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COMMENTARY

RELATIONSHIPS BETWEEN EICOSANOIDS AND CANNABINOIDS

ARE EICOSANOIDS CANNABIMIMETIC AGENTS?

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The first indication that there might be a relationship between cannabinoids and eicosanoids appeared in the literature more than 20 years ago [1]. In the intervening period, both fields have grown considerably, and, during that time, evidence has slowly accumulated suggesting that there is, in fact, a connection between these two classes of biologically active substances. Two general hypotheses have been put forth as the basis for such a relationship. One, which is largely theoretical, rests on the structural similarities between both classes of molecules [2]. The other, which is of a functional nature, states that many of the actions of the cannabinoids can be best understood by their abilities to regulate eicosanoid biosynthesis [3]. The second hypothesis is supported by a number of experimental observations utilizing a variety of systems ranging from subcellular fractions to human subjects.

At one time, the term cannabinoid was understood to mean a group of structurally related substances found in the species *Cannabis sativa*. The most studied member of this group is THC,§ which is the principal psychotropic constituent of the many and varied preparations of this plant (structure shown in Fig. 1). Many synthetic "cannabinoids" have been prepared, motivated by the goal of discovering a structure that will show minimal psychoactivity and still retain some of the very useful medicinal properties of the drug, which have been known almost as long as recorded history. Figure 1 also illustrates several examples of synthetic cannabinoids, and, while some have obvious similarities to THC, others bear no apparent structural relationship. What they do have in common, however, is the ability to bind to the same receptors and to produce a similar spectrum of biological activities [4]. A more relevant term, cannabimimetic, has been suggested [5] to describe these substances that share similar activities rather than a common botanical origin.

Substances now called eicosanoids also evolved from a more limited group, namely, the prostaglandins. Originally a very small class, it was recognized that their common precursor, arachidonic (eicosatetraenoic) acid, served several biosynthetic pathways, giving rise to a large and varied family of potent modulators of cellular activity. A few examples of the more frequently encountered eicosanoids are shown in Fig. 2. Products of eicosatrienoic and eicosapentaenoic acid are also known and considered members of the eicosanoid family, as are the two-carbon higher homologs of the twenty-carbon series.

A metabolite of arachidonic acid called anandamide, which has been found in brain [6], could, at least in a formal sense, be considered both a cannabinoid and an eicosanoid. Its structure is the ethanolamide derivative of arachidonic acid and is shown in Fig. 1. Due to its ability to bind to cannabinoid receptors [7, 8] and because of biological actions similar to many of the cannabinoids [7–9], it has been the object of much interest as a candidate for an endogenous cannabinoid. Furthermore, it now appears that its cellular biosynthesis has much in common with other members of the eicosanoid family [10]. These points will be discussed more fully below.

Receptor binding studies

Specific, high-affinity tissue binding sites are known for both the eicosanoids and the cannabinoids [11–13]. All of the putative receptors for both groups that have been reported thus far are members of the seven-transmembrane superfamily, which are coupled to heterotrimeric GTP-binding proteins. An obvious question arises, namely, can any of the eicosanoids, other than anandamide, bind to either the brain cannabinoid receptor (CB-1) or its peripheral counterpart (CB-2)? A related question concerns possible binding activity for any of the cannabimimetics to eicosanoid receptors. A useful result from such studies could be the identification of other eicosanoic acid moieties that might be coupled to ethanolamine to give novel cannabinoid receptor ligands.

Some effort has been made in this direction but with little success. Using a washed P_2 membrane preparation from rat brain, and [^{3}H]CP-55940 as the ligand, a number of eicosanoids, as well as other molecules, were screened for heterologous displacement [14]. While none of the eicosanoids potently displaced the tritiated ligand, as a group they showed more activity than any of the other molecules. It is interesting to note that among

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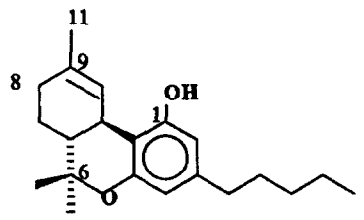
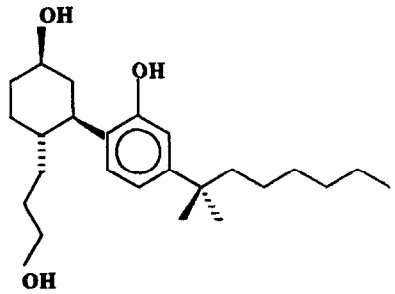
§ Abbreviations: THC, Δ^9-tetrahydrocannabinol; PG, prostaglandin; HETE, hydroxyeicosatetraenoic acid; cAMP, cyclic AMP; LT, leukotriene; EA, ethanolamine; PL, phospholipase; DAG, diacylglyceride; PE, phosphatidylethanolamine; PBQ, parabenzoquinone; EET, epoxyeicosatrienoic acid; and DHET, dihydroxyeicosatetraenoic acid.



Δ^9 - Tetrahydrocannabinol (THC)



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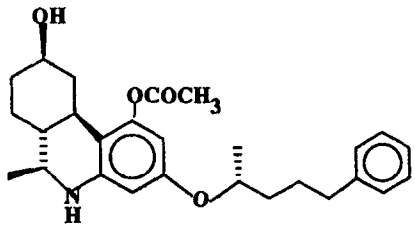

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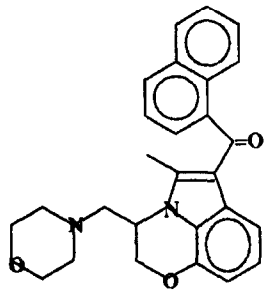

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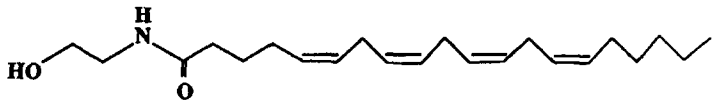

Anandamide

Fig. 1. Examples of exogenous, synthetic and endogenous cannabimimetic agents.

the eicosanoids tested the HETEs were the substances that were most active. By contrast, the prostanoids examined showed virtually no tendency to bind to the rat brain receptor preparation. This is reasonable since, by analogy with anandamide itself, acyclic structures might be expected to be better candidates for novel endogenous cannabinoids.

The above experiment was carried one step further in a recent report [15] where the ethanolamides of several prostaglandins were prepared and screened for binding. Derivatives of PGE_2, PGA_2, PGB_1 and PGB_2 were tested at concentrations of 10 and 100 μM against CP-55940 in the brain membrane preparation. No effect was seen at the higher concentration, and only small amounts of displacement were reported at 10 μM with all four of the PG-ethanolamides. Possibly lower concentrations would have been more effective.

Based on the earlier results with underivatized HETEs, it would probably be more meaningful to examine the binding properties of substances such as 12(*S*)- or 15(*S*)-hydroxyeicosatetraenoic acid ethanolamide. Such molecules would also appear to be better analogs for THC by virtue of the hydroxy group, which, in the case of THC, is generally considered essential for most of its actions. It is interesting to note that anandamide can be metabolized by cytochrome P450 to several oxygenated products, suggesting that such types of compounds may indeed be endogenous substances [16].

Cannabinoid–eicosanoid structural overlap

A comparison of the structures of PGE_2 and levonantradol, a synthetic cannabinoid, revealed some interesting similarities [2]. In particular, it was shown that the distances between the two hydroxyl groups on PGE_2 and the hydroxyl and the acetoxy groups on levonantradol are quite similar. Since these are believed to be important pharmacophores for both substances, the comparison could be of significance. This observation would predict that cannabinoids and PGE_2 might compete for the same binding sites; however, this does not seem to be the case when tested with the brain cannabinoid receptor [14]. It might also be of interest to see whether cannabinoids have any affinity for one or more of the PGE receptors.

In view of the importance of the ethanolamide group in anandamide, it would be more relevant to compare analogous eicosanoid derivatives with the cannabinoids. As has been discussed above, the initial attempt in this direction met with little success [15]. Because of the diverse structural and biological properties of the eicosanoids, it is reasonable to assume that only certain members of the group would show significant similari-

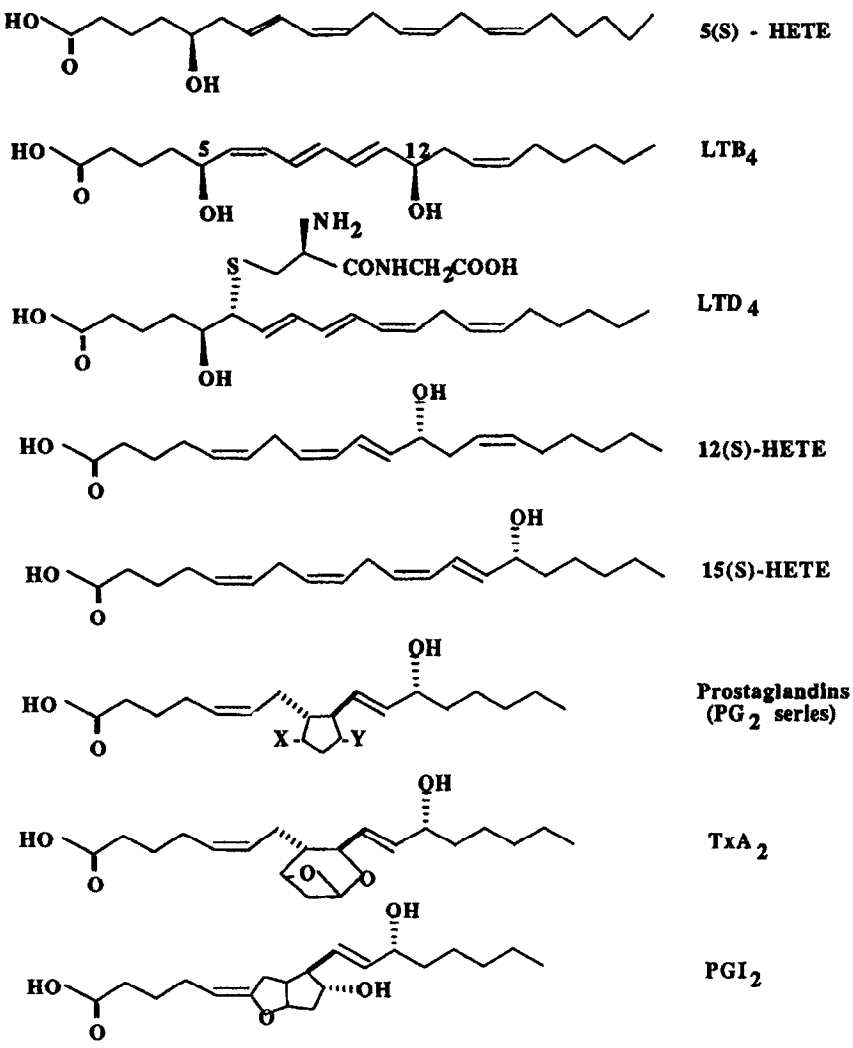

Fig. 2. Structures of several of the more commonly encountered eicosanoids. Abbreviations: HETE, hydroxyeicosatetraenoic acid; LT, leukotriene; Tx, thromboxane; PG, prostaglandin (X,Y = hydroxyl or keto); and PGI_2, prostacyclin.

ties with any of the cannabinoids. Moreover, many pairs of eicosanoids, such as prostacyclin (PGI_2) and thromboxane A_2 (TxA_2), show opposing biological actions in certain systems, emphasizing their heterogeneity. Thus, the results discussed above [14, 15] must be viewed as inconclusive.

We have been interested in seeing whether the stereotopographies of other eicosanoid-ethanolamide derivatives would show similarity to any of the cannabinoids. Two such comparisons are shown in Fig. 3. In the first, a synthetically modified derivative of THC, 11-hydroxy-3-(1′,1′-dimethylheptyl)-Δ^8-THC (Fig. 3a), is compared with anandamide (Fig. 3e). Some structural overlap is seen; however, the important hydroxy pharmacophores in the cannabinoid are not represented in anandamide. This could help explain the generally low potencies thus far reported for anandamide.

A better overlap is obtained when LTB_4-EA (Fig. 3f) is compared with CP-55940 (Fig. b), a potent synthetic cannabinoid that has been used as the tritiated ligand in many of the receptor binding studies. In such a comparison, the 12-hydroxy group of LTB_4-EA overlaps the phenolic hydroxyl of CP-55940 and the terminal hydroxyl of LTB_4-EA is superimposed on the 9β-hydroxyl of the cannabinoid (Fig. 3d). Interestingly, even the side-chain hydroxyl of CP-55940 is in close proximity to the 5-hydroxyl of LTB_4-EA. With regard to this point, our model would predict that a derivative of CP-55940 with a hydroxybutyl side chain should be even more active than the parent compound, since there would be a complete overlap of these groups in such a homolog. This substance is known and, in fact, shows approximately 25% greater potency in the PBQ test for analgesia in mice [18].

The critical nature of the ethanolamide group is evidenced by at least three studies [7, 15, 19], and its relevance to LTB_4 derivatives has also been observed [15]. The dimethylamide of LTB_4 is a known substance, and its low affinity for the brain cannabinoid receptor was reported [15]. What is noteworthy is the fact that it displayed any binding activity. One of the allowable modifications in the anandamide structure is the addition of

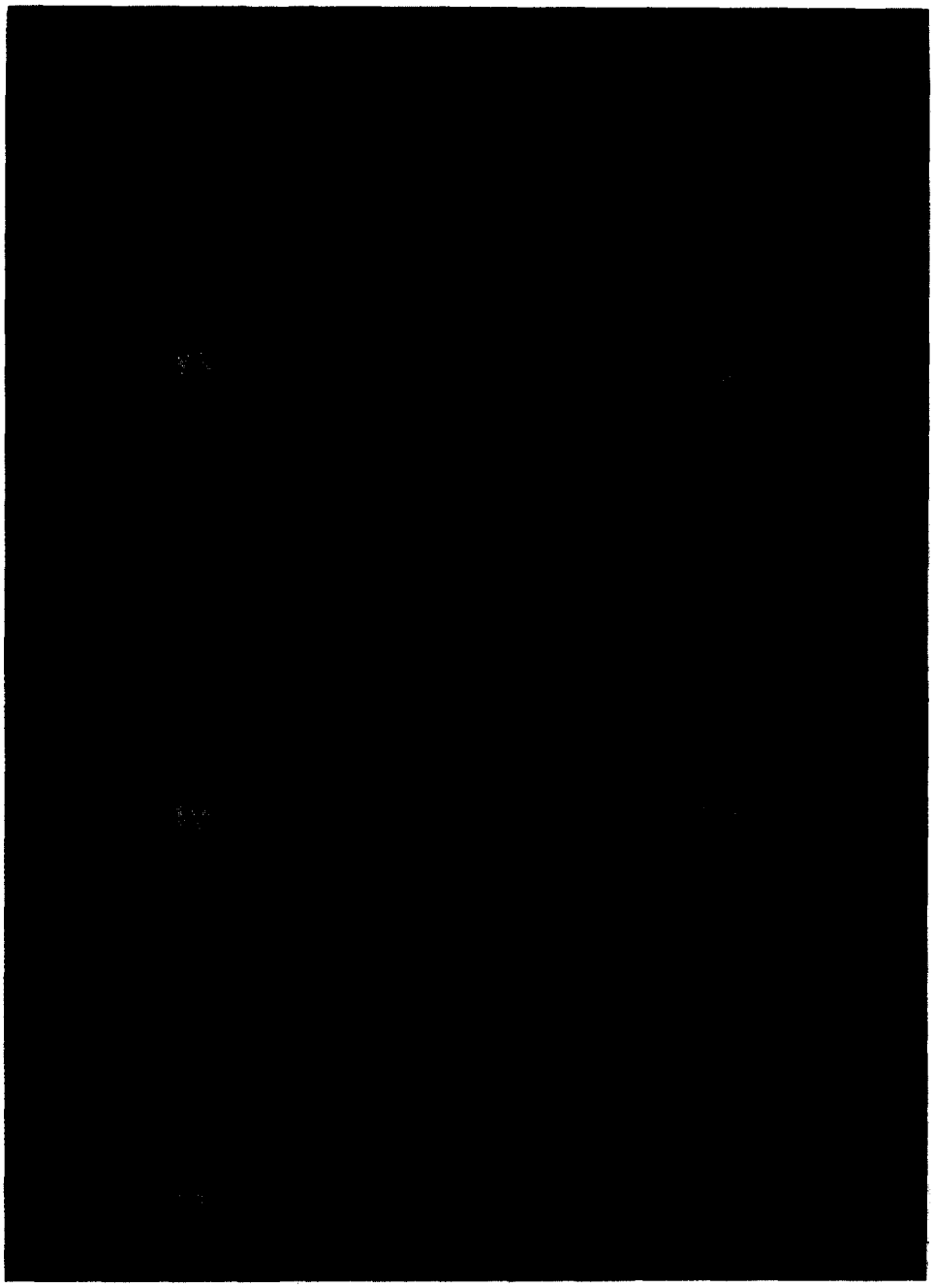

Fig. 3. Comparison of structures of selected cannabinoids and eicosanoids. All structures were generated using simulated annealing through the Biosym Molecular Modeling package. Both A and B were generated using dihedral angles from the crystal structure of THC [17]. To obtain the superimposition of E with A and F with B, individual bonds were rotated. Once a good fit was obtained, the structure was minimized using conjugate gradient minimization. (A) 11-hydroxy-3-(1′,1′-dimethylheptyl)-Δ^8-THC; (B) CP-55940; (C) A + E; (D) B + F; (E) anandamide; and (F) leukotriene B_4-ethanolamide.

a methylene group to the amine portion, which actually causes a small increase in the binding affinity [7]. Such a homolog would provide even better overlap with A (Fig. 3) than anandamide, suggesting that the orientations shown are relevant to the production of cannabimimetic activity. The addition of two methyl groups at the carbon alpha to the carbonyl group in anandamide also increases binding [19] and, interestingly, overlaps the dimethyl group on the ring structure (position 6) of A (Fig. 3).

The potential occurrence of eicosanoid-ethanolamide derivatives, such as LTB_4-EA, poses the vital question of their biosynthetic origins. One possible route would involve amide formation from a particular eicosanoid, e.g., LTB_4, and ethanolamine mediated by a specific synthase. Another pathway could utilize anandamide as the precursor, which would then be subjected to oxidative metabolism either by the known cyclooxygenases, lipoxygenases, etc., or by enzymes specific for anandamide. Some evidence for P450 action on anandamide has

been reported [16]; however, this would seem to be an area where significant findings could be made.

Similarities in biosynthesis between anandamide and the eicosanoids

The most obvious similarity in the biosynthesis of anandamide and the eicosanoids is the requirement for free arachidonic acid (Fig. 4). In the case of the eicosanoids, a large body of literature describes the factors and events that promote the hydrolytic release of arachidonic acid from various phospholipid cellular pools [11]. A common sequence involves receptor-mediated stimulation of either phospholipase C (PLC) or phospholipase D (PLD) to give a DAG intermediate. In the case of a DAG containing arachidonic acid, subsequent lipase action(s) would provide free arachidonate for eicosanoid synthesis. An alternative is possible where DAG acts as a protein kinase C stimulator that ultimately results in cytosolic phospholipase A_2 ($cPLA_2$) action on phospholipid to yield arachidonic acid directly. A number of factors, such as intracellular calcium concentrations and expression levels of the various mediators, provide opportunities for the cell to regulate the process and allow physiological control to be maintained.

In the synthesis of anandamide, much less is known concerning either pathways or regulation. The early findings indicate that mechanisms similar to those for the eicosanoids probably are involved [10]. Moreover, it has been reported that anandamide production is neither CoA nor ATP dependent [20]. THC-stimulated anandamide synthesis in cultured neuroblastoma cells [10] appears to be mediated by phospholipases in a manner similar to THC-stimulated eicosanoid production in other cell types [21]. Whether the biosynthetic processes occur in the same cellular compartments, and compete for the same sources of free arachidonic acid, is not known; however, this is an important point that warrants further investigation. Thus, THC-stimulated anandamide and the classical eicosanoids probably arise through similar mechanisms up to the point where free arachidonic acid is produced (Fig. 4). The pathways then diverge with the eicosanoids following oxidative routes involving a number of alternative mediators. Anandamide, on the other hand, is coupled to ethanolamine by means of a yet to be isolated synthase.

A somewhat different pathway for calcium ionophore-stimulated anandamide synthesis in cultured neurons has been reported recently [22]. The immediate precursor in this system is *N*-arachidonyl-PE, from which anandamide is liberated by the action of a PLD-type mediator. Earlier studies demonstrated the existence of *N*-acylphosphatidylamides containing other long-chain fatty acids [23], and more recently other authors [22] gave evidence for the arachidonyl analog. This somewhat novel mechanism appears to be quite different from the pathway mentioned above for THC-stimulated anandamide synthesis (Fig. 5). The apparently high Ca^{2+} requirement for this route [24] suggests that it may have a greater role in pathological circumstances, such as ischemia, than in situations where anandamide could have a neuromodulatory function. However, the differences may not be as great if the question of the origin of *N*-arachidonyl-PE is considered. Specifically, it is reasonable to assume that free arachidonic acid is required at some stage for its synthesis. Thus, it is conceivable that arachidonate may be released from the 2-position of other phospholipids, either through a diglyceride intermediate or directly mediated by phosphorylated $cPLA_2$. The free arachidonate would then react with PE to produce the *N*-acylated derivative (Fig. 5). This route is supported by the findings of Bachur *et al.* [23] for the synthesis of palmitoylethanolamide in rat liver. It is interesting to note that palmitoylethanolamide, which is found in brain, liver and muscle, has been reported to have antiinflammatory properties [23]. The importance of the various possible pathways may depend on the particular cell type and the agonists or stimuli involved. In any case, these questions should provide the basis for interesting and significant future research.

A further analogy between anandamide and certain eicosanoids is the ability to modulate arachidonic acid metabolism [25]. For example, leukotrienes such as LTC_4 and LTD_4 are able to cause the release of free arachidonic acid from human endothelial cells [26], and hepoxilin A_3, a 12-lipoxygenase product, promotes release in human neutrophils [27]. Prostaglandins such as PGD_2, PGE_2 and PGI_2 can down-regulate release by virtue of their ability to elevate cAMP levels [25, 28] so

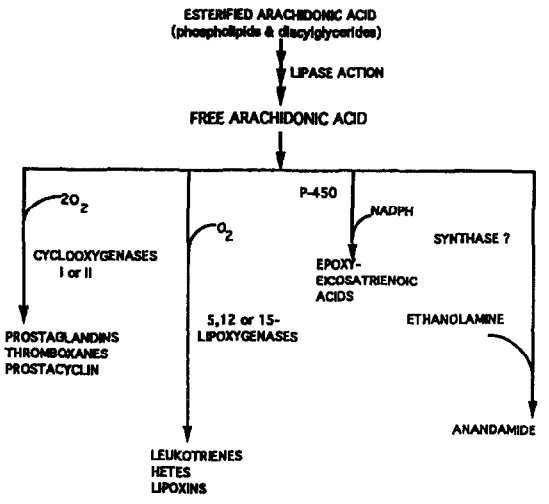

Fig. 4. Biosynthesis of anandamide and the classical eicosanoids.

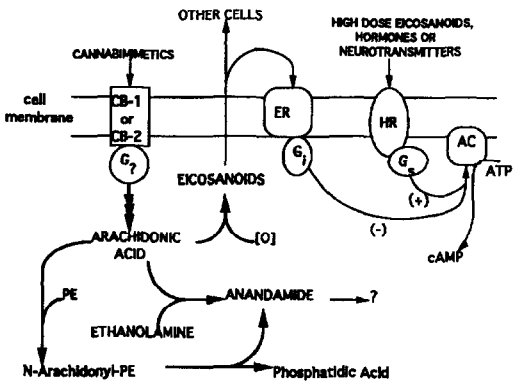

Fig. 5. Proposed mechanism for the action of cannabimimetic agents. Abbreviations: CB, cannabinoid receptor; ER, eicosanoid receptor; HR, hormone receptor; PE, phosphatidylethanolamine; AC, adenylyl cyclase; and G, heterotrimeric GTP binding protein.

that these "feedback" effects may operate in either direction. There is some evidence that anandamide may also participate in similar processes. Wartmann *et al.* [29] observed that, in WI-38 cells, anandamide causes a dose-related release of arachidonic acid that was blocked by pertussis toxin pretreatment. It also promoted a comparable activation of $cPLA_2$ as evidenced by phosphorylation in a gel-shift assay, suggesting that the mechanism was similar to other agonist-stimulated eicosanoid syntheses.

Finally, there seem to be certain parallels between the further transformations of anandamide and arachidonic acid. In particular, the ability to be oxygenated by hepatic P450s is common to both substances [11, 16]. It is not known whether the same P450 isotypes are involved in both situations; however, it would not be too surprising if this turns out to be the case. Deutsch and Chin [30] reported that anandamide is rapidly hydrolyzed back to ethanolamine and arachidonic acid. This raises the possibility that some of the oxygenated anandamides could serve as precursors for eicosanoids such as the EETs, hepoxilins, and DHETs. The role of such a novel route for eicosanoid production would only be a subject of conjecture at this point.

Regulatory effects on adenylyl cyclase

Both cannabinoids and eicosanoids are able to modulate the activity of adenylyl cyclase. A number of reports have demonstrated that exogenous cannabinoids [31–37], as well as anandamide [7], can inhibit agonist-stimulated cAMP formation in a variety of model systems. Evidence has been published [35] that this action is mediated through an effect on an inhibitory GTP-binding protein (G_i), and structure–activity profiles suggest that such a mechanism may explain the psychotropic properties of cannabinoids [34]. Close correlations between cannabinoid receptor binding and inhibition of agonist-induced adenylyl cyclase activity have been observed [34], lending further support to this mechanism.

It has long been known that prostaglandins have a stimulatory effect on adenylyl cyclase [25]. It has also been reported that the stimulation of cyclase by 1 μM PGI_2 can be inhibited by THC, levonantradol and carbachol in neuroblastoma cells [31]. In other systems, such as platelets, more complete dose–response studies revealed that the effect of prostaglandins on cAMP is, in fact, biphasic [38]. At concentrations below 0.5 μM, PGE_2 was inhibitory, whereas higher levels caused activation. Low concentrations (0.1 to 100 nM) of several prostaglandins in the E series were found to inhibit arginine vasopressin-induced adenylyl cyclase activity in rabbit kidney collecting tubule cells [28]. The authors suggested that the eicosanoids act through a PGE receptor coupled to G_i to inhibit the arginine vasopressin effect on its receptor that is coupled to a stimulatory GTP-binding protein (G_s). This latter model is similar to the one proposed for cannabinoid inhibition of hormonally stimulated adenylyl cyclase cited above [31–36].

Based on the foregoing reports, it is possible to suggest a model that could include both the "cAMP hypothesis" and the "prostaglandin hypothesis" in a mechanism for cannabinoid action. Figure 5 outlines this putative mechanism that involves the following sequence of events. The initial step is the binding of a cannabinoid agonist to either the CB-1 or CB-2 receptor, which is coupled to a G-protein ($G_?$) that can activate the release of free arachidonic acid. This could result in the production of any of the classical eicosanoids by an oxidative pathway, or the synthesis of anandamide through synthase-mediated amide formation utilizing arachidonate and ethanolamine. The eicosanoid would then be secreted and bind to a receptor on the same or a nearby cell. At low concentrations, eicosanoid receptors coupled to G_i would be involved, leading to an inhibition of G_s-stimulated adenylyl cyclase activity as has been reported [28, 38]. In situations where high levels of eicosanoid are generated, hormone receptors coupled to G_s may become involved, resulting in stimulation of cAMP synthesis. A feature of such a model is that it might explain the occurrence of biphasic or bell-shaped dose–response relationships frequently seen in cannabinoid pharmacology.

The mechanism described above includes a route that would be expected to be sensitive to inhibitors of cyclooxygenase, one of the mediators of eicosanoid synthesis. Such an effect has, in fact, been reported for high-dose cannabinoid-stimulated adenylyl cyclase activity [39]. Both indomethacin and aspirin, as well as PLA_2 inhibitors, were effective in blocking the cannabinoid response in mouse cerebral cortical homogenates. While this high-dose pathway may not be important in the *in vivo* actions of cannabinoids, it does provide support for the mechanism shown in Fig. 5. In vivo cannabinoid responses such as mouse catalepsy, the hot plate test for antinociception, and others respond to the manipulation of eicosanoid levels [40–44]; however, data on adenylyl cyclase activity in these systems have not been reported. Nevertheless, it seems reasonable to suggest that at least part of the proposed mechanism may account for the observed activities.

Is anandamide an eicosanoid, a cannabinoid, or both?

The question posed above represents more than a matter of semantics; it contains implications for understanding of the character and role of anandamide as a putative neuroregulator. Such a view, that anandamide is a novel eicosanoid, would provide interesting and possibly productive insights into the actions of anandamide in the brain. Because of the similarities of its activities to those of the exogenous cannabinoids, as well as its ability to bind to the cannabinoid receptors, anandamide can be considered a cannabinoid. Its structural features and its biosynthetic origins, however, suggest an eicosanoid character. A comparison of the *in vivo* actions of both groups of substances would reveal a number of similarities. Because of the abundance and variety of these actions it could be argued that purely coincidental properties would result. Nevertheless, the facts are suggestive and worthy of consideration.

A difficulty with, or perhaps an explanation for, the suggested similarities between cannabinoids and eicosanoids is the recent report that THC stimulates anandamide synthesis in intact neuroblastoma cells [10]. Of course it has been known for some time that THC induces the synthesis of classical eicosanoids in a number of experimental models [3]. Thus, it could be suggested that the similarities in activities are a manifestation of the stimulatory action of THC on arachidonic acid metabolism and anandamide synthesis. Left unanswered is the somewhat perplexing issue that THC and anandamide appear to bind to the same sites. Perhaps a major

effect of anandamide, like THC, is to stimulate the production of eicosanoids including anandamide itself.

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