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Newsletter for the Neuroscientist

### **1998 Nobel Prize in Physiology or Medicine awarded to RBI collaborator for work on Nitric Oxide**

RBI congratulates the 1998 Nobel Laureates in Physiology or Medicine: Drs. Robert F. Furchgott, Louis J. Ignarro, and long time RBI collaborator, Ferid Murad. Their individual contributions in identifying and elucidating the role of nitric oxide (NO) as a signaling molecule have stimulated a wealth of basic and applied research.

At RBI, we are proud of our long association with Dr. Murad. In 1994, he authored a *Neurotransmissions* article entitled 'The Role of Nitric Oxide in Modulating Guanylyl Cyclase'. Most recently, he served as a contributor to the 3rd Edition of *The RBI Handbook of Receptor Classification and Signal Transduction*, providing a chart on 'Nitric Oxide Synthases'. Dr. Murad is currently with the University of Texas Medical School, Department of Integrative Biology, Pharmacology and Physiology in Houston. In 1977, he discovered that nitroglycerin and related vasodilators relax smooth muscle cells by releasing NO.

While the Nobel Committee cited the role of NO in the cardiovascular system, the effects of NO in neural tissue are also significant. The May, 1998 *Neurotransmissions* article, 'Nitric Oxide in CNS Physiology and Pathology', provides an excellent review of recent developments in this dynamic field.

RBI is the leading supplier of NO research. Our product line includes inhibitors of nitric oxide synthase (NOS), precursors and cofactors involved in NO biosynthesis research tools, as well as NO donors and scavengers which modulate soluble guanylyl cyclase activity. Additionally, RBI offers polyclonal and monoclonal antibodies to NOS subtypes.

Again, RBI salutes this year's Nobel Laureates in Physiology or Medicine.

For a free reprint of the May 1998 *Neurotransmissions* Article on Nitric Oxide, please contact RBI Technical Service at Tel: 508-651-8151, US Toll Free: 800-736-3690, or e-mail: [tech@resbio.com](mailto:tech@resbio.com)

For more information on RBI's Nitric Oxide products, please return the reply card in this issue, contact Technical Service or visit our website at [www.callrbi.com](http://www.callrbi.com).

For more information on these Nobel Laureates, search the internet at [www.nobel.se/announcement-98/medicine98.html](http://www.nobel.se/announcement-98/medicine98.html).

# Endogenous and Synthetic Cannabinoids and their Receptors

Michelle Glass and Christian C. Felder

**T**he term cannabinoids refers to a group of compounds found in the plant *Cannabis sativa* of which  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC) (RBI Cat. No. T2386) is the principal psychoactive component. *Cannabis* (marijuana) has been used medicinally for over 4,000 years for the treatment of a variety of disorders, including migraine, muscle spasms, seizures, glaucoma, pain and nausea. However, until recently, relatively little was known about the molecular mechanism of action of  $\Delta^9$ -THC in brain and peripheral tissues. In the late 1980's, a pharmacological binding site in brain tissue was discovered, which led in 1990 to the cloning and sequencing of the first cannabinoid receptor, referred to as the CB<sub>1</sub> receptor. Subsequently, a second cannabinoid receptor, referred to as CB<sub>2</sub>, was cloned and sequenced, and shown to have a similar pharmacological profile to the CB<sub>1</sub> receptor. However, the CB<sub>2</sub> receptor appears to be present primarily in the peripheral immune system, and is largely absent from brain.

The discovery of cannabinoid receptors led to the identification of endogenous lipid compounds from brain and peripheral tissues that bind selectively to cannabinoid receptors. Currently, much research effort is being focused on the biosynthesis and metabolism of the so-called endocannabinoids and their role in normal physiological processes. Whether the endocannabinoids represent the only endogenous agonists for the cannabinoid receptors is still subject to debate. However, recent progress in

cannabinoid research has revealed important information about the mechanisms of action of cannabinoids at the cellular level which should broaden our understanding of the normal and pathophysiological role of cannabinoid receptors and lead to the rational development of cannabinoid therapeutic agents.

## Cannabinoid Receptors

Changes in adenylyl cyclase activity provided the first evidence that cannabinoids mediate their effects through an interaction with specific receptor proteins [1]. However, it was the synthesis of novel, high affinity cannabinoid receptor ligands, such as CP 55,940, which are less lipophilic and more potent than  $\Delta^9$ -THC, that greatly facilitated the discovery of specific cannabinoid binding sites [2]. Using [<sup>3</sup>H]-CP 55,940, high levels of cannabinoid binding sites were found to be widely distributed in brain sections from several mammalian species [3]. The CB<sub>1</sub> receptor was subsequently cloned from rat [4], and later from human [5] and mouse [6], and found to belong to the family of G protein-coupled receptors. Later, the CB<sub>2</sub> cannabinoid receptor was cloned from human [7] and mouse tissues [8] and shown to exhibit low amino acid sequence homology with the CB<sub>1</sub> receptor (44% overall, with 68% in the transmembrane spanning regions). Mice bearing genetic deletion of both CB<sub>1</sub> and CB<sub>2</sub> receptors have recently been bred and may provide information about additional members of this receptor family [9,10].

## About the Authors

Michelle Glass received her Ph.D. in Neuropharmacology from the University of Auckland, New Zealand and then undertook post-doctoral research with Christian Felder at the National Institute of Mental Health. She is currently working in the Laboratory of Cell Biology at the National Institute on Deafness and Other Communication Disorders in Rockville, Maryland, USA.

Christian Felder received his Ph.D. in Biochemistry from Georgetown University in Washington D.C. He joined Julius Axelrod's lab at the National Institute of Mental Health as a Staff Fellow in 1987, became a Senior Staff Fellow in 1990, and Chief of the Unit on Cellular and Molecular Signaling in 1993. He recently joined Eli Lilly and Company where he is a Research Scientist in the Neuroscience Division of Lilly Research Laboratories in Indianapolis, Indiana, USA.

## Endogenous and Synthetic Cannabinoids (cont.)

The initial temptation to classify CB<sub>1</sub> receptors as neuronal, and CB<sub>2</sub> receptors as peripheral, has proven to be overly simplistic. The distribution of CB<sub>1</sub> receptors has been very well characterized in rat [11] and human brain [12,13], and their localization correlates well with the known effects of cannabinoids on memory, perception and the control of movement. CB<sub>1</sub> receptors are highly expressed in the hippocampus, association cortex, cerebellum and basal ganglia. In comparison, these receptors are either sparsely distributed or absent from brain stem, medulla and thalamus, which might explain the general lack of life threatening effects associated with marijuana abuse. The CB<sub>1</sub> receptor has also recently been identified in several peripheral tissues, including testis, small intestine, urinary bladder, vas deferens, cerebral vascular smooth muscle cells, in addition to being localized pre-synaptically on sympathetic nerve terminals [14]. CB<sub>1</sub> receptor mRNA has been observed in the adrenal gland, heart, lung, prostate, bone marrow, thymus and tonsils [15,16], although an absence of CB<sub>1</sub> mRNA has been reported in some of these peripheral regions [17]. In contrast, CB<sub>2</sub> receptors are found in the marginal zone of the spleen, tonsils, immune cells (B-cells, monocytes, T-cells, etc.) and possibly in primary cultures of rat microglia [14,18].

### Signal Transduction Mechanisms

Despite similarities in their ligand recognition, CB<sub>1</sub> and CB<sub>2</sub> receptors vary considerably in their coupling to signal transduction mechanisms. While both receptors inhibit adenylyl cyclase via pertussis toxin-sensitive G-proteins

(G<sub>i/o</sub> family), CB<sub>1</sub> receptors have also been recently shown to stimulate cyclic AMP formation under certain conditions [19]. Furthermore, activation of CB<sub>1</sub> receptors, unlike that of CB<sub>2</sub> receptors, has been shown to block N- and P/Q-type calcium channels [20,21] and to activate inwardly rectifying potassium channels [20]. Blockade of N-type calcium channels may be the mechanism by which cannabinoids inhibit acetylcholine release in hippocampus [22,23], norepinephrine release at sympathetic nerve terminals [24] and centrally in the hippocampus, cortex and cerebellum [25], and glutamate release in cultured hippocampal neurons [26].

Many of the intracellular effects of cannabinoids can be explained by their ability to activate G<sub>i/o</sub> proteins, thereby inhibiting cyclic AMP accumulation. For example, attenuation of inducible nitric oxide synthase gene expression, and nitric oxide production by cannabinoids, occurs, at least in part, through the inhibition of cyclic AMP signaling. This, in turn, may lead to cannabinoid receptor-mediated inhibition of immune function, a process believed to be mediated via CB<sub>2</sub> receptors [27,28]. The inhibition of calcium currents observed following activation of CB<sub>1</sub> receptors is pertussis toxin-sensitive, but independent of cyclic AMP inhibition, thereby suggestive of a direct G protein mechanism; perhaps through interaction with  $\beta\gamma$  subunits. Stimulation of potassium channels is also pertussis toxin-sensitive and thought to be mediated by inhibition of cyclic AMP accumulation [29]. Again, other mechanisms, such as direct modulation by G protein  $\beta\gamma$  subunits, may be involved.

Cannabinoid receptor agonists have also been shown to stimulate mitogen-activated protein (MAP) kinases via a G protein, but not via a cyclic AMP dependent mechanism [30]. It is possible that MAP kinase activation is an intermediate step in the cannabinoid receptor-mediated induction of the transcription factor Krox 24 [31,32], increased AP-1 DNA-binding activity and Fos-related antigen activity [33].

### **Cannabinoid Receptor Pharmacology**

In an effort to separate the psychoactive and medicinal properties of cannabinoids, the pharmacology of cannabinoid receptors has been extensively studied. Several non-selective cannabinoid receptor agonists have been synthesized (Figure 1), including analogs of the tetrahydropyran structure of  $\Delta^9$ -THC [34], such as the highly potent agonist (-)-11-OH- $\Delta^8$ -THC-dimethylheptyl (HU 210) [35] and nabilone [36]. Structurally diverse agonists are represented by several non-classical cannabinoids, such as CP 55,940, WIN 55,212-2 (RBI Cat. No. **W-102**) [37] and levonantradol [38]. Recently, several promising compounds have been developed which show some CB<sub>2</sub> receptor receptor selectivity; these include JWH 015 [39], 1-deoxy HU 210 [40] and the indole analog indomethacin morpholinylamine (RBI Cat. No. **I-151**) [41].

Several cannabinoid receptor antagonists have also been described in the literature (Figure 1). The first CB<sub>1</sub> receptor antagonist to be synthesized, SR141716A, is based on the aminoalkylindole structure [42]. Two other potent CB<sub>1</sub> antagonists have also been produced; AM 630, a pyrazole type structure [43], and LY 320,135, a substituted benzofuran [44].

Recent studies have demonstrated that SR 141716A and AM 630 possess inverse agonist properties at the CB<sub>1</sub> receptor [45-47], suggesting a high level of spontaneous activity of this receptor. Finally, SR 144528 has recently been described as the first, highly potent and selective CB<sub>2</sub> cannabinoid receptor antagonist [48].

### **Endogenous Cannabinoids**

The discovery of the CB<sub>1</sub> receptor prompted the search for an endogenous cannabinoid receptor agonist. Reasoning that an endogenous agonist may have similar hydrophobic properties as synthetic or exogenous cannabinoid receptor agonists, Devane, Mechoulam, and co-workers focused their search on organic solvent extracts of porcine brain. Their hypothesis proved correct when they discovered a lipid molecule, arachidonyl-ethanolamide, that displaced specific binding of a radiolabeled cannabinoid agonist in rat brain membranes and functionally inhibited an electrically-induced twitch response in mouse vas deferens [49]. The compound was termed 'anandamide' based on 'ananda' the Indian Sanskrit word for bliss, and its amide-containing chemical structure. The structure was shown to be arachidonic acid coupled to ethanolamine through an amide linkage (Figure 1). Anandamide (RBI Cat. No. **A0580**) levels were first measured in porcine whole brain [49], and subsequently in sheep and cow whole brain [50], rat testis [51] and the brain and periphery of rat and humans [52]. The demonstration of anandamide in spleen suggested that it might be an endogenous agonist for CB<sub>2</sub> cannabinoid receptors, although levels were below those measured in brain [52].

## Endogenous and Synthetic Cannabinoids (cont.)

When evaluated at the biochemical level, anandamide demonstrated almost identical effects to those observed in response to classical cannabinoid agonists. These effects included displacement of cannabinoid agonist binding from CB<sub>1</sub> and CB<sub>2</sub> receptors, inhibition of adenylyl cyclase activation, inhibition of N-type calcium currents via CB<sub>1</sub> receptor activation and stimulation of cannabinoid receptor-independent mobilization of arachidonic acid and calcium [53]. Behavioral effects seen with Δ<sup>9</sup>-THC, such as hypothermia, analgesia, hypomobility and catalepsy, were also mimicked by anandamide [54,55]. However, relatively high concentrations of anandamide were required to exert these behavioral effects, probably due to rapid metabolism. The development of metabolism-resistant forms of anandamide [56], in particular R(+)-methandamide (RBI Cat. No. **M-186**) (Figure 1) has permitted higher potencies to be demonstrated [57,58].

The relatively low affinity of anandamide for CB<sub>1</sub> cannabinoid receptors suggested the possibility that other, more potent endogenous agonists might exist for the CB<sub>1</sub> receptor in brain, or conversely, that higher affinity binding sites may exist, in addition to the CB<sub>1</sub> receptor. Thus, considerable effort has been focused on the possible existence of other endogenous cannabinoid receptor agonists. One such putative endocannabinoid, 2-arachidonylglycerol (2-AG) (RBI Cat. No. **A-261**, see figure 1), was first isolated from canine gut [59] and later identified in mouse neuroblastoma cells [60,61] and rat brain [62]. Like anandamide, 2-AG has a relatively low affinity for CB<sub>1</sub> and CB<sub>2</sub> receptors and its distribution has yet to be well-characterized [63]. Recently, 2-AG was shown to be released from brain neurons follow-

ing stimulation with the calcium ionophore ionomycin, possibly through activation of phosphatidylinositol-specific phospholipase C [64]. In these neurons, 2-AG inhibited forskolin-stimulated cyclic AMP accumulation through an interaction with CB<sub>1</sub> receptors, but displayed a relatively low affinity. However, in brain samples, levels of 2-AG were found to be approximately 200 times higher than anandamide [64].

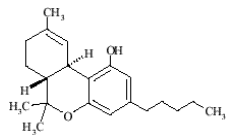
A variety of fatty acid ethanolamides, possessing carbon chain lengths of 14-22, have also been identified in rat testes, and brain tissue from sheep, cow, rat and pigs. [50,51,65]. However, none of the fatty acid ethanolamides, except for anandamide, display any significant affinity for the cannabinoid receptors. It remains to be determined if these molecules have their own biological activity and binding sites. Very recently, evidence for a peripheral palmitoyl-ethanolamide receptor, similar to the CB<sub>2</sub> receptor, was presented [66] increasing the likelihood that fatty acid ethanolamides represent a family of transmitters, each with a distinct receptor.

### Synthesis and Metabolism of Endogenous Cannabinoids

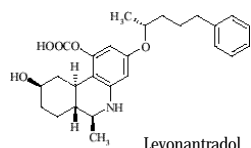
It has been hypothesized that anandamide might reside in the membrane in the form of a phospholipid precursor, which is released following activation of an appropriate phospholipase or related enzyme. The most accepted pathway is based on the early studies of Schmidt *et al.* [67] and has been demonstrated in rat testes and brain [68]. This pathway involves the phospholipase D-catalysed hydrolysis of a transient phospholipid precursor (Figure 2). The formation of N-arachidonylphosphatidylethanolamide occurs through an acyl transferase

**FIGURE 1. Structures of Cannabinoid Receptor Agonists and Antagonists**

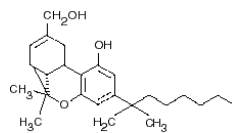
**Non-selective agonists**



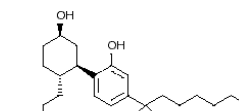
$\Delta^9$ -Tetrahydrocannabinol  
(RBI Cat. No. 2386)



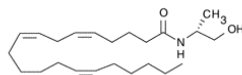
Levonantradol



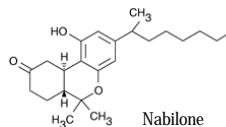
HU 210



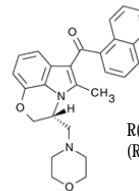
CP 55,940



R(+)-Methanandamide  
(RBI Cat. No. M-186)

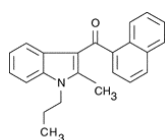


Nabilone



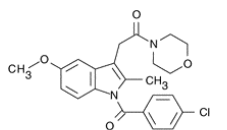
R(+)-WIN 55,212-2 Mesylate  
(RBI Cat. No. W-102)

**CB<sub>2</sub>-selective agonist**



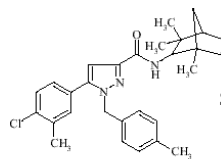
JWH-015

**CB<sub>2</sub>-preferring ligand**



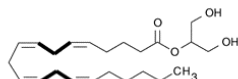
Indomethacin morpholinylamide  
(RBI Cat. No. I-151)

**CB<sub>2</sub>-selective antagonist**

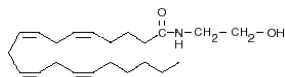


SR 144528

**Endocannabinoids**

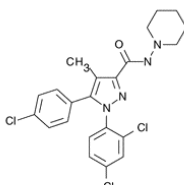


2-Arachidonylglycerol  
(RBI Cat. No. A-261)

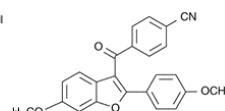


Anandamide  
(RBI Cat. No. A0850)

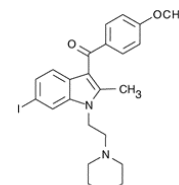
**CB<sub>1</sub>-selective antagonists**



SR 141716A



LY-320,135



AM 630

in response to increased calcium concentrations. The acyl transferase moves arachidonic acid from the first or possibly second position of a donor phospholipid to form an amide bond at the ethanolamine head group in the third position of phosphatidylethanolamine. Release of anandamide would then occur following activation of phospholipase D. An earlier suggestion that concurrent stimulation of phospholipase A<sub>2</sub> and D would release both arachidonic acid and ethanolamine which could then be combined by a 'synthase' enzyme to produce anandamide

[69,70] has been demonstrated to be physiologically unlikely [71].

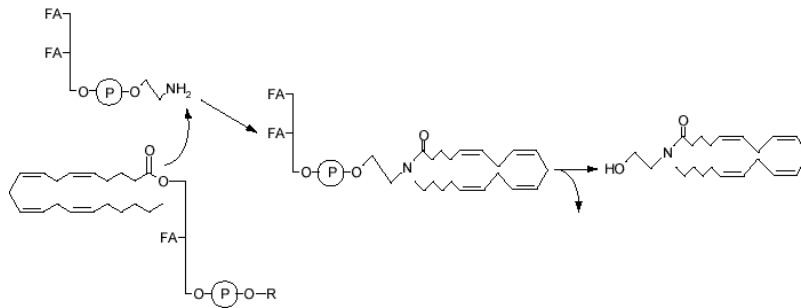
As a putative neurotransmitter, the removal of anandamide may occur through either uptake or metabolism. Enzymatic degradation of anandamide has been observed by an aminohydrolase that also degrades a sleep-inducing oleamide. This enzyme has been isolated and cloned from rat liver [72] and has been called "fatty acid amide hydrolase" (FAAH). Recombinant FAAH has been found to efficiently catalyze the hydrolysis of 2-AG

## Endogenous and Synthetic Cannabinoids (cont.)

and, recently, inhibitors of FAAH have been developed [73-75]. The relative lack of selectivity of FAAH may have important physiological implications for its regulation by natural fatty acid amides and esters that do not bind to cannabinoid receptors, but are recognized by the enzyme. The co-release of other substrates, along with anandamide and 2-AG, may compete for the same hydrolytic enzyme thereby increasing the levels of the endocannabinoids and potentiating their actions, as recently shown for oleamide [76].

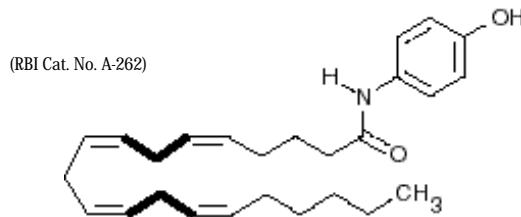
FAAH activity has been demonstrated in the cytoplasm of neurons and therefore diffusion or uptake of anandamide into the cytoplasm is a prerequisite for degradation. Selective and saturable anandamide uptake by a sodium- and energy-independent mechanism has been observed in cortical neurons in primary culture [77]; however, little progress has been made on identification of this carrier protein. An inhibitor of anandamide uptake, AM 404 (Figure 3), has recently been developed and should aid research into this process [78].

**FIGURE 2. Anandamide Synthesis Pathway**



**Proposed pathway for the biosynthesis of anandamide. Increase in intracellular calcium trigger the transfer of arachidonic acid from the *sn*-1 position of an arachidonic acid containing phospholipid to the amine group of phosphatidylethanolamine to form N-arachidonoyl-phosphatidylethanolamine. Hydrolysis by phospholipase D (PLD) releases arachidonylethanolamine (anandamide) and phosphatidic acid.**

**FIGURE 3. AM 404 Anandamide Transport Inhibitor**



### Summary

Cannabinoid research has seen considerable progress in the last ten years following discovery of two distinct cannabinoid receptors, referred to as CB<sub>1</sub> and CB<sub>2</sub>, and two endogenous lipid cannabinoid receptor agonists, anandamide and 2-AG. Significant progress has also been made towards understanding the biosynthesis and metabolism of anandamide. Novel compounds are now becoming available, such as receptor selective agonists and antagonists, and inhibitors of anandamide uptake and breakdown. It is anticipated that the availability of these tools will lead to a greater understanding of the physiological role of endocannabinoids, which will play a key role in understanding the physiology of the anandamide system and in the development of cannabinoid-based therapeutics.

### References

1. Howlett, A.C., Fleming, R.M., *Mol. Pharmacol.* **26**, 532-538 (1984).
2. Johnson, M.R., Melvin, L.S., "The discovery of non-classical cannabinoid analgesics." In: *Cannabinoids as Therapeutic Agents* (Ed. Mechoulam, C.R.) pp. 121-145; CRC Press, Boca Raton (1986).
3. Herkenham, M., Lynn, A.B., Little, M.D., et al., *Proc. Natl. Acad. Sci., USA* **87**, 1932-1936 (1990).
4. Matsuda, L.A., Lolait, S.J., Brownstein, M.J., et al., *Nature* **346**, 561-564 (1990).
5. Gerard, C.M., Mollereau, C., Vassart, G., et al., *Biochem. J.* **279**, 129-134 (1991).
6. Chakrabarti, A., Onaivi, E.S., Chaudhuri, G., *DNA Sequence* **5**, 385-388 (1995).
7. Munro, S., Thomas, K.L., Abu-Shaar, M., *Nature* **365**, 61-65 (1993).
8. Shire, D., Calandra, B., Rinaldi-Carmona, M., et al., *Biochem. Biophys. Acta* **1307**, 132-136 (1996).
9. Buckley, N., Bonner, T., Zimmer, Z., et al., *National Institute of Health, Bethesda, Maryland, USA. Personal Communication*.
10. Buckley, N.E., Mezey, E., Bonner, T., et al., *Symposium on the Cannabinoids, International Cannabinoid Research Society, Vol.1*, p.57, Burlington, Vermont, USA (1997).
11. Herkenham, M., Lynn, A.B., Johnson, M.R., et al., *J. Neurosci.* **11**, 563-583 (1991).

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12. Glass, M., Faull, R.L.M., Dragunow, M., *Neuroscience* **77**, 299-318 (1997).
13. Westlake, T.M., Howlett, A.C., Bonner, T.I., et al., *Neuroscience* **63**, 637-652 (1994).
14. Pertwee, R.G., *Pharmacol. Ther.* **74**, 129-180 (1997)
15. Galieue, S., Mary, S., Marchand, J., et al., *Eur. J. Biochem.* **32**, 54-61 (1995).
16. Rice, W., Shannon, J.M., Burton, F., et al., *Eur. J. Pharmacol.* **327**, 227-232 (1997).
17. Schatz, A.R.E., Lee, M., Condie, R.B., et al., *Toxicol. Appl. Pharmacol.* **142**, 278-287 (1997).
18. Kearn, C.S., Hillard, C.J., *Symposium on the Cannabinoids, International Cannabinoid Research Society, Vol.1*, p.61, Burlington, Vermont, USA (1997).
19. Glass, M., Felder, C.C., *J. Neurosci.* **17**, 5327-5333 (1997).
20. Mackie, K., Lai, Y., Westenbroek, R., et al., *J. Neurosci.* **15**, 6552-6561 (1995).

## Endogenous and Synthetic Cannabinoids (cont.)

### References (Cont.)

21. Mackie, K., Hille, B., *Proc. Natl. Acad. Sci., USA* **89**, 3825-3829 (1992).
22. Gifford, A.N., Ashby, C.R., *J. Pharmacol. Exp. Ther.* **277**, 1431-1436 (1996).
23. Gessa, G.L., Mascia, M.S., Casu, M.A., *et al.*, *Eur. J. Pharmacol.* **327**, R1-R2 (1997).
24. Ishac, E.J.N., Jiang, L., Lake, K.D., *et al.*, *Br. J. Pharmacol.* **118**, 2023-2028 (1996).
25. Schlicker, E., Timm, J., Görg, T., *et al.*, *Symposium on the Cannabinoids, International Cannabinoid Research Society, Vol.1*, p.63, Burlington, Vermont, USA (1997).
26. Shen, M.X., Piser, T.M., Seybold, V.S., *et al.*, *J. Neurosci.* **16**, 4322-4334 (1996).
27. Jeon, Y.J., Yang, K.H., Pulaski, J.T., *et al.*, *Mol. Pharmacol.* **50**, 334-341 (1996).
28. Coffey, R.G., Yamamoto, Y., Snella, E., *et al.*, *Biochem. Pharmacol.* **52**, 743-751 (1996).
29. Deadwyler, S.A., Hampson, R.E., Mu, J., *et al.*, *J. Pharmacol. Exp. Ther.* **273**, 734-743 (1995).
30. Bouaboula, M., Poinotchazel, C., Bourrie, B., *et al.*, *Biochem. J.* **312**, 637-641 (1996).
31. Bouaboula, M., Bourrie, B., Rinaldi-Carmona, M., *et al.*, *J. Biol. Chem.* **270**, 13973-13980 (1995).
32. Glass, M., Dragunow, M., *NeuroReport* **6**, 241-244 (1995).
33. Porcella, A., Gessa, G.L., Pani, L., *Eur. J. Neurosci.* **10**, 1743-1751 (1998).
34. Martin, B.R., Compton, D.R., Prescott, W.R., *et al.*, *Drug Alcohol Depend.* **37**, 231-240 (1995).
35. Mechoulam, R., Feigenbaum, J.J., Lander, N., *et al.*, *Experientia* **44**, 762-764 (1988).
36. Ward, A., Holmes, B. *Drugs* **30**, 127-144 (1985).
37. Eissenstat, M.A., Bell, M.R., D'Ambra, T.E., *et al.*, *J. Med. Chem.* **38**, 3094-3105 (1995).
38. Razdan, R.K. *Pharmacol. Rev.*, **38**, 75-149 (1986).
39. Griffin, G., Fernando, S.R., Ross, R.A., *et al.*, *Eur. J. Pharmacol.* **339**, 53-61 (1997).
40. Huffman, J.W., Yu, S., Showalter, V., *et al.*, *J. Med. Chem.* **39**, 3875-3877 (1996).
41. Gallant, M., Dufresne, C., Gareau, Y., *et al.*, *Bioorg. Med. Chem. Lett.* **6**, 2263-2268 (1996).
42. Rinaldi-Carmona, M., Barth, F., Heaulme, M., *et al.*, *FEBS Lett.* **350**, 240-244 (1994).
43. Pertwee, R., Griffin, G., Fernando, S., *et al.*, *Life Sci.* **56**, 1949-1955 (1995).
44. Felder, C.C., Joyce, K.E., Briley, E.M., *et al.*, *J. Pharmacol. Exp. Ther.* **284**, 291-297 (1998).
45. Bouaboula, M., Perrachon, S., Milligan, L., *et al.*, *J. Biol. Chem.* **272**, 22330-22339 (1997).
46. MacLennan, S.J., Reynen, P.H., Kwan, J., *et al.*, *Br. J. Pharmacol.* **124**, 619-622 (1998).
47. Landsman, R.S., Makriyannis, A., Deng, H.F., *et al.*, *Life Sci.* **62**, PL109-PL113 (1998).
48. Rinaldi-Carmona, M., Barth, F., Millan, J., *et al.*, *J. Pharmacol. Exp. Ther.* **284**, 644-650 (1998).
49. Devane, W.A., Hanus, L., Breuer, A., *et al.*, *Science* **258**, 1946-1949 (1992).
50. Schmid, P.C., Krebsbach, R.J., Perry, S.R., *et al.*, *FEBS Lett.* **375**, 117-120 (1995).
51. Sugiura, T., Kondo, S., Sukagawa, A., *et al.*, *Biochem. Biophys. Res. Commun.* **218**, 113-117 (1996).
52. Felder, C.C., Nielsen, A., Briley, E.M., *et al.*, *FEBS Lett.* **393**, 231-235 (1996).
53. Felder, C.C., Glass, M., *Annu. Rev. Pharmacol. Toxicol.* **38**, 179-200 (1988).
54. Fride, E., Mechoulam, R., *Eur. J. Pharmacol.* **231**, 313-314 (1993).
55. Crawley, J.N., Corwin, R.L., Robinson, J.K., *et al.*, *Pharmacol. Biochem. Behav.* **46**, 967-972 (1993).
56. Abadji, V., Lin, S.Y., Taha, G., *et al.*, *J. Med. Chem.* **37**, 1889-1893 (1994).
57. Burkey, R.T., Nation, J.R., *Exp. Clin. Psychopharm.* **5**, 195-202 (1997).
58. Romero, J., Garcia-Palmero, E., Lin, S.Y., *et al.*, *Life Sci.* **58**, 1249-1257 (1996).
59. Mechoulam, R., Ben-Shabat, S., Hanus, L., *et al.*, *Biochem. Pharmacol.* **50**, 83-90 (1995).

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# New Products from RBI

## ADENOSINES / PURINERGICS

- M-227 MRS 1191**  
*Selective A<sub>3</sub> adenosine receptor antagonist; selective for both human and rat. Sold under license from the National Institutes of Health.*
- M-228 MRS 1220**  
*A<sub>3</sub> Adenosine receptor antagonist; selective for human vs. rat. Sold under license from the National Institutes of Health.*

## ADRENERGICS

- C-247 Cyclazosin hydrochloride**  
*α<sub>1B</sub> Adrenergic receptor antagonist.*

## BENZODIAZEPINES

- Z-105 Zopiclone**  
*(Imovane)*  
*Benzodiazepine receptor agonist.*

## CHOLINERGICS

- R-130 RJR-2403 hemigalactarate**  
*Nicotinic acetylcholine receptor agonist which displays CNS activity.*

## ENDOTHELINS

- J-107 JKC 301**  
*(Cyclo-[D-Asp-Pro-D-Ile-Leu-D-Trp])*  
*ET<sub>A</sub> Endothelin receptor antagonist.*

## ENZYME INHIBITORS

- M-270 Milrinone**  
*Phosphodiesterase III inhibitor.*



Products introduced since the last issue of *Neurotransmissions*.

## EXCITATORY AMINO ACIDS

- A-267 (S)-AMPA zwitterion**  
*Active enantiomer of (RS)-AMPA zwitterion (Cat. No. A-136); potent agonist at the AMPA subclass of ionotropic glutamate receptors.*
- A-266 (R)-AMPA zwitterion**  
*Inactive enantiomer of (RS)-AMPA (Cat. No. A-136).*
- A-263 AIPA**  
*Selective kainate receptor agonist.*
- B-171 1-BCP**  
*Centrally acting AMPA receptor modulator; crosses the blood brain barrier.*
- C-237 L-CCG-1**  
*Potent group II metabotropic glutamate receptor agonist.*
- C-271 CX546**  
*Positive AMPA receptor modulator; more potent ampakine than 1-BCP (Cat. No. B-171).*
- F-154 Felbamate**  
*Anticonvulsant agent that acts as an antagonist at glutamate or kainate receptors and an agonist at GABA receptors.*

## DOPAMINERGICS

- P-233 PD 168,077 maleate**  
*D<sub>4</sub> dopamine receptor agonist.*




## HISTAMINERGICS

- F-134 S(+)-α-Fluoromethylhistidine hydrochloride**  
*(S(+)-α-FMH)*  
*Histidine decarboxylase inhibitor. Distributed exclusively by RBI (US Patent No. 5,030,645).*

## INHIBITORY AMINO ACIDS

- T-200 TPMPA**  
*Selective GABA<sub>C</sub> receptor antagonist.*  
*Sold in the USA under exclusive license from the University of California.*

## ION CHANNEL MODULATORS

- D-221** **L-cis-Diltiazem hydrochloride**  
 Blocks cyclic nucleotide-gated cation channels.  
(Not to be confused with cis-Diltiazem hydrochloride  
(Cat. No. D-112) which blocks L-type Ca<sup>2+</sup> channels.)
- I-158** **Ivermectin**  
(22,23-Dihydroavermectin B1)  
Positive allosteric modulator of  $\alpha 7$  neuronal nicotinic  
acetylcholine receptor; also modulates glutamate-  
GABA-activated chloride channels.
- V-118** **Valinomycin**  
(Valinomycin)  
Potassium ionophore which uncouples oxidative phos-  
phorylation, induces apoptosis in murine thymocytes,  
inhibits NGF-induced neuronal differentiation and  
antagonizes ET-induced vasoconstriction.

## NEUROPEPTIDE Y

- B-174** **BIBP 3226**  
synthetic, > 99% purity  
Selective non-peptide Y<sub>1</sub> Neuropeptide Y receptor  
antagonist.

## OPIOIDS


- M-231** **3-Methoxynaltrexone hydrochloride**  
Putative antagonist of heroin/morphine-6b-glu-  
curonide-induced opioid activity.
- N-213** **[Phe<sup>1</sup>-Ψ(CH<sub>2</sub>-NH)-Gly<sup>2</sup>]Nociceptin(1-13)-NH<sub>2</sub>**  
synthetic; >97% purity  
Selective nociceptin receptor antagonist.
- N-215** **Nocistatin**  
Endogenous 17 amino acid peptide which antagonizes  
the effects of nociceptin (Cat. No. N-184).

## SEROTONERGICS




- B-173** **BRL 54443 maleate**  
Potent 5-HT<sub>1E/1F</sub> serotonin receptor agonist.

## SIGNAL TRANSDUCTION

### Adenylyl/Guanylyl Cyclase Products

- I-159** **Isoliquiritigenin**  
(2',4,4'-Trihydroxychalcone)  
Soluble guanylyl cyclase activator and aldose  
reductase inhibitor.
- N-211** **NS 2028**  
Specific soluble guanylyl cyclase inhibitor.
- Y102** **YC-1**  
 NO-independent activator of soluble guanylyl cyclase.

### Apoptosis Products

- A-279** **Anisonomycin**  
(Flagecidin)  
 Activates p54 (SAPKs).
- D-220** **Doxorubicin hydrochloride**  
(Adriamycin hydrochloride)  
 Apoptosis inducer.
- E-167** **Ebselen**  
(PZ51)  
 Apoptosis inhibitor.

### Chemotactic Products

- F-140** **F-Met-Leu-Phe**  
(N-Formyl-Met-Leu-Phe; FMLP)  
synthetic; >97% purity. Chemotactic peptide;  
stimulates several cytoplasmic events leading  
to chemotaxis in neutrophils.

### Protein Kinase/Phosphatase Related Products

- C-272** **Cantharidin**  
(Cantharidine)  
Inhibitor of phosphatases 1 and 2A.
- G-153** **Geldanamycin**  
(GDM)  
Blocks the activities of signaling proteins requiring  
interaction with Hsp90 for proper functioning.
- K-114** **Kemptide**  
synthetic; >97% purity  
Peptide substrate for protein kinase A  
(cAMP-dependent protein kinase).
- P-255** **Piceatannol**  
(3,3',4,5'-Tetrahydroxy-trans-stilbene)  
Inhibits the non-receptor tyrosine kinases,  
Syk and Lck; preferentially inhibits Syk over Lck.
- R-126** **Radicalol**  
(R2146)  
Inhibits Ras-MAP kinase pathway by selectively  
depleting cells of Raf; inhibits Src tyrosine kinase  
activity.




# New Products from RBI

## SIGNAL TRANSDUCTION (cont.)

### Protein Kinase/Phosphatase Related Products (cont.)

- U-120 U0126**  
*Specific inhibitor of MEK1 and MEK2 (MAP kinase kinase; MAPKK); also inhibits constitutively active, mutant form of MEK.*

### Miscellaneous Signaling Products

- F-141 Fumagillin**  
*(Fugillin)  
Angiogenesis inhibitor.*
- L-140 IPA**  
 *(L- $\alpha$ -Lysophosphatidic acid)  
Endogenous agonist for the LPA receptor; putative ligand for EDG-2 and EDG-4.*
- M-273 Minocycline hydrochloride**  
*(Minocin hydrochloride)  
Basement membrane protease inhibitor; inhibits endothelial cell proliferation and angiogenesis.*
- M-274 2-Methoxyestradiol**  
*Angiogenesis inhibitor.*
- O-122 Oleamide**  
 *(Oleic acid amide)  
Sleep inducing brain lipid which allosterically modulates GABA<sub>A</sub> receptors and potentiates serotonin receptor responses.*
- P-251 L- $\alpha$ -Phosphatidylethanolamine**  
 *Membrane phospholipid.*

## Chemokines from RBI



RBI now offers an extensive line of 48 Chemokines and anti-Chemokine antibodies: including human, mouse, and rat recombinant chemokines as well as anti-human chemokine antibodies.

Antibodies are validated for Western Blot, ELISA, Immunohistochemistry and Neutralization techniques. Chemokines are validated for use in cell culture assays.

Contact the RBI Technical Service Department or your RBI Distributor for additional information, including detailed Product Data Sheets.

### Nitric Oxide Products

- M-230 S-Methyl-L-thiocitrulline acetate**  
*Potent inhibitor of NOS; more potent than L-thiocitrulline (Cat. No. T-173).*

### Receptor Modulating Agent

- P-253 Phenylarsine oxide**  
*(PAO; Arzene)  
Blocks internalization of cell surface receptors; metabolic poison.*

### Sphingolipid Signaling Pathway Products

- F-142 Fumonisin B1**  
*Inhibitor of sphingosine-N-acetyltransferase.*
- S-196 Sphingomyelinase**  
*(Sphingomyelin phosphodiesterase)  
Initiates the formation of sphingomyelin-based second messengers.*
- S-195 Sphingosine-1-phosphate**  
*(D-erythro-Sphingosine-1-phosphate)  
EDG-1 receptor ligand; involved in cellular signaling; putative lipid second messenger.*

## New Antibodies from RBI



RBI now offers an expanded line of antibodies to meet the needs of Neuroscience researchers. New specificities are listed on pages 15-17.

Antibodies have been validated for a variety of techniques including immunohistochemistry, immunoblotting, ELISA, immunoprecipitation and RIA.

Contact the RBI Technical Service Department or your RBI Distributor for additional information including detailed Product Data Sheets.



Products introduced since the last issue of *Neurotransmissions*.

## ***New Antibodies from RBI***

Cat. No.	Antibodies To:	Size
<b>Neuronal Enzymes, Synaptic Proteins</b>		
D-216	Dopamine $\beta$ -hydroxylase (C-terminal)	30 $\mu$ g
D-217	Dopamine $\beta$ -hydroxylase (N-terminal)	40 $\mu$ g
S-193	Synapsin I	10 $\mu$ g
<b>Receptors</b>		
A-268	A <sub>1</sub> Adenosine receptor	100 $\mu$ l
A-269	A <sub>2A</sub> Adenosine receptor	100 $\mu$ l
A-270	$\alpha_1$ Adrenergic receptor	100 $\mu$ l
A-271	$\alpha_{2A}$ Adrenergic receptor	100 $\mu$ l
A-272	$\beta_1$ Adrenergic receptor	100 $\mu$ l
D-218	Dihydropyridine receptor ( $\alpha_1$ subunit), clone 1A	100 $\mu$ l
D-219	Dihydropyridine receptor ( $\alpha_2$ subunit), clone 20A	100 $\mu$ l
I-157	Inositol 1,4,5-trisphosphate receptor (Type I) (IP3R)	100 $\mu$ l
M-264	N-Methyl-D-aspartate R2A glutamate receptor (NMDA R2A)	10 $\mu$ g
M-265	N-Methyl-D-aspartate R2B glutamate receptor (NMDA R2B)	10 $\mu$ g
M-266	N-Methyl-D-aspartate R2C glutamate receptor (NMDA R2C)	10 $\mu$ g
M-272	M <sub>2</sub> Muscarinic acetylcholine receptor, clone 31-1D1	100 $\mu$ l
R-128	Ryanodine receptor, clone C3-33	100 $\mu$ g
R-129	Ryanodine receptor, clone 34-C	100 $\mu$ l
<b>Neuropeptides</b>		
E-162	$\beta$ -Endorphin	200 $\mu$ l
E-163	Endothelin, clone ET-1/58	200 $\mu$ l
E-164	Endothelin	200 $\mu$ l
E-165	Leu-enkephalin	500 Tests
E-166	Endothelin-1, clone TR.ET.48.5	100 $\mu$ l
<b>Cell Structure</b>		
P-246	PSD95, clone 7E3-1B8	100 $\mu$ l

Contact RBI Technical Service for a free detailed "Applications Brochure" for the new RBI Antibodies and Product Data Sheets that include a complete listing of antibody forms, species reactivity, applications and titers for individual procedures.

### ***New Antibodies from RBI (Continued)***

Cat. No.	Antibodies To:	Size
<b>Signal Transduction Agents: Ca<sup>2+</sup> Associated Proteins, Protein Kinases/Phosphatases, Apoptosis Related Agents,</b>		
C-265	CaM Kinase II ( $\alpha$ subunit), clone 6G9	100 $\mu$ l
C-266	$\mu$ -Calpain (domain III), clone 9A4H8D3	100 $\mu$ l
C-267	$\mu$ -Calpain (domain II), clone 2H2A7C2	100 $\mu$ l
C-268	m-Calpain (domain III/IV), clone 107-82	100 $\mu$ l
C-269	Calpain (28 kDa subunit), clone 156	100 $\mu$ l
C-270	Calpastatin, clone 1F7E3D10	100 $\mu$ l
F-136	Focal adhesion kinase	200 $\mu$ l
J-103	c-Jun N-terminal kinase	200 $\mu$ l
M-267	MAP kinase kinase	200 $\mu$ l
M-268	MAP kinase kinase 4	200 $\mu$ l
M-269	p38 MAP kinase	200 $\mu$ l
P-235	Phosphatidylinositol 3-kinase	200 $\mu$ l
P-236	Protein kinase C- $\beta_1$	200 $\mu$ l
P-237	Protein kinase C- $\beta_1$ , clone PK-B13	200 $\mu$ l
P-238	Protein kinase C- $\beta_2$	200 $\mu$ l
P-239	Protein kinase C- $\beta_2$ , clone PK-B26	200 $\mu$ l
P-240	Protein kinase C- $\gamma$	200 $\mu$ l
P-241	Protein kinase C- $\gamma$ , clone PK-G4	200 $\mu$ l
P-242	Protein kinase C- $\delta$	200 $\mu$ l
P-243	Protein kinase C- $\epsilon$	200 $\mu$ l
P-244	Protein kinase C- $\zeta$	200 $\mu$ l
P-245	Protein kinase C- $\eta$	200 $\mu$ l
P-248	Poly(ADP-ribose) polymerase, clone C-2-10	50 $\mu$ l
R-127	Rsk1 (p90 <sup>rsk</sup> )	200 $\mu$ l
S-194	S6 kinase (p70 <sup>s6k</sup> )	200 $\mu$ l
<b>Nitric Oxide Synthases</b>		
N-217	Nitric oxide synthases, universal	100 $\mu$ l
N-218	Nitric oxide synthase, universal, clone NOS-3F7-B11-B5	200 $\mu$ l

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## New Antibodies from RBI (Continued)

Cat. No.	Antibodies To:	Size
<b>Ion Transporters</b>		
A-274	H <sup>+</sup> /K <sup>+</sup> ATPase (β subunit), clone 2G11	100 μl
N-216	Na <sup>+</sup> /Ca <sup>2+</sup> Exchanger, clone C2C12	100 μl
A-276	Na <sup>+</sup> /K <sup>+</sup> ATPase (α subunit), clone M7-PB-E9Mouse	200 μl
A-277	Na <sup>+</sup> /K <sup>+</sup> ATPase (α1 subunit), clone M8-P1-A3	200 μl
A-278	Na <sup>+</sup> /K <sup>+</sup> ATPase (β1 subunit), clone M17-P5-F11	200 μl
A-275	Na <sup>+</sup> /K <sup>+</sup> ATPase (α1 subunit), clone 9A-5	100 μl
A-273	Na <sup>+</sup> /K <sup>+</sup> ATPase (α3 subunit), clone XVIF9-G10	100 μl
N-216	Na <sup>+</sup> /Ca <sup>2+</sup> Exchanger, clone C2C12	100 μl
<b>Transcription Factors</b>		
F-137	cFos	200 μl
F-138	FosB	200 μl
F-139	Fra2	200 μl
J-104	cJun	200 μl
J-105	JunD	200 μl
J-106	JunB	200 μl
P-249	Peroxisome proliferator activated receptor (PPAR)	100 μl
P-250	Peroxisome proliferator activated receptor (γ2 isoform) (PPARγ2)	100 μl
<b>Chemokines, Cytokines, Growth Factors</b>		
E-160	Eotaxin	100 μg
M-253	MCP-1	100 μg
M-254	MCP-2	100 μg
M-255	MCP-3	100 μg
M-256	MIP-1α	100 μg
M-257	MIP-1β	100 μg
R-125	RANTES	100 μg
G-151	GROα	1 mg
I-155	IL-8	100 μg
I-156	IP-10	100 μg
S-192	SDF-1α	100 μg

Contact RBI Technical Service for a free detailed "Applications Brochure" for the new RBI Antibodies and Product Data Sheets that include a complete listing of antibody forms, species reactivity, applications and titers for individual procedures.

## Cannabinoids: Practical Problems and Solutions

*Graeme Griffin*

The low aqueous solubility and high lipophilicity of various cannabinoid receptor ligands present several potential problems associated with the use of these compounds in day-to-day experiments. This brief review will address some of the experimental practices that have been adopted to counter these problems.

The low solubility of these compounds in water necessitates the use of an alternative solvent. The most commonly used approach is to dissolve these compounds in ethanol, in which the majority will remain stable for long periods of time if protected from light and stored at low temperatures (-20°C). The major exception to this general rule appears to be the endogenous cannabinoids, particularly anandamide (RBI Cat. No. **A-176**), whose shelf life may be limited to approximately a month, even when undertaking these precautions (unpublished observation). Another approach is to dissolve these compounds in an alternative organic solvent, such as dimethyl sulphoxide (DMSO), or into a solvent/saline combination [1]. However, the use of any organic solvent will have detrimental effects upon the cell, tissue or whole animal being studied, and careful control experiments must be performed in order to determine its influence on the observed response so that its effects can be minimized.

In addition to their poor aqueous solubility, cannabinoid receptor ligands also have a tendency to adhere to laboratory

plastic/glassware. They may also precipitate out of solution when placed in an aqueous environment and, therefore, some means must be used to ensure the accurate delivery of the drug to the desired site of action. Although many researchers have found direct dilutions of ethanol stock solutions to be adequate, others have found it necessary to also include a carrier molecule. Several molecules have been used for this purpose. These range from bovine serum albumin (BSA), usually fatty-acid free, to organic solvents such as Tween 80 and DMSO or oil/ethanol/saline mixtures, depending on the type of experiment being conducted. Examples of these approaches would include the use of BSA in radioligand binding assays, adenylyl cyclase assays and electrophysiological experiments [2,3]. The level of BSA used varies, but is normally in the range of 1-50 mg/ml of buffer. Although BSA is one of the more inert vehicles available, its use still involves exposure of the tissue to the organic solvent in which the drug is dissolved, and certain experimental models may be highly sensitive to even very low concentrations of ethanol. Accordingly, another approach to minimize or even eliminate the presence of ethanol in the experiment, is to use a vehicle such as Tween 80 or DMSO (often in a 2:1 vehicle:drug ratio by weight). Combination of the drug/ ethanol solution with one of these vehicles allows for the evaporation of the ethanol (usually under nitrogen or vacuum) before dilution in saline. This approach has been used, for example,

### About the Author

Graeme Griffin received his undergraduate degree in Pharmacology from the University of Aberdeen, Scotland in 1988. He remained in the Department of Biomedical Sciences at the University of Aberdeen for his Ph.D. studies, investigating the pharmacology of cannabinoids under the supervision of Roger Pertwee. He is presently engaged in postdoctoral research within the laboratory of Mary Abood in the Department of Pharmacology and Toxicology at the Medical College of Virginia, Richmond where he is investigating the functional coupling of cannabinoid receptors.

## Cannabinoid and Related Products from RBI

RBI CAT. NO.	Unit Name		
<b>A-262</b>	<b>AM 404</b> Anandamide transport inhibitor; enhances receptor-mediated anandamide responses.	<b>C-243</b>	<b>CB<sub>2</sub> Cannabinoid receptor, human</b> (CHO-K1)
<b>A-261</b>	<b>2-Arachidonyl glycerol</b> (2-AG) Endogenous cannabinoid receptor ligand.	<b>I-151</b>	<b>Indomethacin morpholinylamide</b> (BML-190) CB <sub>2</sub> Cannabinoid receptor ligand.
<b>A-231</b>	<b>Arachidonyl trifluoromethyl ketone</b> Inhibits anandamide hydrolysis <i>in vitro</i> ; inhibits phospholipase A <sub>2</sub> .	<b>M-186</b>	<b>R(+)-Methanandamide</b> > 96% purity Congener of anandamide that displays higher affinity for the cannabinoid receptor. R(+)-Methanandamide possesses stability to aminopeptidase hydrolysis and cannabinometric properties <i>in vivo</i> .
<b>C-217</b>	<b>CB<sub>1</sub> Cannabinoid receptor, human</b> (HEK-293)		

See p. 7, figure 1 for additional products  
For more information contact our Technical Service Department.

with *in vitro* organ bath experiments using various tissues and with brain slice recordings [4,5].

*In vivo* studies also require careful planning as the quantities of drugs used are often much higher than in *in vitro* studies and because ethanol has its own unique profile of effects at high concentrations. A common approach to this problem is to administer the drug in an oil/ethanol/saline mixture. An oil that is commonly used to dissolve cannabinoids is the polyoxyethylated vegetable oil emulphor (available as Alkmulphor), usually in a 1:1:18 ratio, with ethanol and saline [6].

Another unavoidable aspect associated with the use of cannabinoid receptor ligands is that their high lipophilicity will tend to partition these compounds into cell lipid compartments, especially at high concentrations. In order to reduce these non-specific membrane perturbation effects, many researchers use these compounds at concentrations that do not exceed 10 µM. Fortunately, with the development of selective cannabinoid receptor antagonists, the determination of a specific cannabinoid receptor-mediated effect, versus a membrane perturbation effect, is now a much simpler task.

Lastly, the endocannabinoid, anandamide, is somewhat unique amongst cannabinoid receptor ligands in that it has been demonstrated to be susceptible to tissue metabolism. In several studies, including radioligand binding assays, *in vitro* smooth muscle preparations and *in vivo* whole animal experiments [4,7,8], the use of various amidase inhibitors has been shown to increase the affinity and/or efficacy of anandamide. Although several inhibitors have been investigated, phenylmethyl-

sulphonyl fluoride (PMSF; Cat. No. **P-252**), a non-specific amidase inhibitor, is the most effective blocker of anandamide breakdown. Other well characterized inhibitors shown to be effective *in vitro* are arachidonyl trifluoromethyl ketone (Cat. No. **A-231**) [9] and methyl arachidonyl fluorophosphate [10]. As an alternative approach, several compounds have also been produced that act as metabolically stable anandamide analogs, notably R(+)-methanandamide (Cat. No. **M-186**).

In summary, although the lipophilic nature of cannabinoid receptor ligands has proven cumbersome in the past, there are a number of techniques that can be used to minimize this problem. The careful use of solvents and drug delivery systems, coupled with the inclusion of suitable controls, now provide the means with which cannabinoids, their receptors and their signaling mechanisms may be successfully studied.

(continue to page 22)

Frequently Asked Questions received by RBI's Technical Service Group

**Q.** In what solvent is this product soluble, what is the solubility and how do I solubilize it?

**A.** The RBI Catalog entry is the best source for finding the solubility of a product in a particular solvent. An approximate maximum value for the solubility is given whenever known. For example, "Soluble in DMSO (5 mg/ml)." These values are usually empirically determined for the product by analysis of the product in the RBI laboratory or extracted from a published source such as the *Merck Index*. Please note that the actual solubility of the product in the investigator's hands may vary  $\pm 20\%$  from the listed value.

In the catalog entry, if the solvent is not followed by a specific value, a statement such as "Slightly soluble in..." or "Soluble in..." is given. These terms indicate specific ranges of solubilities. The definition of these statements are listed below:

Insoluble - less than 0.1 mg/ml

Slightly soluble - approximately 1 mg/ml

Moderately soluble - between 1 and 5 mg/ml

Soluble - between 5 and 10 mg/ml

Freely soluble - greater than 10 mg/ml

In general, RBI makes the following recommendations for solubilization of its products:

- The product should first be solubilized using one of the recommended solvents listed in the RBI Catalog or on the Product Data Sheet. The solvents RBI evaluates are distilled water, 100% anhydrous DMSO, 100% ethanol, 100% methanol, dilute aqueous acid (0.1 N HCl) or dilute aqueous base (0.1 N NaOH).
- Upon solubilization, the solution may then be diluted to a desired concentration with buffer, saline or media as needed. If the product is soluble in aqueous media, it is best to solubilize it in water or dilute aqueous base or acid before diluting with buffer, saline or media, as the product's solubility directly in these solvents may be significantly lower than that in the recommended solvents.
- To aid in solubilization, the solution may be heated slightly to about 37-40°C, and/or sonicated for 5 -10 minutes. Sonication should be monitored closely, as it may heat the solution considerably.

For advice or information on the solubilization or use of any RBI product, please contact RBI Technical Service at 508-651-8151 or by email at [tech@resbio.com](mailto:tech@resbio.com).

## Cannabinoids: Practical Problems and Solutions (cont.)

### References

1. Stella, N., Schweitzer, P., Piomelli, D. *Nature* **388**, 773-778 (1997).
2. Devane, W.A., Dysarz, F.A., Johnson, M.R., et al. *Mol. Pharmacol.* **34**, 605-613 (1988).
3. Griffin G., Atkinson P.J., Showalter V.M., et al. *J. Pharmacol. Exp. Ther.* **285**, 553-60 (1998).
4. Pertwee, R.G., Fernando, S.R., Griffin, G., et al. *Eur. J. Pharmacol.* **272**, 73-78 (1995).
5. Collins D.R., Pertwee R.G., Davies S.N. *Br. J. Pharmacol.* **115**, 869-70 (1995).
6. Compton, D.R., Aceto, M.D., Lowe, J. et al. *J. Pharmacol. Exp. Ther.* **277**, 586-594 (1996).
7. Childers, S.R., Sexton, T., Roy, M.B. *Biochem. Pharmacol.* **47**, 711-715 (1994).
8. Compton, D.R., Martin, B.R. *J. Pharmacol. Exp. Ther.* **283**, 1138-1143 (1997).
9. Koutek, B., Prestwich, G.D., Howlett, A.C. et al. *J. Biol. Chem.* **269**, 22937-22940 (1994).
10. Deutsch, D.G., Omeir, R., Arreaza, G., et al. *Biochem. Pharmacol.* **53**, 255-260 (1997).

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