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

Raphael Mechoulam and Lumir Hanuš

Hebrew University Medical Faculty, Jerusalem, Israel

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
certainly seen in psychiatric patients, but any relationship of the physiolog-
cal 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’Shaughnessy, 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’Shaughnessy 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 tra-
ditions. 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’Shaughnessy in any of the numerous contemporary publications
on this topic.

The reports by O’Shaughnessy 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 inflam-
mation – but its use in psychiatric cases appears to have been minimal.

Donovan (1845) confirmed many of O’Shaughnessy’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).
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, Δ⁹-tetrahydrocannabinol (Δ⁹-THC; Gaoni and Mechoulam, 1964). Numerous additional cannabinoids were isolated by column chromatography and their structures were elucidated. The major ones were cannabidiol (CBD), cannabiol, 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 Δ⁹-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, cannabiol has some activity and Δ⁸-THC, which is a very minor component, parallels Δ⁹-THC activity, although it is somewhat less potent. Since 1964 thousands of papers on the chemistry, pharmacology, metabolism and clinical effects of Δ⁹-THC and related synthetic compounds have appeared.

A comparison of the somatic and behavioural effect of Δ⁹-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,
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 $\Delta^8$-THC alone accounts for the activity of cannabis. Thus we showed that in rhesus monkeys, $\Delta^9$-THC alone and $\Delta^8$-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 $\Delta^8$-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; Turkaniš 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\textsubscript{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; Guimarães 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, cannabiol 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 Δ⁹-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 stereoselectivity. This was not initially thought to be the case with cannabinoids. Synthetic (+)-Δ⁹-THC showed some cannabinimetic activity when compared with that of natural (−)-Δ⁹-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...
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 com-
pared with the chronic effects of anaesthetics at low dose levels. The action of
 cannabinoids hence could be explained without necessarily postulating the exis-
tence of a specific cannabinoid receptor and of an endogenous mediator of cannabi-
noid 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
 cannabionoid 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 CB1, was cloned
(Matsuda et al., 1990; Gerard et al., 1991). A peripheral receptor (CB2) was iden-
tified in the spleen (Kaminski et al., 1992; Munro et al., 1993). Surprisingly, the
CB2 receptor has only 44% chemical homology with the CB1 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 CB1 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 assump-
tion 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 rep-
resented the first example of a purified brain constituent which exhibited most of
the properties of Δ9-THC (Devane et al., 1992b).
We named the active constituent anandamide, based on the Sanskrit work \textit{ananda}, meaning bliss, and on its chemical nature (Fig. 1.1). This constituent inhibited the specific binding of $[^3\text{H}]$ HU-243 in a manner typical of competitive ligands with a $K_i$ value of $52 \pm 1.8$ nmol/l. Surprisingly, this value is almost identical to that of $\Delta^9$-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).

\textbf{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).

\textbf{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
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Figure 1.2 Endocannabinoids.

(with in vivo antagonistic activity) at the CB1 receptor and a full agonist at the peripheral CB2 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
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.,