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COMMENTARY

SEARCH FOR ENDOGENOUS LIGANDS OF THE CANNABINOID RECEPTOR

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The literature in chemistry and pharmacology on plant cannabinoids and their synthetic analogs, and on their clinical effects, is vast and well reviewed [1–4]. Since the first cannabinoid receptor and the first endogenous ligand (anandamide) were described in 1988 and 1992, respectively [5, 6], a sizable amount of data on them has also accumulated. Yet, we still know nothing of the roles the cannabinoid receptors and their endogenous ligands play in the body. In the present review, we describe the road leading to the discovery of the anandamides, and their biochemical and pharmacological properties; we also speculate on their physiological roles.

The cannabinoids

Many of the actions of cannabis preparations were known in antiquity. For example, in the Assyrian Empire, which disintegrated around 627 B.C., cannabis was used "to take away the mind" as well as to treat neurological conditions. Although the use of cannabis, like that of opium, continued over the millennia, research on it over the past 150 years has lagged behind that of the opiates. This was probably due to the chemical nature of the active constituent: while morphine could be easily separated, purified and crystallized as a salt, Δ^9-THC§ (Fig. 1) is present in a complicated mixture of related compounds (cannabinoids) from which it was separated only when appropriate techniques became available. Indeed, Δ^9-THC was obtained in pure form and its structure was elucidated only in 1964 [7]. With the advent of facile synthetic routes and of labeled cannabinoids, Δ^9-THC metabolism, and that of the closely related Δ^8-THC, could be investigated. Numerous, largely monohydroxylated products were found to be the primary metabolites [8]. The 11-hydroxy-Δ^9- and -Δ^8-THCs (Fig. 1) appear to be the predominant components. They exhibit essentially the same pharmacological profiles as the parent compounds. The next metabolic steps lead to the respective carboxylic acids (Fig. 1), which are not

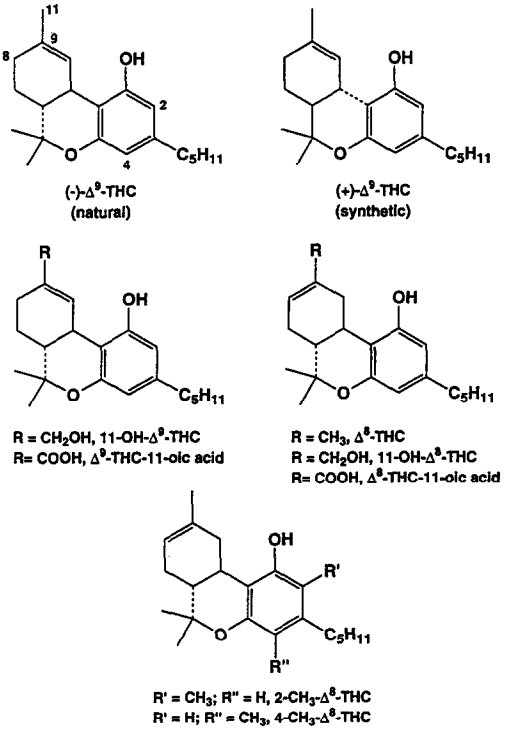

Fig. 1. Structures of the enantiomers of Δ^9-THC and related compounds.

cannabimimetic; however, they persist in the body for many weeks [8, 9].

Although the cannabinoids exert pharmacological effects on numerous organ systems, the primary interest has been in their effects on the central nervous system [3, 10]. In humans, they produce initial euphoria followed by periods of sedation. Often characterized as hallucinogens, they rarely produce visual or auditory hallucinations. Cannabis is best characterized for its alterations in sensory perception, frequently interfering with estimated time elapse and impairment of short-term memory. There have been numerous efforts to characterize cannabis-induced motor impairment. The general conclusion is that high doses impair performance of highly complex tasks, whereas low doses appear to

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§ Abbreviations: THC, tetrahydrocannabinol; SAR, structure–activity relationship; PMSF, phenylmethylsulfonyl fluoride.

have little influence on performance of simple tasks. Cannabinoids have also been studied extensively in numerous animal models with many of the actions apparently mimicking those in humans. Some of the most notable actions include motor stimulation and depression, hypothermia, analgesia, catalepsy, and a discriminative stimulus. In addition, unique behavioral syndromes have been described in dogs and monkeys.

The mode of action of the cannabinoids has been discussed since the mid-1970s. Lawrence and Gill [11] pointed out that in many respects the pharmacology of cannabis paralleled that of the general anesthetics without, however, producing surgical anesthesia. The high lipophilicity of THC was assumed to underlie many of its pharmacological actions. The absence of actual anesthesia was explained by assuming that "there is too great a physicochemical disparity between it and biological membranes into which it is inserted for a volume fraction to be achieved sufficient to produce the phenomenon of full anesthesia."

Some experimental work has given support to these ideas. It was found that Δ^9-THC increased the fluidity of the liposome bilayer in a manner comparable to that produced by the steroid anesthetic alphaxolone and the volatile anesthetic halothane. However, Δ^9-THC produced considerably less fluidization than alphaxolone, thus explaining the lack of clinical anesthesia. The psychotropically inactive cannabidiol was found to produce an opposite effect—a decrease of the molecular disorder of the lipid bilayer. It was concluded that "as the liposomal membrane is apparently able to discriminate between the various cannabinoids in a way similar to the nerve cell, it is unnecessary to postulate the existence of a more complex macromolecular receptor substance to account for the observed structure/activity relationships" [11]. However, other properties, such as the SARs and some biochemical effects (in particular the inhibition of adenylyl cyclase activity; see below) pointed in a different direction.

The SARs in the cannabinoid series have been investigated in considerable depth (for reviews see Refs. 1, 2 and 12). Minor changes in structure have been shown to cause major changes in activity. Thus, 2-methyl-Δ^8-THC is a potent cannabimimetic, whereas 4-methyl-Δ^8-THC lacks activity (Fig. 1); hexahydrocannabinol (9-methyl equatorial) is much more active than the epimer in which the 9-methyl is axial. Such major changes of activity following relatively minor chemical modification are usually seen with compounds whose mode of action is via receptors rather than by changes in membrane fluidization. Recently, Reggio *et al.* [13] proposed a model of steric interference by cannabinoids at the receptor site, which can explain many of the SAR observations.

The complex nature of the behavioral and neurochemical effects of cannabinoids makes it highly plausible that these agents exert an array of actions on the brain, some of which could include non-specific effects on membranes as discussed above. The fact that sedation is produced by numerous classes of centrally acting compounds with no apparent common neurochemical action serves as an illustration. Additionally, cannabinoid analogs that are quite potent in some, but not all, of their effects [2] have been synthesized.

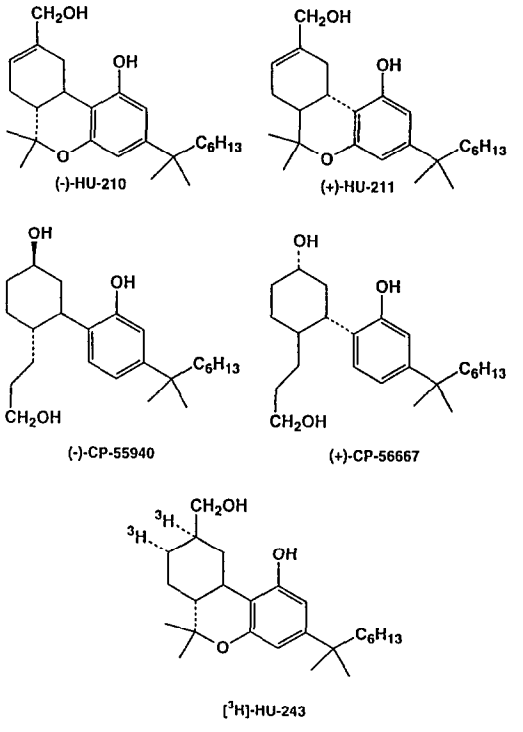


Fig. 2. Structures of the enantiomers of HU-210 and CP-55940, along with the structure of HU-243.

Compounds acting via receptors, which being protein-based are asymmetric, generally exhibit very high enantioselectivity. Originally, the cannabinoids were not found to possess this property. The unnatural (+)-THCs (Fig. 1) were shown to be up to 15% as active as their (−)-enantiomers in many pharmacological tests, exemplified by the naturally occurring Δ^9-THC (for a review, see Ref. 14). Most of the early work on THC enantioselectivity was done with (+)-THCs synthesized from commercial (+)-pinene. As this material is seldom stereochemically pure, and most publications dealing with cannabinoid SAR do not indicate that additional purification of the commercial material was undertaken, it is plausible that at least some of the activity seen with (+)-THC was due to the presence of (−)-THC. However, several pairs of enantiomers with essentially total separation of activity have been reported. The non-classical cannabinoids CP-55940 (Fig. 2) and CP-55244 [which are (−)-enantiomers] were shown to be *ca.* 10 and 33 times more potent than Δ^9-THC, respectively, in a variety of *in vivo* tests, whereas their (+)-enantiomers, CP-56667 (Fig. 2) and CP-55243, showed no activity at doses *ca.* 50 and 250 times higher [15]. Of particular interest is the pair (−)-HU-210 and (+)-HU-211 (Fig. 2). These compounds possess the classical cannabinoid

structure. The (−)-enantiomer HU-210 is 100–800 times more potent than Δ^9-THC in the same battery of tests used for the non-classical cannabinoids mentioned above; (+)-HU-211 is *ca.* 2000 times less active than the (+)-enantiomer. In drug discrimination assays in pigeons, (+)-HU-211 was inactive at doses 4500 times higher than the ED_{50} of the (−)-enantiomer [1, 16, 17].

More recently, the cannabimimetic activity of the two enantiomers of the primary 11-hydroxy metabolites of (−)- and (+)-Δ^8-THC was evaluated in drug discrimination assays in pigeons. Activity by the (−)-enantiomer was noted at a level (ED_{50} 0.17 mg/kg) similar to that of Δ^9-THC. No cannabimimetic activity was seen after administration of the (+)-enantiomer [18].

On the basis of the SAR work, the high enantioselectivity, and the biochemical effects observed, such as those on adenylyl cyclase (see section on the cannabinoid receptor), by the mid-1980s it was generally assumed that cannabinoids act on receptors, presumably novel ones, but some effects—predominantly non-specific ones—may be exerted via other mechanisms. A detailed analysis of the evidence for the existence of a cannabinoid receptor has been published [19].

The cannabinoid receptor

The first direct evidence for a cannabinoid receptor came when a study from Howlett's laboratory demonstrated that radiolabeled CP-55940 bound to brain membranes in a highly specific and selective manner, features that are characteristic of a receptor [5]. Behaviorally, active cannabinoids, including Δ^9-THC, exhibited high affinity for this site, lending credence to its being the cannabinoid receptor. Subsequently, this receptor was characterized using other radiolabeled ligands that all appeared to be binding to the same site. Δ^9-THC has been reported to compete with these ligands with K_i values of approximately 40 nM [20]. Howlett *et al.* [21] examined an impressive array of other centrally acting compounds and found none that acted at the cannabinoid receptor. It has now been well established that there is an excellent correlation between the pharmacological potencies of cannabinoids in several behavioral tests and their affinity for this binding site [22]. While it is reasonable to speculate that multiple cannabinoid receptors may exist in the central nervous system, evidence has not yet been forthcoming.

The anatomical distribution of the receptor throughout the brain has been determined by autoradiography [23], as well as the localization within the neuron. The binding sites are very dense in the basal ganglia and cerebellum. High receptor densities in the extrapyramidal motor system and the cerebellum are consistent with the actions of cannabinoids on many forms of movement. The hippocampus, a brain region demonstrating relatively dense binding of cannabinoids, has been shown to be involved in coding sensory information and storing memory. Therefore, the effects of cannabinoids on cognition and memory may be due to the relatively dense receptors in the hippocampus and cortex. The low density of cannabinoid receptors in medullary nuclei is consistent with the lack of lethal effects of marijuana in humans.

Convincing evidence for a cannabinoid receptor was provided by Matsuda *et al.* [24], who isolated a clone from a rat brain library that had a high degree of homology with G-protein coupled receptors. The discovery that the distribution of mRNA of the clone paralleled that of the cannabinoid receptor, as reported by Herkenham *et al.* [23], led them to conclude that they had cloned the cannabinoid receptor. Subsequently, a human cannabinoid receptor was cloned, which had almost identical homology with the rat clone [25].

All evidence points to the brain cannabinoid receptor as being coupled to G-proteins. Ligand binding at the cannabinoid receptor was found to be reduced by the non-hydrolyzable guanine nucleotide analog Gpp (NH)p [5]. The most likely candidate for a second messenger system is adenylyl cyclase [20]. Numerous laboratories have demonstrated that cannabinoids inhibit adenylyl cyclase both *in vivo* and *in vitro* most likely by interaction with G_i [19, 20]. However, it does not appear that the effects of cannabinoids are confined to adenylyl cyclase. Electrophysiological studies in neuroblastoma cells indicate that cannabinoids inhibit an ω-conotoxin-sensitive, high voltage-activated calcium channel, an effect that is blocked by the administration of pertussis toxin and independent of the formation of cAMP [26]. It was hypothesized that N-type calcium channels were affected since the L-type calcium channel blocker nitrendipine failed to alter the effect of the cannabinoids. It has also been reported recently that calcium influx stimulates the release of an endogenous factor that displaces cannabinoid binding [27].

Although multiple cannabinoid receptors have not been identified in brain, a peripheral receptor that is structurally different from the brain receptor has been identified [28]. This cloned receptor is expressed in macrophages in the marginal zone of the spleen. This peripheral receptor has 44% homology with the brain receptor (68% homology if only the transmembrane regions are considered). Based upon a limited pharmacological evaluation of this receptor, these investigators concluded that the recognition site of this peripheral receptor is different from that of the brain receptor. However, this assumption is based largely upon the observation that cannabinol and Δ^9-THC are nearly equipotent in binding to the peripheral receptor, whereas cannabinol has an affinity for the brain receptor which is much less than that of Δ^9-THC. Kaminski *et al.* [29] have also demonstrated cannabinoid receptor binding in mouse spleen cells, as well as the presence of mRNA for the cannabinoid receptor. However, they suggest that this receptor is associated with T-cells [30] and is similar to the receptor found in brain [29]. Additional characterization of these receptors in spleen will be needed to determine whether they are distinct entities and to ascertain their functional roles. The discovery of these cannabinoid receptors in spleen raises the possibility that other receptor subtypes with entirely unique functional roles may exist.

The anandamides

The probe. The classical approach for the isolation of active constituents from natural sources—be they from plant or animal tissues—is based on chromatographic separation monitored by a suitable *in vivo* or *in vitro* test. The standard assay for new receptor agonists (or antagonists), including natural ones, is the displacement of a labeled probe bound to the appropriate receptor. This route was indeed followed in the isolation of anandamide. First, a new probe that was based on the highly active HU-210 was developed (see above). This compound, which has a typical THC-like structure, a K_D in the picomolar range, and high enantioselectivity in both pharmacological activity and binding, was an ideal candidate for labeling, thus producing a novel probe. This was achieved by *enantiospecific* reduction of the double bond in HU-210 with tritium, leading to [^{3}H]HU-243, as shown in Fig. 2 [31]. Its non-labeled form engenders Δ^9-THC-like responding in pigeon drug discrimination (ED_{50} = 0.002 mg/kg) at the potency level of HU-210, and binds to the cannabinoid receptor with a K_i of 45 pM, significantly lower than even that of HU-210 (K_i = 181 pM).

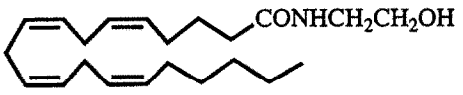
Isolation of anandamides. All plant or synthetic cannabinoids are lipid-soluble compounds. Hence, the procedures employed for the isolation of endogenous ligands by the Jerusalem group were based on the assumption that such constituents are also lipid-soluble, an assumption that ultimately proved to be correct. Porcine brains were extracted with organic solvents, and the extract was chromatographed according to standard protocols for the separation of lipids [6]. The fractions obtained were screened for cannabinoid activity on the basis of their ability to displace radiolabled HU-243 in a centrifugation-based ligand binding assay. The choice of assay was a lucky one. The more generally used filtration assay requires larger amounts of protein that may contain high levels of amidase, which is known now to cause a breakdown of anandamide [32]. A further problem was the lability of anandamide: although purity increased on repeated chromatographies, the amounts of anandamide diminished rapidly. In another publication [33], an improvement of the separation procedure was reported. After precipitation of the inactive phospholipids with acetone, the extract was chromatographed once only with a large number of small fractions collected.

The original publication reported the identification of one active compound only [6]. In a second paper, two additional constituents (shown to be closely related to the first anandamide in both structure and activity, see below) were reported [33]. While the original anandamide was identified by direct assays of fractions from chromatographic separations, the two additional ones were first obtained by synthesis of several compounds biogenetically related to anandamide. These synthetic compounds were tested for binding activity. Two compounds, homo-γ-linolenylethanolamide and docosatetraenylethanolamide (see Fig. 3), exhibited activity. They were compared by TLC and GC–MS and were shown to be identical to the compounds obtained from porcine brain by liquid chromatography.

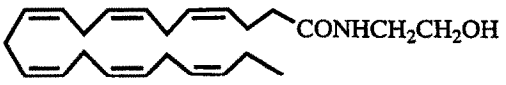
Structure elucidation and synthesis. The isolation of minute amounts of a labile natural product from a complicated mixture poses problems for the structural elucidation due to minor impurities of other frequently related constituents, traces of materials originating from the plastic labware, or even traces of solvents tenaciously binding to the natural product. After laborious elimination of such impurities, the structure of anandamide was deduced from MS and NMR measurements [6]. High-resolution MS suggested the elemental composition $C_{22}H_{37}NO_2$ (m/z 347.2762), which indicated the presence of five double bonds (or rings). The first structural clue was the observation that the NMR peaks at δ 5.30–5.45 ppm, presumably due to double bond protons, were coupled (meaning that they are close in space) with peaks at δ 2.75–2.90 ppm, which were assumed to be signals of doubly allylic protons. Such protons, along with their couplings and the specific ratio of vinylic to doubly allylic protons, are typically observed in all-*cis*, non-conjugated polyunsaturated fatty acids, such as linoleic and arachidonic acids. The odd-number molecular weight suggested that anandamide was a nitrogen derivative of such a fatty acid. MS spectra supplied additional data. Collision-induced dissociation (CID) measurements of the MH^+ ion (m/z 348), obtained from direct exposure chemical ionization, gave several major significant ions at m/z 287, 62 and 44. The m/z 62 ion had an elemental composition C_2H_8NO, which best fits a protonated ethanolamine ion, $HOCH_2CH_2NH_3^+$; the m/z 44 ion could represent dehydrated ethanolamine (protonated form); the m/z 287 ion corresponded to MH^+ less ethanolamine. The existence of an ethanolamine moiety was supported by the MS of a trimethylsilyl derivative (TMS) of anandamide. A m/z 419 ion indicated the formation of a mono TMS derivative and hence the existence of a single hydroxyl group.

The NMR and MS data led to the conclusion that anandamide is the ethanolamide of a C-20 fatty acid with four unconjugated double bonds, presumably arachidonic acid. This deduction was proved by a straightforward synthesis [6]. Arachidonic acid was converted into its acyl chloride with oxalyl chloride, and the arachidonyl chloride thus obtained was reacted with ethanolamine, leading to synthetic arachidonylethanolamide. This synthetic product was identical to natural anandamide in its infrared, NMR and MS spectra and in its ability to inhibit the twitch response of isolated vasa deferentia. Synthetic anandamide bound to the cannabinoid receptor with a K_i of 39 nM, which was essentially identical to that of the natural anandamide.

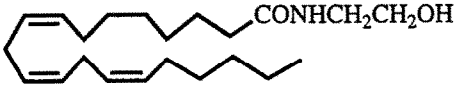
Nomenclature. The first ligand isolated from brain, arachidonylethanolamine, was named anandamide on the basis of the Sanskrit word "ananda" meaning bliss and from the chemical nature of the compound (an amide) [6]. In view of the existence of several related unsaturated fatty acid ethanolamides that bind to the brain cannabinoid receptor, it has been suggested [33] that the entire group of active compounds be named "anandamides" with each individual member identified with the parent fatty



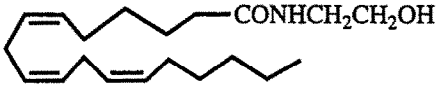
Anandamide
(anandamide, 20:4, n-6)
Ki = 39.0 ± 5.0 nM (Hebrew Univ.)
543 ± 83 nM (NIH)



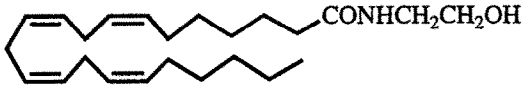
Anandamide (22:6, n-3)
Ki = 383±25 nM (Hebrew Univ.)
12,200 ± 500 nM (NIH)



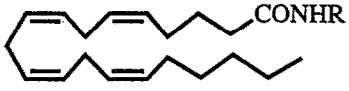
Homo-γ-linolenylethanolamide
(anandamide, 20:3, n-6)
Ki = 53.4 ± 5.5 nM (Hebrew Univ.)
598 ± 264 nM (NIH)



Anandamide (18:3, n-6)
Ki = 4,600±300 nM (Hebrew Univ.)
>41,400 ± 6,000 nM (NIH)



7,10,13,16-Docosatetraenylethanolamide
(anandamide, 22:4, n-6)
Ki = 34.4 ± 3.2 nM (Hebrew Univ.)
848 ± 102 nM (NIH)



Anandamide analogs (20:4, n-6)
R = $CH_2CH_2CH_2OH$, Ki = 364 ± 95 nM (NIH)
R = $CH(CH_3)CH_2OH$, Ki = 1,300 ± 100 nM (NIH)
R = $CH_2CH(OH)CH_3$, Ki = 4,000 ± 1,600 nM (NIH)

Fig. 3. Structures of anandamide and several synthetic analogs. The NIH values were described previously [34]; some of the Hebrew University values were determined as a part of this commentary.

acid (indicated in parentheses) following the generally accepted fatty acid shorthand designation. The anandamide derived from arachidonic acid is anandamide (20:4, n-6); that from homo-γ-linolenic acid—anandamide (20:3, n-6); and the one from docosatetraenoic acid—anandamide (22:4, n-6).

Structure–activity relationships of anandamides

Both the fatty acid moiety and the ethanol amide chain have been modified and the new compounds have been tested for cannabinoid activity.

Changes in the fatty acid moiety. As mentioned above, the Jerusalem group isolated and tested three naturally occurring anandamides: anandamide (20:4, n-6); anandamide (20:3, n-6) and anandamide (22:4, n-6). Their respective K_i values (39.0 ± 5.0, 53.4 ± 5.5 and 34.4 ± 3.2 nM) suggested that they were active [6, 33]. The same compounds tested by the NIH group (by a different methodology) gave values of 543 ± 83, 598 ± 264 and 848 ± 102 nM [34]. Additional compounds with changes in the fatty acid moiety have been tested by both groups ([34]: Hanuš L and Mechoulam R, unpublished observations). The results are presented in Table 1. The binding affinities obtained by these two groups are quite different, which is at present unexplained. The binding methodologies were not the same,

Table 1. Receptor binding affinities of various anandamides (fatty acid ethanolamides)*

Anandamide†	K_i (nM)	
	Our values	NIH‡
20:4, n-6	39.0 ± 5.0	543 ± 83
20:3, n-6	53.4 ± 5.5	598 ± 264
22:4, n-6	34.4 ± 3.2	848 ± 102
22:6, n-3	383 ± 25	12,200 ± 500
18:3, n-6	4,600 ± 300	>41,400 ± 6000
18:2, n-6	>25,000	
16:0	>25,000	
18:3, n-3	>25,000	
20:5, n-3	200–400	
20:2, n-6	1500 ± 200	
20:1, n-9	>1000	
20:3, n-3	>10,000	

* Data are expressed as means ± SEM of at least three experiments.

† Fatty acid shorthand nomenclature. For example, anandamide is 20:4, n-6 where 20 denotes the number of carbons in the backbone, 4 indicates the number of unsaturated bonds, and n-6 specifies that the first double bond begins at C-6 from the non-acidic end of the molecule.

‡ Values from Ref. 34.

which may account for these differences. In the method used by the NIH group, some enzymatic breakdown of the anandamides may be taking place, as Childers *et al.* [32] found that the enzyme inhibitor phenylmethylsulfonyl fluoride (PMSF) increased the binding affinity of anandamide almost 50-fold.

Several tentative conclusions can be drawn from the limited results obtained thus far: (a) at least three double bonds on the fatty acid chain are required for pronounced activity; (b) if the first double bond is on the third (rather than the sixth) carbon atom from the non-acidic end of the fatty acid, activity is strongly reduced; (c) highest potencies are observed with the C-20 and C-22 polyunsaturated acids. It should be stressed that presently the number of examples is too limited to allow firm conclusions.

Changes in the amino ethanol moiety. The NIH group [34] has reported that substitution of a propanol for the ethanol moiety results in active compounds—the straight chain being most active, the branched chain propanolamides being less active (see Fig 3). Replacement of the hydroxyl group with a fluorine atom increases the binding potency nearly 10-fold (Razdan R K, Martin B R and Compton D R, unpublished observations).

Biological activity. Anandamide has been examined in mice following peripheral administration and found to exhibit THC-like properties in producing hypomotility, hypothermia, antinociception and catalepsy [35]. It is less potent than Δ^9-THC by 4- to 20-fold, depending upon the pharmacological measure [36]. Anandamide also has a shorter duration of action than Δ^9-THC, which may be due to metabolic factors. Deutsch and Chin [37] have shown that anandamide is degraded rapidly by an amidase, which can be blocked by PMSF. Efforts in our laboratory in Richmond to produce THC-like responding in rat drug discrimination have thus far proven to be difficult. However, some animals do perceive anandamide to be Δ^9-THC-like.

Vogel *et al.* [38] have reported that anandamide is a specific cannabinoid agonist and exerts its action directly via cannabinoid receptors. Anandamide specifically binds to membranes from cells transiently (COS) or stably (Chinese hamster ovary) (CHO) transfected with an expression plasmid carrying the cannabinoid receptor DNA but not to membranes from control non-transfected cells. Moreover, anandamide inhibited the forskolin-stimulated adenylyl cyclase in transfected cells and in cells that naturally express cannabinoid receptors (N18TG2 neuroblastoma) but not in control non-transfected cells. As with exogenous cannabinoids, inhibition by anandamide of the forskolin-stimulated adenylyl cyclase was blocked by treatment with pertussis toxin.

Felder *et al.* [34] have reported similar findings. Anandamide inhibited forskolin-stimulated cAMP accumulation in CHO cells expressing the human cannabinoid receptor but not in CHO cells expressing a muscarinic receptor. This response was blocked by pertussis toxin. N-type calcium channels were inhibited by anandamide in N-18 neuroblastoma cells. Surprisingly, anandamide was found to release arachidonic acid and intracellular calcium in both types of cells, indicating an action in which the cannabinoid receptor was not involved. This group concluded that anandamide fulfills the essential criteria for establishing that it is an endogenous agonist for the cannabinoid receptor and that "the cannabinoid/anandamide receptor appears to exhibit the properties of an inhibitory receptor except for its inability to modulate the release of arachidonic acid in the CHO cell model as shown for other inhibitory receptors."

Mackie *et al.* [39] have shown that the inhibition of N-type calcium channels is voltage dependent and *N*-ethylmaleimide sensitive and that anandamide appears to act as a *partial* agonist at the cannabinoid receptor, which is not the case with some synthetic cannabinoids examined. These data indicate that results from studies with Δ^9-THC cannot always be extrapolated for anandamide.

In a study aimed at the identification of endogenous substances that regulate L-type calcium channels, Johnson *et al.* [40] isolated anandamide from lyophilized calf brain. Synthetic anandamide inhibited [^{3}H]1,4-dihydropyridine binding to rat cortex membranes with an estimated IC_{50} value of 10–20 μM and a Hill coefficient >2. They concluded that anandamide interacts with the dihydropyridine binding site in a non-competitive fashion. It will be of interest to find out whether tricyclic cannabinoids cause related effects and the relevance of these observations. However, it is important to point out that others have reported that L-type channels are not involved in cannabinoid action, but rather have implicated N-type calcium channels [26, 39].

THC can induce certain endocrine changes including stimulation of adrenocortical function [10]. Weidenfeld *et al.* [41] have found that intracerebroventricular injection of anandamide (50–150 μg/rat) significantly increases the serum levels of ACTH and corticosterone in a dose-dependent manner and causes a pronounced depletion of CRF-41 in the median eminence. These data suggest that anandamide parallels THC in activating the hypothalamo-pituitary adrenal axis via mediation of a central mechanism that involves the secretion of CRF-41.

It is of interest that the caudate-putamen of adrenalectomized rats contains 50% higher levels of mRNA for the cannabinoid receptor than the controls. This increase could be counteracted by dexamethasone [42]. Taken together with the findings of Weidenfeld and colleagues, it seems possible that the corticoid and anandamide systems could be mutually regulating. However, numerous criteria have to be satisfied before such an assumption will be acceptable.

Dopaminergic regulation of cannabinoid receptor mRNA levels in rat caudate putamem has also been recorded [43]. Interactions between the dopaminergic system and the cannabinoid-anandamide systems are well known [44–47]. Recently, it has been reported that anandamide acts in a fashion similar to that of other cannabinoids to enhance GABAergic transmission [48]. It seems that the anandamide systems, like most other receptor-transmitter systems in living organisms, interacts quite extensively with other mediators. It may be more than a curious coincidence that in patients with Huntington's

disease both D1 and cannabinoid receptors are lost in the substantia nigra [49].

A look into the future

The cannabinoid receptors are present in high concentrations (relative to most other receptors) in certain brain areas. Their ligands, the anandamides, parallel the psychoactive cannabinoids in many of their biological effects, most of which are receptor mediated. It seems reasonable to assume that these receptors and the anandamides play a physiological role. However, we cannot attribute any specific role to the anandamide system at present. At best we can surmise.

Cannabinoid receptors are present in the cerebellum and the basal ganglia—areas associated with coordination of movement. Since cannabis is known to affect coordination, is it possible that this type of activity is within the physiological profile of the anandamide system? Can we expect that certain movement disorders are due to excess of, or lack of, sufficient levels of anandamide activity? Following the same line of thought, short-term memory can be expected to be associated with the same system.

Marijuana is consumed for its effects on mood, perceptions and emotions. In view of the high concentration of receptors in the limbic system, a reasonable assumption may be that the anandamide system is involved in the biochemical translation of such processes.

The presence of cannabinoid receptors outside the CNS is an even greater enigma when one considers their physiological roles. Until the appropriate ligand—an anandamide or some other type of molecule—and more peripheral effects have been described, it may be impossible to attribute, even tentatively, a physiological role to this system.

With the accumulation of additional knowledge, we expect the anandamide system to take its place alongside the other, better established receptor-transmitter systems in the periphery and the CNS.

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