From *gan-zi-gun-nu* to anandamide and 2-arachidonoylglycerol: the ongoing story of cannabis

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1 Introduction

The use of *Cannabis sativa* goes back to a far away past.[1] The Assyrians who ruled large parts of the Middle East for nearly a millennium, about 3000 years ago, have left us a pharmaceutical legacy on hundreds of clay tablets. Cannabis was one of the major drugs of their pharmacopoeia. Apparently they named the plant according to its use: *qunnabu* seems to have been used in certain rites; *azallu* may have been the medical term as well as hemp; *gan-zi-gun-nu* has been translated as 'the drug that takes away the mind'—certainly a more picturesque definition than the present day 'cannabimimetic'.[1] *Papaver somniferum*—the opium plant—was another one of their important drugs. After three millennia opium and cannabis, their preparations and derivatives, are still the most widely used illicit drugs in most parts of the world.

Research on *Cannabis sativa* has always lagged behind that on *Papaver somniferum*. While morphine was isolated from opium early in the 19th century, tetrahydrocannabinol was not fully identified until 1964; the first opiate receptors were described in the 1970s but those of the cannabinoids were not discovered for another 20 years. The first endogenous opiates were isolated in the 1970s; the endocannabinoids, in the 1990s. Why the discrepancy? The reasons are both technical and conceptual.

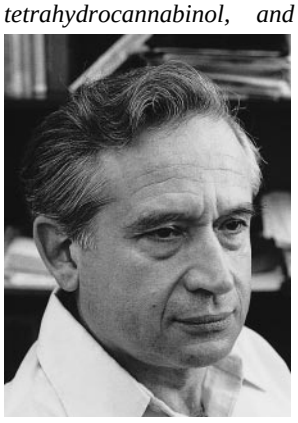
On the technical side one finds that morphine forms numerous, easily isolable, crystalline salts, while, even as early as the 19th century, the active compounds(s) in cannabis were known to be present as a complex oily mixture. In the 1930s and early 1940s Todd in the UK and Adams in the US reisolated cannabinol, which is probably an artefact and not an original natural product, and elucidated its structure. Cannabidiol (CBD), an inactive constituent, was isolated, although its structure remained in doubt. However the active constituent was not identified.[2]

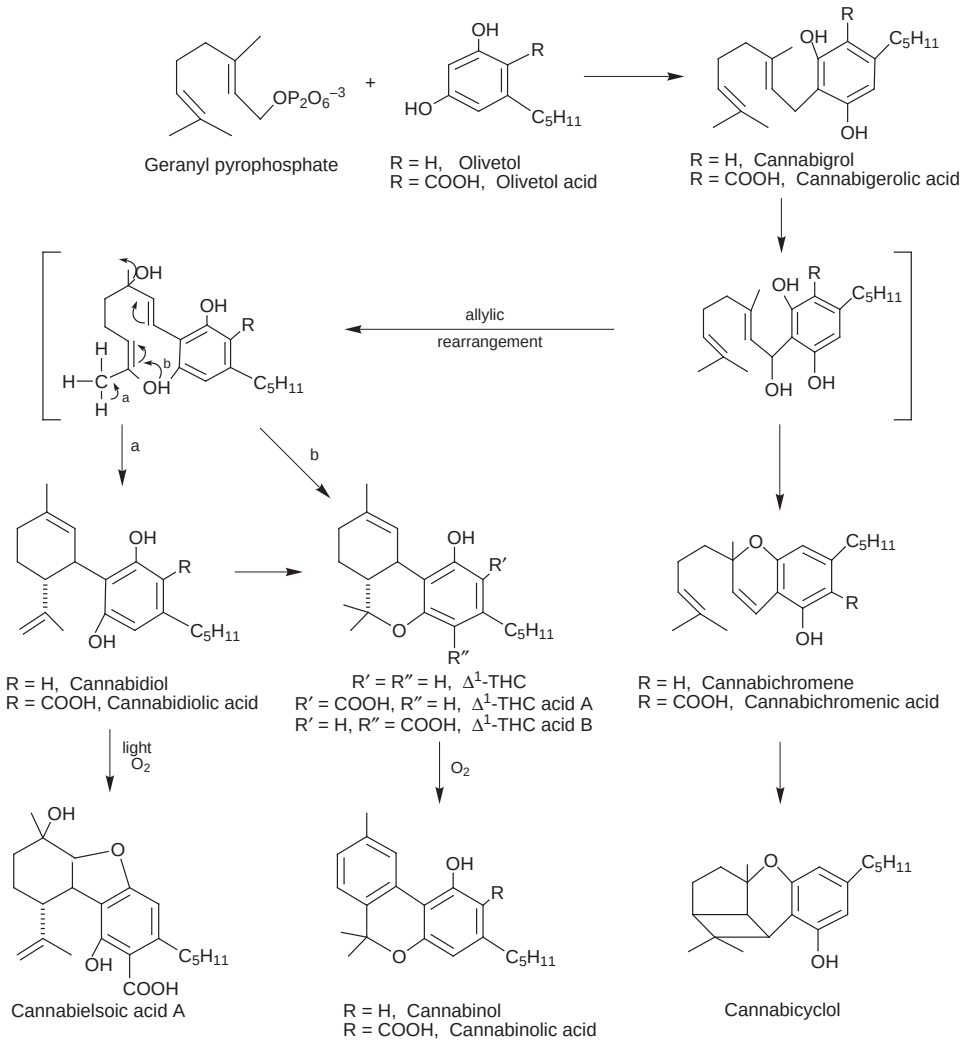
In the early 1960s we took a new look at the problem. By then better chromatographic techniques had evolved and we were able to separate numerous new cannabinoids—a term which we suggested then and which has received wide acceptance. Some of the cannabinoids which were isolated by our and other groups during that period are presented in Scheme 1.[2] Their structures and relative stereochemistry were elucidated by the then novel techniques of NMR and mass spectrometry. The absolute stereochemistry of Δ^1-THC and of CBD and hence of all other cannabinoids with which they had been chemically related was established by chemical correlation.[3]

The natural cannabinoids were assayed for psychotropic activity by Edery on rhesus monkeys: only one constituent, Δ^1-tetrahydrocannabinol (Δ^1-THC),[4] now usually designated Δ^9-THC, showed potent activity. The rhesus monkeys became sedated and sleepy after an i.v. dose of 0.5 mg kg^{-1} THC. None of the other constituents showed any activity except cannabinol, which was much less potent. Δ^6-THC (now usually named Δ^8-THC), which is present in very low concentrations, was slightly less active than Δ^1-THC. When a mixture of the major constituents was administered to monkeys, all the activity could be attributed to Δ^1-THC alone.[5] This picture has not changed much over the last three decades.

Today the number of natural cannabinoids is about 70. Most of them are variations on the structures represented in Scheme 1. The carboxylic acids are found in the plant in higher concentrations than the neutral cannabinoids and it is possible

Raphael Mechoulam obtained his PhD degree in organic chemistry under the direction of F. Sondheimer at the Weizmann Institute in Rehovot. After a post doctorate with S. W. Pelletier at the Rockefeller Institute in New York he returned to the Weizmann Institute where he initiated several projects in the chemistry of Natural Products. In 1966 he established a Laboratory (later a Department) of Natural Products at the Hebrew University in Jerusalem where he has been since. During 1979–1982 he was Rector (academic head) of the University but later succeeded in disengaging himself from administration and returned to research and teaching. His scientific interests are in the chemistry and biological activity of natural products and in synthetic drugs based on natural products. He is particularly proud of having achieved the identification of the active principle in Cannabis sativa, *tetrahydrocannabinol, and of the first endocannabinoids, anandamide, in the brain, and 2-arachidonylglycerol, in the gut. A synthetic cannabinoid HU-211 (HU being Hebrew University) produced in his laboratory has been shown to be an NMDA antagonist and a TNF antagonist and has now successfully completed Phase I and II in clinical trials aimed at reduction of the neuronal trauma produced by closed head injury.*





Scheme 1 Natural cannabinoids and their putative biogenesis. For details see text.

that they represent the actual natural products, the neutral cannabinoids being formed by decarboxylation, possibly in part in the plant itself, but mostly after the plant material has been dried to obtain marijuana or hashish.[2] Δ^1-THC acid is found in the plant in both the A and B forms.[6]

2 Biogenesis of plant cannabinoids

The isolation of cannabigerol indicated that cannabinoids presumably follow the standard pathway of monoterpenoid biosynthesis (Scheme 1).[2] Recently the enzyme that condenses geranyl pyrophosphate with olivetol carboxylic acid to give cannabigerolic acid was identified.[7] As expected the monoterpene does not condense with olivetol, indicating that, as mentioned above, the neutral cannabinoids are presumably formed by decarboxylation.

We have suggested that cannabigerol on oxidation (not necessarily by addition of a hydroxy group, although the latter is indicated on Scheme 1 for convenience), rearrangement and multiple cyclizations will lead to CBD and THC.[2] Recently it was shown that this pathway is only partly correct. An oxidation–cyclization enzyme was purified by the Shoyama group.[8] It converts cannabigerolic acid into Δ^1-THC acid, but not into CBD acid. The existence of such an enzyme may explain the existence of *Cannabis* strains which contain THC, but no CBD, although the latter constituent is usually more prevalent than THC in most strains.The rest of the known cannabinoids may be formed from the above mentioned cannabinoids (see Scheme 1). It is possible that some of the 'natural' cannabinoids are actually artefacts formed on oxidation (cannabielsoic acid, for example) or on photochemical cyclization (cannabicyclol).

3 Metabolism

Several groups, including ours, almost simultaneously identified the major primary route of cannabinoid metabolism—hydroxylation at the allylic C-7 position.[9,10] Later work by several groups showed that Δ^1-THC, Δ^6-THC, CBD and CBN are hydroxylated (or oxygenated) by many animal species, including man, at most allylic positions as well as on the side chain. The relevant positions are indicated by arrows in Fig 1.

Many monohydroxylated (or mono-oxygenated) THC metabolites are pharmacologically active. Some of these metabolites (in particular 7-hydroxy-Δ^1-THC and 6-hydroxy-Δ^1-THC) contribute to the activity observed *in vivo*.[9–11]

The monohydroxylated products can undergo further hydroxylations as well as oxidations to the corresponding 7-oic acids which have no THC-type activity.[10,12] The side chain can also be cleaved and oxidized giving mono- or polycarboxylic acids. Considerable metabolic species specificity exists, although the general pathways apparently are similar.[10]

Two types of secondary metabolites have been identified.[10] The first are esters of fatty acids with cannabinoids or primary metabolites of cannabinoids. These compounds are less polar than the natural cannabinoids. A second, much more abundant

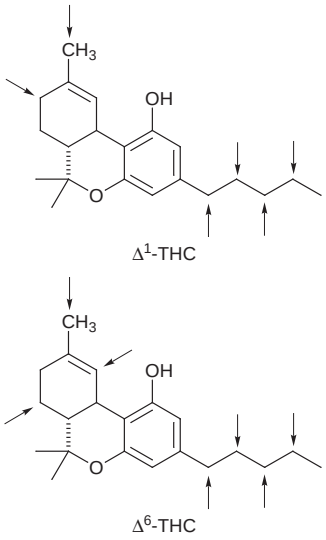


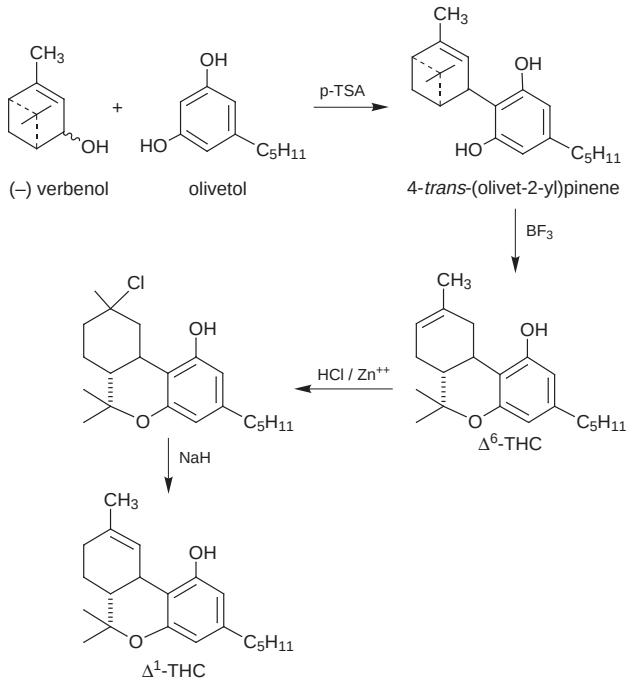
Fig. 1 Oxidation at allylic positions and on the side chain—the primary route of cannabinoid metabolism.

type of secondary metabolites, are the glucuronides of cannabinoids. These glucuronides form a significant portion of the water soluble cannabinoids which represent most of the cannabinoid excretion products in urine. In man only *ca.* 10% of the cannabinoid excretion is by this route. The rest is through the faeces.[10]

4 Synthesis

The first total synthesis of Δ^1-THC was reported over 30 years ago (see Scheme 2).[13] As starting material it uses pinene, which, being available in both enantiomeric forms, may lead to either the natural (−)-Δ^1-THC or its enantiomer. Of synthetic interest is the isomerization of the double bond in the Δ^6 position to the Δ^1 one, which makes use of an internal dehydrochlorination by a phenolic group. The same synthetic pathway (without the double bond rearrangement) has been used for the synthesis of (−)-7-hydroxy-Δ^6-THC, 1,1-dimethylheptyl homolog (HU-210), an important, highly potent agonist, and its enantiomer, HU-211, which is in clinical trials as an antitrauma drug (see below).[14]

A second, more facile, total synthesis was reported in 1969 (Scheme 3).[15] However, it leads to the natural (−) series only. It is used for the preparation of Dronabinol, the synthetic Δ^1-



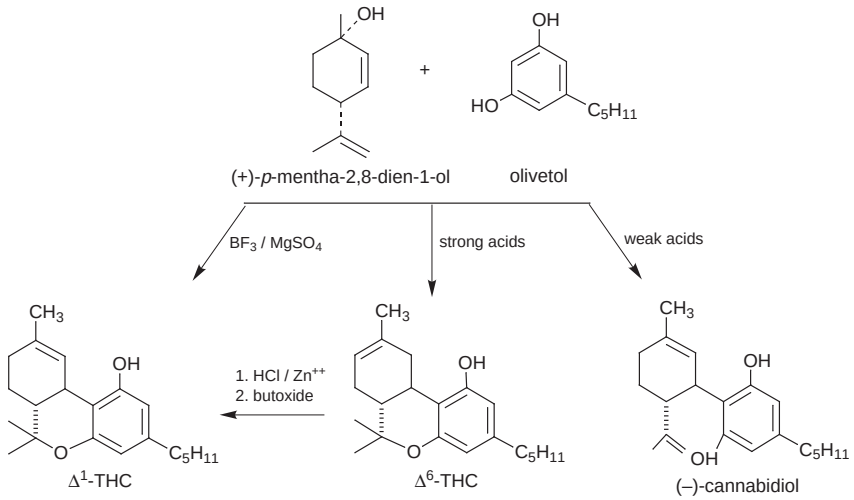
Scheme 2 Total synthesis of THCs (see ref. 13).

THC, marketed as a medicinal agent (see below). CBD can also be prepared *via* the same route.

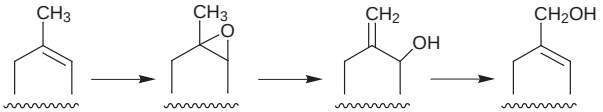
Cannabichromene has been prepared by oxidative cyclization of cannabigerol;[16] photolysis of cannabichromene in the presence of a sensitizer led to cannabicyclol.[17] The natural cannabinoid acids can be prepared by carboxylation with methylmagnesium carbonate of the respective neutral cannabinoid.[18]

The syntheses of the major metabolites proved to be more complicated. The first metabolite to be synthesized, 7-hydroxy-Δ^6-THC, made use of an allylic rearangement to place the hydroxy group in the 7 position[9] (Scheme 4). This synthetic pathway has been employed in numerous other variations of this procedure. Many other THC metabolites have also been synthesized.[19] It is surprising that the CBD metabolites have not been prepared so far in practical yields.

The synthetic field up till 1980 is covered by an excellent and detailed review by Razdan.[19] No major synthetic advances were reported over the next decade. However, recently several new groups have reported new and interesting approaches. Tius has described the application of novel synthetic techniques for the synthesis of cannabinoids as well as a bifunctional cannabinoid



Scheme 3 Total synthesis of THCs (see ref. 15).



Scheme 4 A widely used strategy for hydroxylation of the C-7 position in THCs.

ligand,[20] while Evans has used a Diels–Alder approach using a cationic bis(oxazoline) Cu complex to obtain (−)-Δ^1-THC in four steps.[21]

5 Molecular basis of cannabinoid action

With the clarification of the basic chemical aspects of cannabis, and the development of synthetic methods for most cannabinoids, interest focused on cannabinoid pharmacology and cellular effects. Although during the 1970s and early 1980s we learned much on the neurochemistry, the neurophysiology and the overt behavioral effects,[22,23] the molecular basis of THC action remained an enigma. As mentioned above conceptual problems hampered work in this direction. One of these was the presumed lack of stereoselectivity.[24] Compounds acting through a biomolecule—an enzyme, a receptor or a gene—generally show a very high degree of stereoselectivity. This was supposedly not the case with cannabinoids. Synthetic (+)-Δ^1-THC showed some cannabimimetic activity compared with that of natural (−)-Δ^1-THC.[24] This observation was not compatible with the existence of a specific cannabinoid receptor and hence of a cannabinoid mediator. However in the mid 1980s it was established that cannabinoid activity is highly stereoselective and that the previous observations resulted from separation problems.[25] Unfortunately, this presumed low degree of stereoselectivity delayed research aimed at the identification of a receptor–mediator cannabinoid system.

A second problem was pointed out by Paton who assumed that 'underlying much of the pharmacology of cannabis is the high lipophilicity of its active principles which is responsible for the slowness of its kinetics, its cumulation, [and] its persistence'.[22] Hence Δ^1-THC should be considered, according to Paton, to belong to the group of biologically active lipophiles, its effects should be compared with the chronic effects of anesthetics at low dose levels, and the action of cannabinoids could be explained without postulating the existence of a specific cannabinoid receptor and of an endogenous mediator of cannabinoid action.

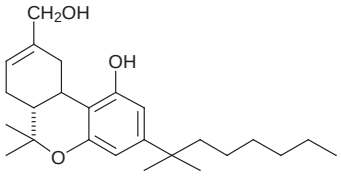
Gill compared the activity of cannabinoids and of steroid anesthetics on the order parameter of spin-labelled liposomes.[26] He reported that the steroid anesthetic alfaxolone increased the fluidity of a liposome bilayer in a manner comparable to that produced by the volatile anesthetic halothane. The same effect was observed with (−)-Δ^1-THC, while cannabinol and CBD decreased the molecular disorder of the lipid bilayer. He concluded that: 'The molecular perturbation produced by the psychoactive cannabinoids is qualitatively the same as that produced by the general anesthetics, namely, an increased fluidization and disordering of the lipid phase of the cell membrane.' However not all cannabinoids followed the same pattern.

Tamir and Lichtenberg in our laboratory found that while the cannabimimetic (−)-Δ^1-THC and its dimethylheptyl analog were most effective in fluidizing membranes, the inactive CBD and the dimethylheptyl homolog of (+)-Δ^1-THC had an opposite effect. This relationship fits the known structure–activity relationship (SAR) of cannabinoids. However the potent cannabimimetic Nabilone showed effects similar to those of CBD, rather than those of THC, which contradicted the predictions.[27]

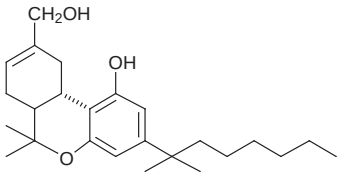
By the mid 1980s it became clear that the membrane perturbation theory of cannabis action represents at best only part of the picture. The structure–activity relationships established that small changes in the THC molecule could lead to significant changes in activity—a situation incompatible with a non specific mode of action. Thus, introduction of a methyl group on the aromatic ring next to the phenolic group retained activity, while the same modification next to the ether group eliminated activity.[28] The very high stereospecificity of cannabinoid action (see below) also pointed towards a more specific mechanism.

Makriyannis has recently again reviewed the evidence and has concluded that 'although the cellular membrane may not be the principal target for cannabinoid activity, it nevertheless plays a role in the mechanism of action'.[29]

The first solid indication that cannabinoids act through receptors was brought forward by Howlett's group. Howlett and Fleming, using the neuroblastoma N18TG2 cell line as a model system, demonstrated that cannabinoids interact with the adenylate cyclase second messenger pathway in an inhibitory fashion. The level of potency of a variety of cannabinoids to inhibit adenylate cyclase paralleled cannabinoid effects in animal models and in humans.[30] Stereospecificity was also demonstrated using the HU-210 and HU-211 enantiomers.[31] (−)-HU-210 was several orders of magnitude more potent in inhibition of cAMP accumulation and adenylate cyclase activity than the enantiomeric (+)-HU-211. This line of research culminated in the discovery in the brain of specific, high affinity cannabinoid binding sites, whose distribution is consistent with the pharmacological properties of psychotropic cannabinoids.[32] Shortly thereafter Matsuda *et al.* cloned this cannabinoid receptor which is now designated CB_1.[33] A peripheral receptor (CB_2) was identified in the spleen.[34] Surprisingly the CB_2 receptor has only 44% chemical homology with the CB_1 receptor. (For reviews covering various aspects of the cannabinoid receptors see ref. 35).



HU-210



HU-211

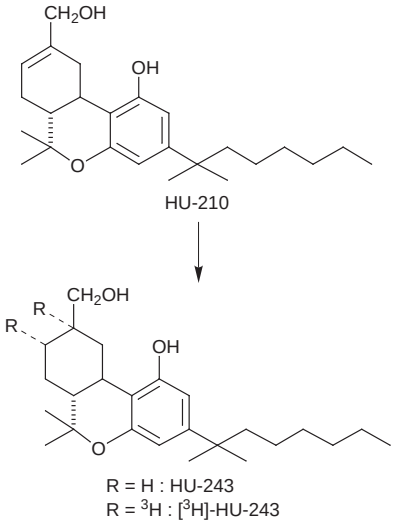
Fig. 2

6 Anandamide

We assumed that the presence of a specific cannabinoid receptor indicates the existence of endogenous specific cannabinoid ligands that activate these receptors. The fact that a plant tricyclic terpenophenol binds to this receptor could be viewed perhaps as a quirk of nature.

The standard assay for new receptor agonists is the displacement of a labeled probe bound to the appropriate receptor. This route was followed in the isolation of anandamide. First, a new probe that was based on the highly active HU-210 was prepared.[36] We found that catalytic hydrogenation of HU-210 led to the formation of two dihydroepimers both of which bind to CB_1. The pure equatorial epimer HU-243 could be obtained using a mixture of Kagan and Wilkinson's catalyst or with [*S*-BINAP], the Noyori catalyst.

The ability of HU-243 to bind to the cannabinoid receptor was assessed in a synaptosomal membrane preparation derived from rat whole brain, using a centrifugation assay. Displacement studies indicated that it was highly potent, binding to the cannabinoid receptor with a K_i of 45 pM. HU-243 is apparently the most active cannabinoid known so far. By comparison, HU-210 has a K_i of 181 pM and Δ^1-THC is a thousand times less potent, with a K_i of 46 nM. When the reduction was performed with tritium the desired labeled probe [^{3}H]HU-243 was obtained. This is an almost ideal probe as it has the typical THC-type cannabinoid structure, a K_D in the picomolar range and high stereospecificity with respect to both pharmacological activity and binding (see Scheme 5 for structures).



Scheme 5 Preparation of the labeled ligand HU-243, used for endocannabinoid isolation.

To screen for endogenous cannabinoid compounds, we tested the ability of fractions from porcine brain extracts to displace [^{3}H]HU-243 in a centrifugation-based ligand binding assay.[37] All plant or synthetic cannabinoids are lipid-soluble compounds. Hence the procedures employed for the isolation of endogenous ligands by our 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.

A major problem encountered in the isolation of anandamide was its lability: although purity increased on repeated chromatography, the amounts of anandamide diminished rapidly. Ultimately, we isolated a fraction which gave one spot on TLC. It eluted mainly as one main peak on gas chromatography–mass spectrometry (GCMS). This compound represented the first example of a purified brain constituent which exhibited most of the properties (see below) of Δ^1-THC. We assumed that it is a natural ligand for the cannabinoid receptor. In a later publication, an improvement of the separation procedure, namely initial precipitation of the inactive phospholipids with acetone, was reported.[38]

We named the active constituent anandamide—based on the Sanskrit word 'ananda' meaning bliss and on its chemical nature (see below) (Fig. 3). This constituent inhibited the specific binding of [^{3}H]HU-243 in a manner typical of competitive ligands with a K_i value of 52 ± 1.8 nM. Surprisingly, this value is almost identical to that of Δ^1-THC in this system (K_i = 46 ± 3 nM).[37]

In addition to the specific binding to the cannabinoid receptor it seemed to us of considerable importance to determine the activity of natural anandamide in an additional bioassay. Pertwee *et al.* had reported that cannabinoids inhibit the twitch response of murine isolated vas deferens (the secretory duct of

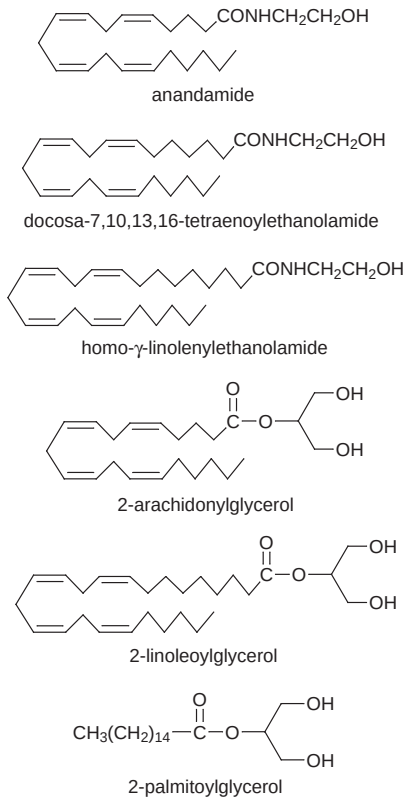


Fig. 3 The natural endocannabinoids.

the testicle) caused by electric current. This assay is not specific for cannabinoids; it had previously been extensively used in opioid research. With cannabinoids it had been shown to be stereospecific and sensitive. Thus, 0.15 nM of (−)-HU-210 decreased the twitch height in the mouse vas deferens by 50%, while the enantiomeric (+)HU-211 had no inhibitory effect up to 30 nM. Δ^1-THC decreased the twitch height by 50% at 6.3 nM. We assumed that this assay was suitable for anandamide in particular in view of its sensitivity and of the minute amounts of natural material available. Indeed, anandamide elicited a concentration-dependent inhibition of the twitch response, decreasing the twitch height by 50% at a concentration of 90 nM.[37]

The laborious isolation procedures described above led to minute amounts of purified material, at best several hundred micrograms of natural anandamide. The isolation of very small amounts of a natural constituent from a complicated mixture inevitably poses problems for the elucidation of structure, owing to the presence of minor impurities associated with the isolation process.

The structure of anandamide was deduced from NMR and MS measurements.[37] The initial indication was by high-resolution MS which suggested the elemental composition $C_{22}H_{37}NO_2$ (*m/z* 347.2762), showing the presence of five double bond equivalents. No ultraviolet absorption above 220 nm, consistent with a conjugated system, was noted. The first indication of the structure was the observation that the NMR peaks at δ 5.30–5.45, presumably due to double-bond protons, were coupled with peaks at δ 2.75–2.90 which we assumed to be signals of doubly allylic protons. Such protons, their couplings and the ratio of vinylic to doubly allylic protons are typically observed in all-*cis*, non-conjugated polyunsaturated fatty acids such as linoleic and arachidonic acids. At this point we assumed that we had a *N*-derivative of such a fatty acid. MS spectra supplied additional data which clarified the structure. 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 of 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); *m/z* 287 ion corresponds to MH^+ less ethanolamine. The existence of an ethanolamine moiety was supported by the MS of a trimethylsilyl derivative (TMS) of anandamide. An *m/z* 419 ion indicated formation of a mono TMS derivative and hence the existence of a single hydroxy group.[37] Additional MS and NMR data are available in the original publication.[37]

The NMR and MS data led to the assumption that anandamide is the ethanolamide of a C_{20} fatty acid with four unconjugated double bonds, presumably arachidonic acid. This assumption was proved by a simple synthesis.[37] Arachidonic acid was converted into its acyl chloride with oxalyl chloride and in a second step the arachidonyl chloride was reacted with ethanolamine, leading to synthetic arachidonoylethanolamide 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 binds to the CB_1 cannabinoid receptor with a K_i of 39 ± 5 nM, that is only slightly lower than that of natural anandamide (K_i of 52 ± 1.8 nM), presumably owing to minor impurities in the natural product.[37]

In addition to anandamide, two other unsaturated fatty acid ethanolamides were isolated from pig brain: homo-γ-linolenylethanolamide and docosatetraenylethanolamide (Fig. 3). They bind to the brain cannabinoid receptor with K_i essentially identical to that of arachidonoylethanolamide.[38] We suggested that the acylethanolamides that bind to the brain cannabinoid receptor be named 'anandamides' with each individual member identified with the parent fatty acid (indicated in parenthesis) following the generally accepted fatty acid shorthand designation: hence the anandamide derived from arachidonic acid is anandamide (20 : 4, *n*-6), that from homo-γ-linolenic acid is anandamide (20 : 3, *n*-6), and that from docosatetraenoic acid is anandamide (22 : 4, *n*-6). When just anandamide is mentioned, it is generally understood to mean the anandamide (20 : 4, *n*-6).

7 2-Arachidonoylglycerol (2-Ara-Gl)

The identification of a second cannabinoid receptor (CB_2) in immune cells[35] led us to look for the presence of additional active endogenous ligands in the gut and later in the spleen, an organ with well established immune functions, again using a fractionation guided by a binding assay. Canine gut or mouse spleen was extracted with methanol, and the extract was chromatographed on a silica gel column to yield a fraction that was found to bind to CB_1. The active fraction consisted mainly of three compounds which, on the basis of MS measurements, were assumed to be the 2-arachidonoylglycerol (2-Ara-Gl), 2-palmitoylglycerol (2-Palm-Gl) and 2-linoleoylglycerol (2-Lino-Gl). This assumption was shown to be correct by direct comparison with synthetic compounds.[39] 2-Ara-Gl was later isolated from brain.[40] The structures of the three 2-acylglycerol esters are presented in Fig. 3.

2-Ara-Gl parallels anandamide in *in vitro* and *in vivo* activity, while 2-Lino-Gl and 2-Palm-Gl showed neither binding activity to CB_1 or CB_2 cannabinoid receptors nor *in vivo* cannabinoid effects in mice. However, both 2-Lino-Gl and 2-Palm-Gl separately or together (in the ratio present in the spleen) potentiated the apparent binding of 2-Ara-Gl to CB_1 and CB_2. The mixture of the three monoglycerides is also more potent than 2-Ara-Gl in the inhibition of adenylyl cyclase in cells transfected with DNA for either CB_1 or CB_2 cannabinoid receptors. The same type of 'entourage' effect was observed in several *in vivo* tests which are commonly used with cannabinoids.[41]

This 'entourage' effect is in part due to inhibition of 2-Ara-Gl enzymatic hydrolysis by cells. Previously, we and others had shown that fatty acid amides, which have no affinity for CB_1 receptors, inhibit anandamide metabolism,[42] thus potentially leading to increased levels of endogenous anandamide available for cannabinoid receptor activation. This inhibitory effect may be the basis of the *in vivo* cannabimimetic actions observed by us for the fatty acid amide oleamide, a putative sleep factor.[42] However other mechanisms cannot be excluded, for example inhibition of endocannabinoid uptake by the cell.

These results may also be of general importance. Biologically active natural products, from either plant or animal origin, are in many instances accompanied by chemically related, though biologically inactive, consituents. Very seldom is the biological activity of the active constituent assayed together with inactive 'entourage' compounds. Investigations of the effect of the active component in the presence of its 'entourage' compounds may lead to results that differ from those observed with the active component only.

This type of synergism may play a role in the widely held (but not experimentally based) view that in some cases plants are better drugs than the natural products isolated from them.

8 Structure–activity relationships of THC derivatives and of endocannabinoids

SAR for psychotropic (CB_1) activity of THC derivatives, were established years ago and have withstood the erosion of time.[25,43] The central rules are: *a*, modifications of the side chain lead to significant changes of activity, the dimethylheptyl moiety increasing activities sharply; *b*, the phenolic group has to be free; *c*, the C_1 position has to be substituted by a hydroxy, a methyl or a hydroxymethyl group; *d*, alkyl substitution at C_4' retains activity, but such a modification at C_6' eliminates activity; *e*, as mentioned above the (−)-(3*R*,4*R*) enantiomers only possess psychotropic activity.

Rules for CB_2-associated action have not been formulated yet. However apparently they differ from the above rules. Thus, the phenolic group may be etherified[44] or it can even be eliminated[45] with retention of activity.

Numerous bicyclic and heterocyclic cannabinoid derivatives have been synthesized, mostly by companies. A SAR analysis of these compounds is beyond the scope of this short review. We would like to point out just a few which are of particular importance: CP-55940 and WIN-55212-2 are extensively used cannabinoid agonists, SR-141716A, is a CB_1 antagonist and SR-144528, is a CB_2 antagonist (see Fig. 4 for structures).[46]

Since the identification of anandamide as an endocannabinoid, numerous groups have reported on the SAR in this series. Mostly three types of modifications have been made:
(A) Changes in the amide moiety in the arachidonoyl (20 : 4, *n*-6) series.
(B) Changes of the acyl moiety leading to a variety of *N*-acylethanolamines.
(C) Changes in the length and branching of the end pentyl chain of anandamide.

All analogs synthesized have been tested for binding to CB_1.[47–52] Other assays are rarely reported and it is yet unknown whether there is a parallelism between binding and *in vivo* activities. There is considerable overlap in the data of the various groups, hence the references indicated above are not assigned to each modification.

The structure–activity relationships observed in the A class modifications were as follows:
(1) *N*-Monoalkylation, up to a branched pentyl group, leads to significant binding. For anandamide-type compounds with *N*-acyl moieties, the regularities in binding were as follows: *n*-C_5H_{11} < branched C_5H_{11} < $CH(CH_3)CH_2CH_3$ (*R* or *S*) < *n*-C_4H_9 < $C(CH_3)_3$ < CH_3 < C_2H_5 < $C(CH_3)_2$ < *n*-C_3H_7. The last two compounds were the most active in these homologous series.

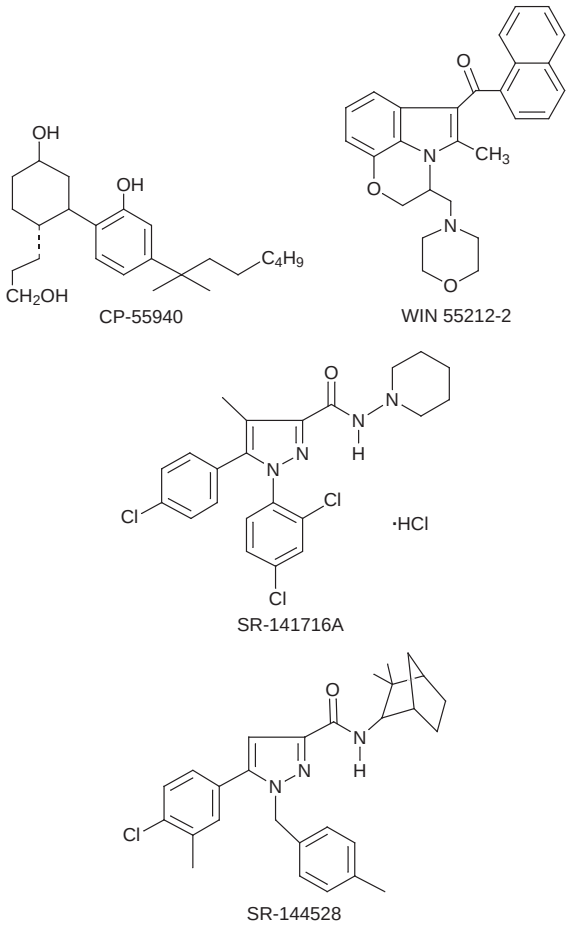


Fig. 4 Heterocyclic cannabinoid agonists and antagonists of cannabinoid receptors.

(2) *N*,*N*-Dialkylation, with or without hydroxylation on one of the alkyl groups, leads to elimination of activity.
(3) Hydroxylation of the *N*-monoalkyl group at the ω carbon atom retains activity, though it may be somewhat lower compared to the parent *N*-alkyl group.

However, (*R*)-(+)-arachidonoyl-1-hydroxy-2-propylamide [(*R*)-methandamide][50] was considerably more potent than anandamide and was more stable to enzymatic hydrolysis. Significant variation in activity was noted between the *S* and *R* compounds.
(4) Alkylation of the carbon adjacent to the nitrogen atom with a methyl group retains activity as compared to anandamide, while alkylation with an isobutoxy group on the same position leads to inactivation.
(5) Anandamide phosphate is less active than the parent alcohol. Oxidation of the primary alcohol in anandamide to a carboxylic acid led to inactivation. Its methyl ester was also inactive. Replacement of the hydroxy substituent with a fluorine led to a potent compound with K_i values about two times lower than that of anandamide.
(6) Methylation or dimethylation of the α carbon adjacent to the carbonyl group retains the level of binding in the case of anandamide; however α-monomethylation or α,α-dimethylation of *N*-propyl derivatives potentiated binding and led to very active compounds. Alkylation of the α carbon with ethyl or isopropyl groups leads to much less active compounds.

B Class modifications, namely variations of the acyl moiety, have not been thoroughly investigated, however some tentative generalizations have been established.
(1) In the 20 : *x*, *n*-6 series, *x* has to be 3 or 4; two double bonds only lead to inactivation.
(2) In the *n*-3 series, the limited data suggest that the derived ethanolamide are either inactive or less active than related compounds in the *n*-6 series.

C Class modifications at the pentyl end of the side chain, corresponding to the pentyl side chain of the tricyclic cannabinoids, can be summarized as follows:
(1) Increasing the length of the end pentyl chain increases the binding affinity.
(2) Branching of the end pentyl chain in anandamide leads to potent analogs, the dimethylheptyl (DMH) analog being the most potent in the series.
(3) Conversion of the pentyl group at the end of the fatty acid chain into a DMH group, in several *N*-alkylated analogs of anandamide, led to a considerable potentiation of activity.

The above observations are compatible with the SAR of tricyclic cannabinoids.

9 Biosynthesis and inactivation of the endocannabinoids

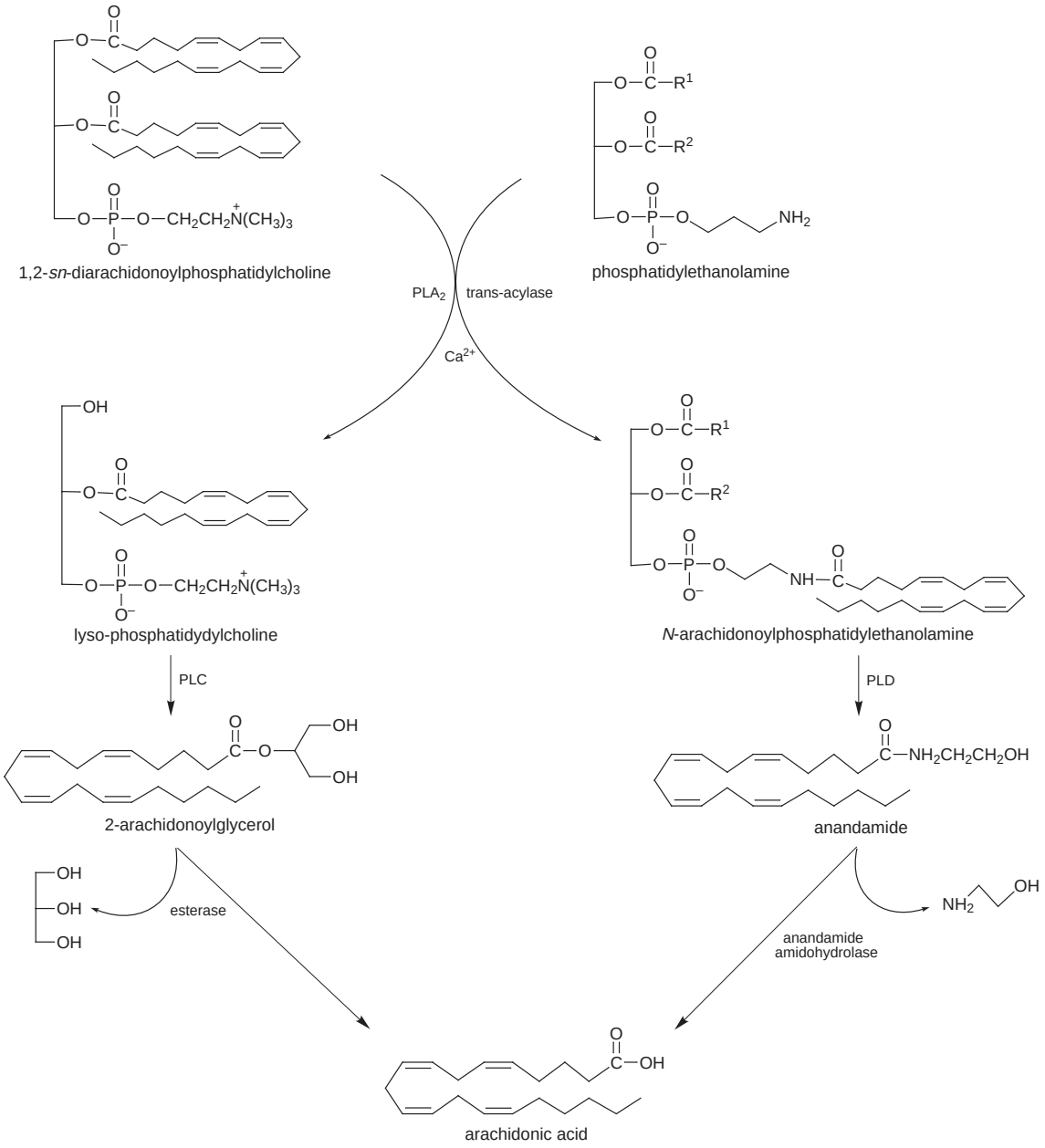
Soon after the identification of anandamide it was prosposed that this amide is formed simply by enzymatic condensation of arachidonic acid with ethanolamide. An enzyme, a synthase, was indeed identified.[53] However the levels of ethanolamine needed seem to be too high for an *in vivo* reaction and evidence was brought forward that this endocannabinoid is actually formed following a pathway previously proposed for other fatty acid ethanolamides, namely the initial formation of *N*-acylphosphatidylethanolamine (NAPE).[54a] Indeed primary cultures of neurons contain detectable levels of NAPE. The biosynthesis of NAPE itself is stimulated by intracellular levels of calcium and is potentiated by a protein kinase. Enzymatic hydrolysis of NAPE by phospholipase D yields anandamide (Scheme 6).[54b]

The biosynthesis of 2-Ara-Gl is also dependant on calcium influx into cells. Enzymatic hydrolysis of diacylglycerol (DAG) seems to be the most important route, although the phospholipase C hydrolysis of phophatidylcholine or phosphatidyl inositol has also been noted (Scheme 6).[55] The intermediacy of DAG, a second messenger associated with stimulation of the activity of protein kinase C, is a further example of the propensity of biological systems of using existing constituents for various purposes.

Anandamide is inactivated in central neurons by both reuptake and enzymatic hydrolysis. The uptake takes place in part at least by transport by carrier proteins.[56] Administration of AM-404, an inhibitor of anandamide uptake, indeed causes potentiation of its action.[57] Some of the anandamide, and apparently the entire 2-Ara-Gl, reuptake is by passive diffusion through the cell membrane.[41,58] The recapture of 2-Ara-Gl is partly inhibited by other endogenous acylglycerols and is part of the 'entourage effect' (see above). Within the cell both anandamide and 2-Ara-Gl are enzymatically hydrolysed to arachidonic acid and ethanolamine or glycerol, respectively. The amidase that hydrolyses anandamide has been cloned.[59] It also hydrolyses oleamide, a sleep inducing factor and has been named fatty acid amide hydrolase (FAAH). This enzyme also works 'in reverse': in the presence of high concentrations of arachidonic acid and ethanolamine or oleic acid and ammonia it forms anandamide or oleamide respectively. It is doubtful whether this reaction has biological significance. Surprizingly FAAH also hydrolyses 2-Ara-Gl.[60] However this ester is also broken down in cells which do not contain FAAH, hence apparently nonidentified lipases can contribute to this reaction.

10 Pharmacology of THC and of the endocannabinoids

Activation of either CB_1 or CB_2 initiates similar, but not identical, transduction pathways.[61] A well studied initial step is the inhibition of adenylyl cyclase *via* an inhibitory G protein.[62] Δ^1-THC binds to both receptors with similar affinity. However, in contrast to its capacity to serve as an agonist for the CB_1 receptor and to inhibit adenylyl cylcase through it, Δ^1-THC was only able to induce a slight inhibition of adenylyl cyclase at the



Scheme 6 Biosynthesis and degradation of anandamide.

CB_2 receptor. Morever, Δ^1-THC antagonizes the agonist-induced inhibition of adenylyl cyclase mediated by CB_2. Therefore, it has been suggested that Δ^1-THC constitutes a weak antagonist for the CB_2 receptor.[63] Similar effects have been observed with anandamide.[61] Contrary to results with CB_1, anandamide did not inhibit significantly CB_2-coupled adenylate cyclase activity in transfected cells. These results characterize the CB_2 receptor as a functional and distinctive member of the cannabinoid receptor family. CB_1, but not CB_2, activation inhibits N- and Q-type calcium channels and enhances inwardly rectifying potassium channels.[46,64] The inhibition of N-type calcium channels may cause a decrease of neurotransmitter release of acetylcholine, noradrenaline and glutamate in some tissues. However, in some cases stimulation rather than inhibition of the cyclase has been recorded. Cannabinoids activate MAP (mitogen-activated protein), and presumably through it, cause the induction of the transcription factor Krox 24.[46,64]

Some of the effects may be CB_1 and CB_2 receptor independent.[64,65] Cannabinoid stimulation of arachidonic acid release, possibly the inhibition of L-type calcium channels, and modulation of serotonin and NMDA transmembrane signalling do not proceed through CB_1 or CB_2, as these effects are not blocked by their receptor antagonists. However the mechanism is still obscure. These observations may lead to the discovery of additional receptors or novel pathways.

A detailed discussion of cannabinoid signalling mechanisms is beyond the scope of this review. Numerous recent reviews address this topic.[33,46,65]

The pharmacology of cannabis, THC and related cannabinoids have been the topic of intensive research over several decades and the results have been summarized in several excellent reviews.[22–24] First we would like to stress that THC-like substances do not parallel in their activity any of the CNS-active drugs, such as cocaine, morphine, phenytoin, amphetamine or atropine, and that "one [cannnot] readily find another substance so 'contradictory', capable of taming yet producing aggressiveness, of both enhancing and depressing spontaneous activity, of being an anticonvulsant yet generating epileptiform cortical discharges."[66] At low doses a mixture of depressant and stimulatory effects are noted, while at higher doses CNS depression predominates.[24] During the early depressive state even a mild stimulus may cause hyper-reflexia. Thus, in a group of sedated mice, sudden noise can cause some mice to jump,

which will produce a 'popcorn' effect of all other mice doing the same. However high doses of THC invariably cause sedation and, in rodents, catalepsy. In dogs one sees ataxia, which however is rarely, if ever, seen in monkeys or man, who usually become sleepy.

The standard animal assay for cannabis action is a tetrad of tests, none of which is specific for cannabinoids but, together, they are strongly indicative.[67] They are the ring immobility (catalepsy) test, which measures the percent of time mice remain motionless on a ring and the open field test, which measures horizontal (locomotor) and vertical (rearing) activity. Hypothermia and antinociception (hot plate latency) are also measured. A further test is the dog ataxia.[22,24] Δ^1-THC (0.2 mg kg^{-1}) injected i.v. causes static ataxia, depressed activity and the tails are typically tucked. Assays based on drug discrimination are at present the most specific *in vivo* tests available.[68] In these types of assays rats or pigeons are trained to emit one response when trained with Δ^1-THC and an alternative response when trained without drug. Compounds which resemble THC in its action will lead the animal to perform as if it were under the influence of THC.

The effects of cannabis and THC in man are quite well known to the general public. Changes in perception are frequently amongst the first to be noted.[22,24] Distances are difficult to estimate with objects being either too small or too large, walls may seem advancing or receding. With large doses 'a lively 3-D effect' of objects, such as a face, are seen.[22] Indeed recently Emrich *et al.* found that a three-dimensional inversion illusion in volunteers under the influence of THC resembled that seen in schizophrenia.[69] Alterations in the sense of time are one of the most regular effects of cannabis. Together, the distortions of time and distance are some of the major dangers of cannabis use. Other effects of cannabis, or THC, involving perception are changes in color, shape, pattern and contrast, increase in auditory sensitivity, as well as various effects, or perceived effects, involving emotions. On moderate intoxication memory impairment, depersonalization and mood changes are seen. While in most cases these mood changes are defined as pleasant and are certainly one of the major reasons for cannabis use, in many cases acute panic reactions or even psychosis are noted. However a specific 'cannabis psychosis' does not exist. Several psychotic cases of cannabis intoxication were recorded from South Africa.[70] Cannabis resin (dagga) from South Africa frequently contains no cannabidiol.[2] This constituent has been shown to be anxiolytic both in animals and man,[71] and to reduce the anxiety reaction caused by THC.[72] Hence one can surmise that the presence of cannabidiol in marijuana or hashish reduces the number of psychotic cases; however experimental proof is not available.

THC produces significant hypothermia in man but the doses required are above those that produce behavioral effects.[73] HU-210 causes a very potent hypothermic effect in the rat.[74] THC causes a decrease in heart rate and hypotension in animals, although in man usually an increase in heart rate is found.[22,24] In view of the hypotensive effect of endocannabinoids and their possible role as endogenous modulators of blood pressure[75] (see below) these early observations may be of considerable significance and importance.

Tolerance to some, but not to all, effects of THC is easy to produce in animals. However high tolerance is seldom seen in cannabis smokers, presumably because of the relatively low levels of THC generally consumed. Withdrawal symptoms generally are not seen on cessation of THC administration in animals, however administration of a cannabinoid antagonist to mice may cause effects which can be viewed as withdrawal symptoms.[76] The relevance of this observation to humans has been challenged.[77]

Shortly after the identification of anandamide as an endocannabinoid, its effects in the mouse 'tetrad'—inhibition of motor activity, catalepsy, hypothermia and hypoalgesia—were described.[78] As expected, they paralleled the THC effects. A flood of comparisons between anandamide and THC then followed. Amongst the comparisons reported were inhibition of the dopaminergic nigrostriatal system,[79] interference with learning and memory,[80] drug discrimination,[81] activation of the hypothalamo–pituitary–adrenal axis,[82] decrease in prolactin release by the hypothalamus,[83] hypotension and bradycardia,[75] and immune modulation.[84] However a number of differences between anandamide and THC have been noted. Thus, surprisingly, anandamide is a partial agonist in some *in vivo* and *in vitro* assays, while THC is a full agonist.[85,86]

Very low doses of anandamide inhibit THC effects both *in vivo* and *in vitro*;[86] a stimulatory (rather than inhibitory) effect on the tetrad and on phagocytosis has been observed in related experiments.[87] It is possible that very low doses of anandamide exert their effects through activation of a CB_1 receptor G-stimulatory protein rather than the G-inhibitory protein pathway generally observed.[86] This early suggestion has received experimental support.[88] In view of the very low levels of anandamide in the brain it is quite possible that some of the actual physiological effects of anandamide in man are stimulatory rather than inhibitory.

Anandamide inhibits the fertilizing capacity of sea urchin sperm;[89] in rodents it can prevent implantation of the embryo in the uterus.[90] Whether anandamide has a role in mammalian reproduction is still to be determined.

The role of anandamide on the regulation of blood pressure and vasodilation is being very actively investigated (for a review see ref. 91). In hemorrhagic shock for example, macrophages produce and release anandamide which causes a sharp decrease in blood pressure.[92] It has been proposed that anandamide may represent the long sought after endothelium-derived hyperpolarizing factor (EDHF) which may be one of the regulators of blood pressure.[91]

Anandamide acts also *via* biological routes not associated with the cannabinoid receptors. For example, anandamide, but not THC, inhibits gap-junction permeability.[93] Direct, gap-junction transport of ions or organic molecules between cells in an important biological pathway and intervention in this process may have pharmacological implications.

It is surprising that very little pharmacological work on the second endocannabinoid, 2-Ara-Gl, has been done so far. In shock, blood platelets release 2-Ara-Gl.[94a] We have found that carbachol, which is known to liberate a hyperpolarizing substance from the vascular endothelium *via* activation of the muscarinic receptor, causes a potent increase of 2-Ara-Gl levels in rat aorta (from about 0.7 nmol g^{-1} wet weight to about 3.7 nmol g^{-1} wet weight). 2-Ara-Gl (12 mg kg^{-1}) causes a short lived decrease of blood pressure in the mouse on i.v. administration, from 122 to 82 mm kg^{-1} in mean arterial pressure which waned after 18 minutes. As 2-Ara-Gl is rapidly hydrolysed *in vivo*, we tested a stable 2-Ara-Gl analog, namely 2-arachidonyl glyceryl ether, in which the labile ester moiety of 2-Ara-Gl is replaced by a stable ether one. This synthetic ether reduced the blood pressure more potently, from 135 mm Hg to 72 mm Hg which lasted for at least 30 minutes.[94b] We have also found that rat hearts on ischemia increase their production of 2-Ara-Gl about twofold. These observations indicate that 2-Ara-Gl may play a role in the cardiovascular system.

11 Cannabinoids as therapeutic agents

The cannabis plant has a long history in medicine.[1] The identification of the plant constituents made possible their examination in various pharmacological screens. Most of the work reported deals with THC and CBD.

THC is an approved drug against nausea and vomiting caused by cancer chemotherapy.[95] Unfortunately at the doses required to suppress these effects about one third of the patients report serious side effects, such as mood changes, for example.

Therefore its use has remained limited. A much larger number of patients seem to smoke cannabis which is claimed to cause less side effects, possibly due to the direct administration by smoking, or to the presence of cannabidiol (see above).

The antiemetic activity of cannabinoids, the mechanism of which is still obscure, is not mediated *via* the cannabinoid receptors. Hence non-cannabimimetic cannabinoids may prevent emesis without causing THC-type effects. HU-211 (see below) is indeed a potent antivomiting agent in pigeons, although it does not bind to the cannabinoid receptors.[96]

We have noted that in mice the response to THC develops gradually and does not reach maximal potency until adulthood possibly due to the gradual development of the receptors.[97] THC administered to mice shortly after birth exhibits essentially no activity. We assumed that, if this observation is relevant for humans, young children will not experience THC-type effects while the antiemetic potential might be preserved.[98] We chose Δ^6-THC, rather than the marketed Δ^1-THC, as it is less psychotropic, much less expensive than Δ^1-THC to produce and is much more stable than Δ^1-THC. We started a clinical trial with a low dose (5 mg m^{-2}) which in some adults already causes psychotropic effects. None was observed in the children. Hence, the antiemetic dose was increased from 5 mg m^{-2} up to 18 mg m^{-2}. At this very high dose, which cannot be administered to adults due to side-effects, children exhibited no psychotropic effects. Vomiting or nausea caused by the cancer chemotherapy was completely eliminated.[98]

One of the symptoms of AIDS is loss of weight. Many patients smoke marijuana as it is an appetite stimulant. Several clinical studies have given support to this use. In one such study the effects of Δ^1-THC (dronabinol) (2.5 mg, twice a day) on appetite, weight, nausea and mood were examined in 139 AIDS patients over a six-week period.[99] After 4 weeks the weight was stable in the treated patients but was lower in the placebo recipients. The data indicated that dronabinol caused increased appetite in about one third of the patients. The authors of this study conclude that 'dronabinol is a safe and effective treatment for anorexia in patients with weight loss due to AIDS. By improving appetite and mood, decreasing nausea, and stabilizing weight, dronabinol may significantly improve the quality of life of patients infected with HIV'.

Multiple sclerosis is a slowly progressive disease with exacerbations and remissions over many years. Its symptoms include spasticity, lack of balance, tremor, muscle pain, slurred speech as well as anxiety and depression. Numerous drugs are used but none is ideal. Numerous patients consume cannabis as it is believed that some symptoms are ameliorated. Several animal and small scale studies in humans have given support to this use.[95]

In an animal model (named experimental autoimmune encephalomyelitis, EAE), rats are administered CNS tissue or myelin basic protein. Within 10–12 days the animals develop symptoms reminiscent of multiple sclerosis. The animals are observed over a period of several weeks. In one of these studies, Lyman *et al.* noted that Δ^1-THC decreased EAE inflammation, and led to much reduced effects and that the time of the appearance of the MS effects was delayed.[100] In a second study Δ^6-THC was employed. Again the drug significantly reduced the incidence and severity of neurological deficit in two strains of rats.[101]

In a clinical trial (double blind, placebo-controlled) a group of nine patients were administered up to 10 mg Δ^1-THC. Some patients felt that they were better able to walk. The authors measured deep tendon reflexes, muscular resistance to stretch of the legs and abnormal reflexes, and found improvements.[102]

In a recent report, Brenneisen *et al*. administered orally Δ^1-THC (10–15 mg) to two patients, and compared its effects with those of Δ^1-THC hemisuccinate administered by suppositories.[103] Both treatments reduced spasticity and rigidity and improved walking in objective measurements.

Consroe *et al.* have reported and analysed the answers to a questionnaire mailed to MS patients who use cannabis.[104] Most of the patients reported strong improvement after cannabis in spasticity, in sleep onset, and in awakening at night, as well as reduction of leg pain at night and of tremor. The patients also reported improvement in anxiety and depression as well as in spasticity when awaking in the morning and on walking. There was only minor improvement in memory loss, in faecal incontinence and in constipation. This difference in reported symptom improvement indicates that the effects are apparently not placebo ones. Hence this report should be considered a good basis for an initiation of clinical trials.

A recent report by the British Medical Association concludes: 'Cannabinoids may have a potential use for patients with spastic neurological disorders such as MS and spinal cord injury. Such patients often have distressing symptoms which are not well controlled with available drugs. Carefully controlled trials of cannabinoids in patients with chronic spastic disorders which have not responded to other drugs are indicated. Such trials merit a high priority.'[105] Maybe the Assyrians were justified in using Cannabis as a drug against neurological diseases?

THC on systemic administration, or on smoking, reduces intraocular pressure, the major symptom of glaucoma.[106] However when administered directly into the human eye no effect is noted. Systemic use causes, of course, THC-like effects which are not acceptable to most glaucoma patients hence its use is minimal. The antiglaucoma effect apparently is not mediated by the cannabinoid receptors and antiglaucoma cannabinoid-type drugs which are less liposoluble than THC can possibly be prepared and can therefore be administered as eye drops, which may not necessarily cause THC-type side effects. No work on the structure–activity relationships (SAR) in this area has been reported. Anandamide also reduces intraocular pressure.[107] However an initial small increase was noted.

THC causes bronchodilation and may possibly represent an opening to new approaches.[108] Yet, again, there are no SAR publications.

In the past cannabis was used for specific kinds of pain (migraine for example).[1] However it is not a potent antinociceptive agent. Numerous cannabinoid modifications, mostly heterocyclic, have been reported and assayed for analgesic activity.[109] A clear cut separation between cannabimimetic effects and antinociception has not been established as THC elicits antinociception mostly through the central CB_1 receptor. In addition THC stimulates the release of opioid (dynorphin) peptides thus leading to kappa opioid antinociception.[110] Indeed cannabinoid antinociception is blocked by opioid antagonists.[111] THC has been shown to augment morphine activity[112] and such a combination may be of therapeutic use.

Nearly 10 years ago we showed that HU-211, a synthetic (+)-(3*S*,4*S*) THC-type enantiomer, has no psychotropic activity.[113] However we observed that it exhibits pharmacological effects typical of *N*-methyl-D-aspartate (NMDA) receptor antagonists.[114] Binding studies with known noncompetive NMDA receptor antagonists showed that HU-211 blocks NMDA receptors stereospecifically by interacting apparently with a site close to, but distinct from, that of noncompetitive NMDA receptor antagonists. The NMDA receptor is one of the subreceptors of glutamate, which is now well-established as the transmitter at most excitatory synapses in the mammalian CNS. In numerous disease conditions (cerebral ischaemia for example) or on brain trauma, overactivation of the NMDA receptor is noted. It causes increased influx of Ca^{2+} ions into cells leading to their death. While numerous NMDA antagonists have been tested, none has as yet successfully passed the clinical tests because of various side-effects.

HU-211 blocks the lethal Ca^{2+} uptake by primary neuronal cultures of rat forebrain.[115] Furthermore, HU-211 protects rat neuronal cultures against NMDA-mediated glutamate tox-

icity,[116] and suppresses production of tumor necrosis factor (TNF), a cytokine with a wide variety of biological effects, some of which, such as septic shock and fever are extremely dangerous.[117]

The above results led to numerous *in vivo* investigations. HU-211 was found to block NMDA-induced tremor, convulsions and death in mice.[114] Shohami and collaborators and Biegon *et al.* reported that some effects of closed head injuries in rats could be significantly improved by HU-211 administration.[118] Thus HU-211 administered up to 4 h after closed head injury resulted in significant reduction of oedema formation, improvement of clinical status, and of spatial memory. The integrity of the blood brain barrier (BBB) is severely broken down on brain injury. HU-211 was found to improve BBB integrity. Accumulation of Ca^{2+} in several brain regions takes place on closed head injury. HU-211 attenuated such accumulation, indicating that the proposed mechanism of action—blockage of the NMDA-operated Ca^{2+} channel—is most probably correct. This compound also inhibits up to 90% of the tumor necrosis factor surge seen after closed head injury in rats. For more detailed reviews on the activity of HU-211 see ref. 119.

A Phase I trial with HU-211 in volunteers (doses up to 100 mg) has been completed. In such trials possible toxic effects are monitored. No undesirable CNS or other effects were noted. Phase II trials in several hospitals in Israel in cases of CNS trauma have been completed. Significant reductions in intracranial pressure were noted and Glasgow Outcome Scale results (an indication of general improvement) have indicated an increase in favourable outcome.[120]

12 Conclusions

Within the past 20 years cannabis research has gradually evolved from chemical investigations of plant constituents, their synthesis and metabolism to investigations of the physiological basis of their activity. This research has led to the discovery of specific receptors and of endogenous ligands which, not surprisingly, differ chemically from the plant constituents. The discovery of this biological system has opened new pathways towards our understanding of numerous physiological processes such as blood pressure regulation, neuroprotection, memory and possibly emotions. These advances were made possible through successful collaboration between chemists, biochemists, pharmacologists and physiologists which obviously is the trend of the future. And, maybe, novel drugs based on the tricyclic cannabinoids or the endocannabinoids will be introduced, with actions ranging from CNS trauma and stroke to inflammation, regulation of blood pressure and neuroprotection.

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