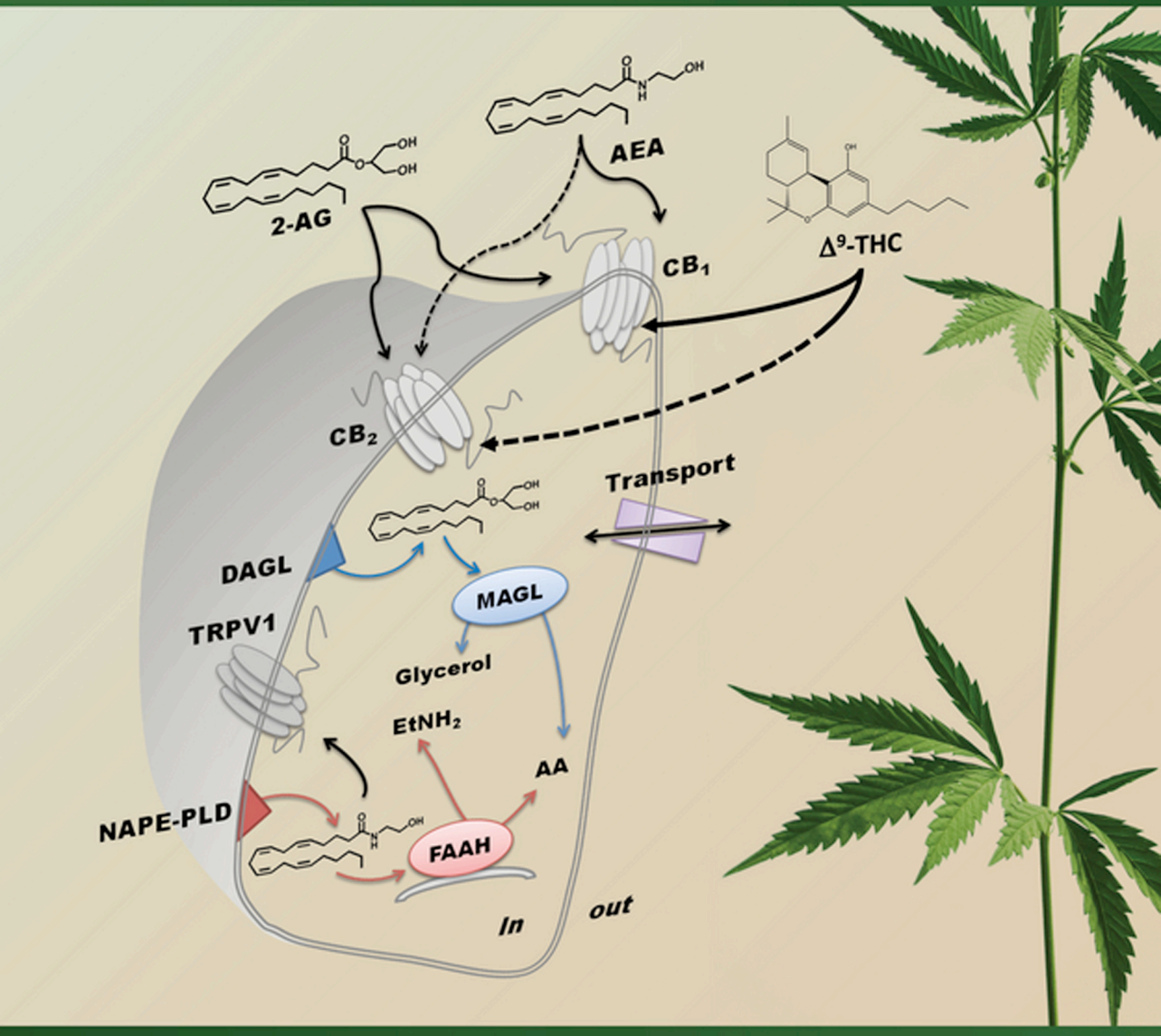
Cannabinoids

EDITOR VINCENZO DI MARZO



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To Raphael ‘Raphi’ Mechoulam, the ‘father of cannabinoid research’, and a dear friend, who never stops amazing me and is still short of just one important prize, at least thus far.

To Geoffrey W. Guy who realised the dream of many scientists in this field by making the development of a cannabinoid-based medicine possible.

To William A. ‘Bill’ Devane, the inventor of the name ‘anandamide’, and an important player in its discovery as well as in that of cannabinoid receptors, whom I have not forgotten.

To Adriana and Marta, for having had to indirectly endure 7-days-a-week cannabinoid research for 21 and 17 years of their lives, respectively, and yet always being several orders of magnitude more important for me.

In memoriam

I would like to remember Ester Fride, Billy R. Martin and J. Michael Walker who have made fundamental contributions to cannabinoid research and, very sadly, are no longer among us.

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Preface

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When hearing the word 'cannabinoid', even the layman immediately knows that this must have to do with the *Cannabis* plant and its various psychotropic preparations, such as marijuana and hashish, which undoubtedly still represent the most widely used drug in the Western world after nicotine and alcohol. Yet, the recreational use of cannabis is only one of several that mankind has found for this plant over many centuries. Unlike other plants used as sources of substances of abuse, hemp has in fact accompanied human progress in many of its aspects, and different varieties of *Cannabis* have been used, among other things, as a source of 'inspiration' in religious rites, a strong fibre for ropes and fabric, and as medicinal preparations, thus helping in at least four fundamental aspects of human life since its early origins: religion, health, manufacture and recreation.

The medicinal use of cannabis probably originates in ancient China, nearly 4000 years ago. Although the earliest written reference to the use of hemp against pain and inflammation is the Chinese Rh-Ya (1500 BC), the 'red emperor' Shen Nung (2838–2698 BC), who is considered the father of all herbalists, is alleged to have documented its use in his book *The Herbal*. More recent evidence for the use of cannabis, for example against various inflammatory and painful conditions, can be found in the ancient Egyptian, Indian, Greek and Roman pharmacopeias, but also in medieval Islamic medicine; whereas the Irish physician William O'Shaughnessy is credited with introducing the therapeutic use of cannabis to Western medicine in the 1830s (O'Shaughnessy, 1838–1840). Despite this centuries old, mostly anecdotal, history of medicinal use, it was only during the 1960s, with the explosion of marijuana abuse in Western countries, that major efforts were made to identify the chemical components of this preparation that could be responsible for its psychotropic activity. Thus, the first studies on the mechanism of action of cannabis were initiated to explain its psychotropic effects and, in some cases, to substantiate its purported dangerousness, rather than its medicinal actions. This potential bias has somewhat influenced research on cannabinoids for many decades, but nevertheless led first to the discovery of the psychotropic component of cannabis, Δ^9-tetrahydrocannabinol (THC), and later to the identification of specific plasma membrane, G protein-coupled receptors for this compound, named 'cannabinoid receptors'. Then followed their endogenous ligands, the endocannabinoids and their

metabolic enzymes – that is the whole 'endocannabinoid system'. This signalling system is currently regarded by many as a fundamental pro-homeostatic regulatory system involved in all physiological and pathological conditions in mammals (Pacher and Kunos, 2013).

A major player in the discovery of the endocannabinoid system – through having led studies towards first the chemical identification of THC and later its pharmacological characterisation, the development of tools that allowed the discovery of its receptors, and finally to the isolation of the first endogenous ligands of such receptors, anandamide (Devane et al., 1992) – Raphael Mechoulam had to be the author of the first chapter of this celebrative book. Universally recognised as the 'father of cannabinoid research', Prof. Mechoulam reviews the milestones in this field, and then describes two topics that represent new trends of high potential therapeutic importance: the physiological role of some anandamide-related mediators, that is the fatty acid amides of amino acids, and the pharmacology of the most abundant non-psychotropic cannabinoid, cannabidiol (CBD). Indeed, the discovery of anandamide triggered interest in other endogenous lipids that do not necessarily act via cannabinoid receptors and are just emerging as important actors in mammalian physiology. On the other hand, non-psychotropic cannabinoids, such as CBD, have been neglected in the past due to the socio-political urgency to focus research on Δ^9-THC, and only now are coming out as potential contributors to the medicinal properties of cannabis. This is also witnessed by the recent approval of Sativex®, a combination of botanical extracts enriched in THC and CBD in a 1 : 1 ratio, used to effectively relieve pain and spasticity in multiple sclerosis (Podda and Constantinescu, 2012).

The second chapter of this book is by Allyn Howlett and her colleagues, Lawrence Blume and Khalil Eldeeb. Prof. Howlett is another 'pivot' in cannabinoid research as, among other things, she coordinated the first studies leading to the identification of specific binding sites for THC in the brain (Devane et al., 1988). She and her co-authors review here the crucial experimental steps that led to this discovery, and the latest developments on how such receptors work in terms of their intracellular signalling and regulation and inactivation by other proteins, which are all aspects of the endocannabinoid system to which Prof. Howlett has provided fundamental contributions during the last 20 years. It goes without saying that a full understanding of cannabinoid receptor function is of paramount importance for the future development of new therapies obtained by targeting these proteins.

The third chapter of the book still covers biochemical aspects of the endocannabinoid system, although focusing on the enzymes that regulate the tissue levels of the endogenous cannabinoid receptor ligands, or 'endocannabinoids', and related lipid mediators. Such enzymes are currently the focus of attention from many pharmaceutical companies, based on the assumption that the pharmacological manipulation of endocannabinoid levels should produce safer therapeutic actions than the direct targeting of receptors. The chapter is authored by Prof. Mauro Maccarrone, one of the major contributors to our current understanding of endocannabinoid biochemistry, and his collaborator, Filomena Fezza. The authors cover important aspects of

the enzymes that biosynthesise and degrade the two major endocannabinoids, anandamide and 2-arachidonoylglycerol (2-AG), such as the diacylglycerol lipases, on the one hand, or the fatty acid amide hydrolase and monoacylglycerol lipase, on the other hand. They also discuss other important enzymes involved in the metabolism of endocannabinoid-related mediators, as well as emerging catabolic pathways for endocannabinoids.

A crucial step in the dissection of the role played by the various 'endocannabinoid proteins', be they receptors or enzymes, in basically all aspects of mammalian physiology and pathology (Pacher and Kunos, 2013) has been the development of both 'global' and 'conditional' genetically modified mice in which such proteins have been inactivated or overexpressed. Beat Lutz and his group have played a fundamental role in these studies over the last 13 years. In his chapter, he reviews how the genetic dissection of the endocannabinoid system has not only illuminated, to the careful eye, the function played by this pleiotropic regulatory system under both physiological and pathological conditions, but also shown how THC exerts its pharmacological effects in mammals. Prof. Lutz also wisely calls for caution against the use of the genetic approach without combining it with other experimental strategies.

One of the earliest functions to be postulated (Di Marzo et al., 1998), the physiological role as an endogenous pro-homeostatic regulator that helps re-establishing the 'steady state' after its perturbation by acute or chronic pathological challenges, such as after cellular or psychological stress, is currently the most widely recognised 'systemic' function of the endocannabinoid system. Cecelia Hillard has authored seminal studies on how stress and endocannabinoids are intimately linked. Together with her colleagues, Qing-song Liu, XiaoQian Liu, Bin Pan, Christopher J. Roberts and Leyu Shi, she reviews here the effect of chronic unpredictable stress exposure on several components of the endocannabinoid signalling system in various brain regions, as well as on cannabinoid CB_1 receptor-mediated regulation of GABA release in the prelimbic region of the medial prefrontal cortex. These data show how the endocannabinoid system plays a vital role in the regulation of the impact of stress on the brain and body, and identify this system as a potential target for the treatment of many stress-related dysfunctions, such as depression and post-traumatic stress disorders.

Indeed, by being the most abundant G protein-coupled receptor in the mammalian brain, and coupled to inhibition of neurotransmitter release from presynaptic terminals, cannabinoid CB_1 receptors are ideally located to play their pro-homeostatic role also in many neurological disorders characterised by neurotransmitter unbalance. On the other hand, by being upregulated in glial cells during inflammatory conditions, and coupled to inhibition of inflammatory cytokine release, cannabinoid CB_2 receptors are ideal candidates to tone down neuroinflammation during such disorders (Velayudhan et al., 2013). This evidence is elegantly reviewed here by Javier Fernandez-Ruiz, perhaps the researcher that has most contributed to our current knowledge of the role of the endocannabinoid system in neuroinflammatory disorders, together with Mariluz Hernández and Yolanda García-Movellán. Importantly,

Prof. Fernandez-Ruiz and his colleagues also discuss the role of this signalling system in other disorders that, at least in part, originate from, or are amplified by, brain dysfunctions, including: neuropathic pain, psychiatric disorders, addictive disorders, nausea and vomiting, sleep disorders, brain tumours and feeding disorders, thus making Chapter 6 of this book probably one of the most comprehensive reviews on endocannabinoids and CNS function and dysfunction that has appeared thus far in the literature on this topic.

Brain and gut, it is a fact, share many signals, and endocannabinoids make no exception. In fact, the beneficial effects of cannabis on diarrhea have been known for centuries (O'Shaughnessy, 1838–1840). Paolo Caraceni, Francesca Borrelli, Ferdinando Giannone and, particularly, Angelo Izzo have played a seminal role in our understanding of endocannabinoid function in the gut and review here state-of-the-art data on the adaptive changes that the endocannabinoid system undergoes in response to gastrointestinal and liver disturbances. They also describe potential areas of therapeutic interest in which cannabinoids and endocannabinoid-based drugs might be used in the near future, such as gastrointestinal reflux disease, irritable bowel syndrome, inflammatory bowel disease, colon cancer and chronic liver diseases, thus providing, again, one of the most comprehensive review articles on this subject to date.

This celebrative book could not be concluded without some reflections on how the use of the correct nomenclature can contribute to tone down the potential general feeling of confusion that might be engendered by the quick succession of discoveries in the rapidly expanding field of cannabinoid research. Having suggested in the past some names that have then met with general approval in the field, I thought I could be entitled to write a chapter on 'cannabinoid nomenclature'. Together with Luciano De Petrocellis, we have tried to describe the history of cannabinoid research and its most important milestones in parallel with the sequential appearance of various names and definitions which have been, and still are, used. This is not a trivial issue for many reasons, including the fact that, as mentioned above, there is an ever increasing interest towards: (i) abundant non-THC cannabinoids from various cannabis varieties, and (ii) endocannabinoid-related endogenous mediators. These chemical entities, unlike THC and 2-AG, respectively, do not have as their main molecular mechanism of action the ability to interact with cannabinoid CB_1 and CB_2 receptors, and for this reason too the nomenclature developed so far in the cannabinoid field (Pertwee et al., 2010) cannot be easily applied to these compounds.

In conclusion, the present book celebrates a very intense half-century of cannabinoid research since THC's discovery in 1964, as well as its impact not only on our understanding of basic physiology, but also on therapeutic drug development. First, with the use of THC to combat cachexia and emesis in cancer and AIDS patients (Martin and Wiley, 2004), then with the development of the first endocannabinoid system-based drug for obesity – the CB_1 inverse agonist rimonabant, subsequently withdrawn from the market due to psychiatric side-effects that might have been avoided with a more careful choice of the target patient and indication (Di Marzo and

Després, 2009); and, lastly, with the development and marketing of Sativex® (Podda and Constantinescu, 2012) (yes indeed, back to the plant!), for which an approval to also treat cancer pain is currently being sought. The contributors, to whom I am extremely grateful for having provided eight top-class chapters, have also opened a window on what could be the potential future outcomes of the next half-century of experimental efforts, in terms of both basic and medical research. We must now only wait and see if all the expectations will be met in the end.

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1

Looking ahead after 50 years of research on cannabinoids

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1.1 Summary

My lab has been involved in research on cannabis and endogenous cannabinoids for 50 years. In this overview I first summarise some of our work over these decades. Then, on the basis of previous research, I speculate on a few of the pathways cannabinoid investigations may follow in the future. Two possible research trends are discussed:

1. Cannabidiol – effects and mechanisms.
2. Fatty acid amides of amino acids and related endogenous molecules – biological roles.

1.2 Introduction

Cannabis research has a long and convoluted history. The first chemical endeavours were published in the 1840s. Around the end of the nineteenth century, crystalline cannabinol acetate was obtained after acetylation of an extract of hashish. Its structure was elucidated in the 1930s, when cannabidiol (CBD) was also isolated, but only a partial structure for it was put forward. Roger Adams and Alexander Todd published numerous, mostly synthetic, papers on cannabis and found that some synthetic tricyclic compounds had cannabis-like activity in dogs. Loewe (1950) summarised the pharmacological work on cannabis extracts and synthetic compounds carried

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out over a century. For early reviews, with an emphasis on the chemical aspects, see Mechoulam and Gaoni (1967a) and Mechoulam (1973).

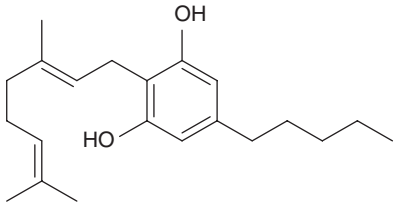
Clinical research with cannabis was also undertaken in the nineteenth century. In the 1840s, the psychiatrist J. J. Moreau conducted a clinical experiment in which he administered hashish to humans. His volunteers, including Moreau himself, experienced 'occurrences of delirium or of actual madness … '. He concluded that 'There is not a single, elementary manifestation of mental illness that cannot be found in the mental changes caused by hashish … ' (Moreau, 1973). Marijuana users today mostly report different effects. One can only wonder what amounts were administered by Moreau to his volunteers.

Modern pharmacological and clinical research is done with precise doses of active compounds. The absence of a well-established chemical basis of cannabis until the mid 1960s, made biological and clinical research with it of very limited value. Novel approaches to elucidate the chemistry of cannabis, in order to proceed with biological evaluations, were badly needed.

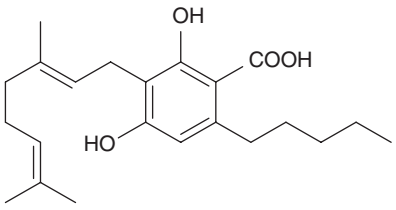
I started research on cannabis in 1963. Initially I assumed that the project would be completed within a few years. Today – 50 years later – my group is still looking at various aspects of cannabis chemistry and pharmacology.

As methods for both separation and structural elucidation by physical techniques were, in the early 1960s, considerably more advanced than those employed by Adams and Todd in the 1930s and 1940s, we assumed that we could solve some of the problems previously encountered. I started with re-isolation of cannabidiol (CBD) by a series of column chromatographies and the elucidation of its structure by NMR, a technique which had just been introduced in organic chemistry (Mechoulam and Shvo, 1963). Then Yehiel Gaoni joined me on the project and we approached the problem of isolation of the active compound (or compounds). We needed biological feedback to identify the active material. Habib Edery and Yona Grunfeld in the nearby Institute for Biological Research had a group of rhesus monkeys which, luckily for us, were rapidly sedated on administration of some chromatographic fractions isolated from cannabis. We concentrated our work on these fractions, and in 1964 we reported that we had identified a single active compound, Δ^9-tetrahydrocannabinol (THC) and had elucidated its structure (Gaoni and Mechoulam, 1964). Later we reported its total synthesis and absolute configuration (Mechoulam et al., 1967; Mechoulam and Gaoni, 1967b). Over the next few years we isolated numerous additional cannabinoids – a term we coined for this group of compounds. Cannabigerol, cannabichromene, cannabicyclol, cannabidiolic acid and cannabielsoic acid among them. None of them showed THC-like activity, and we finally stated that ' … except for THC, no other major active compounds were present in the analyzed sample of hashish' (Mechoulam et al., 1970, 1976). Over the years, dozens of new cannabinoids, mostly minor constituents, have been identified in various cannabis strains (Figure 1.1). None has shown marijuana-like activity.

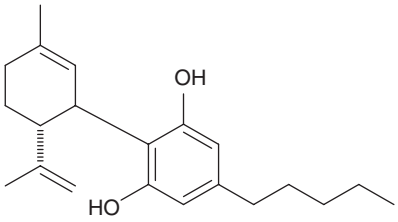
The next step followed in our laboratory was investigation of the metabolism of cannabinoids. Together with colleagues in the USA, UK, Sweden and later Japan we



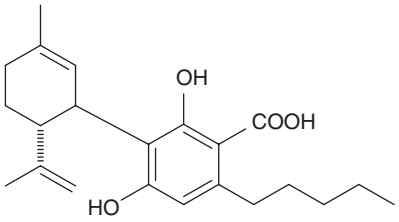
Cannabigerol (CBG)



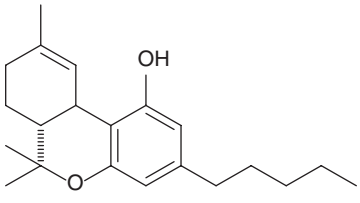
Cannabigerolic acid (CBGA)



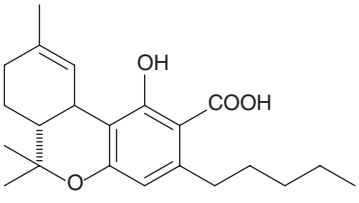
Cannabidiol (CBD)



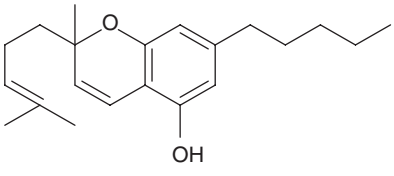
Cannabidiolic acid (CBDA)



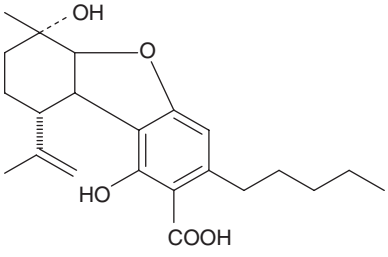
Δ^9-Tetrahydrocannabinol (Δ^9-THC)



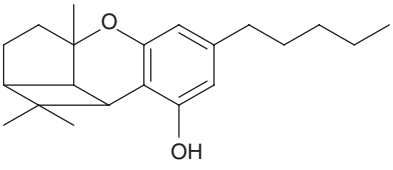
Δ^9-Tetrahydrocannabinolic acid (Δ^9-THCA)



Cannabichromene (CBC)



Cannabielsoic acid A



Cannabicyclol (CBL)

Figure 1.1 Cannabinoids within *Cannabis sativa*

elucidated several metabolic pathways. By now several groups had become involved in cannabinoid investigations and four groups simultaneously reported the first steps of the metabolism of THC! (Mechoulam et al., 1976).

For about two decades after the isolation of THC numerous groups, including ours, worked on the pharmacology of cannabinoids. The major contribution by my group was the discovery that THC activity is stereospecific, which indicated that apparently THC acts on a biological entity – be it an enzyme or a receptor (Mechoulam et al., 1987, 1988). Indeed, in the mid 1980s Allyn Howlett's group reported the existence of a receptor (Devane et al., 1988). As receptors obviously exist for activation by endogenous ligands and not by exogenous plant materials, we went ahead looking for such agonists. While we did not believe that they would resemble plant cannabinoids in their structure, we assumed that they should be – like the plant cannabinoids – lipid molecules. Hence the techniques we used were those followed for lipids. Bill Devane, who had just taken his PhD degree with Allyn Howlett and had joined my group as a post doc, took this project upon himself. The basic idea was to prepare a potent radiolabelled receptor ligand, bind it to Howlett's receptor (later named the CB_1 receptor) and then try to displace it with lipid brain fractions. Such fractions were to be purified, ultimately leading to a pure brain constituent – an endogenous receptor ligand. The first step was surprisingly easy. We reduced the highly potent (−)-11-hydroxy-THC-dimethylheptyl (HU-210), which we had synthesised a few years previously, to obtain an even more potent (−)-11-hydroxy-hexahydrocannabinol (Figure 1.2) (Devane et al., 1992a). It is presumably still the most potent cannabinoid known. Then this reduction reaction was repeated with tritium and the tritiated material was bound to the receptor found in pig brain. We decided to use pig brains as we understood that pig biochemistry is close to human biochemistry. At this point we were joined by Lumir Hanus, a post doc from Brno in the Czech state. Devane and Hanus extracted the brains with petroleum ether and indeed obtained active fractions by silica gel chromatography. However, as soon as active fractions were purified, they started to lose their activity. We know now that this was due to the lack of stability of the endogenous cannabinoid ligand. Ultimately we had a miniscule amount of material which seemed pure and we succeeded in obtaining NMR and mass spectra, which led to the correct structure (Devane et al., 1992b). We named it anandamide

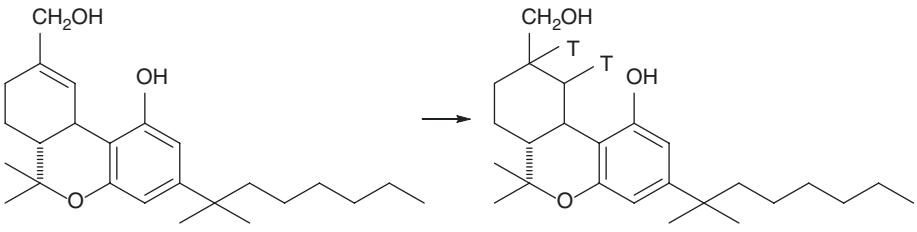


Figure 1.2 Preparation of labelled ligand used for isolation of anandamide

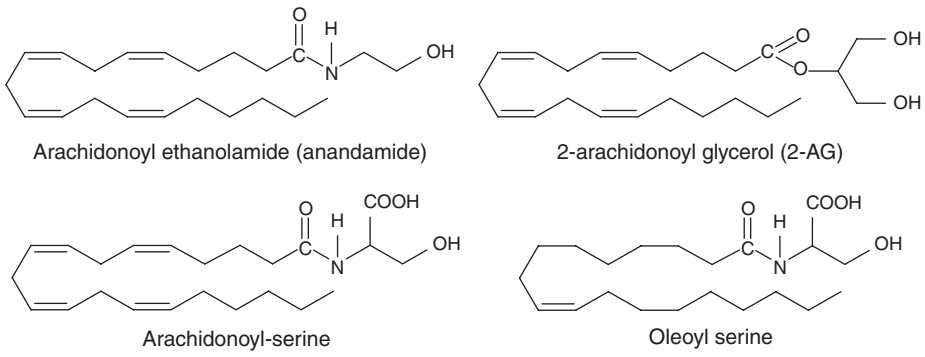


Figure 1.3 Endocannabinoids and related endogenous molecules

and synthesised it. In its receptor binding and initial pharmacological activity it paralleled THC (Fride and Mechoulam, 1993; Vogel et al., 1993; Smith et al., 1994). Later we identified in intestines a second major endogenous cannabinoid, 2-arachidonoylglycerol (2-AG) (Mechoulam et al., 1995). For structures of these endocannabinoids and related endogenous molecules, see Figure 1.3.

Over the next few years we investigated the structure–activity relationships of the endocannabinoids (Sheskin et al., 1997) and together with numerous groups looked into the pharmacology of anandamide and 2-AG. Some of the more significant results reported were the identification of an entourage effect, namely the potentiation of endocannabinoid action by related endogenous molecules (Ben-Shabat et al., 1998); the biphasic effect of anandamide (Sulcova et al., 1998); the synthesis of a specific potent CB_2 agonist (Hanus et al., 1999); the protective effect of 2-AG in brain trauma (Panikashvili et al., 2001); the enhancement of heart resistance to the arrhythmogenic effects of epinephrine by anandamide (Ugdyzhekova et al., 2000); the importance of 2-AG in suckling (Fride et al., 2001) and so on. Numerous groups have investigated the involvement of the endocannabinoids in a large number of physiological systems and in a long list of diseases (Pacher and Mechoulam, 2011). Indeed, recently Pacher and Kunos (2013) stated: '… modulating [the] endocannabinoid sysem activity may have therapeutic potential in almost all diseases affecting humans' – a courageous statement!

Our original publication on anandamide has been cited over 3000 times and that on 2-AG over 1000 times. However, to the best of my understanding, neither anandamide nor 2-AG have ever been administered to humans. We should compare this with the almost immediate administration of insulin to patients after its discovery in the 1920s!

I would like to present some ideas in two areas where I expect to see progress – in cannabidiol (CBD) chemistry and pharmacology and in fatty acid amides of amino acids (FAAAs) and related compounds. It is of course impossible to predict the pathways of future research in a rapidly evolving scientific area. If a

cannabinoid – be it synthetic or natural – becomes a widely used drug, we shall certainly see considerable endeavour in therapeutics. Likewise, advances in epigenetics, if they are associated with the endocannabinoid system, will presumably attract considerable attention and will become a centre of major interest.

1.3 Cannabidiol (CBD)

While structurally CBD is a rather simple compound, its biological effects are widely spread and yet it is essentially non-toxic. It has very low affinity to both cannabinoid receptors, but has been shown to alter THC activity. Over 30 years ago, Brady and Balster (1980) reported that CBD antagonises the effects of THC on operant behaviour in rhesus monkeys. More recently, it was reported that while acute intoxication with THC (or with cannabis that contains high levels of THC and low levels of CBD) impairs cognitive function, the cannabinoid spray Sativex (a 1 : 1 ratio of CBD : THC) at low doses reduces some of the THC effects including subjective ratings of intoxication and cognitive impairment (Robson, 2011; Schoedel et al., 2011; Wade et al., 2004). Furthermore, recent studies in humans have shown that smoking CBD-enriched marijuana does not lead to the deficits of prose recall that are caused by CBD-poor cannabis, and users of CBD-rich cannabis have better preserved recognition memory compared to users of CBD-poor cannabis (Morgan et al., 2012).

Taffe (2012) has shown that in monkeys, THC impairs spatial working (short-term) memory, consistent with research in rodents showing that spatial working memory is much more vulnerable to disruption by THC than is reference (long-term) memory (Mechoulam and Parker, 2013). Recently Taffe's group presented direct evidence that in monkeys CBD can oppose the cognitive impairing effects of some, but not all, forms of behavioural and memory disruption by THC (Wright et al., 2013).These data strengthen the view that medicinal cannabis containing reasonably high levels of CBD may be a better drug than cannabis with low levels of CBD or of pure THC alone. Indeed, Van et al. (2008) have shown that CBD affects the discriminative stimulus and place conditioning effects of THC and Zuardi et al. (2012) have determined the dose ratios of the two compounds that can lead to the interaction of CBD in the actions of THC.

CBD does not cause THC-like psychoactivity. In animal assays it has been reported to be neuroprotective, to have anti-anxiety, anti-emetic and anti-nausea effects, to lower autoimmune reactions (in diabetes type 1 and rheumatoid arthritis), to have anti-cancer properties and, being a general anti-inflammatory agent, to affect inflammation associated with numerous conditions, including those of the central nervous, gastrointestinal and the cardiovascular systems (Mechoulam et al., 2009). This therapeutically positive list is growing all the time. In human volunteers and patients it has been shown to have anti-anxiety, anti-epileptic and

anti-schizophrenic properties (Mechoulam et al., 2009; Leweke et al., 2012), although the doses needed may be rather high. It is quite unusual that a single compound should have so many therapeutic effects and this suggests that it may act on some general, basic biochemical pathway.

CBD is known to act through numerous specific mechanisms. The Hillard group has demonstrated that CBD enhances adenosine signalling through inhibition of uptake. Indeed CBD binds to the equilibrative nucleoside transporter with a $K_i <$ 250 nM. This mechanism may explain, in part at least, the anti-inflammatory action of CBD (Carrier et al., 2006). It is known that in vivo CBD decreases TNF-α production in lipopolysaccharide (LPS)-treated mice (Malfait et al., 2000). This effect is reversed with an A2A adenosine receptor antagonist and abolished in A2A receptor knockout mice.

Numerous CBD actions proceed through the serotonergic 5-HT1A receptor . Thus, CBD significantly reduces the infarct volume induced by middle cerebral arterial occlusion. This neuroprotective effect of CBD is inhibited by WAY100135, a 5-HT1A receptor antagonist. The cerebral blood flow increased by CBD was also partially reversed by WAY100135. CBD exerts robust neuroprotective effects in vivo in piglets, modulating excitotoxicity, oxidative stress and inflammation. These results suggest that the neuroprotective and other effects of CBD in many cases proceed through the serotonergic 5-HT1A receptor (Pazos et al., 2013). Some additional recent examples: 5-HT1A receptors play a role in the CBD anti-anxiety effects (Gomes et al., 2011) and even in the anti-aversive effects of CBD on panic attack-like behaviours evoked in the presence of a wild snake (Twardowschy et al., 2013). It is involved in some motor effects of CBD (Espejo-Porras et al., 2013) as well as in the attenuation by CBD of vomiting and nausea-like behaviour (Rock et al., 2012), in the amelioration of cognitive and motor impairments in bile-duct ligated mice by CBD (Magen et al., 2010), in inhibition of the reward-facilitating effect of morphine (Katsidoni et al., 2013); in the CBD-induced attenuation of behavioural and cardiovascular responses to acute restraint stress in rats (Resstel et al., 2009) and so on.

CBD decreases the Th17 inflammatory autoimmune phenotype (Kozela et al., 2013). It is of interest that both CB_2 and 5HT1A receptors are implicated in this effect. CBD also inhibits marble-burying behaviour, a model for depression, in which involvement of CB_1 receptors was noted (Casarotto et al., 2010), as well as the hyperphagia induced by CB_1 receptor agonists (Scopinho et al., 2011).

Another mechanism through which CBD exerts its effects is its potent anti-oxidative action. This non-enzymatic reaction is typical of resorcinols. Hampson et al. (2000) have shown that CBD, presumably due to its lipophilic nature, is better than vitamin C in prevention of hydroperoxide-induced damage. More recently, Fernández-Ruiz et al., (2013) summarised evidence that that the potent anti-oxidative action of CBD may partly explain its neuroprotective effects in Parkinson's disease and possibly in cerebral ischaemia-reperfusion.

Further mechanisms through which CBD exerts its action are agonism to the TRPV channels (De Petrocellis et al., 2012; Qin et al., 2008), glycine receptors (Xiong et al., 2012), GPR55 antagonism, PPARγ receptor agonism, intracellular Ca^{2+} increase and others. For a review, see Campos et al. (2012).

As mentioned above, this wide range of activities and of mechanisms suggests that CBD may have the capacity to affect basic physiological mechanisms rather than just a specific site. Epigenetic effects by cannabinoids seem plausible since anandamide is known to induce DNA methylation of keratinocyte-differentiating genes by increasing DNA methyltransferase 1 (DNMT-1) activity (Paradisi et al., 2008). The same group has now reported that treatment of differentiated human keratinocytes cells with CBD significantly increased DNA methylation of keratin 10 gene. In addition, CBD increased global DNA methylation levels by selectively enhancing DNA methyltransferase DNMT1 expression, without affecting DNA methyltransferases DNMT 3a, 3b or 3L (Pucci et al., 2013). Vogel's group has recently shown that the CBD anti-inflammatory effects are mediated mainly by downregulating the expression of proinflammatory genes and upregulation of genes encoding negative regulators of NF-κB and AP-1 transcriptional activities (Juknat et al., 2013). They have also shown that CBD affects the expression of genes involved in zinc homeostasis in BV-2 microglial cells (Juknat et al., 2012). The McAllister group has recently reported that CBD is an inhibitor of Id-1 gene expression in aggressive breast cancer cells (Soroceanu et al., 2013).

Are the above described – and presumably many additional – activities of CBD based on its epigenetic actions? A possible and enticing example is schizophrenia. CBD has been shown to ameliorate the symptoms of this disease in patients (Leweke et al., 2012). Neuregulin-1is a common marker gene known to be upregulated in schizophrenia, while being silenced through methylation (Weickert et al., 2012), As mentioned above, it has been shown that CBD increases DNA methylation levels by selectively enhancing DNA methyl transferase 1 expression in certain skin cells. Does CBD affect schizophrenia symptoms through a similar route on neuregulin-1?

THC action mimics that of anandamide and 2-AG. Does CBD mimic an as yet unknown endogenous compound with a wide spectrum of activity based on its possible DNA methylation properties?

1.4 Fatty acid amides of amino acids and related compounds

A very large number of fatty acid amides of amino acids and related compounds have been identified by targeted lipidomics in the mammalian body (Tan et al., 2009, 2010) and, surprisingly, in *Drosophila* (Tortoriello et al., 2013). Many compounds of the same types have been reported (see for example Milman et al., 2006; Hansen 2010; Smoum et al., 2010). Except for anandamide, 2-AG, and