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The cannabinoid system: from the point of view of a chemist

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This book is about cannabis (marijuana) and psychotic illnesses; more specifically, it outlines how our increasing understanding of cannabis itself, the effects of cannabis on the brain and psychic functions and of the cannabinoid system can inform our understanding of the relationships between cannabis and psychosis. This chapter serves as an introduction to this topic, with a brief historical overview of the psychic effects of cannabis, followed by an exposition on the cannabinoid system.

Cannabis and mental illness

J. J. Moreau, the first nineteenth-century psychiatrist with an interest in psychopharmacology, described in great detail his experiments with hashish (Moreau, 1973). He took the drug himself and asked his students to follow his example. He also administered it to his patients. By modern standards the doses used were enormously high. The effects on one of his assistants, who swallowed 16 g of an extract – presumably containing several hundred milligrams of tetrahydrocannabinol (THC), which we know today to be the major psychotropic principal of cannabis – were intense agitation, incoherence, delirium and hallucinations. On the basis of numerous such experiments, Moreau declared that 'there is not a single, elementary manifestation of mental illness that cannot be found in the mental changes caused by hashish, from simple manic excitement to frenzied delirium, from the feeblest impulse, the simplest fixation, the merest injury to the senses, to the most irresistible drive, the wildest delirium, the most varied disorders of feelings'. He considered hashish intoxication to be a model of endogenous psychoses, which could offer an insight into the nature of psychiatric diseases. Some of the effects described by Moreau – obsessive ideas, irresistible impulses, persecutory delusions and many others – are

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certainly seen in psychiatric patients, but any relationship of the physiological and biochemical basis of cannabis action to that of mental disease is still questionable.

About the same time O'Shaughnessy in India experimented with charas – the local brand of cannabis – as a therapeutic drug (O'Shaugnessy, 1841; 1843). He administered small doses of charas to dogs and 'three kids'. The dogs 'became stupid and sleepy', 'assumed a look of utter and helpless drunkenness', and 'lost all power of the hinder extremities'. As to the kids, 'In one no effect was produced; in the second there was much heaviness and some inability to move; in the third a marked alteration of countenance was conspicuous, but no further effect.' In none of these, or several other experiments, was pain or any degree of convulsive movement observed. These experiments apparently convinced O'Shaugnessy that 'no hesitation could be felt as to the perfect safety of giving the resin of hemp an extensive trial in the cases in which its apparent powers promised the greatest degree of utility', and clinical trials were initiated.

Ethanol extracts (tincture) of cannabis resin were administered to patients with rheumatism, tetanus, rabies, infantile convulsions, cholera and delirium tremens. These diseases were chosen in order to confirm well-established local medical traditions. In the case of rheumatism two out of three cases were 'much relieved . . . They were discharged quite cured in three days after'. In both cases the huge doses caused side-effects such as catalepsy or uncontrollable behaviour, which today would be considered unacceptable. Further trials with lower doses gave closely analogous effects: 'alleviation of pain in most – remarkable increase of appetite in all – unequivocal aphrodisia, and great mental cheerfulness. The disposition developed was uniform in all'. O'Shaughnessy also noted that cannabis was a potent antivomiting agent. This property was rediscovered about 120 years later; no credit has been given to O'Shaugnessy in any of the numerous contemporary publications on this topic.

The reports by O'Shaugnessy were received with considerable interest. Gradually Indian hemp became an accepted drug in therapy, originally in England and later, to a limited extent, in other European countries and in North America (Mechoulam, 1986). Cannabis was used in a variety of conditions – mostly in pain and inflammation – but its use in psychiatric cases appears to have been minimal.

Donovan (1845) confirmed many of O'Shaugnessy's observations, in particular the potent anti-inflammatory effects. He also observed the effect of causing hunger and suggested its use in anorexia. However, he does not seem to have done any work in this direction.

Russell Reynolds recorded that cannabis is 'absolutely successful for months, without any increasing dose, in cases of senile insomnia'. In mania cannabis was 'worse than useless'. He found no effect in depression (Reynolds, 1890).

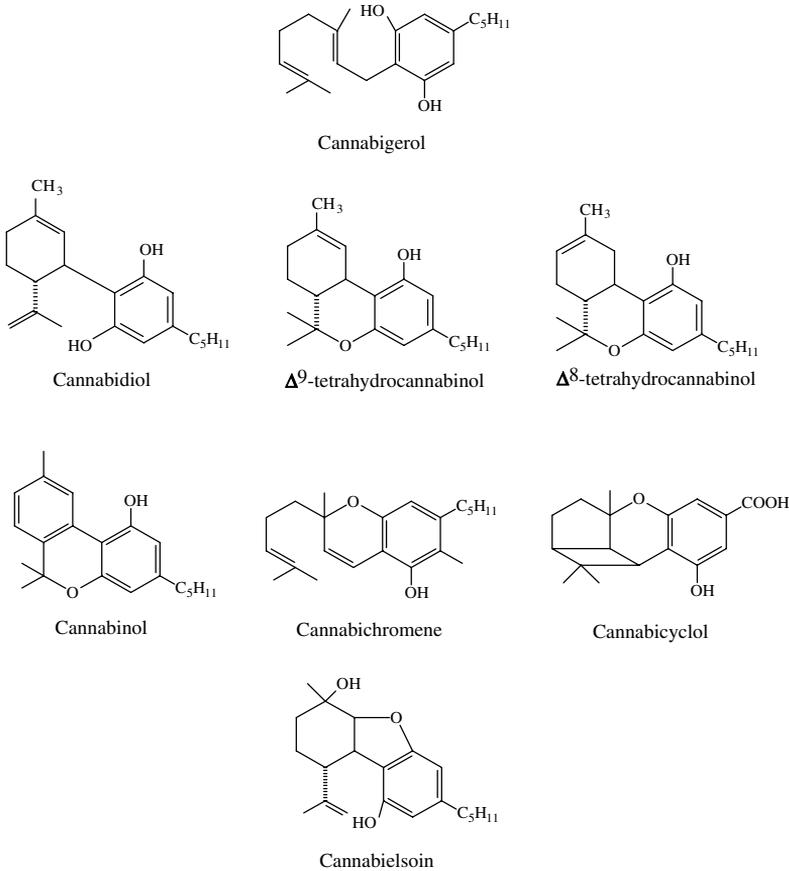**3**   **The cannabinoid system: a chemist's point of view**

Numerous nineteenth-century physicians, mainly in the UK, confirmed the anti-inflammatory effects of Indian cannabis. Good results were also seen with persistent headaches and as calmatives. The main problem seems to have been the lack of consistency of therapeutic results. It is known today that THC undergoes oxidation with ease. While fresh imported Indian charas was effective initially, it probably lost its potency gradually (Mechoulam, 1986).

Understanding cannabinoid chemistry

A comparison between the chemistry of opium and cannabis, the two major illicit drugs in most of the world, can perhaps explain the lag in research and therapeutic use of these natural products. The active constituent of opium, morphine, was easily identified early in the nineteenth century as it is an alkaloid which forms isolable crystalline salts. It was introduced in medical practice shortly thereafter. By contrast, the active constituent of cannabis, in spite of numerous trials, could not be isolated and identified. We know today that the active THC is present in a mixture of many, chemically closely related, terpeno-phenols which are difficult to separate and purify.

In the late 1930s and early 1940s Roger Adams, in the USA (Adams, 1941–1942), and Alexander Todd, in England (Todd, 1946), made significant progress in cannabinoid chemistry, but the active constituent was not isolated and further research in this field was abandoned. Our group renewed work on cannabis in the early 1960s and, using novel separation techniques, which by then had been developed, we were able to identify in hashish many new cannabinoids, including the major psychotropic constituent, Δ^9-tetrahydrocannabinol (Δ^9-THC: Gaoni and Mechoulam, 1964). Numerous additional cannabinoids were isolated by column chromatography and their structures were elucidated. The major ones were cannabidiol (CBD), cannabinol, cannabigerol and cannabichromene (Mechoulam, 1973; Turner *et al.*, 1980; Fig. 1.1). The rest were exiguous. All the purified compounds were tested in rhesus monkeys (Mechoulam and Edery, 1973). Only Δ^9-THC showed psychotropic activity: the monkeys became sedated, indifferent to the environment, and decline of aggression was noted. The effects were dose-dependent. CBD, cannabigerol and cannabichromene had no THC-like activity. However, cannabinol has some activity and Δ^8-THC, which is a very minor component, parallels Δ^9-THC activity, although it is somewhat less potent. Since 1964 thousands of papers on the chemistry, pharmacology, metabolism and clinical effects of Δ^9-THC and related synthetic compounds have appeared.

A comparison of the somatic and behavioural effect of Δ^9-THC in human subjects and in monkeys has been made (Mechoulam and Edery, 1973). Both species have comparable threshold effective doses (50 µg/kg), dose-dependent effects,

Cannabigerol

Cannabidiol Δ^9-tetrahydrocannabinol Δ^8-tetrahydrocannabinol

Cannabinol   Cannabichromene   Cannabicyclol

Cannabielsoin

Figure 1.1 Major cannabinoids in marijuana.

impairment of motor coordination and of performance, redness of the conjunctiva,
loss of muscle strength, heart rate increase and slow movements. Unfortunately,
due to legal–ethical considerations, very little further work on monkeys, either with
the plant cannabinoids or with the endogenous cannabinoids (see later), has been
done over the last few decades.

Some studies indicate that Δ^9-THC alone accounts for the activity of cannabis.
Thus we showed that in rhesus monkeys, Δ^9-THC alone and Δ^9-THC together
with several of the major cannabis components (in a ratio found in the crude drug)
caused the same effects (Mechoulam *et al.*, 1970). A more recent study in healthy
volunteers came to the same conclusion (Wachtel *et al.*, 2002). However, marijuana
users insist that smoked cannabis and Δ^9-THC administered orally do not have
identical action (Grinspoon and Bakalar, 1997). Smoking is a more efficient and
rapid route of administration and maybe this is the main reason for the differences

observed; the presence of additional non-psychotropic constituents may also be of importance.

Cannabidiol

Most of the non-psychotropic cannabinoids have only been examined cursorily for their biological effects. However, there is renewed interest in CBD. In view of its putative action in anxiety and schizophrenia (see below), its pharmacological effects are discussed here in some detail.

CBD was first isolated from the cannabis plant in the late 1930s and early 1940s (Todd, 1946). Its structure was elucidated in 1963 (Mechoulam and Shvo, 1963). The chemistry of CBD was recently reviewed (Mechoulam and Hanuš, 2002). No detailed pharmacological work was reported on CBD until the early 1970s, except that it had no THC-like activity in vivo (Mechoulam and Edery, 1973). Then, by a strange coincidence, two groups, at almost the same time, reported that CBD reduces or blocks convulsions produced in animals by a variety of procedures (Carlini *et al.*, 1973; Turkanis *et al.*, 1974). It was also found to enhance the anticonvulsant effects of diphenylhydantoin and phenobarbital. Since then a considerable amount of research has been done in this area (for a review, see Consroe, 1998). The anticonvulsive activity of CBD differs from that of THC. While the effects of THC can be blocked by cannabinoid receptor antagonists (see below), those of CBD are not affected (Wallace *et al.*, 2001). Apparently the anticonvulsive action of CBD is not mediated through these receptors. The research over the last few decades indicates that CBD is inactive in animal models of absence seizures produced by electroshock or chemical shock. However, it is active against cortical focal seizures produced by electrical stimulation or application of convulsant metals, as well as in generalized maximal seizures produced by electroshock (Consroe, 1998).

A double-blind clinical trial with CBD on 15 patients with secondary generalized epilepsy with temporal focus was undertaken in Brazil in 1980. Most of the patients remained essentially free of convulsions or demonstrated partial improvement in their clinical condition (Cunha *et al.*, 1980). This clinical trial has not been repeated since then, presumably due to the large amounts of CBD required (200–300 mg/day).

CBD causes reduction of cytokine production in in vitro assays and in mice (Watzl *et al.*, 1991; Srivastava *et al.*, 1998). These reports led to a recent study on its effect on collagen-induced arthritis in mice, a model of human rheumatoid arthritis (Malfait *et al.*, 2000). CBD was shown to block the progression of the disease. CBD has also been reported to block nausea in a rat model based on conditioned rejection (Parker *et al.*, 2002).

CBD is mildly sedative in mice: its ED_{50} is 4.7 mg/kg, compared to 1.3 mg/kg for chlorpromazine (Pickens, 1981). It also increased the entry ratio (open/total

number of entries) in the elevated plus maze test, which is a widely accepted assay for anxiety (Onaivi *et al.*, 1990; Guimaraes *et al.*, 1990).

CBD blocks the anxiety produced by THC, or by a simulated public-speaking test, in normal subjects (Zuardi *et al.*, 1982; 2002). However, the antianxiety effect observed is less than that of diazepam. Carlini and Cunha (1981) also reported that CBD caused longer sleep in insomniacs than those on placebo.

South African cannabis, known as dagga, contains very low levels of CBD (Field and Arndt, 1980) and, not surprisingly, its effects seem to differ considerably from those seen in Europe, America or the Middle East, where users smoke cannabis (marijuana and hashish) with high levels of CBD. Rottanburg *et al.* (1982) have reported that South Africans, after smoking dagga, frequently exhibit psychosis with hypomanic features. While this effect could be due to the high doses apparently consumed, it is also possible that the absence of CBD in dagga could be the reason. This conjecture is supported by more recent work. Zuardi *et al.* (1991) have shown that CBD is active in animal models predictive of antipsychotic activity. On the basis of the positive results observed, a single-case clinical trial was undertaken (Zuardi *et al.*, 1995). A patient with schizophrenia was administered CBD (up to 1.5 g/day). Improvement was noted in all items of a standard rating scale, and was close to the improvement seen with haloperidol. Leweke *et al.* (2000) have reported that while nabilone (a cannabinoid agonist) causes impairment of binocular depth inversion, a visual phenomenon also noted in schizophrenics, CBD reduced this impairment. A clinical trial is in progress evaluating the antipsychotic activity of CBD (Gerth *et al.*, 2002).

Cannabichromene, cannabigerol, cannabinol and the minor plant cannabinoids have not been investigated in any depth and it is quite possible that some of them may have a pharmacological profile close to that of CBD.

The endocannabinoids

Between 1964, when the active principal of cannabis was identified, and the mid-1980s, thousands of papers were published on the biochemistry, pharmacology and clinical effects of Δ^9-THC. Its mechanism of action, however, remained an enigma. Mainly conceptual problems hampered work in this direction. One of these was the presumed lack of stereoselectivity. Compounds acting through a biomolecule – an enzyme, a receptor or a gene – generally show a very high degree of stereo-selectivity. This was not initially thought to be the case with cannabinoids. Synthetic $(+)$-Δ^9-THC showed some cannabimimetic activity when compared with that of natural $(-)$-Δ^9-THC. 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

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and that the previous observations resulted from separation problems (Mechoulam *et al.*, 1988; Howlett *et al.*, 1990).

A second conceptual problem was the assumption that the cannabinoids belong to the group of biologically active lipophiles and that their effects should be compared with the chronic effects of anaesthetics at low dose levels. The action of cannabinoids hence could be explained without necessarily postulating the existence of a specific cannabinoid receptor and of an endogenous mediator of cannabinoid action.

The first solid indication that cannabinoids act through receptors was brought forward by Howlett's group. Howlett and Fleming, using the neuroblastoma N18TH2 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 (Howlett and Fleming, 1984).

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 (Devane *et al.*, 1988). Shortly thereafter this cannabinoid receptor, which was designated CB_1, was cloned (Matsuda *et al.*, 1990; Gerard *et al.*, 1991). A peripheral receptor (CB_2) was identified in the spleen (Kaminski *et al.*, 1992; Munro *et al.*, 1993). 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 Felder and Glass, 1998; Howlett, 1998; Piomelli *et al.*, 2000; Di Marzo *et al.*, 2002; Pertwee and Ross, 2002.)

Anandamide

We assumed that the presence of a specific cannabinoid receptor indicates the existence of endogenous specific cannabinoid ligands that activate these receptors.

In order to isolate the putative endogenous cannabinoids we first synthesized a tritium-labelled probe [³H] HU-243, which binds to the CB_1 receptor (Devane *et al.*, 1992a). To screen for endogenous cannabinoid compounds, we tested the ability of fractions from porcine brain extracts to displace [³H] HU-243 in a ligand-binding assay. 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. We isolated a fraction which eluted mainly as one main peak on gas chromatography-mass spectrometry (GC-MS). This compound represented the first example of a purified brain constituent which exhibited most of the properties of Δ^9-THC (Devane *et al.*, 1992b).

We named the active constituent anandamide, based on the Sanskrit work *ananda*, meaning bliss, and on its chemical nature (Fig. 1.1). 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 nmol/l. Surprisingly, this value is almost identical to that of Δ^9-THC in this system ($K_i = 46 \pm 3$ nmol/l; Devane *et al.*, 1992b).

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.* (1992) had reported that cannabinoids inhibit the twitch response of murine vas deferens (the secretory duct of the testicle) caused by electric current. Indeed, anandamide elicited a concentration-dependent inhibition of the twitch response, decreasing the twitch height by 50% at a concentration of 90 nmol/l (Devane *et al.*, 1992b).

Anandamide also activates VR1 receptor (Di Marzo *et al.*, 2002) and possibly other, not yet well defined receptors (see below).

Arachidonoylglycerol (2-AG)

The identification of a second cannabinoid receptor (CB_2) in immune cells 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 fractionation guided by a binding assay. The active fraction consisted mainly of three compounds – 2-arachidonoylglycerol (2-AG), 2-palmitoylglycerol (2-palm-G) and 2-linoleoylglycerol (2-lino-G: Mechoulam *et al.*, 1995). The structure of 2-AG is presented in Figure 1.2.

2-AG parallels anandamide in in vitro and in vivo activity, while 2-lino-G and 2-palm-G showed no binding activity to either CB_1 or CB_2. However, both 2-lino-G and 2-palm-G separately or together (in the ratio present in the spleen) potentiated the apparent binding of 2-AG to CB_1 and CB_2 (Ben-Shabat *et al.*, 1998). The same type of 'entourage' effect was observed in several in vivo cannabinoid tests (see, for example, Panikashvili *et al.*, 2001). This 'entourage' effect is in part due to inhibition of the enzymatic hydrolysis of 2-AG by cells.

2-AG was later isolated from brain (Sugiura *et al.*, 1995).

Additional endocannabinoids

Besides anandamide, several additional acylethanolamides which bind to the CB_1 receptor have been found in porcine brain but biological work with them has been limited (Hanuš *et al.*, 1993). For structures, see Figure 1.2.

Recently two new types of endocannabinoids, noladin ether and virodhamine, were identified (Hanuš *et al.*, 2001; Porter *et al.*, 2002). Noladin ether binds well to the CB_1 receptor and weakly to CB_2. It causes sedation, hypothermia, intestinal immobility and mild antinociception in mice. Virodhamine is a partial agonist
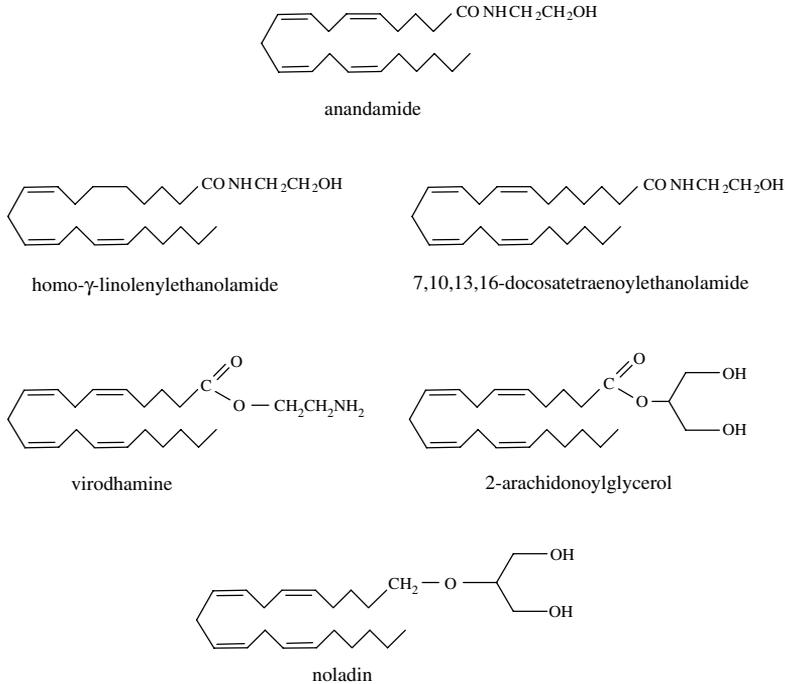
Figure 1.2 Endocannabinoids.

(with in vivo antagonistic activity) at the CB_1 receptor and a full agonist at the peripheral CB_2 receptor.

Both anandamide and 2-AG undergo the whole gamut of enzymatic transformations leading to prostaglandin, thromboxane and leukotriene-type endocannabinoid derivatives (Kozak and Marnett, 2002; van der Stelt *et al.*, 2002). However, it is as yet unknown whether these derivatives are formed in the mammalian body and represent a part of the endocannabinoid system.

Biosynthesis and inactivation of the endocannabinoids

The biosynthesis and metabolism of the endocannabinoids have been discussed in detail in numerous reviews (Mechoulam *et al.*, 1998; Di Marzo *et al.*, 1999; Hillard, 2000; Schmid, 2000; Giuffrida *et al.*, 2001; Sugiura *et al.*, 2002). Hence they are only outlined here (Figs 1.3 and 1.4).

Anandamide is formed following a pathway previously proposed for other fatty-acid ethanolamides, namely the initial formation of *N*-acylphosphatidyl-ethanolamine (NAPE). 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

Figure 1.3 Pathways for the biosynthesis and degradation of 2-arachidonoylglycerol (2-AG).

by phospholipase D yields anandamide. This endocannabinoid is not stored in the cells but is formed mainly when needed.

The biosynthesis of 2-AG is also dependent on calcium influx into cells. Enzymatic hydrolysis of diacylglycerol (DAG) seems to be the most important route, although the phospholipase C hydrolysis of phosphatidylcholine or phosphatidyl inositol has also been noted. 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 for using existing constituents for various purposes (Sugiura *et al.*, 2002).

Anandamide is inactivated in central neurons by both reuptake and enzymatic hydrolysis. Administration of AM-404, an inhibitor of anandamide uptake (Beltramo *et al.*, 1997), indeed causes potentiation of its action. It is not clear whether the uptake of the endocannabinoids is a passive diffusion process or whether carrier proteins are also involved. The reuptake of 2-AG is partly inhibited by other endogenous acylglycerols and is part of the 'entourage' effect (see above). For a recent review on the cellular transport of endocannabinoids and its inhibition, see Fowler and Jacobsson (2002).

Within the cell, anandamide and 2-AG are enzymatically hydrolysed to arachidonic acid and ethanolamine or glycerol respectively. The fatty-acid amide hydrolase (FAAH: Deutsch *et al.*, 2002) which hydrolyses anandamide has been cloned. It also hydrolyses oleamide, a sleep-inducing factor (Boger *et al.*, 1998; Fowler *et al.*,