-CD40 antibodies can induce resting murine B cell cultures to divide and switch isotypes from IgM and IgD to IgG_1 and IgE. Anti-CD40 stimulates B cell proliferation while IL-4 induces class switching. In the current study, C57BL/6 mouse splenic B cells were purified by negative selection and cultured with B cell activators in the presence or absence of CP55,940 in the concentration range of 0.5 to 10 μM. The cells were cultured for up to 10 days and analyzed at different days by flow cytometry using fluorescent-labeled antibodies to detect the surface expression of either IgM or IgE on the B cells. Cells treated with CP55,940 showed a concentration-dependent increase in the percent expressing IgE by day 5 in culture. These results suggest that the cannabinoids bias toward antibody immunity by directly affecting B cell differentiation and maturation. The mechanisms of this effect have yet to be determined.

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PRELIMINARY IMMUNOLOGICAL CHARACTERIZATION OF CB_1/CB_2 NULL MICE

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The role of CB_1 and CB_2 in immune modulation by cannabinoids is poorly understood. Selective antagonists for CB_1 and CB_2, although useful, have been reported to possess their own biological activity, which can putatively confound the interpretation of results when employed as antagonists. In the present study an alternative approach to assess the role of CB_1 and CB_2 in immune modulation by cannabinoids was employed, genetically engineered CB_1/CB_2 null mice. Utilizing CB_1/CB_2 null mice and wild-type counterparts, basic immunological cellular population profiles were determined using flow cytometry; no differences were detected in $CD3^+CD4^+$ or $CD3^+CD8^+$ T cell subpopulations, nor were there any differences in $CD3^+$, $CD19^+$ or the pan-macrophage marker, $F4/80^+$, cell populations between wild-type C57Bl/6J mice and CB_1/CB_2 null mice. For a global analysis of the immunological response, in vivo and in vitro antibody forming cell (AFC) response assays were performed. Oral administration of Δ^9-tetrahydrocannabinol (Δ^9-THC) suppressed the in vivo T cell-dependent IgM AFC response against sheep erythrocytes (sRBC) in the wild-type mice but not in the CB_1/CB_2 null mice. Further, the response of the vehicle treated CB_1/CB_2 null mice was significantly greater than that in wild-type mice. This contrasts the results of the Tcell-dependent in vitro AFC response in which the magnitude of the AFC response was markedly lower in CB_1/CB_2 null than in wild-type mice. In contrast, no difference in the in vitro polyclonal AFC response between wild-type and CB_1/CB_2 null mice was observed to either lipopolysaccharide *E. coli* 128 or *S. typhosa*. In a one-way mixed lymphocyte reaction, Δ^9-THC at 10μM or 15μM equally suppressed the response from CB_1/CB_2 null and wild-type splenocytes. The dichotomy between the in vivo and in vitro responses to T cell-dependent antigens point toward a compensatory mechanism existing in vivo which remains to be elucidated. Further, this work indicates that CB_1 and/or CB_2 are involved in Δ^9-THC mediated suppression of the anti-sRBC IgM in vivo AFC response while neither CB_1 nor CB_2 appear to be involved in the Δ^9-THC-mediated suppression of the mixed lymphocyte reaction.

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CANNABINOID INTERACTION WITH LIPOPOLYSACCHARIDE-INDUCED FEVER

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It is generally thought that fever is induced by the action of various pyrogenic cytokines produced mainly by mononuclear phagocytes activated by invading pathogenic microorganisms and/or their product, e.g., bacterial endotoxin lipopolysaccharide (LPS). Because of the well established immunosuppressive effects of cannabinoids, we have investigated the effect of the cannabinoid receptor agonist aminoalkylindole, (+)-WIN55,212-2 [(4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6H-pyrrolo[3,2,1ij]quinolin-6-one] (WIN55,212-2) in lipopolysaccharide (LPS)-induced fever. Radio transmitters (Mini-Mitter), implanted under anesthesia 5-7 days before testing, were used to measure body temperature (Tb). Intraperitoneal (i.p.) injection of adult male Sprague-Dawley rats with 50 μg/kg of LPS (E. *coli*, 0111:B4) induced a biphasic fever, with the first peak at 180 min and the second at 300 min post-injection. Pretreatment with non-hypothermic doses of the cannabinoid receptor agonist WIN55,212-2 (0.5-1.5 mg/kg, i.p.) significantly reduced LPS-induced fever. However, pretreatment with the inactive enantiomer WIN55212-3 (1.5 mg/kg, i.p.) did not affect the LPS-induced fever. The present report extends the anti-inflammatory role of cannabinoids previously reported and shows for the first time that the cannabinoid system can affect the development of LPS-induced fever.

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CANNABIGEROL-DIMETHYL HEPTYL (CBG-DMH), A SYNTHETIC CANNABINOID WITH HYPOTENSIVE AND VASORELAXANT PROPERTIES

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Cannabigerol-dimethyl heptyl (CBG-DMH) a non-psychotropic synthetic homolog of cannabigerol, a natural product, causes hypotension in pentobarbital anaesthetized rats in doses of 5mg/kg without affecting the heart rate. Cannabidiol dose-dependently inhibits the hypotensive effect of CBG-DMH and Abn-CBD but not of the CB_1 receptor agonist (−)-Δ^9-THC.

CBG-DMH produces endothelium-dependent vasodilation of rat isolated abdominal aortic rings. Vasorelaxation by CBG-DMH, which is pertussis toxin-sensitive, is inhibited by CBD (1 μM) and O-1918 (1 μM), but not by the nitric-oxide synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (300 μM), SR 141716A (CB_1 receptor antagonist; 1 μM) SR144528 (CB_2 receptor antagonist; 1 μM) or by the vanilloid VR1 receptor antagonist capsazepine(10 μM). Cannabidiol itself does not bind to CB_1 or CB_2 receptors and does not cause vasorelaxation at concentrations up to 30 μM.

CBG-DMH also suppresses generation of nitric oxide (NO) and formation of tumor necrosis factor alpha (TNF-α) by murine macrophages.

In conclusion, CBG-DMH relaxes the rat abdominal aorta by endothelium-dependent activation of a target coupled to Gi/Go protein. The relaxation is cannabidiol-sensitive and does not involve CB_1 and CB_2 receptors. Its effects may be either receptor-independent or related to the Abn-CBD sensitive receptor. The anti-inflammatory properties in addition to the lack of behavioral activity make this compound a potential candidate for further investigations that will lead to cannabinoid-based pharmacotherapy.

PREJUNCTIONAL NEURAL CANNABINOID RECEPTORS IN ISOLATED PREPARATIONS OF HUMAN AND RABBIT VAS DEFERENS AND PROSTATE

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We studied *in vitro* preparations of human and rabbit vas deferens and prostate to assess the functional importance of cannabinoid CB_1 and CB_2 receptors in these tissues. (-) Cannabidiol and (+)WIN55,212-2 (WIN), both CB agonists, as well as the CB_1 and CB_2 receptor selective antagonists, SR141716 and SR144528 respectively, were tested.

Macroscopically normal specimens of human vas deferens were obtained from patients undergoing radical prostatectomy for clinically localized prostate cancer and not treated with any neo-adjuvant anti-androgen. The prostatic portion of the vas deferens as well as prostate were removed from male rabbits (New Zealand White). After removing the serosa and subserosa layers, muscle strips were dissected longitudinally and mounted in a 20 ml organ-bath containing warm (37°C) Krebs solution aerated with 95%O_2 and 5%CO_2, under constant tension of 1g. Isotonic, twitch-like contractions were evoked by sub-maximal electrical field stimulation (EFS). Cumulative concentration-response curves for the agonists were obtained, expressing their inhibitory response as per cent of maximal effect against atropine (1μM) added to each strip at the end of the experiment. EFS essentially activated human and rabbit cholinergic neurons, since atropine or tetrodotoxin, abolished the evoked contractions.

In human vas deferens, WIN and (-)Cannabidiol (10nM-10μM) inhibited the contractions with a biphasic response (WIN IC_{50s}, 19 nM and 10μM, maximal inhibitions of 30 and 85%; (-)Cannabidiol $IC_{50s,}$ 20nM and 6.3μM, maximal inhibition 40 and 75%). SR141716 and SR144528 (both at 0.1μM) had no intrinsic effect, but reduced the high affinity response of both WIN(1μM) by 36 and 61% and (-)cannabidiol (1μM) by 27 and 35%, respectively. Both antagonists had no significant effect on the low affinity response of the agonists.

In rabbit vas deferens, WIN (10nM-10μM) inhibited the contractions with an IC_{50} of 9 μM (maximal inhibition 96 %). SR141716 and SR144528 (both at 0.1μM) had no intrinsic effect but antagonized the inhibitory effect of WIN (pK_B 7.2 and 7.9 respectively). (-)Cannabidiol, also inhibited the contractions (IC_{50} 1.6 μM, maximal inhibition 98%) and this effect was antagonized by SR141716 (pK_B 7.2) and SR144528 (pK_B 7.5). In the rabbit prostatic tissue preparations, WIN inhibited the contractions with the same potency as in the vas deferens (IC_{50}: 15 μM, maximal inhibition 85%). SR141716 and SR144528 (both at 0.1μM) had no intrinsic activity and, as in the vas deferens, antagonized the WIN inhibitory effect (pK_B 7.2 and 7.5, respectively).

The antagonism of the high affinity response of WIN or (-)cannabidiol by SR141716 and SR144528 provide the evidence of pre-junctional neural cannabinoid CB_1 and CB_2 receptors inhibiting acetylcholine release in the smooth muscle of human and rabbit peri-prostatic vas deferens and prostate.

INTERACTION OF CP55940 AND SR141716 WITH MORPHINE IN THE MYENTERIC PLEXUS-LONGITUDINAL MUSCLE PREPARATION

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Cannabinoids and opioids are both well established inhibitors of the amplitude of electrically evoked contraction of guinea-pig myenteric plexus-longitudinal muscle preparation (MPLM; Leslie, **1987**, Pharmacol. Rev. 39(3), 197-249; Pertwee, **2001**, Gut 48, 859-867). SR141716, the CB_1 receptor specific antagonist, has been shown to independently increase the amplitude of electrically evoked contraction of the MPLM. This study examines opioid inhibition in the presence of the cannabinoid ligands CP55940 and SR141716.

Ileum was obtained from Heston-2 guinea-pigs of both sexes (300-500g) and experiments performed as described by Begg *et al.* (**2002**, Br. J. Pharmacol. 137(8), 1298-1304). Supermaximal electrical stimuli were applied at a frequency of 0.1 Hz, pulse duration 0.5 ms. Morphine was dissolved in water and cumulative concentration response curves established with 5 min dosing intervals. CP55940 and SR141716 were dissolved in ethanol and added 30 min prior to morphine.

Morphine, added cumulatively, inhibited electrically-evoked contractions producing a maximal inhibition of 39.8 ± 5.8 % at a concentration of 1 μM (n = 9). The non-specific CB receptor agonist CP55940, when added alone, inhibited contractions by 12.0 ± 2.9 %, 26.8 ± 5.6 % and 40.8 ± 7.5 % at doses of 1, 10 and 100 nM respectively. 0.1 nM CP55940 displayed no inhibition. Cumulative dose-response curves to morphine, performed after incubation with 1 and 10 nM CP55940, displayed additive interactions (when measured independently of CP55940 inhibition, morphine caused inhibition and produced maximal inhibition at the same concentrations as in the absence of CP55940, and produced similar degrees of maximal inhibition). However, pre-incubation with 100 nM CP55940 abolished morphine inhibition. Interestingly, inhibition produced by 100 nM CP55940 (40.8 ± 7.5 %) was significantly less than that produced by 1 μM morphine in the presence of 10 nM CP55940 (62.7 ± 3.4 %; $P < 0.05$ Student's t-test). The CB_1 receptor antagonist SR141716 (100 nM) increased the amplitude of contraction by 80.3 ± 24.7 % (n = 7). Morphine caused inhibition and produced maximal inhibition at the same concentrations as in the absence of SR141716. The degree of maximal inhibition produced by morphine increased in the presence of SR141716 (67.8 ± 5.1 % of SR141716 treated contraction size; $P < 0.01$ Student's t-test), reducing the amplitude of contraction to a level similar to that produced by morphine in the absence of SR141716.

The additive interaction of CP55940, at concentrations of 1 and 10 nM, with morphine suggests inhibition occurs through independent mechanisms. However, the abolishment of morphine inhibition caused by 100 nM CP55940, coupled with the lower inhibition evoked by 100 nM CP55940 and 1 μM morphine when compared with 10 nM CP55940 and 1 μM morphine, suggests higher concentrations of CP55940 act via an alternate mechanism to interact with the morphine induced effects. The increased degree of inhibition evoked by morphine in the presence of SR141716, when compared to the unchanging degree of inhibition evoked by morphine in the presence of low concentrations of CP55940, is consistent with cannabinoid intersection with opioid signalling through multiple mechanisms in the guinea-pig MPLM preparation.

INHALED MARIJUANA SMOKE ALTERS MITOCHONDRIAL FUNCTION IN AIRWAY EPITHELIAL CELLS IN VIVO

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Chronic bronchitis and inflammation of the upper airways are commonly observed among habitual marijuana smokers even though they smoke only a limited number of cigarettes. We previously demonstrated that *in vitro* exposure of lung epithelial cells to whole marijuana smoke or to tetrahydrocannabinol (THC) decreased cellular ATP levels. Examination of mitochondria in these cells using the fluorescent potentiometric probe, JC-1, revealed a loss of mitochondrial membrane potential following even brief exposure to THC. This loss in function recovered very slowly after THC was removed and was attenuated by cyclosporin A, suggesting a role for the mitochondrial permeability transition pore. Tar extracts prepared from the smoke of marijuana cigarettes (3% THC) also impaired mitochondrial membrane potential whereas tar extracts prepared from tobacco or placebo marijuana cigarettes (0% THC) produced minimal effects. In order to evaluate these findings for their potential impact on the lung, we set-up an *in vivo* smoke inhalation model in which rats were exposed to smoke from these different types of cigarettes. Using a cross-flow nose-only inhalation system, a single 20 min exposure to marijuana smoke produced blood THC levels ranging from 2-15 ng/ml and lung levels of 46-76 ng/g wet wt tissue. Values varied as a function of total smoke consumed and demonstrated that inhaled THC is highly concentrated within lung tissue as compared to blood. The impact of smoke exposure on mitochondrial function in airway/alveolar epithelial cells was also evaluated using the JC-1 probe. After smoke inhalation, JC-1 was instilled into the lung by intratracheal infusion. Fresh tissue sections were then evaluated for red/green JC-1 fluorescence by microscopy. Red fluorescence, characteristic of normal mitochondrial function, was greatly diminished throughout the lungs of rats exposed to marijuana smoke. These results suggest that inhalation of marijuana smoke deposits high concentrations of THC within lung tissue and may have deleterious effects on airway/alveolar epithelial cell energetics *in vivo*.

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THE EFFECT OF CANNABIDIOLIC ACID (CBDA) AND CANNABIDIOL (CBD) IN THE GASTROINTESTINAL TRACT OF *SUNCUS MURINUS*

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We have previously shown that cannabidiolic acid (CBDA) significantly reduces motion-induced emesis in *Suncus murinus* (Cluny *et al., J Pharm Pharmacol*, 56 (Suppl), 236). The aim of the present study was to investigate the effect of CBDA and its decarboxylation product, cannabidiol (CBD), on the gastrointestinal tract of *Suncus murinus*.

Segments (1 cm length) taken from the intestine (2-4 cm (proximal) and 5-7 cm (central) distal to the pyloric sphincter and 2cm proximal to the anal region (terminal)) of adult female Japanese House musk shrew, *Suncus murinus* (50.2 ± 2.1 g) were mounted in 20 ml organ baths containing Krebs' solution (37 °C, aerated with 95 % O_2, 5 % CO_2) and left to equilibrate for 60 min and washed every 20 min. The resting tension was maintained at 0.5 g and contractions were recorded isometrically using a paired experimental design. The concentration response curves to CBD and CBDA (10 nM – 30μM) were established, cumulatively, in non-contracted and, non-cumulatively, in tissues pre-contracted with electrical field stimulation (EFS) (60 sec, 4.0 Hz, 30 V, 0.5 ms pulse duration). Changes in tension due to the application of drugs and EFS were expressed as the mean ± s.e.mean (n = 4-6) of the response (g) or of EFS-induced contraction, respectively, and analysed using the paired Student's t-test.

The application of CBD and CBDA (10 nM – 30μM) alone did not induce a contraction response in any tissue at any concentration examined. However, CBD at concentrations higher than 1 μM significantly ($P < 0.05$, 0.01, 0.001) attenuated the tension in all tissues examined. Indeed at 30μM the effect of CBD was to induce a relaxation response. The application of CBDA at concentrations higher than 1 μM also significantly ($P < 0.01$, 0.001) attenuated the tension in tissues taken from the central and terminal regions of the intestine. When CBDA was applied at 30 μM a significant ($P < 0.01$, 0.001) relaxation response appeared in all tissues examined. When CBD and CBDA (10 nM – 30μM) were applied 30 min prior to the application of EFS, the responses to EFS were comparable to those in vehicle treated tissues.

In summary both CBD and its cannabinoid carboxylic acid precursor, CBDA, caused a relaxant effect on the isolated intestine, however, both compounds failed to modify the EFS-induced contractions in the *Suncus murinus* gastrointestinal tract.

INHIBITION OF SALIVARY SECRETION BY ACTIVATION OF CANNABINOID RECEPTORS

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The submandibular gland (SMG) is one of the major salivary glands and its secretion is controlled by the autonomic nervous system. End secretory units, called acini, are continuous with a ductal system. Two major signal transduction pathways are implicated in salivary gland cell functions, one involves the generation of cAMP and the other the breakdown of plasma membrane polyphosphoinositides, diacylglycerol and inositol triphosphate. Moreover, increases in the intracellular level of cAMP stimulate various salivary functions, including salivary flow rate and secretion of proteins. Interestingly, most cannabinoid receptors (CB-r) are coupled to G inhibitory protein and respond by inhibiting the activity of adenylyl cyclase. Since it is known that marihuana use decreases saliva secretion, we hypothesized that cannabinoid receptors should be located in salivary glands and could mediate this effect. Therefore, immunohistochemical and physiological studies on SMG of adult male rat were performed. The results showed that both, CB_1-r and CB_2-r are present in SMG and that they are mainly localized to the ductal system. The *in vitro* studies incubating SMG in Krebs-Ringer containing the compounds to be tested demonstrated that in the presence of anandamide, forskolin-induced increase of cAMP content was significantly reduced. Moreover, this effect was partially blocked by CB_1-r and CB_2-r antagonists (AM251 and AM630, respectively), indicating that both receptors are implicated in SMG salivary secretion. Furthermore, in experiment *in vivo* intraglandularly injected of AEA, inhibited noradrenaline and metacholine-stimulated saliva secretion injected via femoral vein. The inhibitory effect of AEA was prevented significantly by previous injections of AM251 and AM630 intraglandularly. The addition of the antagonists *in vitro* or the injection into salivary gland *in vivo* did not affect the cAMP levels nor salivary flow rates. Therefore, we conclude that endocannabinoids control saliva secretion acting through CB_1-r and CB_2-r localised in SMG.

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INVOLVEMENT OF ENDOCANNABINOIDS AND PALMITOYLETHANOLAMIDE IN INTESTINAL DISORDERS WITH INFLAMMATORY COMPLICATIONS: HUMAN STUDIES

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There is increasing evidence from animal studies in favour of a protective role of cannabinoid CB_1 receptors and endocannabinoids against inflammatory conditions of the gut (Izzo et al., Br. J. Pharmacol., **2001**; Massa et al., J. Clin. Invest. **2004**). However, no data have been reported yet in humans showing that the endocannabinoid system is activated during pathological conditions. Here we report the results of studies aiming at establishing a correlation between endocannabinoid levels in human intestinal mucosa and various pathological conditions, including ulcerative colitis, diverticular disease and celiac disease, all characterized by inflammatory hallmarks.

Biopsies were obtained in agreement with Italian healthcare rules after informed consent from the subjects, during endoscopic evaluation on the colonic mucosa from control patients, submitted to either colonoscopy or gastroscopy for either haematochezia, colon carcinoma screening or abdominal pain, or on the inflamed mucosa from untreated patients with: 1) mild to moderate ulcerative colitis at the first diagnostic work-up (6 males and 2 females, average age 49.8); 2) diverticulosis operated for different complications at the "San Raffaele Hospital" (7 males and 6 females, average age 64); and 3) celiac disease (3 males and 7 females, average age 17) before treatment and 4 patients after gluten-free diet and histological remission (1 males and 3 females, average age 32). A small aliquot (10-15 mg wet weight) of each biopsy sample was kept at –80°C until processing, whereas the rest of the samples was generally subjected to histological analysis. Since tissue aliquots were too small to be weighed without being defrosted, anandamide (AEA), palmitoylethanolamide (PEA) and 2-arachidonoyl glycerol (2-AG) contents, measured by means of isotope dilution-LC- MS, were compared by ANOVA followed by the Bonferroni's post-hoc test.

The levels of AEA were significantly (from 2 to 2.6-fold) increased in the biopsies from diseased patients belonging to all groups. In the case of celiac disease patients, histological remission after gluten-free diet was accompanied by a striking decrease of AEA concentrations back to control levels. The levels of 2-AG were never significantly altered, except for patients with diverticular disease, where the amounts of this compound were decreased by 34%. PEA levels were increased only in biopsies from patients with celiac disease and ulcerative colitis. Regarding this latter disorder, the human data were partly confirmed by analyses carried out on the large intestine from rodents treated with DTNB, where previous findings (Massa et al., J. Clin. Invest. **2004**) showed that CB_1 receptors and endogenous AEA are protective against inflammation. In rats treated with DTNB, the intestinal levels of AEA, but not PEA, were significantly increased. These findings strongly suggest that in humans, as in rodents, endocannabinoids and PEA are overproduced during inflammatory conditions of the gut, possibly to play a protective function.

DISTRIBUTION OF THE CB_2 RECEPTOR IN ENTERIC NERVES OF THE RAT ILEUM

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The actions of the CB_1 receptor have been characterized in the gut of guinea-pig, rat, mouse and humans. The CB_1 receptor is widely distributed in the myenteric and submucosal plexuses in guinea-pig and rat. The actions of CB_1 agonists are well established; they decrease neuronal excitablity reducing transmitter release, thereby attenuating motility and secretion. However, a recent study by Mathison *et al.,* (BJP;142 (8):1247-54), demonstrated that in LPS-inflammed rats, the inhibitory actions of CB_1 receptor agonists were abolished. Unexpectedly, CB_2 agonists which have no actions in control animals, now exerted an inhibitory action on motility, reversible by a CB_2 antagonist, indicating they may be upregulated under pathophysiological conditions. Little is known about the localization and function of CB_2 receptors in the gut, therefore the aim of the present study is to investigate the distribution of CB_2 receptors in the rat ileum.

RNA was isolated from rat tissues using Trizol, cDNA was generated and RT-PCR reaction products imaged using gel electrophoresis. Whole mounts of rat ileal tissue were used for immunofluorescence staining of neuronal markers and CB_2 receptors and imaged using confocal microscopy.

RT-PCR studies demonstrate there are CB_2 receptors present in both muscle and mucosal layers of the rat ileum. The PCR product was sequenced and was found to match the spleen product obtained using the same CB_2 primers. Moreover, preliminary real-time RT-PCR data indicates that the highest level of CB_2 receptor mRNA is located in the myenteric plexus. In immunohistochemical studies, CB_2-like immunoreactivity partially co-localised with the neuronal marker PGP 9.5 and the presynaptic marker synaptotagmin indicative of neuronal location. In addition, CB_2-like immunoreactivity does not colocalise with the glial marker GFAP.

These data confirm the presence of CB_2 receptors in the rat ileum, and demonstrate possible neuronal localization. These findings are important as CB_2 modulation of gut motility has recently been described in may prove to be a novel therapeutic target in gastrointestinal disease.

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HUMAN SKIN PERMEATION OF JWH-018 AND O-1812, TWO SYNTHETIC CANNABINOIDS

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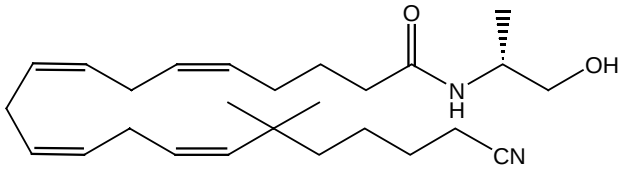
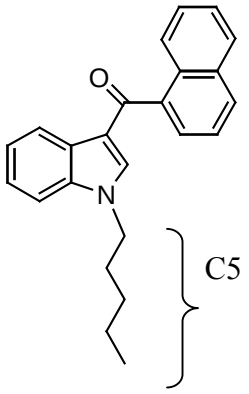
Cannabinoid dosage form development research is very important, because of the unique hydrophobicity and stability challenges encountered with endogenous cannabinoid-based compounds and other compounds that exhibit pharmacological activity at CB_1 and CB_2 receptors. In this study, two synthetic cannabinoids, JWH-018 and O-1812, were investigated for their human skin permeation rates in order to evaluate their feasibility for transdermal delivery. JWH-018 is an aminoalkylindole of therapeutic interest because it is more potent than Δ^9-THC (Aung et al., Drug and Alcohol Dependence, 60 (**2000**) 133-140), and less drug may have to be delivered across the skin to achieve a therapeutic effect. O-1812 is a metabolically stable anandamide derivative of therapeutic interest because it has shown some anticonvulsant activity in animal models (Wallace et al., European Journal of Pharmacology, 452 (**2002**) 295-301).



O-1812
MW 414.62



JWH-018
MW 341.45

In vitro diffusion studies of JWH-018 and O-1812 were conducted using split-thickness human abdominal skin in flow-through diffusion cells. The receiver solution consisted of HEPES-buffered Hanks' balanced salt solution containing 0.5% Brij-98. The drug disposition in the skin at the end of the 48 hour experiment was also determined. Samples were analyzed for drug content by high pressure liquid chromatography with ultraviolet detection.

The *in vitro* fluxes of JWH-018 and O-1812 across human skin were 5.01± 0.78 and 8.35 ± 0.76 nmol/cm^2/h, with mean lag times of 7.5 and 6.2 h, respectively. The mean drug contents in the skin were found to be 0.12 and 0.49 μmol/g of skin for JWH-018 and O-1812, respectively. The target therapeutic flux (transdermal delivery rate) of Δ^9-THC is 9.6 nmol/cm^2/h. JWH-018 may be near its target therapeutic flux, since it is much more potent than Δ^9-THC. The results of the present study indicated that significant levels of JWH-018 and O-1812 could be delivered via the transdermal route. Acknowledgment:

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ELEVATED CEREBROSPINAL OLEOYLETHANOLAMIDE LEVELS IN HEALTHY SLEEP DEPRIVED VOLUNTEERS

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Oleoylethanolamide (OEA) is a naturally occurring amide of oleic acid and ethanolamine that activates the peroxisome proliferator-activated receptor-α (PPAR- α). Treatment with different PPAR-α activators, such as hypolipidemic fibrates as well as monosaturated and polyunsaturated fatty acids (PUVA) induces an increase in activity of major antioxidant enzymes in brain and protects the brain against noxious biological reactions by anti-oxidant and anti-inflammatory mechanisms. This study was aimed to investigate the effect of sleep deprivation as a stressful condition on the production of fatty acid ethanolamides (FAEs) in cerebrospinal fluid (CSF) and serum from healthy volunteers. Subjects were lumbar punctured before and after sleep deprivation using a non-traumatic lumbar puncture procedure. Each time peripheral blood was collected as well. Alertness was monitored by wrist actigraphy, using "Actiwatch 2000", a piezoelectric transductor recording wrist activity with 240 motions per minute to the maximum. Thus, sleep deprivation was controlled. Quantification of the fatty acid ethanolamides was done by isotope dilution high-performance liquid chromatography coupled with mass spectrometry (HPLC/MS) after lipid extraction. We found a significant increased level of OEA in CSF after 24 hours of sleep deprivation in healthy volunteers whereas the levels of anandamide (AEA) and palmitoylethanolamide (PEA) were not significantly affected. These findings suggest that increased levels of OEA observed during sleep deprivation may be considered as a potential neuroprotective factor through its activation of PPAR-α.

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THE GENOTOXICOLOGY OF SATIVEX®

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The objective of these studies was to determine the genotoxic potential of the Botanical Drug Substances (BDSs) present in the cannabis-based medicine, Sativex®.

A 1:1 mix of the BDSs Tetranabinex® (THC BDS) and Nabidiolex® (CBD BDS) were tested in the standard battery of genotoxicology assays: Bacterial Mutation Assay (Ames Test), Mammalian Cell Mutation Assay (MCMA), Unscheduled DNA Synthesis (UDNAS) and the Mouse Micronucleus Test (MMT).

Concentrations of 1:1 Tetranabinex® : Nabidiolex® (Test Article, TA) up to 5000 µg/plate were evaluated in the BMA (Ames Test). There was no evidence of mutagenic activity was seen at any dose level in either mutation test.

The dose levels evaluated in the MCMA in the absence of the S9-mix were: 0.5, 1, 1.5, 2.5, 5, 7, 9, 10, and 15 µg/ml. The doses evaluated in the presence of the S9-mix were: 1, 2.5, 5, 7, 10 and 15 µg/ml of TA. In the absence of the S9-mix, there were no observed increases in mutant frequency (which were outside the historical control range with Day_0 relative survival in excess of 10%) after treatment with the TA. In the presence of S-9 mix, increases in mutant frequency were observed but these were at levels of high toxicity.

In the UDNAS assay, when treated once via oral gavage with the TA, at doses of 50mg/kg or 125mg/kg, male and female rats did not produce group mean Net Grain counts (NNG) >0.4 nor were there >1% of cells found in repair at either dose. Thus, there was no evidence of induction of UDS in hepatocytes isolated *ex vivo* approximately 12-14 or 2-4 hours after dosing.

In the MMT, groups of seven male mice were given 2 doses of TA, each dose approximately 24 hours apart. Doses of 60, 95 or 150mg/kg/day were administered to the males by oral gavage at a dose volume of 10ml/kg. There were no statistically significant differences in micronucleus frequency in animals treated with TA at any dose, compared to the vehicle control group. There was no statistically significant decrease in the PCE/NCE ratio in animals treated with TA at any dose, compared to the vehicle control group.

It is concluded that the principal active substances in Sativex®, Tetranabinex® and Nabidiolex®, show no evidence of:

- mutagenic activity in the bacterial mutation assay (either in the presence or absence of S-9 mix) at doses up to 5000µg / plate.
- mutagenic potential in the *in vitro* gene mutation assay (either in the presence or absence of S-9 mix) at doses up to 15µg/ml.
- genotoxic activity as demonstrated in the unscheduled DNA synthesis assay at doses up to 125mg/kg
- clastogenicity or aneugenicity at doses up to 150mg/kg/day.

THE EFFECTS OF CANNABIS EXTRACTS TETRANABINEX® AND NABIDIOLEX® ON HUMAN CYTOCHROME P450-MEDIATED METABOLISM

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The objective of this study was to determine the effect of Tetranabinex® (THC Botanical Drug Substance (BDS)) & Nabidiolex® (CBD Botanical Drug Substance (BDS)) and 1:1 % (v/v) mixture of Tetranabinex® & Nabidiolex® on human CYP450-mediated metabolism**.**

The effects of the 3 test articles (TAs) on 5 cytochrome-P450 isoenzymes (CYP1A2, CYP2C9, CYP2C19, CYP3A4 and CYP2D6) were investigated. Tetranabinex®, Nabidiolex® and 1:1 mixture (10 concentrations) were incubated at 37°C with human liver microsomal protein, buffer, enzyme co-factor solution and CYP450-selective substrates. The extent of metabolism of each CYP450-selective substrate in the presence and absence of the TAs was compared. The concentration range of TAs under investigation was = 0, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30 & 100 µM.

IC_{50} was defined as the concentration of inhibitor that affords 50% inhibition of the control activity under the stated assay conditions. The results of the effects of the 3 TAs on the 5 isoforms are presented in Table 1.

Table 1 – IC_{50} concentrations for Inhibition of Cytochrome P450 isoforms

	IC_{50}					
Cytochrome P-450 Isoform	**Tetranabinex®**		**Nabidiolex®**		**1:1 Tetranabinex® : Nabidiolex®**	
	µM	**ng/ml**	**µM**	**ng/ml**	**µM**	**ng/ml**
CYP1A2	40	12,579	14	4,403	12	3,774
CYP2C9	44	13,837	72	22,642	20	6,289
CYP2C19	34	10,692	9	2,830	7	2,201
CYP2D6	125	39,309	84	26,416	38	11,950
CYP3A4	26	8,176	7	2,201	6	1,887

Under these in vitro conditions, it appears that at high concentrations, CBD has greater inhibitory potential than THC, and that this results in the inhibitory potential which may be associated with a 1:1 mixture of the TAs. However, in human clinical studies, therapeutic doses of Sativex®, (a cannabis-based medicine containing Tetranabinex® and Nabidiolex® in a 1:1 ratio), produce human plasma levels of THC and CBD of approximately 5-10ng/ml (*ca.* 15-30nM).

THC is a relatively weak inhibitor of CYP3A4 and a weak inhibitor of CYP1A2, CYP2C9 and CYP2C19. THC is not an inhibitor of CYP2D6. CBD is a relatively potent inhibitor of CYP2C19 and CYP3A4 activity and a relatively weak inhibitor of CYP1A2. CBD is a weak inhibitor of CYP2C9 and CYP2D6.

Based upon the IC_{50} values obtained in this study (without pre-incubation), it is unlikely that either THC or CBD would contribute to cytochrome P450-derived inhibitory drug-drug interactions in vivo considering the far lower levels of both components observed in plasma during clinical studies (some 440-fold lower than the most potent interaction (IC_{50}) observed in this study).

THE EFFECTS OF CANNABIS EXTRACTS TETRANABINEX® & NABIDIOLEX® ON HUMAN CYCLO-OXYGENASE (COX) ACTIVITY

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The objective of this study was to determine the effect of cannabis-based extracts Tetranabinex® (THC Botanical Drug Substance (BDS)) & Nabidiolex® (CBD Botanical Drug Substance (BDS)) on cyclo-oxygenase (COX-1 & COX-2) activity in vitro compared to Indomethacin.

The effects of test articles (concentration range = 0.1 ng/ml to 1 mg/ml), or vehicle (1 % v/v dimethyl sulfoxide) on the activities of COX-1 and COX-2 in human whole blood, and COX-2 in A549 cells were determined on 4 separate occasions using a standard method. On each study day, the 'control' formation of TxB_2 or PGE_2 was assessed as the mean of six determinations. For each experiment the effects of the compounds were calculated and represented as % control using the mean control value.

The effects of test agents on COX-1 and COX-2 activity are detailed in Tables 1& 2. Results in Table 1 are expressed as % control and shown as Mean ± S.E.M. (n=4) from which IC_{50} values were calculated.

Log_{10} [TA] (mg/ml)	-7	-6	-5	-4	-3	-2	-1	0
[TA] (ng/ml)	0.1	1	10	100	1000	10,000	1 x 10^5	1 x 10^6
COX-1 activity in human whole blood (TxB_2, % control)								
Nabidiolex™	105±8	94±9	96±9	88±1	85±3	68±7	60±7	45±6
Tetranabinex™	102±3	96±6	82±5	77±8	61±6	55±4	46±4	21±4
Indomethacin	94±10	73±18	56±16	22±8	3±1	1±1	1±1	1±1
COX-2 activity in A549 cells in human whole blood (PGE_2, % control)								
Nabidiolex™	100±1	92±4	86±4	71±4	73±5	64±6	64±5	52±2
Tetranabinex™	100±2	94±8	88±6	92±8	84±9	78±7	74±8	59±5
Indomethacin	95±8	79±10	78±7	46±8	36±10	30±12	16±9	4±3

Tetranabinex® and Nabidiolex® were found to be weak inhibitors of both human COX-1 (IC_{50} = 400,000 ng/ml; and 1,490,000 ng/ml respectively) and COX-2 (IC_{50} = 15,000 ng/ml; and 10,650,000 ng/ml respectively). Indomethacin, used as a positive control, produced IC_{50} values of 10.6ng/ml (COX-1) and 105ng/ml (COX-2).

Despite Tetranabinex® showing a mathematical selectivity towards COX-1, this is unlikely to be of clinical relevance *in vivo.* Given that therapeutic doses of Sativex® produce human plasma levels in the 5-10ng/ml range it is highly unlikely that either BDS would produce COX-1 or COX-2 inhibition in clinical practice.

Neither Tetranabinex® nor Nabidiolex® would be likely to cause much gastrointestinal toxicity as both were weak COX inhibitors, particularly in comparison to indomethacin.

ADVERSE EVEBTS OF CANNABIS: A SYSTEMATIC REVIEW OF PUBLISHED CASE REPORTS

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Recent legislative changes have resulted in the legalization of cannabis for medical use in Canada. Under present regulations, patients must apply to the federal government for authorization to possess or cultivate cannabis. The support of a physician is also required. Currently, a family practitioner may support an application by a terminally ill patient, but a specialist is required for other medical conditions. However, herbal cannabis is not approved as a drug in Canada. Physicians are therefore being asked to support patients' use of cannabis in the absence of conventional safety and efficacy information. The safety of cannabis is also a concern for insurers, policy makers, researchers (including their ethics boards) and regulators.

A systematic review of all case reports published was performed to generate a summary of reported adverse events and form a database for future clinical trials concerning medicinal cannabis use. A systematic search was conducted using the databases MEDLINE, CINAHL, EMBASE, and PsycINFO, using the following search terms: "Bhang", "Charas", "cannabis", "cannabinoids", "dagga", "Ganja", "hashish", "hemp", "marijuana", "marihuana", "human" and "case report". The search was limited to papers published in English and French.

A total of 266 adverse event cases were identified, reported in 141 articles published between **1962** and **2004**. The age of cases ranged from newborn to 60 years old. Adverse psychiatric events were the most frequently reported event, constituting 31.2% of the total cases reported. Nervous system and respiratory system disorders were the second and third most frequently reported, 19.2% and 12.8%, respectively. Potential biases inherent in this systematic review limit the generalizability of these findings. A large, high-quality epidemiologic study with a well-designed control group is required to provide needed information on the safety of cannabis when used for medical purposes.

Key words: cannabis, safety study, adverse events, systematic review, case reports, MedDRA.

ELECTROPHSYIOLOGICAL EVIDENCE OF ALTERED NEURAL SYNCHRONY IN CANNABIS USE: IMPLICATIONS FOR THE CANNABINOID MODEL OF PSYCHOSIS

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Cannabis use has emerged as a risk factor for the development of schizophrenia (SZ), and SZ itself may be influenced by altered endocannabinoid dynamics. Deficits in neural synchrony, which may be critically involved in perceptual integration and cognition, is one possible mechanism whereby cannabinoids might exert their psychotomimetic effects. The current experiment therefore examined the effect of cannabis use on electroencephalographic (EEG) neural synchronization to auditory and visual stimulation. Subjects passively observed periodic sensory stimuli (auditory click-trains (20, 30, and 40 Hz) and photic flickers (18 and 25 Hz)) while EEGs were recorded. Synchronization was quantified via EEG spectral power (Fourier analysis). Clinical interviews and psychometric questionnaires were also administered in order to determine possible relationships between EEG brain measures and the perceptual/personality correlates of cannabis use. Cannabis users showed decreased EEG power at the auditory stimulation frequency of 20 Hz and in the harmonic of 20 Hz (40 Hz). Within the cannabis group, 20 Hz power values were negatively correlated with the amount of self-reported cannabis use. In the visual condition, a significant gender X group interaction occurred at 18 Hz of stimulation, and age of onset of cannabis use positively correlated with 18 Hz spectral power values. Finally, current cannabis users demonstrated increased schizotypal personality characteristics as assessed via the schizotypal personality questionnaire (SPQ), which positively correlated with total years of cannabis use. These data provide evidence for neural synchronization deficits in cannabis users. This finding, along with the observed increased rates of schizotypy in cannabis users, suggests that cannabis' perceptual, cognitive, and psychotomimetic effects may be related to its ability to modulate synchronized oscillations in neural networks.

THE ENDOCANNABINOID ANANDAMIDE IN CSF IS RELATED TO THE PATTERNS OF CANNABIS USE IN FIRST-EPISODE SCHIZOPHRENIA

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Background: Cannabis use has been considered a risk factor for relapse and an influencing factor on the pattern of psychotic symptoms in schizophrenic patients. The underlying neurobiological mechanisms for these long standing clinical observations remain conjectural. This study investigates the influence of previous and present cannabis use on the recently suggested adaptive role of the endocannabinoid system in first-episode, antipsychotic-naive schizophrenic or schizophreniform psychosis.

Methods: Concentrations of the centrally acting endocannabinoid anandamide were measured in cerebrospinal fluid (CSF) and serum of acute psychotic patients (n = 47) and of healthy volunteers (n = 81) by HPLC/MS. Psychopathology in patients, patterns of cannabis use, and urine drug screenings in both groups were assessed independently.

Results: Acute paranoid psychotic patients with less than 5 times of cannabis use lifetime and no acute use (n = 25) show significantly higher levels of anandamide in CSF than comparable healthy volunteers (n = 55; P = .000). They also differ significantly from those patients with a history of more than 20 times but no recent use of cannabis (n = 9; P = .001). Levels of anandamide in CSF were reversely correlated to psychotic symptoms depending on the history of cannabis use.

Conclusions: The pattern and history of cannabis use in acute, first-episode, antipsychotic-naive schizophrenia yields a selective and significant association to anandamide in CSF. This if of particular importance as anandamide is suggested to play an adaptive role in acute paranoid psychosis and may enhance our understanding of the underlying neurobiology.

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EFFECTS OF ESTROUS CYCLE ON FAAH ACTIVITY IN MOUSE BRAIN

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Previous work has demonstrated that there are sex differences in the behavioral effects of exogenously administered cannabinoids (Tseng and Craft, **2001**). This finding stimulated research into whether there are differences in the endogenous cannabinoid system, including the levels, effects, and regulation of the endocannabinoid anandamide (AEA). Brain AEA content is regulated by catabolism by the enzyme fatty acid amide hydrolase (FAAH). Given that it has been shown that AEA levels vary across the estrous cycle (Rimmerman, et al., **2004**), and that FAAH expression is upregulated by progesterone in peripheral lymphocytes (Maccarrone et al., **2001**), we hypothesized that progesterone might also regulate brain FAAH activity and that this regulation is a key factor in the effects of estrous stage on brain AEA content. The purpose of the current study was to determine whether brain FAAH activity varies with changes in progesterone levels across the estrous cycle in mice. Female mice were divided into four groups based on estrous stage. Kinetic assays for FAAH activity in tissue from mouse brain demonstrated that there was a trend toward an increase in Vmax for FAAH activity during proestrous, the time when progesterone levels are highest, although this did not reach statistical significance. The Km of FAAH for AEA was not affected by estrous stage. The potential relationship between FAAH regulation of AEA in the brain and progesterone levels have considerable impact for issues such as the role of AEA and FAAH in premenstrual syndrome as well as post-partum depression. FAAH activity has also been implicated in anxiety, as inhibition of FAAH results in a decrease in anxiety-like behavior in response to stressful situations (Kathuria et al., **2003**). Additional research examining sex differences in FAAH regulation and AEA content in the brain in response to estrous cycle and sex hormone levels is needed to adequately address these issues.

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REGULATION OF ANANDAMIDE (AEA) SYNTHESIS AND CANNABINOID RECEPTORS (CB_1/CB_2) EXPRESSION DURING THE ESTROUS CYCLE IN THE RAT

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Marijuana consumption has negative effects on female fertility and early pregnancy. On the other hand, the fact that the uterus has the major concentration of AEA registered in mammals tissues suggests that this endocannabinoid might have a role in female reproductive physiology. Spontaneous abortion is one of the main causes of early pregnancy loss in women. Due to the fact that high concentrations of AEA are toxic both for implantation and embryo development, it has been postulated that AEA is a modulator of the crucial processes that occurs during early pregnancy. It has been observed that the acute treatment with AEA suppresses the luteinizing hormone pre – ovulatory peak, thus inhibiting ovulation. Besides, the chronic exposure to AEA causes altered estrous cycles and affects the structures of the pre – ovulatory follicles. It is well known that AEA interact with two types of receptors: CB_1 and CB_2.

The aim of the present work was to determine AEA synthesis (conversion of [^{14}C]-arachidonic acid and ethanolamine in [^{14}C]-AEA) and the expression of CB_1 and CB_2 receptors (western blot) during the estrous cycle in the rat uterus and oviduct. Thus, rats of the Wistar strain were sacrificed in different days of the estrous cycle (proestrous, estrous, metaestrous and diestrous) and the uterus and oviduct were obtained.

AEA synthesis was detectable during the four days of the estrous cycle both in the uterus and the oviduct. The highest production of AEA was detected during estrous in the uterus (1.40 ± 0.13 nmoles AEA/mg prot/1h, $p<0.01$) and during estrous (10.40 ± 0.20/1h, $p<0.001$) and proestrous (10.80 ± 0.30, $p<0.001$) in the oviduct.

CB_1 and CB_2 expression was present in all the days analyzed. In the uterus, CB_1 remained constant while CB_2 diminished during estrous ($p<0.05$). During proestrous in the oviduct the expression of CB_1 was maximum ($p<0.05$) and of CB_2 was minimum ($p<0.01$).

These results indicate that AEA synthesis and the expression of CB_1/CB_2 are present both in the rat uterus and oviduct and that they are regulated during the estrous cycle, suggesting that its modulation during ovulation and early pregnancy by important mediators, chiefly sexual hormones, prostaglandins, nitric oxide, might affect female fertility.

THE EFFECT OF THE PERIPHERAL CANNABINOID RECEPTOR ON BIRTH RATE, SEX AND GENOTYPIC RATIO OF MICE OFFSPRING

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The recent discovery of cannabinoid receptors in the uterus and early embryo stage suggest the involvement of the cannabinoid system in reproductive biology. Two types of cannabinoid receptors discovered, the central cannabinoid receptor (CB_1) and the peripheral cannabinoid receptor (CB_2), are expressed in mouse preimplantation embryos and are the focus of study in the field of developmental biology. This particular study examined the effect of CB_2 on reprodutive outcome. We specifically investigated the effect of CB_2 on the birth rate, sex and genotypic ratio. We analyzed offspring from diverse CB_2 wild-type, CB_2 heterozygous and CB_2 knockout mice breeding pairs. One thousand four hundred and twenty nine 3 week-old offsprings were counted, sexed and genotyped by tail DNA analysis using polymerase chain reaction. Of the nine genotypic breeding pairs, the CB_2 wildtype breeding pair exhibited the highest mean for the number of offspring per litter. Compared to the CB_2 wildtype breeding pair, the breeding between CB_2 heterozygous mother and CB_2 wildtype father showed significantly less birth rate. The sex ratio within the offspring from each genotypic breeding pairs was 1:1 regardless of birth rate. Breeding between CB_2 heterozygous mother and CB_2 knockout father generated significantly more CB_2 heterozygous offspring than CB_2 knockout offspring. We conclude that CB_2 expression plays a role in determining the fate of offspring litter size and genotype, and thus, has a role in reproduction.

REGULATION OF ANANDAMIDE (AEA) SYNTHESIS AND CANNABINOID RECEPTORS (CB_1/CB_2) EXPRESSION DURING PREGNANCY IN THE MOUSE UTERUS

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Although exposure to exocannabinoids is associated with adverse pregnancy outcome, little is known about the biochemistry, physiology, and consequences of endocannabinoids in normal pregnancy.

In this study, we determined anandamide (AEA) synthesis (conversion of [^{14}C]-arachidonic acid and ethanolamine in [^{14}C]-AEA) and the expression of CB_1 and CB_2 receptors (western blot) during pregnancy in mouse uterus. Thus, Balb-C mice were sacrificed in different days of pregnancy (5, 8, 11, 13, 15, 18 and 19) and non-pregnant mice in diestrus were used as controls.

AEA synthesis was detectable throughout whole pregnancy and in non-pregnant uterus. The production of this endocanabinoid was greater in non-pregnant mice uteri (3,2 ± 0,3 nmol AEA/mg tissue/h) than in pregnant (day 5: 0.5 ± 0,1 $p<0.001$). Anandamide synthesis in the middle pregnancy was significantly higher than in either early pregnancy or the days before labor.

CB_1 and CB_2 expression was present in all the days analyzed. CB_1 and CB_2 expression diminished at implantation. CB_2 remained constant during the rest of the pregnancy while CB_1 diminished the days before labor.

Anandamide (10^{-6}M) inhibited uterine nitric oxide (NO) synthase activity while a nitric oxide donor (NOC-18, 2mM) was able to augment anandamide synthesis, and this effect was blocked by hemoglobine (20ug/ml).

These findings suggest that successful pregnancy implantation and progression requires low levels of AEA. These results also show that anandamide participates in normal mouse pregnancy interacting with NO pathway, probably by exerting a negative feedback on NO. This work highlights a possible role for endogenous cannabinoids during pregnancy.

ALTERED CEREBELLAR SYNAPTIC ACTIVITY FOLLOWING CHRONIC Δ^9-THC EXPOSURE

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Chronic cannabinoid exposure results in tolerance to cannabinoid-induced locomotor effects, which are mediated by the cannabinoid receptors (CR1Rs) located in motor control region, such as cerebellum. Our experiments were aimed to investigate the neurophysiological and molecular adaptive responses to chronic Δ^9-tetrahydrocannabinol (Δ^9-THC) exposure occurring in the cerebellum. In order to investigate the role of the Ras/ERK pathway in cannabinoid tolerance, we focused on a genetically engineered mouse model (RasGRF1 null mice) (Brambilla et al., **1997**), that do not develop behavioural tolerance after chronic treatment with Δ^9-THC (Rubino et al., **2004**). In cerebellar cortex, Purkinje cells (PCs) receive excitatory inputs from parallel fibers (PFs), the axon of granule cells (GC). Acute activation of CB_1Rs located presynaptically suppresses PF synaptic inputs to PCs. Electrophysiological experiments have been carried out in PCs to determine the properties of evoked synaptic transmission in mice exposed chronically to Δ^9-THC (10 mg/Kg, 2 daily s.c. injections, 4.5 d treatment) or to its vehicle. Electrical stimulation of PFs evokes excitatory postsynaptic currents (EPSCs), allowing the study of excitatory synaptic transmission by means of whole-cell patch-clamp recordings. We used PF-PC synaptic transmission features, including the characteristic form of short-term plasticity paired-pulse facilitation (PPF), to assess an altered functionality of excitatory synaptic connections. Our results show a decreased PPF in tolerant mice chronically exposed to THC (PPF at 50 ms stimulus interval; vehicle, 2.19±0.05, n=11; THC, 1.86±0.04, n=11; $p<0.05$), indicating an increased release probability after chronic cannabinoid treatment. This modification of synaptic plasticity is not seen in mice lacking RasGRF1 gene, (PPF at 50 ms stimulus interval; vehicle, 2.04±0.07, n=8; THC, 1.98±0.05, n=13), suggesting that the Ras/ERK pathway plays an important role in both synaptic plasticity and tolerance. In the cerebellum, chronic THC administration results in CB_1R downregulation and uncoupling from Gi/o proteins. Preliminary results indicate that inhibition of PF-PC synaptic transmission induced by CP55940 is decreased in THC-treated mice, as compared to vehicle-treated animals (% EPSCs inhibition, CP55940 20 μM: vehicle, 84±5 %, n=5; THC, 61±5 %, n=5), showing a reduced efficacy of CB_1R-mediated signalling in THC-exposed mice. Further experiments are now in progress exploring the effects of chronic THC exposure on different forms of cerebellar synaptic plasticity. We are also performing proteomics analysis on cerebellum to identify proteins involved in the adaptive processes underlying cannabinoid addiction. Our results indicate that chronic Δ^9-THC exposure modulates cerebellar PF-PC synaptic plasticity. This might contribute to the behavioural tolerance to cannabinoid-induced locomotor effects observed following chronic cannabinoid administration.

EFFECTS OF RIMONABANT (SR141716) ON ALCOHOL ABSTINENCE IN WISTAR RATS

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Recent studies have postulated that the endocannabinoid system might play a regulatory role in physiological processes altered in addictive states. Thus, there is evidence on an involvement of this system in genetic vulnerability, seeking behavior, dependence, relapse and other phenomena elicited by different types of habit-forming drugs, such as alcohol, opioids, cocaine or tobacco. Even, recent evidence suggest that the blockade of CB_1 receptors might serve to reduce craving for different substances such as nicotine or alcohol. However, there is less evidence concerning the possibility that the blockade of CB_1 receptors might be also beneficial to attenuate withdrawal symptoms elicited by the interruption of regular abuse of these substances. In the case of alcohol, this hypothesis is supported by the reduction of withdrawal syndrome observed in CB_1 receptor mice [Racz et al., J. Neurosci. 23, 2453-2458 (**2003**)]. We have examined the potential of rimonabant (SR141716) to reduce the intensity of the abstinence caused by the interruption of chronic alcohol exposure in Wistar rats subjected to alcoholization for 10 days through a constant rate of alcohol (7.2%) in the drinking water. We found that abstinent rats developed a withdrawal syndrome characterized by the occurrence of anxiety (measured in the plus-maze test) and, to a lesser extent, motor hyperactivity (measured in a computer actimeter). The administration of rimonabant, however, reduced the anxiogenic effect caused by the alcohol abstinence, increasing the number of visits to the open arms and the time spent in each visit. This was, however, an effect found exclusively in abstinent rats, since the administration of rimonabant to control rats produced the expected anxiogenic effect. The anxiolytic effect produced by rimonabant in abstinent rats might be related to a correction of GABA and, to a lesser extent, dopamine deficits generated by alcohol abstinence in several brain regions related to emotional and motor responses, without affecting serotonin transmission. In summary, rimonabant might be efficacious to attenuate withdrawal signs associated with the alcohol abstinence. This effect was presumably due to normalization of GABA and, to a lesser extent, dopamine transmission in emotion-related and motor-related areas. It is also possible that it is related to an attenuation of the stress response activated by the interruption of regular alcohol abuse.

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ENDOCANNABINOID INVOLVEMENT IN ETHANOL DRINKING BEHAVIOR IN ALCOHOL PREFERRING P RATS – IS DOPAMINE INVOLVED?

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Cannabinoids acting at CB_1 receptors activate the mesolimbic dopaminergic reward system, and this mechanism is thought to underlie the role of endocannabinoids in alcohol (EtOH) and drug seeking behavior. Here we used alcohol preferring P rats and their non-preferring counterparts (NP rats) in a two-bottle, free-choice paradigm to test the effect of the CB_1 antagonist rimonabant on voluntary EtOH drinking. A single i.p. does of 3 mg/kg rimonabant dramatically reduced EtOH intake and preference in P rats, but had no effect on the much lower intake and preference in NP rats. To test the role of dopamine in this effect, we measured the release of dopamine (DA) and its metabolite DOPAC in the nucleus accumbens shell of conscious, freely moving rats using in vivo microdialysis and electrochemical detection. EtOH (4g/kg i.p.) caused no significant change in DA release in either P rats or in normal Sprague-Dawley (SD) rats, but elicited a small but significant increase in DOPAC, suggesting that DA released may have been metabolized during the collection period. To prevent metabolism, rats were treated with the MAO inhibitor pargyline (70mg/kg ip), which resulted in a ~3-fold increase in DA and a near-complete disappearance of DOPAC in the dialysate in both P and SD rats. Subsequent administration of EtOH caused a further significant increase in DA release (2.8-fold in SD, 1.8-fold in P). Injection of pargyline-pretreated rats with 3 mg/kg rimonabant did not influence DA levels in the dialysate and did not affect the increase in DA caused by a subsequent injection of EtOH (3.5-fold in SD and 2.3-fold in P). These findings suggest that rimonabant reduces voluntary EtOH drinking by a mechanism other than modulation of EtOH-induced DA release in the nucleus accumbens.

DIFFERENTIAL ROLE OF CB_1 AND OPIOID RECEPTORS IN THE REINSTATEMENT OF HEROIN-SEEKING BEHAVIOUR AND CANNABINOID INTAKE FOLLOWING EXTINCTION

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Cannabinoids and opioids have been shown to interact in the expression and modulation of reward-related events as well as relapsing phenomena. We recently demonstrated that primings with cannabinoid receptor (CB_1) agonists can trigger relapse to heroin in rats with a previous history of heroin self-administration (SA).

In the present follow up study we tested the effect of the opioid antagonist naloxone (NX) and CB_1 antagonist SR141716A (SR) on (*i*) cannabinoid-induced resumption of heroin-seeking behaviour, (*ii*) long-lasting effect of cannabinoid primings on heroin-seeking reinstatement and (*iii*) intake of the CB_1 agonist WIN55,212-2 following heroin SA in a drug substitution test (i.e. with no drug primings).

Results of this study show that (*i*) cannabinoid-induced reinstatement of heroin-seeking behaviour is significantly attenuated by either SR (0.3 mg/kg) or NX (1 mg/kg), while completely prevented by the simultaneous administration of the two antagonists. Notably, (*ii*) the residual long-lasting effect of cannabinoid primings on heroin-seeking reinstatement is not affected by SR or NX pretreatment. Finally, (*iii*) as trained rats are switched from heroin (30 μg/inf) to WIN55,212-2 (12.5 μg/kg/inf) SA, operant responding rapidly declines on the first day of drug substitution. However, when rats are given access to the CB_1 agonist not before 7, 14 or 21 days of extinction, they promptly resume operant behaviour and do self-administer WIN55,212-2 in a time-dependent manner. Intriguingly, cannabinoid intake in abstinent rats is significantly attenuated by NX while fully prevented by SR administration.

Taken together, the present findings strengthen previous hypothesis of a strict functional interaction between the cannabinoid and opioid receptors in the modulation of central mechanisms regulating relapsing phenomena, suggesting that the neural substrates triggering relapse to drug-seeking after chronic drug consumption may be different from those underlying drug-taking behaviour.

THE ABUSE POTENTIAL OF THE ENDOCANNABINOID TRANSPORT INHIBITOR AM404: SELF-ADMINISTRATION BY SQUIRREL MONKEYS

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Therapeutic application of cannabis-based medicines has been stalled by the fact that Δ^9-tetrahydrocannabinol (THC) and other cannabinoid agonists suffer from a narrow therapeutic window between desired clinical effects and unwanted psychic side-effects (Iversen, **2003**). Pharmacological manipulation of the endocannabinoid system by drugs that inhibit the inactivation of endocannabinoids has been suggested as a safer and more subtle approach for the treatment of pain and neuropsychiatric disorders (e.g., Kathuria et al., **2003**). We have previously shown that THC maintains high rates of i.v. self-administration behavior in squirrel monkeys with or without a history of pre-exposure to other drugs (Tanda et al., **200**0; Justinova et al., **2003**). Here we investigated whether the inhibitor of anandamide and 2-AG transport, AM404, maintains persistent self-administration behavior in monkeys with different self-administration histories and whether cannabinoid CB_1 and/or vanilloid VR1 receptors are involved in the mediation of any observed reinforcing effects of AM404. In squirrel monkeys with a history of anandamide self-administration, we substituted injections of different doses of AM404 (1–100 μg/kg/injection; n=3) for either 40 μg/kg injections of anandamide or vehicle. In monkeys with a history of cocaine self-administration (n=3), we also substituted AM404 for either 30 μg/kg injections of cocaine or vehicle. Each dose of AM404 was studied for five consecutive sessions with five sessions of anandamide, cocaine or vehicle substitution between each dose-testing. Using a 10-response, fixed-ratio schedule with a 60-s time-out after each injection of drug during daily 1-hr sessions, we found that self-administration behavior of squirrel monkeys was persistently maintained by AM404. Dose-response curves had an inverted-U shape, with peak response rates averaging about 1 response/sec at a dose of 10 μg/kg/injection of AM404. When vehicle was substituted for AM404, self-administration behavior immediately decreased to low levels and was rapidly reinstated when AM404 injections were again available. AM404 self-administration behavior was partially, but significantly, reduced by pre-session treatment with the cannabinoid CB_1 receptor antagonist rimonabant (SR141716, 0.1 mg/kg i.m. for 5 sessions), but remained above vehicle substitution levels. The vanilloid VR1 receptor antagonist capsazepine had no statistically significant effect on AM404 self-administration at a 0.01 mg/kg dose (i.m. for 5 sessions), which did not disrupt responding for intravenous injections of 30 μg/kg of cocaine or food pellets in two control groups of monkeys (n=3-4). Thus, the endocannabinoid transport inhibitor AM404 functioned as an effective reinforcer (comparable to THC, anandamide and cocaine under identical conditions) in non-human primates under a fixed-ratio schedule of drug injection. These effects appeared to be partially mediated by cannabinoid CB_1 receptors, but not vanilloid VR1 receptors. Our findings suggest that medications which promote the actions of endocannabinoids throughout the brain by inhibiting their membrane transport have a potential for abuse. It remains to be seen whether medications such as FAAH inhibitors, which augment CB_1 signaling only in certain regions of nervous system, would be self-administered in a similar manner.

CHOLINERGIC MODULATION OF THE DISCRIMINATIVE STIMULUS EFFECTS OF Δ^9-TETRAHHYDROCANNABINOL (THC) IN RATS

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Systemic administration of the main active ingredient in cannabis, Δ^9-tetrahydrocannabinol (THC), has been reported to increase or to decrease extra-cellular levels of acetylcholine in several brain areas suggesting an involvement of the cholinergic system in the psychotropic effects of cannabis. In this study, we first investigated whether drugs acting at either nicotinic or muscarinic receptors could modulate the discriminative effects of THC. In rats that had learned to discriminate 3mg/kg of THC from vehicle injections, the nicotinic agonist nicotine (0.1-0.56 mg/kg s.c.) and the muscarinic agonist pilocarpine (0.3-3 mg/kg i.p.) did not produce THC-like effects but they both potentiated the discriminative effects of low doses of THC. Neither the nicotinic antagonist mecamylamine (1-5.6 mg/kg i.p.) nor the muscarinic antagonist scopolamine (0.01-0.1 mg/kg i.p.) altered the discriminative effects of THC but they blocked the potentiation induced by nicotine and pilocarpine, respectively. In order to further investigate the nicotinic receptor subtypes involved in the discriminative effects of THC, we then made use of selective antagonists for beta-2 or alpha-7 subunits. The selective alfa-7 nicotinic receptor antagonist MLA (0.3-3 mg/kg i.p.) but not the selective beta-2 nicotine antagonist DHBE (1-18 mg/kg s.c.) significantly reduced the discriminative effects of THC. However, both MLA and DHBE reversed the potentiation induced by nicotine. Our results demonstrate cholinergic system involvement in the discriminative-stimulus effects of THC. Furthermore, our results with selective nicotinic antagonists suggest that potentiation or reduction of THC's effects may depend on different mechanisms.

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INVESTIGATING CANNABINOIDS AND BEHAVIORAL SENSITIZATION TO PSYCHOSTIMULANTS

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Following repeated administration of psychostimulants such as cocaine or amphetamine, animals develop a sensitized locomotor response to subsequent drug exposures. This *behavioral sensitization* has been related to specific neural adaptations which occur in response to the drug treatment, including altered characteristics of plasticity of synaptic transmission. Such processes, predominantly involving the mesocorticolimbic dopamine system and its target nuclei, are believed to also underlie a similar and parallel sensitization of incentive value, akin to drug craving in humans. Enhanced locomotion and behavioral stereotypies in test animals may therefore be reflective of increased motivational salience for the sensitizing drug.

The nucleus accumbens (NAc) is a brain area important for the rewarding effects of psychostimulants, as well as certain aspects of behavioral sensitization. Excitatory synaptic transmission in the NAc is acutely modulated by cannabinoid receptor activation, and endocannabinoids (eCBs) act to induce long-term depression (LTD) of synaptic inputs from prefrontal cortex. Interestingly, cocaine sensitization is correlated to a form of LTD at these synapses, and there is evidence that even single doses of cocaine can alter expression of eCB-dependent synaptic plasticity in the NAc. The present study investigates the importance of CB_1 receptors in behavioral sensitization to cocaine.

Behavioral sensitization consists of two dissociable phases – induction and maintenance. Pretreating C57Bl6 mice with either Δ^9-THC (10 mg/kg, i.p.) or SR141716A (3 mg/kg, i.p.), 30 min prior to daily cocaine (15 mg/kg, i.p., for 5 days), we have not observed any significant alteration in the development of sensitized locomotor responses to a challenge dose of cocaine given at day 14. However, in preliminary experiments the *maintenance* of previously established cocaine sensitization was effectively extinguished in mice treated subsequently with a regimen of SR141716A (3 mg/kg, i.p. for 5 days). Thus mice receiving the CB_1 antagonist showed diminished responsiveness to cocaine on a final challenge (day 21), whereas control littermates injected similarly with drug vehicle (70% saline/20% DMSO/10% Tween-80) continued to exhibit sensitized locomotor activity in response to cocaine. Although drugs were administered systemically, this finding appears to correlate with others' observations that the NAc is critical for the maintenance of cocaine sensitization, but less important for its induction. We note, however, that a high degree of variability was observed in initial responses to cocaine and in the timecourse of cocaine sensitization across subjects, lending caution to our interpretations. Experiments are underway to utilize i.v. injections to more reliably induce behavioral sensitization with less variability in the delivery of all compounds. Experiments with amphetamine will also be discussed.

LACK OF CB_1 CANNABINOID RECEPTOR PRODUCES CHANGES IN MDMA PHARMACOLOGICAL EFFECTS

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Interactions between cannabinoids and other drugs of abuse, such as opioids and nicotine have been previously reported. However, the role played by the cannabinoid system in the pharmacological effects of psychostimulants remains unclear. The aim of the present study was to evaluate the possible involvement of the CB_1 cannabinoid receptor in the responses induced by MDMA by using knockout mice lacking this cannabinoid receptor.

Changes in locomotor activity, body temperature and anxiety-like responses were evaluated in CB_1 knockout mice and their wild-type littermates after a single injection of MDMA (5, 10 and 20 mg/kg i.p.). Acute MDMA administration increased locomotor activity in wild-type mice, but this response was significantly attenuated in knockout mice. In addition, the increase in body temperature induced by MDMA (20 mg/kg i.p.) was also reduced in knockout animals. Finally, the administration of MDMA (10 mg/kg i.p.) induced an anxiogenic-like response in the elevated-plus maze, being this effect completely abolished in mice lacking CB_1 receptors.

In conclusion, these results show that the cannabinoid system through the CB_1 cannabinoid receptor modulates the acute pharmacological effects of MDMA. Further studies are being developed in our laboratory to investigate the participation of CB_1 cannabinoid receptors in the addictive properties of MDMA.

THE TRABECULAR MESHWORK AS A TARGET FOR MODIFICATION BY CANNABINOIDS

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Intraoccular pressure (IOP) reflects the balance between aqueous humor inflow and outflow and is altered by changes in outflow facility. An important component of the outflow pathway is the trabecular meshwork (TM). Pharmacological alterations in TM cell actin organization and contractility can change aqueous humor outflow facility and alter IOP (Peterson et al., **2000**, Invest. Ophthalmol. Vis. Sci. 41: 1749-1758). Cannabinoids reduce IOP, likely via actions on tissues associated with aqueous humor inflow and outflow, however the tissue-specific signaling effects of these compounds in the eye have yet to be established.

The aim of our study was to determine the effects of CB_1 receptor (CB_1R) activation, and associated signaling pathways in human TM cells. We also investigated how other known contractile agents, such as endothelin-1 (ET-1), which acts on endothelin receptors, may interact with CB_1R activation and signaling.

The TM5 human cell line was used for all experiments. Intracellular [Ca^{2+}] was measured using the fluorescent Ca^{2+} ion imaging with the ratiometric dye, FURA 2-AM (5 μM). Western Blot analysis and immunocytochemistry was performed to determine the effects of the synthetic cannabinoid agonist, WIN55,212-2 (WIN), on myosin light chain (MLC) and extracellular signal regulated kinase (ERK1/2).

Measurement of $[Ca^{2+}]_i$ revealed that WIN (1-100 μM) elevated $[Ca^{2+}]_i$ in TM cells in a dose-dependant manner. Co-application of a CB_1R antagonist (SR141716A; 10μM) with WIN, or removal of Ca^{2+} from the extracellular solution, blocked the increase in $[Ca^{2+}]_i$ observed with WIN alone. Application of either ET-1 (1-10 nM) or WIN (10 μM) produced a very small or moderate $[Ca^{2+}]_i$ increase, however when both agonists were co-applied an enhanced increase in [Ca^{2+}]i was observed. Western Blot analysis revealed that application of WIN (0.1-100 μM) to the cells increased both MLC and ERK1/2 phosphorylation. WIN-induced MLC phosphorylation was inhibited with SR141716A (0.1-10 μM).

In summary, our results show that activation of the CB_1Rs in the TM5 cell line leads to ERK1/2 activation, an increase in $[Ca^{2+}]_i$ and MLC phosphorylation. Inhibition of both the increase in $[Ca^{2+}]_i$ and MLC phosphorylation by the selective CB_1R antagonist, SR141716A, indicates that these effects are mediated via CB_1R activation. The synergistic response observed with co-application of WIN and ET-1 implies cross talk is occurring between these two G protein coupled receptor signaling pathways. Future experiments will determine if CB_1R-mediated signaling produces alterations in actin cytoskeleton and cell contractility.

ENDOCANNABINOID AND PALMITOYLETHANOLAMIDE LEVELS IN EYES WITH DIABETIC RETINOPATHY OR MACULAR DEGENERATION

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We have previously reported the presence of the endocannabinoids anandamide and 2-arachidonoyl glycerol (2-AG), and of the anandamide congener, palmitoylethanolamide (PEA), in human eye tissues (Matias et al., ICRS Meeting, **2004**), where cannabinoid CB_1 receptors control intra-ocular pressure (IOP) as well as other ocular functions concerned with the retina. While 2-AG was most abundant in the retina, anandamide and PEA levels were found to be highest in the iris. Previous studies showed that the CB_1 receptor is abundantly expressed in the two synaptic layers of the retina in several vertebrate species. By in situ hybridisation and RT-PCR analysis, it was also found that CB_1 receptors are expressed also in the ciliary body, iris and choroid. In contrast, the expression of the cannabinoid CB_2 receptor seems to be limited to the neurosensory retina. Immunohistochemical studies showed also the presence of FAAH in the retina, particularly in the large ganglion cells and in the soma of dopamine amacrine cells and large cells. Finally, recent immunohistochemical data indicate that also the other major molecular target for anandamide, the vanilloid TRPV1 receptor, is expressed in the retinas of rat, cat, and monkey (Yazulla and Studholme, **2004**).

In the present study we quantified by isotope-dilution LC-MS the levels of anandamide, PEA and 2-AG in human cornea, iris, ciliary body, choroid and retina in eyes (N=6-10) from patients with diabetic retinopathy (DR) or macular degeneration (MD), and compared them with those of eyes from control patients. The distribution and net concentrations of the three compounds in control eyes corresponded to those previously reported, with anandamide/PEA or 2-AG being significantly most abundant in the iris or retina, respectively. In eyes with DR, significantly enhanced levels of anandamide were found in the retina (1.8-fold), ciliary body (1.5-fold) and, to a lesser extent, cornea (1.3-fold). Surprisingly, 2-AG levels were significantly higher (3-fold) only in the iris, which is not involved in DR, whereas PEA levels only slightly increased (1.3-fold) in the ciliary body. In eyes with MD, significantly enhanced levels of anandamide were found in the choroid (1.3-fold), ciliary body (1.4-fold) and cornea (1.4-fold), whereas in the retina only a trend towards increase (1.5-fold) was observed. No significant changes in the levels of 2-AG or PEA were detected in eyes with this disease. In our previous study on eyes from patients with glaucoma (Matias et al., ICRS Meeting, **2004**), we reported a selective reduction of 2-AG levels in the ciliary body, and of PEA in the choroid and ciliary body, with no changes for anandamide.

Despite its limitations (i.e. the use of post-mortem samples from patients whose clinical history was not always completely known), this study confirms that endocannabinoids may be involved in ophthalmic disorders. The tissue- and disease-selective nature of the changes observed supports the validity of our studies and suggests that, depending on their molecular targets (CB_1 and TRPV1 for anandamide, CB_1 and CB_2 for 2-AG, unknown for PEA), the compounds analysed here may play different roles in the control of eye function under physiological and pathological conditions.

NEUROPROTECTIVE AND BLOOD-RETINAL BARRIER-PRESERVING EFFECTS OF CANNABIDIOL IN EXPERIMENTAL DIABETES

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Purpose: Diabetic retinopathy is characterized by blood-retinal barrier (BRB) breakdown and neurotoxicity. BRB breakdown has been associated with increases in oxidative stress and expression of pro-inflammatory cytokines. Neurotoxicity involves glutamate-induced oxidative stress and neuronal cell death. P38 MAP kinase, a down stream target of TNF-alpha and oxidative stress has been shown to be a key regulator of vascular permeability and retinal neural cell death. Cannabinoids, generally believed to have properties of anti-inflammation and anti-oxidation, have not been tested in diabetes due to their psychotropic activity. In the present study, the anti-oxidant and anti-inflammatory effects of a non-psychotropic cannabinoid, Cannabidiol (CBD) are examined in experimental diabetes.

Methods: Rats were rendered diabetic by streptozotocin injection and were treated by CBD or vehicle for 1, 2, 4, or 7 weeks. Diabetes-induced retinal cell death was determined by TUNEL. BRB function was measured by quantifying extravasation of BSA-fluorescein. Oxidative stress was determined by an assay for lipid peroxidation and measurement of reactive oxygen species. Expression of retinal ICAM-1, TNF-alpha and p38 MAP kinase were determined by immunohistochemistry, ELISA or Western blot analyses.

Results: Diabetes induced significant increases in oxidative stress, retinal neural cell death and vascular permeability. That effect was associated with activation of p38 MAP kinase and increased levels of TNF-alpha and ICAM-1 expression. CBD treatment significantly reduced oxidative stress and prevented retinal cell death and vascular permeability increase in the diabetic retina. Moreover, diabetes-induced activation of p38 MAP kinase was significantly reduced by CBD. The anti-inflammatory activity of CBD was demonstrated by its effect in reducing the levels of TNF-α and ICAM-1.

Conclusions: These results demonstrate that CBD treatment reduces neurotoxicity and BRB breakdown in diabetic animals through both its activities in anti-oxidation and anti-inflammation by a process that may involve inhibition of p38 MAP kinase.

CANNABINOID CB_1 RECEPTORS IN THE BASAL GANGLIA AND MOTOR RESPONSE TO ACTIVATION OR BLOCKADE OF THESE RECEPTORS IN PARKIN-NULL MICE: RELEVANCE TO PARKINSON'S DISEASE

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The endocannabinoid transmission becomes overactive in the basal ganglia in Parkinson's disease (PD), as reported in patients and animal models of this disease. In the present study, we examined the status of cannabinoid CB_1 receptors in the basal ganglia of female and male *Park-2* knockout mice, a genetic model of PD that progresses with no neuronal death and that may be considered representative of early and presymptomatic parkinsonian deficits. We found an increase in the density of CB_1 receptors in the substantia nigra compared to wild-type animals with no changes in other basal ganglia, although this occurred only in females. Despite this increase, the motor inhibition caused by the acute administration of the cannabinoid agonist Δ^9-tetrahydrocannabinol to *Park-2* knockout female mice was markedly of lesser magnitude compared with the response found in wild-type animals. By contrast, the administration of the CB_1 receptor antagonist SR141716 resulted in a hyperkinetic response in parkin-null mice, response that was almost absent in wild-type animals and that was accompanied by a decrease in tyrosine hydroxylase activity in the caudate-putamen. However, parkin-null male mice exhibited normal levels of CB_1 receptors in the substantia nigra and the remaining basal ganglia, excepting a small decrease in the lateral part of the caudate-putamen, which was associated with an increase in mRNA levels for superoxide dismutase in this structure. Interestingly, they responded to the administration of Δ^9-tetrahydrocannabinol in a way significantly different than parkin-null females, since the motor depression produced by this cannabinoid agonist was significantly greater in *Park-2* knockout mice compared with their wild-type counterparts, and this corresponded with an increase in tyrosine hydroxylase activity in the caudate-putamen. In summary, extending the data obtained in humans and animal models of basal ganglia neurodegeneration, the changes in CB_1 receptors observed in parkin-null mice revealed that they are already produced by dopaminergic dysfunction and would not necessarily require massive destruction of dopamine neurons in the substantia nigra. In addition, these changes would be associated with differences in behavioral responses to cannabinoid agonists or antagonists between *Park-2* knockout and wild-type mice, although parkin-null mice exhibited evident gender-dependent differences for both levels of CB_1 receptors and motor responses to agonists or antagonists.

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FURTHER CHARACTERIZATION OF CANNABINOID CB_2 AND FAAH-POSITIVE GLIA IN CORTICAL REGIONS OF ALZHEIMER´S DISEASE BRAINS

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Previous data indicate that the endocannabinoid system may participate in neuroinflammatory responses. Among these, we have recently reported immunohistochemical data on the presence of cannabinoid CB_2 receptors and fatty acid amide hydrolase (FAAH) on activated glia in Alzheimer´s disease brains. Further, these cells seemed to be linked to specific regions where beta-amyloid (BA) deposition takes place, as they were located proximal to BA-enriched plaques. Thus, while CB_2 receptors were expressed by microglial cells, FAAH was specifically present on astrocytes. As it is currently known that crucial differences exist on the properties of these "activated" cell types, we then focused on the phenotypic characterization of each type of cells expressing CB_2 or FAAH. To that end we employed several specific antibodies against some of the most prototypic markers of activated glia, such as Il-1, MCP-1, and S-100B, to perform double and triple immunohistochemical and immunofluorescent stainings. Our results seem to confirm a link between the expression of CB_2 and FAAH in glial cells located on the vicinity of BA-plaques and the activational process of these nervous cells, that is characteristic of inflammatory processes such as Alzheimer´s disease.

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POST-ISCHEMIC Δ^9-TETRAHYDROCANNABINOL (THC) PROTECTS AGAINST ISCHEMIA-INDUCED NEURONAL INJURY WITH A BELL-SHAPED-DOSE-RESPONSE CURVE

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During the last two decades, it has become clear that exogenous natural and synthetic cannabinoids exert neuroprotective functions in several models of neurotoxicity (Hampson et al., 1998; Nagayama et al. **1999**; Panikashvli et al., **2001**; Braida et al. **2000**, **2003**; van der Stelt et al., **2001**a, **2001**b; Veldhuis et al., **2003**; Marsicano et al., **2003**). About Δ^9-tetrahydrocannabinol (THC), the major psychoactive constituent of marijuana, a neuroprotection either *in vitro* (Hampson et al., **1998**) or *in vivo* (Louw et al., **2000**; Van der Stelt et al., **2001**) in rat cortical neurons and in a model of rat forebrain ischemia has been found, respectively.

To further investigate the effect of THC against neuronal injury *in vivo*, we injected the compound 5 minutes after transient global cerebral ischemia in Mongolian gerbils using a wide range of doses (0.05 - 2 mg/kg i.p.).To quantify the ischemic damage we measured from 1 hour to 7 days after recirculation, electroencephalographic (EEG) mean total spectral power, spontaneous motor activity, cognitive function, rectal temperature and hippocampal neuronal count.

THC antagonized the electroencephalographic flattening of total spectral power and hyperlocomotion on day 7 and 1, respectively, with a dose-dependent bell-shaped curve: the neuroprotective effect was greatest with 1 mg/kg. The same dose, which decreased rectal temperature within the first hour, completely reversed ischemia-induced cognitive deficit, in the passive avoidance test evaluated on day 3.

Experiments are in progress to clarify the mechanism by which THC shows its protective effect through the blockade of CB_1, VR_1vanilloid and opioid system.

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IMMUNOHISTOCHEMICAL TIME-COURSE OF HIPPOCAMPAL CANNABINOID CB_1 RECEPTOR REDISTRIBUTION IN THE RAT PILOCARPINE MODEL OF ACQUIRED EPILEPSY

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Approximately 3% of people will be diagnosed with epilepsy at some time during their life. Cannabinoids have been shown to have cannabinoid receptor type 1 (CB_1R) dependent anticonvulsant effects in several animal seizure models (Wallace et al., **2001**, *Eur J Pharmacol* 428, 51-57; Wallace et al., **2002**, *Eur J Pharmacol* 452, 295-301; Marsicano et al., **2003**, *Science* 302, 84-8). In addition, the endogenous cannabinoid system has been shown to play a role in controlling seizure frequency and duration in the rat pilocarpine model of chronic epilepsy (Wallace et al., **2003**, *J Pharmacol Exp Ther* 307, 129-37). In this model, epileptic animals exhibit a long-term redistribution of CB_1R within the hippocampus (Falenski et al., **2004**, *ICRS* 167). In particular, immunohistochemical analyses have demonstrated selective increases in CB_1R immunoreactivity (IR) in the stratum oriens and radiatum of CA1-CA3, with concomitant decreases in the dentate gyrus molecular layer and stratum pyramidale of CA1-2. However, these studies were conducted on animals that were epileptic for at least 6 months; what is not known are the temporal changes in CB_1R expression that occur during the development of epilepsy. To address this question, our laboratory conducted a time-course study to evaluate changes in CB_1R expression following pilocarpine-induced status epilepticus (SE).

After pilocarpine-induced SE, rats were sacrificed and brains were fixed at a number of different time points including 1 hour, 4 days, 7 days, 14 days, 1 month, and 4 months. Immunohistochemistry was performed on all brains with age-matched controls using a well-characterized N-terminus antibody and modifications of established techniques (Tsou et al., 1998, *Neuroscience* 83, 393-411). Immunohistochemical analysis indicates that there are several notable changes in CB_1R-IR at both the 4 and 7-day time points, including a decrease in overall CB_1R expression throughout the hippocampus, particularly in the hilus, with a qualitative increase in the staining of interneurons. However, at 1 month, when pilocarpine-treated animals begin to display epileptic seizures, CB_1R expression shows the characteristic redistribution that is observed in the chronic epileptic state. These results indicate that the CB_1R and endocannabinoid system may play an important role during epileptogenesis. This study also suggests that this characteristic CB_1R redistribution temporally correlates with the emergence of spontaneous epileptic seizures.

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A ROLE FOR CANNABINOID RECEPTORS IN MODULATING POST-ISCHEMIC HIPPOCAMPAL FUNCTION

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Endocannabinoids are released during cerebral ischemia and have been implicated as neuroprotective agents. We assessed the role of cannabinoid receptors in modulating the response of neurons to *in vitro* ischemia using rat hippocampal slices. Standard extracellular recording techniques (at 32^{o}C) were used to monitor excitatory synaptic transmission. Under control conditions, 15 minutes of ischemia (removal of both oxygen and glucose) markedly depressed the field excitatory postsynaptic potential (fEPSP) slope (8% of the pre-ischemic baseline, n = 24), leading to a partial recovery at 30 minutes post-ischemia (32%; n = 24). This sustained depression of function was primarily NMDA receptor dependent as MK-801 (50 μM) significantly improved recovery of synaptic transmission (61%; n = 8). The CB_1 receptor antagonist AM251 (1 μM) did not affect baseline synaptic transmission but markedly improved recovery of function following the ischemic episode (49%; n=18) and this effect was independent of $GABA_A$ receptors (46%; n=10). Conversely, treatment of slices with the CB receptor agonist WIN55,212-2 (1 μM) resulted in a delayed depression of the fEPSP (to ~50% control) and this effect was inhibited in the presence of AM251 (1 μM). These results are consistent with the idea that endogenous cannabinoids released during an ischemic episode could act to modulate excitatory synaptic transmission.

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REDISTRIBUTION OF HIPPOCAMPAL CB_1 RECEPTOR EXPRESSION AT GLUTAMATERGIC AND GABAERGIC TERMINALS IN EPILEPTIC BRAIN

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An imbalance between excitatory and inhibitory synaptic transmission is believed to be a primary mechanism underlying seizure discharge. Recent studies from our laboratory and others have suggested a role for the endocannabinoid system in regulating neuronal hyperexcitability associated with seizures and have shown that cannabinoids can exert anticonvulsant effects via a cannabinoid receptor type 1 (CB_1R)-dependent pathway (Wallace et al., **2001**, *Eur J Pharmacol* 428, 51-57; Marsicano et al., **2003**, *Science* 302, 84-8). Furthermore, utilizing the rat pilocarpine model of acquired epilepsy, we have shown that the endocannabinoid system is involved in controlling the frequency and duration of epileptic seizures through a CB_1R-dependent mechanism, and that a permanent redistribution of hippocampal CB_1R protein expression occurs with the epileptic phenotype (Falenski et al., **2004**, *ICRS* 167; Wallace et al., **2003**, *JPET*, 307, 129-37). This study was carried out to determine if the reorganization of the CB_1R in the epileptic hippocampus acts to shift the role of the endocannabinoid system towards regulating excitatory and inhibitory synaptic transmission. To address this question, we utilized the rat pilocarpine model of acquired epilepsy and carried out immunohistochemical co-localization analysis of CB_1R with markers for excitatory (glutamatergic) and inhibitory (GABAergic) synapses on control and epileptic rat brain sections.

Four to six months following pilocarpine-induced epilepsy, rats were put under deep anesthesia and transcardially perfused with 4% paraformaldehyde and brains were then processed for cryosectioning. Immunofluorescent co-localization analysis was carried out on control and epileptic brain sections with a specific antibody to CB_1R in combination with antibodies to vesicular glutamate transporter 1 (VGLUT1) or vesicular GABA transporter (VGAT) to evaluate excitatory glutamatergic or inhibitory GABAergic synaptic terminals respectively. When compared to control, epileptic hippocampus showed an increase in CB_1R immunostaining in the stratum oriens and radiatum of the VGLUT positive CA1-CA3 molecular regions. Conversely, CB_1R immunostaining decreased throughout the VGAT positive CA1-CA2 stratum pyramidale. These findings suggest that CB_1R-dependent endocannabinoid regulation of synaptic transmission is increased at glutamatergic and decreased at GABAergic terminals in the epileptic hippocampus. This redistribution of CB_1R would act to suppress excitatory and increase inhibitory synaptic transmission in the epileptic phenotype and may underlie the anticonvulsant properties of cannabinoids in this model.

PHARMACOKINETIC INTERACTIONS OF $\triangle^9$-TETRAHYDROCANNABINOL AND CANNABIDIOL CO-ADMINISTRATION IN GUINEA PIGS

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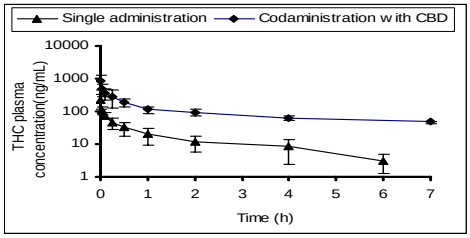
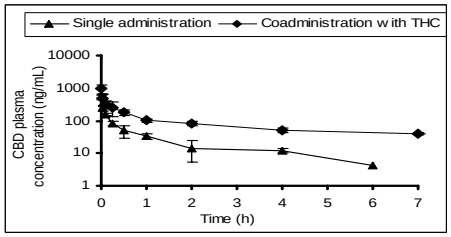
$\triangle^9$-Tetrahydrocannabinol (THC) is used in the treatment of chemotherapy-induced nausea and vomiting in cancer patients and in AIDS cachexia. Cannabidiol (CBD) is a nonpsychoactive cannabinoid and may be a promising treatment for multiple conditions, including multiple sclerosis, rheumatoid arthritis, psychosis, dystonic movement disorders, and epilepsy. Reports show that combinations of cannabinoids may provide benefits that surpass treatment with single cannabinoids. Most of the cannabinoid combination therapy data points to the promise of coadministration of THC and CBD (Karniol et al., Eur. J. Pharmacol. 28 (**1974**) 172-177; Zuardi et al., Psychopharmacology (Berl). 76 (**1982**) 245-250; Bornheim et al., Drug Metab. Dispos 23 (**1995**) 825-831). CBD has been reported to decrease some of the side-effects of THC (Dalton et al., Clin. Pharmacol. Ther., 19 (**1976**) 300-309). However, not much work is published about the pharmacokinetics of these drugs after coadministration. Hence, the purpose of this study was to determine whether a pharmacokinetic interaction occurs between THC and CBD after intravenous administration in guinea pigs.

THC (1 mg/kg) and CBD (1 mg/kg) were administered alone and in combination via the jugular vein in guinea pigs. Blood samples were collected frequently, and plasma samples were assayed for THC and CBD by liquid chromatography-mass spectrometry (LC-MS). A separate study was carried out to determine the pretreatment (30 minutes prior) effects of CBD and THC on the pharmacokinetics of THC and CBD, respectively.





Coadministration of CBD significantly (p<0.05) altered the area under the curve (AUC), maximum concentration (C_{max}), systemic clearance, volume of distribution, and elimination half life of THC compared to THC alone. The THC AUC and C_{max} increased 9-10 fold, and the clearance and volume of distribution decreased by 10 and 4 fold, respectively. CBD pharmacokinetics were also significantly (p<0.05) altered with the coadministration of THC compared to CBD alone, although the parameters were changed to a lesser extent than those for THC. Elimination half lives and mean residence times (MRT) were prolonged by 1.6-2 fold for both drugs. Pretreatment with CBD or THC did not alter the pharmacokinetic parameters of THC and CBD, respectively, as extensively when compared to coadministration, but the parameters were significantly different when compared to single drug administration. In conclusion, a significant pharmacokinetic interaction exists between THC and CBD when administered together in guinea pigs. This potential interaction should be investigated in humans in order to further understand combination cannabinoid therapy.

Acknowledgements: This work was supported by the American Cancer Society (RSG-00-027-04-CDD). THC and CBD were gifts from the NIDA Drug Supply.

THE BIPHASIC EFFECTS OF CANNABINOIDS ON THE GROWTH AND INVASIVENESS OF MULTIPLE HUMAN CANCER CELL LINES

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Cannabinoid compounds can inhibit cell growth and produce apoptosis in multiple types of cancer cell lines, and can reduce the growth of tumors (Guzman **2003**). However, there are also studies demonstrating that certain cannabinoid agonists can stimulate human cancer growth *in vitro* (Velasco et al., **2001**; Sanchez et al., **2003**). These drug effects are concentration dependent: lower concentrations stimulate cell growth whereas higher concentrations inhibit cell proliferation (Hart et al., **2004**). Human cancers are highly heterogeneous and vary in their response to therapeutic treatments. We wanted to determine how this heterogeneity would be reflected in the biphasic response that is produced by cannabinoid agonists. We also wanted to determine whether certain classes of cannabinoid agonists lack biphasic activity and are entirely antiproliferative in their activity. The effects of multiple cannabinoid agonists were compared on cancer cell growth and invasiveness using human glioma and breast cancer cell lines.

Human glioma and breast cancer cell lines were treated for three or seven days with multiple concentrations of Δ^9-THC, WIN55,212-2 (WIN), or cannabidiol (CBD). At the end of the treatment periods, cell proliferation was measured using the MTT assay and corresponding IC_{50} values were calculated. The Boyden chamber invasion assay was used to determine the effect on cannabinoids on the invasiveness of an aggressive human breast cancer cell line.

The cannabinoids compounds killed 100% of the cancer cell lines by days three and seven but with varying potencies. During drug treatments, the IC_{50} values ranged from (0.9 - 2.9 μM). 100 nM of Δ^9-THC produced a small stimulation of cell growth with the glioma cell lines SF126, U373-MG, and U251. This stimulation was not observed with SF126 cells during the three day treatment. However, Δ^9-THC did cause a small stimulation of cell growth in two human breast cancer cell lines (MDA-MB231 and MDA-MB436) when nanomolar concentrations were applied for three days. 100 nM Δ^9-THC also increased the invasiveness of MDA-MB231 cells, but higher concentrations inhibited the invasiveness of these cells. WIN did not demonstrate mitogenic activity in any of the glioma cell lines tested. In contrast, in MDA-MB231 and MDA-MB436 cells 1μM WIN consistently caused a small increase in cell proliferation. Interestingly, 1μM WIN inhibited the invasiveness of MDA-MB231 cells. CBD inhibited cell proliferation in both glioma and breast cancer cell lines and also inhibited the invasiveness of MDA-MB231 cells. CBD did not demonstrate mitogenic activity in any human caner cell lines tested.

Cannabinoid compounds are effective at inhibiting multiple types of human cancer cell lines. The biphasic response of cannabinoids on cell proliferation is dependent on the drug treatment period, the class of cannabinoid compound used, and the cell line studied. The biphasic effect of WIN on breast cancer cell proliferation is not observed on cell invasiveness. CBD is an effective anticancer agent *in vitro* and lacks any mitogenic activity.

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DIFFERENT SENSITIVITY BETWEEN HUMAN GLIOMA CELLS AND PRIMARY GLIA CULTURE TO THE CELLULAR EFFECTS INDUCED BY CANNABIDIOL

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Recently, we reported the ability of the non-psychotropic cannabinoid compound cannabidiol (CBD) in inducing anti proliferative and apoptotic effects on U87 and U373 glioma cells (Massi et al., **2004**) with a non CB_1 and CB_2 receptor -based mechanism. Moreover, in a recent study we also demonstrated that CBD impairs the ability of U87 tumoral cell to migrate under stimulation with conditioned medium (Vaccani et al., **2005**).

Based on this experimental evidence of promising antitumoral effects of CBD, we were interested in evaluating if CBD could alter the viability of primary glia cells in comparison with its effects on tumoral cells.

First, we checked the cytotoxic effects of the cannabinoid compound on primary glia culture in a range from 20 μM to 50 μM. CBD, as expected, in this range exerted a significant reduction of cell viability in tumoral cells whereas in primary glia cells we did not observe any significant decrease in the number of cells.

Since we hypothesised that oxidative stress could be a mechanism by which CBD induces apoptosis in glioma cells , we evaluated using a cytofluorimetric analysis the generation of radical oxygen species (ROS), through a fluorimetric probe named 2',7'-dichlorofluorescein diacetate (DCFH-DA). An in vitro exposure to an active concentration of CBD (25μM) on U87 cells induced a significant 6-fold increase of ROS after 6 h by CBD exposure. Under the same experimental conditions we did not observe any increase in ROS production after CBD treatment in primary glia culture.

Taken together, the present data indicate that oxidative stress may be an important mechanism by which cannabidiol exerts its apoptotic effect on human glioma cells. On the contrary, CBD does not induce neither cytotoxic effect nor ROS production on primary glia culture, indicating that CBD can differently affect tumoral and primary cells.

Concluding, whatever the precise mechanism underlying CBD effects, the present data strengthen the hypothesis of a possible application of CBD as antineoplastic agent not affecting normal tissue.

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TOPOISOMERASE II: SPECIFIC INHIBITION BY A CANNABINOID

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Introduction: Anthracyclines, a large group of quinonoid compounds produced by different strains of streptomyces, exert antibiotic and antineoplasic effects. They are used to treat some forms of cancer. Though highly effective in cancer therapy, these compounds are not selective, acting on cancer and other cells by numerous mechanisms and thus also causing side effects, which limit their use. The development of quinonoid compounds that display antineoplastic and/or anti-angiogenic activity, but are more selective and less toxic is a major therapeutic goal. A new anticancer quinone, HU331, was synthesized from cannabidiol. It shows very high effectivity against human cancer cell lines *in-vitro* and also against *in-vivo* tumor grafts in *nude* mice . The most striking inhibition by HU331 was found in tests with the Raji, Jurkat and HT-29 cells. In *in-vivo* assays the tumors in the HU331 treated groups were half the size of the tumors in the controls, a difference that was highly significant. We now present evidence on its unique mechanism.

Methods: For assaying the mechanisms of HU331-mediated cancer cell death several standard methods were used, such as annexin V binding (for assaying apoptotic cell death), PI DNA staining (for cell cycle analysis), intracellular staining with anti-caspase-3 (for assaying caspases involvement), MTT (proliferation) test with HU331 in presence of CB_1/CB_2 antagonists or ROS scavengers (for assaying free radicals and cannabinoid receptors involvement). The ability of HU331 to inhibit topoisomerases was assayed on puc19 plasmid relaxation. The enantiomer of HU331 was synthesized and tasted alongside HU331 itself.

Results: While doxorubicin and other anthraquinones act by numerous mechanisms, such as apoptosis, blockage of the cell cycle, caspases involvement, ROS activation, inhibition of both topoisomerase I and II, intracellular second messengers activation etc., HU331 is highly selective. It does not cause cell cycle arrest, cell apoptosis and caspase activation. HU331-caused cell death is partially mediated by ROS, as some antioxidants partially prevent it. (+)HU331's potency in killing Jurkat cells was not lower than that of (-)HU331, which means that HU331 does not act through binding to a receptor, but probably enters the cell and acts through its quinone moiety. HU331 has limited influence on topoisomerase I action, but is able to inhibit topoisomerase II even in nanomolar concentrations. In comparative assays HU331 inhibited Jurkat cells growth more than some known anticancer drugs (doxorubicin, mitoxantrone and etoposide)

Conclusion: The cannabinoid quinone HU331, which is more selective and more potent than most known anticancer quinones and also possesses high anti-angiogenic activity, specifically inhibits topoisomerse II and thus has a high potential as a new anticancer drug.

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