

The Endocannabinoid System: Can It Contribute to Cannabis Therapeutics?

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ABSTRACT. Receptors for Δ^9 -tetrahydrocannabinol (THC), cannabis' major psychoactive principle, have been identified in animal tissues. These proteins have a reason to exist because endogenous substances may bind to and functionally activate them, thereby producing pharmacological effects similar to those of THC. Such substances, named "endocannabinoids," have been isolated and several studies have been performed on their pharmacological properties as well as on the molecular mechanisms for their biosynthesis, action and inactivation in animal cells. Within the framework of the ongoing debate on the therapeutic potential of cannabinoid receptor agonists and antagonists, the present article addresses the possibility that our knowledge of the endocannabinoid system may result in the development of new drugs for the treatment of illnesses as diverse as nervous and immune disorders, pain, inflammation and cancer. [Article copies available for a fee from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: <getinfo@haworthpressinc.com> Website: <http://www.HaworthPress.com> © 2001 by The Haworth Press, Inc. All rights reserved.]

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THE ENDOCANNABINOID SYSTEM

Research on the mechanism of action of the psychoactive components of *Cannabis sativa*, the cannabinoids, culminated in the early

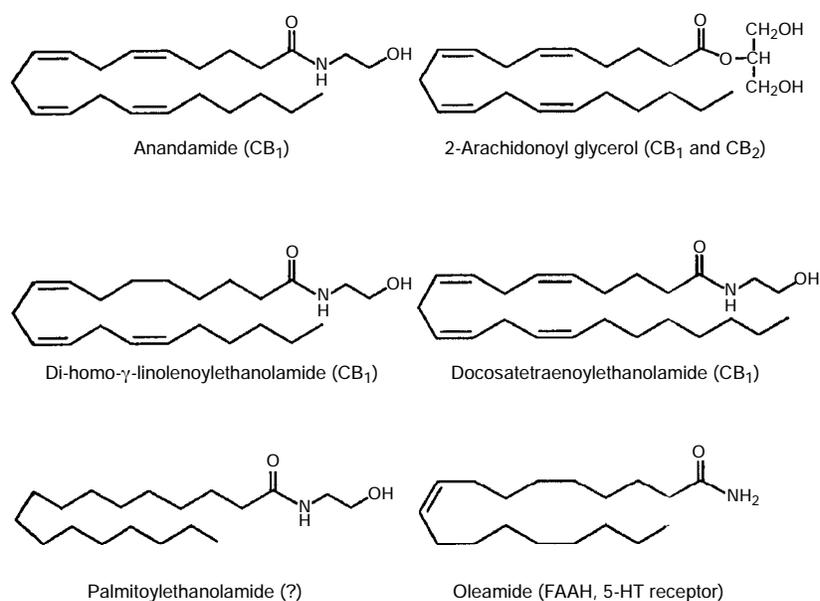
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1990's with the finding of cannabinoid receptors and of their possible endogenous agonists (see Matsuda 1997 and Di Marzo 1998 for reviews) (Figure 1). These molecules, together with the proteins that regulate their activity and/or levels, constitute the "endocannabinoid system." The first subtype of cannabinoid receptors, named CB₁, is widely distributed in both nervous and non-nervous tissues, and is responsible for most of the 'central' actions, and also for some of the peripheral ones, of plant and synthetic cannabinoids. The second subtype of cannabinoid receptors, named CB₂, has been found to date in high levels only in immune tissues and cells and may mediate some of the immune-modulatory effects of the cannabinoids, although little direct evidence for this possibility has been found so far. Evidence for CB₂-like receptors in peripheral nerves has been also described (Griffin et al. 1997). The finding of selective CB₁ and, more recently, CB₂ receptor antagonists (Rinaldi-Carmona et al. 1994, 1998; Felder et al. 1998), and the development of cannabinoid receptor knockout mice (Ledent et al. 1999; Zimmer et al. 1999; Buckley et al., 1999) will

FIGURE 1. Chemical structures and likely molecular targets of the endocannabinoids and other cannabimimetic fatty acid derivatives.



soon provide a definitive answer as to which of the typical pharmacological actions of cannabinoids are mediated by either receptor subtype, and may even support the hypothetical presence of further molecular targets for these compounds. As to the possible endogenous counterparts of the cannabinoids, over the last seven years several fatty acid derivatives have been found to mimic the properties of Δ^9 -tetrahydrocannabinol (THC), cannabis' major psychoactive principle. Not all of these substances, however, have the capability to displace high affinity cannabinoid ligands from selective binding sites in membrane preparations containing the CB₁ or the CB₂ receptor. Anandamide (Devane et al. 1992), the amide of arachidonic acid with ethanolamine, was the first of such compounds to be isolated and received its name from the Sanskrit word for "internal bliss," *ananda*. Next came two polyunsaturated congeners of anandamide (Hanus et al. 1993), and a glycerol ester, 2-arachidonoyl glycerol (2-AG) (Mechoulam et al. 1995; Sugiura et al. 1995). These compounds share the ability to bind to and activate CB₁ and (particularly in the case of 2-AG) CB₂ receptors. Therefore, they induce a series of pharmacological effects *in vitro* and *in vivo* that are, to some extent, similar to those exerted by THC (Hillard and Campbell 1997; Di Marzo 1998; Mechoulam et al. 1998). Hence the name of "endocannabinoids" was proposed for anandamide and 2-AG. Other fatty acid derivatives (Figure 1), such as palmitoylethanolamide and *cis*-9-octadecenoamide (oleamide), do not have high affinity for either of the two cannabinoid receptor subtypes discovered so far, and yet they exhibit pharmacological actions that in some cases are cannabis-like (see Lambert and Di Marzo 1999 for review). The molecular mode of action of these latter compounds, that cannot be referred to as "endocannabinoids," is currently being debated and is possibly due in part to the modulation of either the action or the metabolism of anandamide and 2-AG (Mechoulam et al. 1997; Lambert and Di Marzo 1999).

The study of the pharmacological properties of the endocannabinoids was not limited to confirm for these compounds the same spectrum of activities previously described for THC. Indeed, qualitative and quantitative differences between the action of classical and endogenous cannabinoids became evident since the first studies on these new metabolites (Hillard and Campbell 1997; Di Marzo 1998; Mechoulam et al. 1998). The chemical structure of anandamide and 2-AG (Figure 1), with the presence of hydrolysable amide or ester bonds and

of an arachidonate moiety, raises the possibility that these substances may be metabolized to other bioactive compounds through the several oxidizing enzymes of the arachidonate cascade (Burstein et al. 2000). Moreover, the lack of chiral centers contributes to making these molecules capable, in principle, of interaction with many molecular targets. The endocannabinoids, therefore, are ideal templates for the development of new drugs. Three different pieces of information are necessary in order to understand whether an endogenous substance can represent the starting point for the design of therapeutic agents. First, its pharmacological activity *in vitro* and *in vivo* needs to be thoroughly assessed. Next, the biochemical bases for the biosynthesis, action and degradation of the substance need to be fully understood. Finally, a correlation between the occurrence of particular physiological and pathological conditions and the levels of this metabolite in tissues must be investigated. In this article, I will briefly describe the landmarks in these three aspects of the research on endocannabinoids. I will also provide a few examples of how endocannabinoid-derived molecules might turn out to be useful in the alleviation and cure not only of those illnesses traditionally treated with cannabis preparations, such as inflammation, nausea, diarrhea, and chronic pain, but also for cancer, mental disorders and immune diseases.

ENDOCANNABINOID PHARMACOLOGY: MORE THAN MEETS THE EYE

As mentioned above, anandamide, in some cases, exhibits effects qualitatively and quantitatively different from those of the classical cannabinoids. This may be partly due to the rapid metabolism of this compound both *in vitro* and *in vivo* (Deutsch and Chin 1993; Willoughby et al. 1997), but also to the fact that anandamide is a partial agonist in some functional assays of CB₁ and CB₂ activity (Mackie et al. 1993; Breivogel et al. 1998). Moreover, recent studies seem to suggest that this compound is able to adapt to binding sites within other receptors (Hampson et al. 1998; Kimura et al. 1998; Zygmunt et al. 1999). The selective antagonists developed so far for cannabinoid receptors (Rinaldi-Carmona et al. 1994, 1998) have been and still are useful tools to understand when and where anandamide effects are mediated by these proteins. It is still difficult at this stage to distinguish, among these effects, those with a physiological or therapeutic

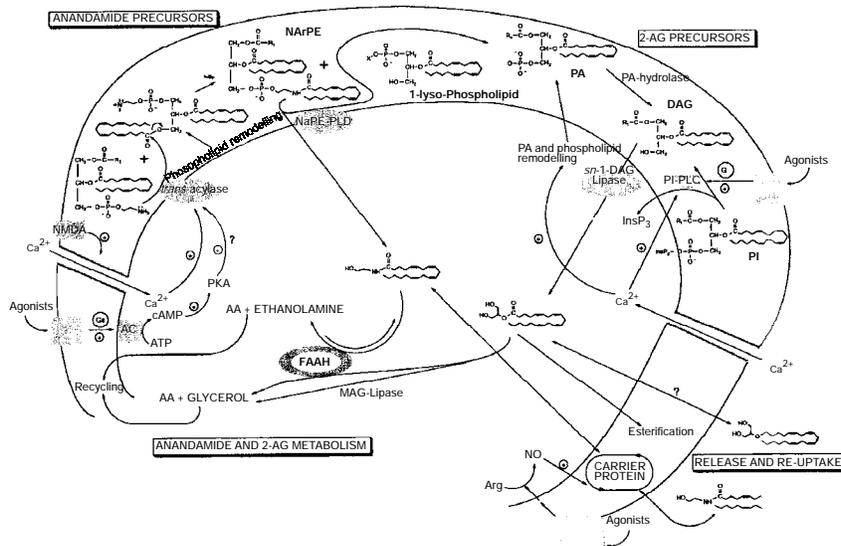
relevance. However, it is possible to speculate based on the range of concentrations necessary to observe a certain effect as compared to the usually low tissue concentrations of anandamide. Thus, in the brain, this metabolite was shown to exert inhibitory actions on learning and memory (Mallet and Beninger 1996; Castellano et al. 1997), to modulate the extra-pyramidal control of motor behavior (Romero et al. 1995), and to protect astrocytes against inflammatory stress (Molina-Holgado et al. 1997). These effects are probably due to the capability of anandamide to induce, via activation of CB₁ receptors, a series of intracellular events resulting in the modulation of neurotransmitter release, action and re-uptake (see Di Marzo et al. 1998b for review). This neuromodulatory action may also underlie anandamide regulation of hormone release at the level of the hypothalamus/pituitary/adrenal axis (Fernandez-Ruiz et al. 1997), as well as the anti-nociceptive effects of the compound through both spinal and supra-spinal mechanisms (reviewed by Martin and Lichtman 1998). In peripheral tissues, anandamide regulates the heartbeat and vascular blood pressure and produces vasodilator effects through several possible mechanisms (recently reviewed by Kunos et al. 2000). The endocannabinoid also relaxes smooth muscle in the gastrointestinal system and reproductive/urinary tract (Pertwee and Fernando 1996; Izzo et al. 1999). Regulation of reproduction also occurs at the level of the sperm acrosome reaction (Schuel et al. 1994) and embryo development and implantation (Paria et al. 1995, 1998). As most of these findings were obtained after the development of the CB₁ receptor antagonist SR141716A (Rinaldi-Carmona et al. 1994), it was possible to demonstrate the intermediacy of this receptor in most of the above effects. Conversely, the involvement of CB₂ receptors in the immune-regulatory effects of anandamide is yet to be fully established (for a recent review see Parolaro 1999), probably due to the only very recent availability of a selective antagonist for these receptors, SR144528 (Rinaldi-Carmona et al. 1998). Finally, anandamide was also found to regulate some key cell functions such as cell proliferation and energy metabolism (De Petrocellis et al. 1998, Guzman and Sanchez 1999), but only in the first case by activating CB₁ receptors. As to 2-AG, only a few pharmacological studies have been performed to date on this compound, possibly because of its limited commercial availability until recently. Apart from its activity in the mouse "tetrad" of tests for cannabimimetic compounds (i.e., analgesia in the "hot-plate" or "tail-

flick" test, immobility on a ring, hypothermia and inhibition of spontaneous activity in an open field [Mechoulam et al. 1995]), this compound shares with THC an immune-modulatory action (Ouyang et al. 1998) and an inhibitory effect on embryo development (Paria et al. 1998) and breast and prostate cancer cell proliferation (De Petrocellis et al. 1998; Melck et al. 2000). 2-AG also induces calcium transients in neuroblastoma \times glioma cells and HL-60 cells (via CB₁ and CB₂ receptors, respectively), an effect that is not efficaciously exerted by anandamide (Sugiura et al. 1999, 2000). Therefore, different pharmacological actions can be observed not only for *endocannabinoids* and *exocannabinoids*, but also for anandamide and 2-AG.

LEVELS OF ENDOCANNABINOIDS IN TISSUES: PHYSIOLOGY AND PATHOLOGY

Biochemical pathways for anandamide and 2-AG biosynthesis and inactivation by intact cells have been identified (see [Hillard and Campbell 1997; Di Marzo 1998; Di Marzo et al. 1998] for reviews) (Figure 2). Mechanisms for the regulation by both physiological and pathological stimuli of the enzymes involved in these pathways have also been found. On stimulation with calcium ionophores, or other calcium mobilizing stimuli, anandamide is produced by neurons and leukocytes from the hydrolysis of a membrane phospholipid precursor, *N*-arachidonoyl phosphatidyl ethanolamine (NArPE). The reaction is catalyzed by a phospholipase D specific for NArPE and other homologous phospholipids. Notably, phospholipase D enzymes are known to be subject to regulation by intracellular mediators (e.g., the diacylglycerols). NArPE, in turn, is produced by the transfer of arachidonic acid from the *sn*-1 position of phospholipids onto phosphatidylethanolamine. The enzyme involved in this case is a *trans*-acylase regulated by calcium and cAMP-induced protein phosphorylation. 2-AG is produced in intact neurons from the hydrolysis of diacylglycerols catalyzed by the *sn*-1 selective diacylglycerol lipase. Diacylglycerols serving as 2-AG precursors are in turn formed from the hydrolysis of either phosphatidylinositol or phosphatidic acid. The enzymes catalyzing these two reactions are a phospholipase C and a phosphatidic acid hydrolase, respectively. There is no evidence that these two enzymes are different from enzymes of the same type responsible for

FIGURE 2. Schematic representation of endocannabinoid biosynthetic and metabolic pathways describes so far in intact cells. Adapted from Di Marzo et al., 1998b. Abbreviations: NMDA, N-Methyl-D-Aspartate; NaPE-PLD, N-acyl-phosphatidylethanolamine-selective phospholipase D; PI-PLC, phosphatidylinositol-selective phospholipase C; PA, phosphatidic acid; DAG, diacylglycerol; AC, adenylyl cyclase; PKA, protein kinase A; MAG, mono-acylglycerol; NO, nitric oxide; AA, arachidonic acid; FAAH, fatty acid amide hydrolase.



the formation of intracellular mediators, and therefore it is likely that they are subject to several regulative mechanisms.

Also the routes leading to endocannabinoid degradation are likely to be tightly regulated (Hillard and Campbell 1997; Di Marzo 1998; Di Marzo et al. 1998b). The major enzyme responsible for anandamide hydrolysis, fatty acid amide hydrolase (FAAH), has been cloned from four species (Cravatt et al. 1996; Giang and Cravatt 1997; Goparaju et al. 1999) and found to contain a proline-rich domain necessary for enzymatic activity (Arreaza and Deutsch, 1999). This domain contains a consensus sequence for recognition by regulatory proteins that may target FAAH to its subcellular location, thereby regulating its activity. FAAH also recognizes as a substrate 2-AG (Goparaju et al. 1998), for which, however, other hydrolytic enzymes have been described. One of these hydrolases, present in rat platelets and macro-

phages, is down-regulated by lipopolysaccharides (LPS) exposed by bacterial walls (Di Marzo et al. 1999).

As the hydrolytic enzymes responsible for the degradation of endocannabinoids seem to be located in intracellular sites (Giang and Cravatt 1997), the internalization of these compounds is necessary for their degradation to occur. A mechanism for the facilitated diffusion of anandamide across the cell membrane has been identified in several cell types. This “carrier” is temperature-dependent, saturable, quite selective for anandamide and some of its analogues, and sensitive to specific inhibitors (Beltramo et al. 1997; Hillard et al. 1997; Di Marzo et al. 1998a; Melck et al. 1999). More importantly, the anandamide carrier is activated by nitric oxide (Maccarrone et al. 1998, 2000), a finding that creates the possibility of regulatory loops between the action of some mediators or pathological stimuli and anandamide inactivation.

The observations described above suggest that the levels of pharmacologically active endocannabinoids in tissues may change during a certain physiological or pathological response and, therefore, that substances interfering with anandamide or 2-AG biosynthesis, action and metabolism may be used as therapeutic agents. However, over the last six years, only a few studies have attempted to correlate endocannabinoid levels with particular physiopathological conditions. Pioneering studies have been carried out in peripheral tissues. Anandamide was produced in the highest levels in the mouse uterus when this tissue is least receptive to the embryo (Schmid et al. 1997). This finding and the observation that anandamide inhibits embryo implantation (Paria et al. 1995, 1998) suggest that a defective regulation of endocannabinoid levels in the uterus may underlie early pregnancy failures. If this is proven to be the case, inhibitors of anandamide synthesis, or CB₁ receptor antagonists, could be used to prevent this clinical problem. Formation of 2-AG in platelets and of both 2-AG and anandamide in macrophages was correlated with septic shock-induced hypotension in rats (Varga et al. 1998). In fact, macrophages and platelets from rats treated with LPS were shown to induce CB₁-mediated hypotension in untreated rats. Likewise, macrophages from rats undergoing hemorrhagic shock produce anandamide and induce hypotension in untreated rats in a fashion sensitive to the CB₁ antagonist SR141716A (Wagner et al. 1997). In this case, THC treatment was found to improve the chances of survival of rats after hemorrhagic shock, whereas

SR141716A appeared to rescue the animals from septic shock. These data underlie the importance of studies on the endogenous cannabinoid system for the development of alternative therapeutic approaches.

In the brain, anandamide, but not 2-AG, was found to be released from the dorsal striatum of freely moving rats and shown to counteract the motor-inducing action of the dopamine D2 receptor agonist quinpirole (Giuffrida et al. 1999). This finding is in agreement with data suggesting for anandamide a role in the extra-pyramidal control of locomotion, possibly at the level of dopamine action (Romero et al. 1995). A more recent study showed that endocannabinoid levels in the external layer of the globus pallidus are inversely correlated with spontaneous motor activity in the reserpine-treated rat, an animal model of Parkinson's disease (Di Marzo et al. 2000a). Out of the six brain regions analyzed, only the globus pallidus—an area which receives CB₁-containing GABAergic terminals from the striatum, and where both classical and endogenous cannabinoids potentiate GABA inhibitory action on movement (Wickens and Pertwee 1993)—was found to contain *increased* amounts of 2-AG concomitantly to the akinesia induced by reserpine-mediated catecholamine depletion in the striatum. Both anandamide and 2-AG levels in the globus pallidus were *reduced* concomitantly to the administration to reserpine-treated rats of dopamine receptor agonists and the subsequent partial recovery of motor behavior. Finally, co-administration to rats of quinpirole and the CB₁-antagonist SR141716A almost totally restored normal locomotion. On the other hand, it was also found that the dyskinesia induced in MPTP-treated monkeys after prolonged treatment with L-dopa, a typical consequence of curing Parkinson's disease in humans with this drug, was alleviated by SR141716A (Fox et al. 1999). These studies suggest that agonists and antagonists of CB₁ receptors may be used advantageously in the future for the treatment of parkinsonian patients. Furthermore, these data reveal the existence of a complex regulatory interplay between the dopaminergic and endocannabinoid systems, according to which activation of dopamine receptors may either activate or inhibit endocannabinoid signaling, and endocannabinoids would either counteract or reinforce dopamine action, depending on the brain region and the pathophysiological situation. Indeed, this interplay may occur also at the level of the limbic system and underlie a role of endocannabinoids in the reinforcement of, or the recovery from, the effects of prolonged drug abuse. In fact, a recent

study showed that chronic treatment of rats with THC results in the down-regulation of cannabinoid receptor binding and signaling in all brain regions analyzed except for the limbic forebrain, where these two parameters were not altered (Di Marzo et al. 2000c). This region was also the only one exhibiting higher amounts of anandamide with respect to vehicle-treated rats. It is possible that dopamine released in the nucleus accumbens following chronic administration with THC (or more potent drugs of abuse, such as morphine and alcohol) (Tanda et al. 1997) stimulates the formation of anandamide in this region, by analogy to what was previously found for the dorsal striatum (Giuffrida et al. 1999). In any event, this finding may suggest the involvement of the endocannabinoid system in motivation and reward, thus opening the way also to the possibility that drugs derived from anandamide and 2-AG be used in the treatment of depression, and related nervous disturbances.

The finding of anandamide and 2-AG in the hypothalamus of rats (Gonzales et al. 1999) and of CB₁ receptors in some nuclei such as the arcuate nucleus and the medial preoptic area (Fernandez-Ruiz et al. 1997) supports the notion, based on the well known appetite-stimulating, anti-emetic and hypothermic properties of THC, that the endocannabinoid system may be involved in the control of hypothalamic functions. Further studies are now required to understand whether endocannabinoid levels can be associated with hyperphagia or anorexia, and be tuned by the several transmitter systems that intervene in the regulation of food intake.

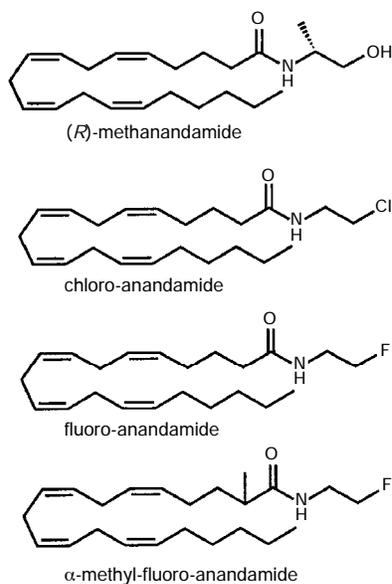
Finally, a possible correlation between anandamide release from neurons of the periaqueductal grey (PAG), a region of the brainstem, and anti-nociception was recently described (Walker et al. 1999). Electrical stimulation of the PAG results in CB₁-mediated analgesia and the release of anandamide in micro-dialysates from this region. Small amounts of the endocannabinoid were released from the PAG also following a nociceptive stimulus such as the injection of formalin into the hindpaw (Walker et al. 1999). The same stimulus does not lead to the local formation of anandamide, 2-AG or palmitoylethanolamide in the hindpaw (Beaulieu et al. 2000). Therefore, it is possible that anti-nociceptive endocannabinoids are formed at a supraspinal level following noxious stimuli. However, it is not clear how the low concentration of anandamide found in PAG microdialysates (~180 pM) can be consistent with the weak analgesic effect observed with this

compound following intrathecal, systemic and, particularly, intra-cerebroventricular administration (Calignano et al., 1998; Martin and Lichtman 1998), or with the high nM concentrations required for this compound to activate CB₁ receptors (Hillard and Campbell 1997).

NEW DRUGS FROM THE ENDOCANNABINOID SYSTEM. CURATIVE OR PALLIATIVE?

From the findings described in the previous sections, it is clear that the discovery of endocannabinoids opens several unprecedented possibilities for the development of new drugs. Firstly, the finding that a novel class of compounds derived from fatty acids and different from classical cannabinoids and aminoalkyl-indoles could activate the cannabinoid receptors stimulated the synthesis of several new endocannabinoid-based compounds (see Martin et al. 1999, for a comprehensive review). Some of these compounds (Figure 3) are several-fold more potent than anandamide and 2-AG at CB₁ receptors, while others are

FIGURE 3. Chemical structures of potent synthetic anandamide analogues with high affinity for CB₁ receptors and/or enhanced metabolic stability.

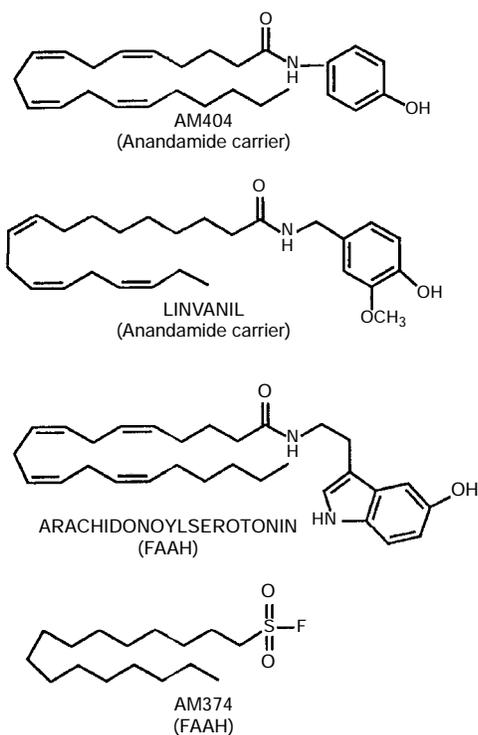


more resistant to enzymatic hydrolysis and can exert longer-lasting pharmacological actions. Secondly, when a cause and effect relationship is established between certain pathological conditions and the levels of endocannabinoids (measured by sensitive analytical techniques as in some of the studies described in the previous section), the application of endocannabinoid-based drugs for the cure of these disorders will be possible. In fact, these studies should provide indispensable hints as to what pathological state can be treated with CB₁ and CB₂ agonists or antagonists. Thirdly, our knowledge of the enzymes regulating endocannabinoid levels will allow us to develop selective inhibitors to be used for those disorders for which a correlation with defective endocannabinoid synthesis or inactivation is clearly demonstrated. Indeed, a few such substances are already available, as in the case of the rather selective inhibitors of FAAH and the anandamide carrier shown in Figure 4. Some of these compounds, such as AM404 and linvanil (two carrier inhibitors) and AM374 (a FAAH inhibitor) have been shown to lower the concentration threshold for anandamide activity both *in vivo* and *in vitro* (Beltramo et al. 1997; Gifford et al. 1999; Maccarrone et al. 2000). These compounds may be useful for those yet-to-be discovered pathological states arising from excessive degradation of endogenous anandamide. Moreover, if ways to target them selectively to peripheral tissues are devised, these compounds may render locally active doses of exogenous anandamide analogues that are devoid of undesired psychotropic activity.

Indeed, the development of new therapeutic agents from the endocannabinoids may provide a way out of the social and legal implications arising from the prescription of medical cannabis, at the center of heated debates in the UK and USA. In fact, given the numerous differences found so far between the pharmacological effects of the endogenous compounds and THC, it is likely that endocannabinoid-like drugs may have beneficial effects by simply compensating for possible malfunctions in the endogenous system, without causing the “high” typical of marijuana intoxication. Indeed, a recent study showed that both anandamide and its metabolically stable analogue (*R*)-methanandamide (Figure 3) do not cause dependence in rats (Acceto et al. 1998).

Finally, one last issue that should be addressed in the future is whether these putative therapeutic agents will be used simply as palliatives, as the history of medicinal cannabis would suggest, or instead

FIGURE 4. Chemical structures of synthetic inhibitors of anandamide inactivation (i.e., facilitated transport into cells or fatty acid amide hydrolase-catalyzed hydrolysis).

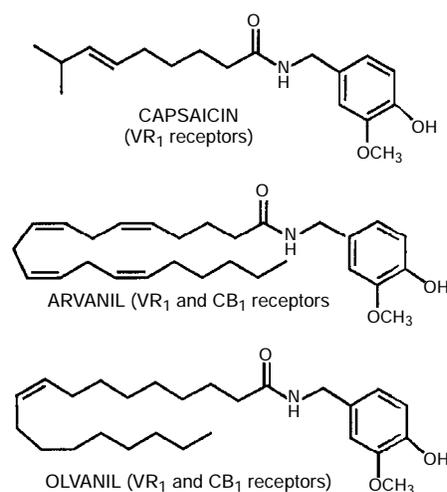


as curative drugs. The answer to this question may come from studies attempting to establish a causative role of a defective endocannabinoid system in some disorders such as, for example, those arising from exaggerated or disrupted immune responses (inflammation, allergy, auto-immune diseases), or from the hyper- or hypo-activity of the dopaminergic or other neurotransmitter systems (schizophrenia, Tourette's syndrome, anorexia, depression) (Consroe 1998). Were such a causative role to be found, metabolically stable endocannabinoids analogues and/or inhibitors of endocannabinoid degradation may contribute to the cure of these diseases. On the other hand, there may be a case for the use of exogenous endocannabinoids also in the treatment of those pathological states that are not necessarily related

to altered endocannabinoid levels and action. One example may be the recent finding of anandamide derivatives with potent anti-proliferative activity against growth factor-dependent breast and prostate cancer cell proliferation (De Petrocellis et al. 1998; Melck et al. 2000; Di Marzo et al. 2000b). One of these compounds, arvanil (Figure 5 and [Melck et al. 1999]) is a structural “hybrid” between anandamide and the widely used pharmacological tool capsaicin (the active principle of hot chiles), and exerts also very potent analgesic actions (Di Marzo et al. submitted). Last, but not least, the capability of endocannabinoids to synergize with opioids and opiates in the treatment of hyperalgesia and chronic pain is being debated (Manzanares et al. 1999).

In conclusion, the road to novel drugs from the endocannabinoid system is still long and unpaved. Although much progress has been done towards the understanding of the chemical bases underlying anandamide molecular recognition by cannabinoid receptors and inactivating proteins, thus leading to new pharmacologically active substances (Figures 3-5), a multi-disciplinary effort will be now required from biochemists, physiologists, pharmacologists and clinicians in order to understand whether and for what disorders these new chemicals can be used as therapeutic agents.

FIGURE 5. Chemical structures and properties of cannabinoid-vanilloid “hybrids.”



REFERENCES

- Aceto, M.D., S.M. Scates, R.K. Razdan, and B.R. Martin. 1998. Anandamide, an endogenous cannabinoid, has very low physical dependence potential. *J Pharmacol. Exp Ther* 287:598-605.
- Arreaza, G. and D.G. Deutsch. 1999. Deletion of a proline-rich region and a transmembrane domain in fatty acid amide hydrolase. *FEBS Lett* 454:57-60.
- Beaulieu, P., T. Bisogno, S. Punwar, W.P. Farquhar-Smith, G. Ambrosino, V. Di Marzo et al. Role of the endogenous cannabinoid system in the formalin test of persistent pain in the rat, *Eur J Pharmacol*, 396:85-92.
- Beltramo, M., N. Stella, A. Calignano, S.Y. Lin, A. Makriyannis, and D. Piomelli. 1997. Functional role of high-affinity anandamide transport, as revealed by selective inhibition. *Science* 277:1094-1097.
- Breivogel, C.S., D.E. Selley, and S.R. Childers. 1998. Cannabinoid receptor agonist efficacy for stimulating [³⁵S]GTP- Δ -S binding to rat cerebellar membranes correlates with agonist-induced decreases in GDP affinity. *J Biol Chem* 273:16865-16873.
- Buckley, N.E., K.L. McCoy, E. Mezey, T. Bonner, A. Zimmer, C.C. Felder et al. 1999. Symposium on the cannabinoids, Burlington, Vermont, ICRS, p. 39.
- Buckley, N.E., K.L. McCoy, E. Mezey, T. Bonner, A. Zimmer, C.C. Felder, M. Glass. 2000. Immunomodulation by cannabinoids is absent in mice deficient for the cannabinoid CB (2) receptor. *Eur J Pharmacol* 396:141-149.
- Burstein, S.H., R.G. Rossetti, B. Yagen, and R.B. Zurier. 2000. Oxidative metabolism of anandamide. *Prostagl Other Lipid Med*, in press.
- Calignano, A., G. La Rana, A. Giuffrida, and D. Piomelli. 1998. Control of pain initiation by endogenous cannabinoids. *Nature* 394:277-281.
- Castellano, C., S. Cabib, A. Palmisano, V. Di Marzo, and S. Puglisi-Allegra. 1997. The effects of anandamide on memory consolidation in mice involve both D1 and D2 dopamine receptors. *Behav Pharmacol* 8:707-712.
- Conroe, P. 1998. Brain cannabinoid systems as targets for the therapy of neurological disorders, *Neurobiol Dis* 5:534-551.
- Cravatt, B.F., D.K. Giang, S.P. Mayfield, D.L. Boger, R.A. Lerner, and N.B. Gilula. 1996. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* 384:83-87.
- De Petrocellis, L., D. Melck, A. Palmisano, T. Bisogno, C. Laezza, M. Bifulco et al. 1998. The endogenous cannabinoid anandamide inhibits human breast cancer cell proliferation. *Proc Natl Acad Sci USA* 95:8375-8380.
- Deutsch, D.G. and S.A. Chin. 1993. Enzymatic synthesis and degradation of anandamide, a cannabinoid receptor agonist. *Biochem Pharmacol* 46:791-796.
- Devane, W.A., L. Hanus, A. Breuer, R.G. Pertwee, L.A. Stevenson, G. Griffin et al. 1992. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 258:1946-1949.
- Di Marzo, V. 1999. Biosynthesis and inactivation of endocannabinoids: relevance to their proposed role as neuromodulators. *Life Sci* 65:645-655.
- Di Marzo, V., T. Bisogno, L. De Petrocellis, D. Melck, P. Orlando, J.A. Wagner et al. 1999. Biosynthesis and inactivation of the endocannabinoid 2-arachidonoyl glycerol in circulating and tumoral macrophages. *Eur J Biochem* 264:258-267.
- Di Marzo, V., T. Bisogno, D. Melck, R. Ross, H. Brockie, L. Stevenson et al. 1998a.

- Interactions between synthetic vanilloids and the endogenous cannabinoid system. *FEBS Lett* 436:449-454.
- Di Marzo, V. 1998. 'Endocannabinoids' and other fatty acid derivatives with cannabimimetic properties: biochemistry and possible physiopathological relevance. *Biochim Biophys Acta* 1392:153-175.
- Di Marzo, V., M.P. Hill, T. Bisogno, A.R. Crossman, and M. Brotchie. 2000a. Enhanced levels of endogenous cannabinoids in the globus pallidus are associated with a reduction in movement in an animal model of Parkinson's disease. *FASEB J* 14:1432-1438.
- Di Marzo, V., D. Melck, T. Bisogno, and L. De Petrocellis. 1998b. Endocannabinoids: endogenous cannabinoid receptor ligands with neuromodulatory action. *Trends Neurosci* 21:521-528.
- Di Marzo, V., D. Melck, L. De Petrocellis, and T. Bisogno. 2000b. Cannabimimetic fatty acid derivatives in cancer and inflammation, *Prostaglandins Other Lipid Mediat* 61:43-61.
- Di Marzo, V., F. Berrendero, T. Bisogno, S. Gonzalez, P. Cavaliere, J. Romero et al. 2000c. Enhancement of anandamide formation in the limbic forebrain and reduction of endocannabinoid contents in the striatum of Δ^9 -tetrahydrocannabinol-tolerant rats. *J Neurochem*, 74:1627-1635.
- Di Marzo, V., C. Breivogel, T. Bisogno, D. Melck, G. Patrick, Q. Tao et al. 2000d. Neurobehavioral effects in mice of *N*-vanillyl-arachidonoyl-amide (arvanil). *Eur J Pharmacol*, in press.
- Felder, C.C., K.E. Joyce, E.M. Briley, M. Glass, K.P. Mackie, K.J. Fahey et al. 1998. LY320135, a novel cannabinoid CB1 receptor antagonist, unmasks coupling of the CB1 receptor to stimulation of cAMP accumulation. *J Pharmacol Exp Ther* 284:291-297.
- Fernandez-Ruiz, J.J., R.M. Munoz, J. Romero, M.A. Villanua, A. Makriyannis, and J.A. Ramos. 1997. Time course of the effects of different cannabimimetics on prolactin and gonadotrophin secretion: evidence for the presence of CB1 receptors in hypothalamic structures and their involvement in the effects of cannabimimetics. *Biochem Pharmacol* 53:1919-1927.
- Fox, S.H., M.P. Hill, A.R. Crossman, and J.M. Brotchie. 1999. On the role of endocannabinoids in L-dopa-induced dyskinesia. *Soc Neurosci Abstr* 25:585.17.
- Gonzalez, S., J. Manzanares, F. Berrendero, T. Wenger, J. Corchero, T. Bisogno et al. 1999. Identification of endocannabinoids and cannabinoid CB₁ receptor mRNA in the pituitary gland. *Neuroendocrinology* 70:137-145.
- Giang, D.K. and B.F. Cravatt. 1997. Molecular characterization of human and mouse fatty acid amide hydrolases. *Proc Natl Acad Sci U S A* 94:2238-2242.
- Gifford, A.N., M. Bruneus, S. Lin, A. Goutopoulos, A. Makriyannis, N.D. Volkow et al. 1999. Potentiation of the action of anandamide on hippocampal slices by the fatty acid amide hydrolase inhibitor, palmitylsulphonyl fluoride. *Eur J Pharmacol* 383:9-14.
- Giuffrida, A., L.H. Parsons, T.M. Kerr, F. Rodriguez de Fonseca, M. Navarro, and D. Piomelli. 1999. Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. *Nat Neurosci* 2:358-363.
- Goparaju, S.K., Y. Kurahashi, H. Suzuki, N. Ueda, and S. Yamamoto. 1999. Ananda-

- mide amidohydrolase of porcine brain: cDNA cloning, functional expression and site-directed mutagenesis(1). *Biochim Biophys Acta* 1441:77-84.
- Goparaju, S.K., N. Ueda, H. Yamaguchi, and S. Yamamoto. 1998. Anandamide amidohydrolase reacting with 2-arachidonoyl glycerol, another cannabinoid receptor ligand. *FEBS Lett* 422:69-73.
- Griffin, G., S.R. Fernando, R.A. Ross, N.G. McKay, M.L. Ashford, D. Shire, J.W. Huffman, S. Yu, J.A. Lainton, and R.G. Pertwee. 1997. Evidence for the presence of CB2-like cannabinoid receptors on peripheral nerve terminals. *Eur J Pharmacol* 339:53-61
- Guzman, M. and C. Sanchez. 1999. Effects of cannabinoids on energy metabolism. *Life Sci* 65:657-664.
- Hampson, A.J., L.M. Bornheim, M. Scanziani, C.S. Yost, A. T. Gray, B.M. Hansen et al. 1998. Dual effects of anandamide on NMDA receptor-mediated responses and neurotransmission. *J Neurochem* 70:671-676.
- Hanus, L., A. Gopher, S. Almog, and R. Mechoulam. 1993. Two new unsaturated fatty acid ethanolamides in brain that bind to the cannabinoid receptor. *J Med Chem* 36:3032-3034.
- Hillard, C.J. and W. B. Campbell. 1997. Biochemistry and pharmacology of arachidonoyl ethanolamide, a putative endogenous cannabinoid. *J Lipid Res* 38:2383-2398.
- Hillard, C.J., W. S. Edgemond, A. Jarrhian, and W.B. Campbell. 1997. Accumulation of N-arachidonoyl ethanolamine (anandamide) into cerebellar granule cells occurs via facilitated diffusion. *J Neurochem* 69:631-638.
- Izzo, A.A., N. Mascolo, R. Capasso, M.P. Germano, R. De Pasquale, and F. Capasso. 1999. Inhibitory effect of cannabinoid agonists on gastric emptying in the rat. *Naunyn Schmiedebergs Arch Pharmacol* 360:221-223.
- Kimura, T., T. Ohta, K. Watanabe, H. Yoshimura, and I. Yamamoto. 1998. Anandamide, an endogenous cannabinoid receptor ligand, also interacts with 5-hydroxytryptamine (5-HT) receptor. *Biol Pharm Bull* 21:224-226.
- Kunos, G., Z. J arai, K. Varga, J. Liu, L. Wang, and J. Wagner. 2000. Cardiovascular effects of endocannabinoids-The plot thickens. *Prostagl other Lipid Med*, in press.
- Lambert, D.M. and V. Di Marzo. 1999. The palmitoylethanolamide and oleamide enigmas: are these two fatty acid amides cannabimimetic? *Curr Med Chem* 6: 757-773.
- Ledent, C., O. Valverde, G. Cossu, F. Petitet, J.F. Aubert, F. Beslot et al. 1999. Unresponsiveness to cannabinoids and reduced additive effects of opiates in CB1 receptor knockout mice. *Science* 283:401-404.
- Maccarrone, M., M. van der Stelt, A. Rossi, G.A. Veldink, J.F. Vliedhart, and A. Finazzi-Agro'. 1998. Anandamide hydrolysis by human cells in culture and brain. *J Biol Chem* 273:32332-32339.
- Maccarrone, M., M. Bari, T. Lorenzon, T. Bisogno, V. Di Marzo, and A. Finazzi-Agro'. 2000. Anandamide uptake by human endothelial cells and its regulation by nitric oxide. *J. Biol. Chem.*, 275:13484-13492.
- Mackie, K., W.A. Devane, and B. Hille. 1993. Anandamide, an endogenous cannabinoid, inhibits calcium currents as a partial agonist in N18 neuroblastoma cells. *Mol Pharmacol* 44:498-503.

- Mallet, P.E. and R.J. Beninger. 1996. The endogenous cannabinoid receptor agonist anandamide impairs memory in rats. *Behav Pharmacol* 7:276-284.
- Manzanares, J., J. Corchero, J. Romero, J.J. Fernandez-Ruiz, J.A. Ramos, and J.A. Fuentes. 1999. Pharmacological and biochemical interactions between opioids and cannabinoids. *Trends Pharmacol Sci* 20:287-294.
- Martin, B.R. and A.H. Lichtman. 1998. Cannabinoid transmission and pain perception. *Neurobiol Dis* 5:447-461.
- Martin, B.R., R. Mechoulam, and R.K. Razdan. 1999. Discovery and characterization of endocannabinoids. *Life Sci* 65:573-595.
- Matsuda, L.A. 1997. Molecular aspects of cannabinoid receptors. *Crit Rev Neurobiol* 11:143-166.
- Mechoulam, R., E. Frider, and V. Di Marzo. 1998. Endocannabinoids. *Eur J Pharmacol* 359:1-18.
- Mechoulam, R., S. Ben-Shabat, L. Hanus, M. Ligumsky, N.E. Kaminski, A.R. Schatz et al. 1995. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol* 50:83-90.
- Mechoulam, R., E. Frider, L. Hanus, T. Sheskin, T. Bisogno, V. Di Marzo et al. 1997. Anandamide may mediate sleep induction. *Nature* 389:25-26.
- Melck, D., T. Bisogno, L. De Petrocellis, H.H. Chuang, D. Julius, M. Bifulco et al. 1999. Unsaturated long-chain N-acyl-vanillyl-amides (N-AVAMs): vanilloid receptor ligands that inhibit anandamide-facilitated transport and bind to CB1 cannabinoid receptors. *Biochem Biophys Res Commun* 262:275-284.
- Melck, D., L. De Petrocellis, P. Orlando, T. Bisogno, C. Laezza, M. Bifulco et al. 2000. Suppression of *trk* and prolactin receptor levels by endocannabinoids leads to inhibition of human breast and prostate cancer cell proliferation. *Endocrinology* 141:118-126.
- Molina-Holgado, F., A. Lledo, and C. Guaza, C. 1997. Anandamide suppresses nitric oxide and TNF-alpha responses to Theiler's virus or endotoxin in astrocytes. *Neuroreport* 8:1929-1933.
- Ouyang, Y., S.G. Hwang, S.H. Han, and N.E. Kaminski. 1998. Suppression of interleukin-2 by the putative endogenous cannabinoid 2-arachidonyl-glycerol is mediated through down-regulation of the nuclear factor of activated T cells. *Mol Pharmacol* 53:676-683.
- Paria, B.C., S.K. Das, and S.K. Dey. 1995. The preimplantation mouse embryo is a target for cannabinoid ligand-receptor signaling. *Proc Natl Acad Sci USA* 92:9460-9464.
- Paria, B.C., W. Ma., D.M. Andrenyak, P.C. Schmid, H.H. Schmid, D.E. Moody et al. 1998. Effects of cannabinoids on preimplantation mouse embryo development and implantation are mediated by brain-type cannabinoid receptors. *Biol Reprod* 58:1490-1495.
- Parolaro, D. 1999. Presence and functional regulation of cannabinoid receptors in immune cells. *Life Sci* 65:637-644.
- Pertwee, R.G. and S.R. Fernando. 1996. Evidence for the presence of cannabinoid CB1 receptors in mouse urinary bladder. *Br J Pharmacol* 118:2053-2058.
- Rinaldi-Carmona, M., F. Barth, M. Héaulme, D. Shire, B. Calandra, C. Congy et al.

1994. SR 141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett* 350:240-244.
- Rinaldi-Carmona, M., F. Barth, J. Millan, J.M. Derocq, P. Casellas, C. Congy et al. 1998. SR 144528, the first potent and selective antagonist of the CB2 cannabinoid receptor. *J Pharmacol Exp Ther* 284:644-650.
- Romero, J., R. de Miguel, E. Garcia-Palomero, J.J. Fernandez-Ruiz, and J.A. Ramos. 1995. Time-course of the effects of anandamide, the putative endogenous cannabinoid receptor ligand, on extrapyramidal function. *Brain Res* 694:223-232.
- Schmid, P.C., B.C. Paria, R.J. Krebsbach, H.H. Schmid, and S.K. Dey. 1997. Changes in anandamide levels in mouse uterus are associated with uterine receptivity for embryo implantation. *Proc Natl Acad Sci USA* 94:4188-4192.
- Schuel, H., E. Goldstein, R. Mechoulam, A.M. Zimmerman, and S. Zimmerman. 1994. Anandamide (arachidonylethanolamide), a brain cannabinoid receptor agonist, reduces sperm fertilizing capacity in sea urchins by inhibiting the acrosome reaction. *Proc Natl Acad Sci USA* 91:7678-7682.
- Sugiura, T., T. Kodaka, S. Nakane, T. Miyashita, S. Kondo, Y. Suhara et al. 1999. Evidence that the cannabinoid CB1 receptor is a 2-arachidonoylglycerol receptor. Structure-activity relationship of 2-arachidonoylglycerol, ether-linked analogues, and related compounds. *J Biol Chem* 274:2794-2801.
- Sugiura, T., S. Kondo, S. Kishimoto, T. Miyashita, S. Nakane, T. Kodaka et al. 2000. Evidence that 2-arachidonoylglycerol but not *N*-palmitoylethanolamine or anandamide is the physiological ligand for the cannabinoid CB2 receptor. *J Biol Chem* 275:605-612.
- Sugiura, T., S. Kondo, A. Sukagawa, S. Nakane, A. Shinoda, K. Itoh et al. 1995. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem Biophys Res Commun* 215:89-97.
- Tanda, G., F.E. Pontieri, and G. Di Chiara. 1997. Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu 1 opioid receptor mechanism. *Science* 276:2048-2050.
- Varga, K., J.A. Wagner, D.T. Bridgen, and G. Kunos. 1998. Platelet- and macrophage-derived endogenous cannabinoids are involved in endotoxin-induced hypotension. *FASEB J* 12:1035-1044.
- Wagner, J.A., K. Varga, E.F. Ellis, B.A. Rzigalinski, B.R. Martin, and G. Kunos. 1997. Activation of peripheral CB1 cannabinoid receptors in haemorrhagic shock. *Nature* 390:518-521.
- Walker, J.M., S.M. Huang, N.M. Strangman, K., Tsou, and M.C. Sanudo-Pena. 1999. Pain modulation by release of the endogenous cannabinoid anandamide. *Proc Natl Acad Sci USA* 96:12198-12203.
- Wickens, A.P. and R.G. Pertwee. 1993. Δ^9 -tetrahydrocannabinol and anandamide enhance the ability of muscimol to induce catalepsy in the globus pallidus of rats. *Eur J Pharmacol* 250:205-208.
- Willoughby, K.A., S.F. Moore, B.R. Martin, and E.F. Ellis. 1997. The biodisposition and metabolism of anandamide in mice. *J Pharmacol Exp Ther* 282:243-247.
- Zimmer, A., A.M. Zimmer, A. Hohmann, M. Herkenham, and T. Bonner. 1999. Increased mortality, hypoactivity and hypoalgesia in cannabinoid CB1 receptor knockout mice. *Proc Natl Acad Sci USA* 96:5780-5785.

Zygmunt, P.M., J. Petersson, D.A. Andersson, H. Chuang, M. Sørsgard, V. Di Marzo et al. 1999. Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature* 400:452-457.

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