Research into cannabinoid medicines may suggest potential therapeutic opportunities

On 10 March a symposium on cannabinoid medicines was held jointly by the Academy of Pharmaceutical Sciences and the Royal Pharmaceutical Society of Great Britain, hosted at the RPSGB. *Pharmacy in Practice* was invited to attend and this review is based largely on information presented at the meeting with some supplemental background information.

The speakers and their presentations

Professor Raphael Mechoulam, The Hebrew University of Jerusalem, Israel — **Cannabis research fifty years on**[1]
Dr Vincenzo Di Marzo, Endocannabinoid Research Group (Italian National Research Council), Italy — **The multiple roles of endocannabinoids**[2]
Dr Jurg Gertsch, Swiss Federal Institute of Technology — **Novel CB2 receptor-selective 'cannabinoids' from plants**[3]
Dr Arno Hazekamp, University of Leiden, Netherlands — **Metabolomics approaches in cannabis research**[4]
Dr José Prieto and Professor Michael Heinrich, University of London, UK. Professor Heinrich gave the presentation on **Cannabis-derived medicines in the treatment of chronic inflammatory conditions**[5]
Professor David Baker, University of London, UK: **The cannabinoid receptors — where do they lead us to?**[6]
Professor John Zajicek, Peninsula Medical School and Derriford Hospital, UK — **Clinical research on cannabis derived medicines**[7]
Professor Rudolf Brenneisen, University of Bern, Switzerland — **Safety of cannabis-based medicines**[8]
Chairs: Professor Michael Heinrich*, University of London, UK and **Professor Tony Moffat,** University of London, UK.
* Professor Heinrich organised the meeting on behalf of the RPSGB and Academy of Pharmaceutical Sciences of Great Britain.


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Marijuana, derived from *Cannabis sativa* has been used for medicinal and recreational purposes for thousands of years, but concerns over its potential for abuse led to its ban in many countries in the 1920s and 1930s. It is illegal to use marijuana in many countries, including the UK, where it has Class C classification under the *Misuse of Drugs Act 1971*.[9] However developments in pre-clinical cannabinoid research alongside clinical trials using existing cannabinoid medicines are beginning to offer new insights into the cannabinoid system and highlight potential therapeutic opportunities.

Plant cannabinoids

The main psychoactive ingredient of *Cannabis*, delta 9-tetrahydrocannabinol (THC), was isolated by Professor Raphael Mechoulam's laboratory at The Hebrew University of Jerusalem in 1964[1,10] (Box 1).[1,3,10–28] To date more than 60 cannabinoids have been extracted from the plant including cannabinol (CBN) and the non-psychoactive component cannabidiol (CBD).[1] The elucidation of cannabinoid structures and their chemical synthesis allowed the identification of cannabinoid CB1[1,15–17] (and subsequently CB2)[1,19,20] receptors, which encouraged a search for endogenous ligands.[1]

Endocannabinoids

In 1992, Professor Mechoulam's team isolated and deduced the structure of an endogenous cannabinoid, N-arachidonoyl-ethanolamine,[1,18] which they termed anandamide (from the Sanskrit 'ananda' meaning bliss).[12] This was followed by the isolation of other endocannabinoids (ECs) 2-arachidonylglycerol (2-AG), noladin ether (2-arachidonylglyercol ether), virodhamine (O-arachidonoyl-ethanolamine) and N-arachidonoyl dopamine (NADA).[12]

Box 1. Historical progress in cannabinoid medicines development[1,3,10–26]

1964	Delta 9-tetrahydrocannabinol (THC) identified as the main psychoactive component of cannabis (Gaoni & Mechoulam)[1,1–12]
1980	Cannabinoids were synthesised, radiolabelled and bound to rat brain membranes[1,11]
1982	UK License granted for antiemetic use of Nabilone®[13] against chemotherapy-induced nausea[3,13]
1986	Drobinol (THC) licensed in US (as antiemetic with chemotherapy and as appetite stimulant for use in HIV)[3,14]
1988	CB1 receptor identified[15,16]
1990	CB1 receptor cloned[17]
1992	Endogenous Ligand (anandamide)[18] and CB2 receptor[19] identified
1993	CB2 receptor cloned[20]
1994–7	Cannabinoid receptor antagonists developed, including rimonabant▼ (SR141716A)[9,21,22]
1995	2-AG was identified in canine gut[23] and rat brain[24]
1996	Fatty acid amide hydrolase role in metabolising endocannabinoids characterised[25]
1998	Endogenous ligands are shown to act as analgesics[12]
1999	Knock out receptor modified mice CB1[26]
2000	Knock out receptor modified mice CB2[27]
2005	Sativex® (cannabis extract, mainly THC and cannabidiol) licensed in Canada for neuropathic pain in multiple sclerosis[3,28]

Dr Vincenzo Di Marzo began his lecture by presenting clear definitions of cannabinoids, endogenous cannabinoids and endocannabinoids. 'Cannabinoids, or 'phytocannabinoids' comprise a series of around 66 metabolites found in *Cannabis sativa* with different activities at receptors in animal tissues (cannabinoid CB1, CB2 and possibly GPR55). Endogenous cannabinoids are cannabinoids found in animal tissues and endocannabinoids are *metabolites* that are chemically different from cannabinoids but have the capability to bind to and activate cannabinoid receptors', he explained.[2]

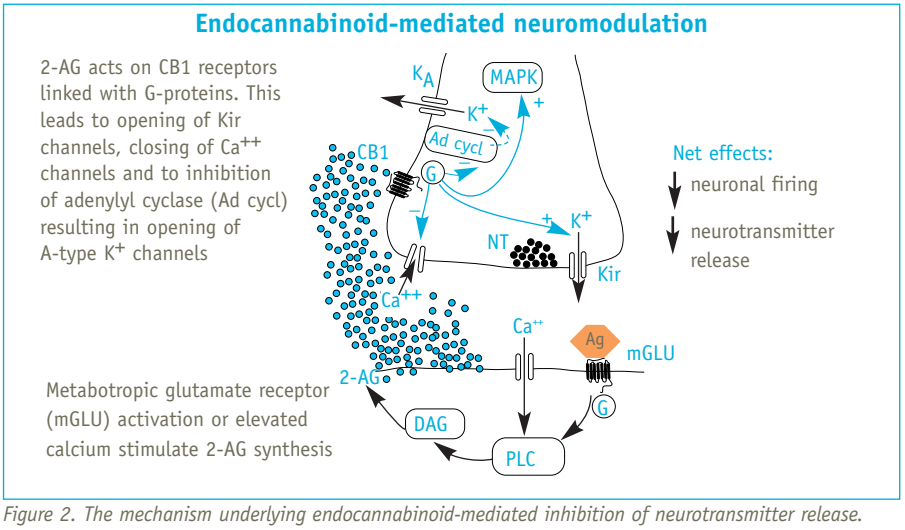

Figure 2. The mechanism underlying endocannabinoid-mediated inhibition of neurotransmitter release. Modified from Dr Di Marzo's presentation.[2] Endocannabinoid (2-AG here) synthesis is stimulated by activation of the post-synaptic neuron. 2-AG is released, transported retrogradely and acts upon CB1 receptors, which are coupled with G-proteins (G). This leads to opening of A-type (K_A) and inwardly rectifying (Kir) potassium channels with potassium efflux, and closing of N and Q/P-type calcium channels, which reduces calcium influx, resulting in a net reduction in neuronal firing and neurotransmitter (NT) release. PLC= phospholipase C; DAG=diacylglycerol; Ca^{++}=calcium; K^+=potassium; MAPK=mitogen-activated protein kinase.

Biosynthesis and degradation of endocannabinoids

The most studied ECs are anandamide[2] and 2-AG.[1,2] These are known to be produced on demand from phospholipid-derived precursors (Figure 1) in response to physiological and pathological stimuli, such as brain injury,[1] neuronal and glial cell death,[1] glutamate release,[1] cerebral ischaemia[1] and raised intracellular cytokines, reactive oxygen intermediates (ROIs) or calcium.[1,2] They act locally, are thought to be reuptaken into nerve terminals by a specific ATP-independent transporter mechanism[29] and are metabolised rapidly.[1,2] Selective inhibitors of EC reuptake and of the inactivating enzyme fatty acid amide hydrolase (FAAH)[25] have been developed, which could be used to prolong their actions at discrete, localised targets.[2] Anandamide has several biosynthetic routes and is degraded solely by FAAH, while 2-AG has one biosynthetic route but several means of degradation (Figure 1). This prompted Dr Di Marzo to postulate the possibility of biosynthetic and degradative redundancy respectively.[2]

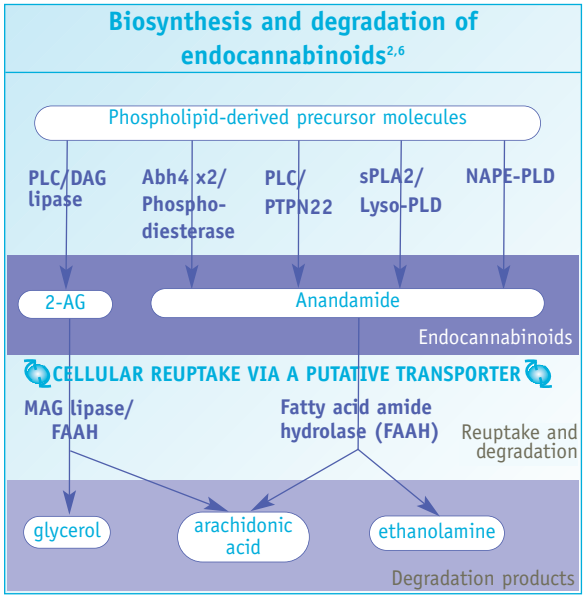

Figure 1. Simplified synthesis and degradation pathways of anandamide and 2-arachidonylglycerol (2-AG). DAG lipase= diacylglycerol lipase; Abh4= alpha/beta hydrolase-4; PLC= phospholipase C; PTPN22 = protein tyrosine phosphatase non-receptor type 22 (lymphoid); sPLA2= phospholipase A2; Lyso-PLD= lysophospholipase D; NAPE-PLD= N-acylphosphatidyl-ethanolamine specific phospholipase D; MAG lipase= monoacylglycerol lipase.

The close correspondence in distribution of the anandamide precursor phosphatidylethanolamine and phosphatidylserine led Professor Mechoulam's team to search for — and find — a new addition to the EC family, arachidonyl L-serine (ARA-S), which has vasodilator actions.[30]

A role for uptake inhibitors?

Both Dr Di Marzo and Professor Baker suggested that an alternative to using direct CB1 or CB2 agonists could be to use FAAH and EC reuptake inhibitors. This, in rather an analogous manner to the actions of certain antidepressants, would tend to raise endogenous EC concentrations, and could potentially be achieved in a target-specific manner.[2,6]

Cannabinoid receptors

EC receptors are known to be coupled with specific G-proteins — inhibiting adenylyl cyclase and activating mitogen-activated protein kinase (MAPK).[1,2] The first cannabinoid receptor, CB1, was identified in 1988[15,16] followed in 1992 by the CB2 receptor[19] (Table 1). Both have been cloned[17,20] and a series of selective agonists and antagonists have been developed with differential selectivity for CB1 and CB2 subtypes (Table 1). Subsequent research has pointed to the existence of additional receptors[31] including the metabotropic GPR55 receptor.

In addition to its actions at CB1 and CB2 receptors anandamide binds to the capsaicin-sensitive, ionotropic vanilloid

TRPV-1 (transient receptor potential vanilloid-1) receptor, which was first proposed to be an EC receptor in 2001.[1,2,32,33] Recent collaborative research from Dr Di Marzo's group show colocalisation of CB1 and TRPV-1 receptors in many regions of mouse brain.[34] Although structurally similar to anandamide, ARA-S is inactive at CB1, CB2 and TRPV-1 receptors. However, it relaxes mesenteric arteries and abdominal aorta *in vitro* by activating an atypical endothelial receptor (Table 1), for which it is thought to be the endogenous ligand.[30]

Mechanisms underlying neuromodulation

ECs are thought to be synthesised post-synaptically in response to neuronal stimulation — perhaps from calcium influx and/or metabotropic glutamate receptor activation — and they act as retrograde messengers to mediate their homeostatic effects.[35] They function as neuromodulators by activating presynpatic metabotropic CB1 receptors, which inhibit adenylyl cyclase and which may cause activation of A-type and inwardly-rectifying potassium channels and/or inactivation of N-type and P/Q-type calcium channels and suppression of neurotransmitter release (Figure 2).[2,35] These effects regulate synaptic strength and have implicated ECs in influencing cognition, motor behaviour and providing neuroprotection.[1,2]

Distribution of cannabinoid receptors

Autoradiographical,[36] *in situ* hybridisation[37] and immunohistochemical[38] mapping studies have allowed characterisation of the anatomical distribution of CB1 and CB2 receptors. The CB1 cannabinoid receptor is found mainly in the central nervous system (CNS), whereas the CB2 receptor is mainly found in the peripheral immune system (Table 2). Professor Mechoulam explained that while CB2 receptors *are* present at a very low level in mice brain (and presumably human brain) they are formed there as a result of brain pathology. This is presumably a protective effect and may represent one of the well-described reactions to damage [Professor Mechoulam, *pers comm*]. The generation of CB1 and CB2 knock-out mice[26,27] have helped define physiological roles for ECs acting at these receptors (Table 2).

Cannabinoids have multiple roles[2]

From the wide distribution of cannabinoid receptors it is not surprising that ECs have a plethora of physiological effects. Some of the better characterised of these are described below along with potential implications for therapeutics.

1. Actions on the central nervous system

Cannabinoids are well-known to exert a range of CNS effects, many of which can be predicted from the receptor distributions. Some of the known CNS actions were highlighted by several speakers.[1,2,6] Some of these will be briefly described below.

■ ECs reduce stress, anxiety and corticosterone production.[2] Findings that in acute stress hypothalamic 2-AG synthesis is reduced and serum corticosterone levels are raised, while with repeated stress 2-AG synthesis is increased

Table 1. Some agonist and antagonists of cannabinoid receptors[1,3,15,18,35,49]

	CB1	CB2	GPR55	TRPV-1	Other receptors	Comments
Phytocannabinoids:						
delta-9-THC	Ag	Ag	Ag			Main psychoactive constituent of cannabis.
Cannabidiol	IA	IA/?*	Ant*		?5HT1-A Ag	Not psychoactive. Blocks adenosine uptake — effects of adenosine mirror those of cannabidiol[1]
beta-caryophyllene	IA	Ag	?	IA		Contained in most plants — anti-inflammatory.[3]
Endocannabinoids:						
Anandamide	Ag	Ag	Ag	Ag		Co-localised with TRPV-1 in many CNS areas.
2-Arachidonylglycerol	Ag	Ag	Ag			
Noladin	Ag	weak Ag				
N-arachidonoyl dopamine	Ag	weak Ag		Ag		Pronociceptive.
Virodhamine	Ant	PA		Ag		
(Palmitoylethanolamide)	IA	IA	Ag			A cannabinomimetic, its analgesic effects are antagonized by CB2 antagonist SR144528.
arachidonoyl L-serine	IA	IA	?	IA	?AER Ag	Vasodilator actions attributed to the new AE receptor.[18]
Synthetic cannabinoids:						
Nabilone®	Ag	Ag				Licensed in UK for chemotherapy nausea/emesis.
Dronabinol (synthetic delta-9-THC)	Ag	Ag				Can be imported for named patients (nausea/emesis).
CP55,940	Ag	Ag				Used radiolabelled for receptor binding assays.
WIN55,212-2	Ag	Ag				
HU-210	Ag	Ag				High-potency mixed agonist.
AM1241	IA	Ag				
HU308	IA	Ag				High potency, anti-inflammatory & immunosuppressant.
SR141716A (Rimonabant▼)	Ant	IA				
AM251	Ant	IA				CB1 antagonist.
SR144528	IA	Ant				Inhibits osteoclast activity *in vitro*.
HU-211	IA	IA			NMDA Ant	Neuroprotective, being tested clinically in head injury.[49]

*Abbreviations: Ag=Agonist; Ant=Antagonist; AER-atypical endothelial receptor; PA=Partial agonist; IA=inactive; ?=unknown. *data indicate that cannbidiol may be an antagonist of GPR55 [Professor Mechoulam* pers comm*].*

and serum corticosterone levels fall, support a role for endocannabinoids in regulating stress.[2,] This inverse relationship occurs because high levels of corticosterone bind to a 'fast' glucocorticoid receptor that stimulates 2-AG synthesis, which exerts retrograde CB1-mediated inhibition of glutamatergic excitation of the hypothalamic paraventricular nucleus (PVN), and this reduces corticosterone production (by negative feedback) through the hypothalamo-hypophyseal-adrenal axis.[2]

■ ECs stimulate food consumption and lipogenesis. Fasting creates a metabolic stress associated with raised circulating glucocorticoids, reduced circulating leptin, raised hypothalamic ECs and hyperphagia.[39] In experiments with fasting animals, leptin blocked glucocorticoid-stimulated EC synthesis in the PVN and suppressed hyperphagia.[2,39] In cases of leptin resistance and obesity EC levels rise and hyperphagia ensues.[2,39]

Rimonabant▼, the CB1 antagonist, reduces EC-mediated hyperphagia.[2] It is licensed as an adjunct to diet and exercise (the mainstay obesity management measures)[40] for the treatment of obesity or overweight patients with associated risk factors, such as type 2 diabetes or dyslipidaemia.[2,21]

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Recent *MeReC* publications reviewed[41,42] the evidence base for rimonabant▼[43,44] including a *Cochrane review* of the four main RCTs.[44] The *Cochrane review*[44] authors concluded that rimonabant▼ produced a modest weight loss of around 5%, but had reservations about the quality of some of the studies, such as high drop-out rates. Pooled data show that almost twice as many discontinuations occurred with rimonabant▼ compared with placebo, because of adverse events. The manufacturer is providing support to help patients but as no long-term data are available it is unclear whether weight loss is maintained in patients who have taken/are taking rimonabant▼ nor whether weight loss is associated with significant reductions in morbidity and/or mortality.[41,42]

CB1 receptors are found in white adipocytes where their activation stimulates lipoprotein lipase activity. Consistent with this, CB1 knock out (-/-) mice have less fat than wild type mice.[2] Dr Di Marzo presented some recent data to show that in obesity, some of the normal negative feedback inhibition on EC synthesis is lost in the brain and in visceral but not subcutaneous adipose tissue.[2,45] This led Dr Di Marzo to suggest the potential of CB1 antagonists, such as rimonabant▼ and taranabant, as preferential blockers of visceral obesity.[2,45]

■ ECs induce sedation and inhibit motor behaviour. Early studies in humans, showed delta-9-THC decreased the time taken to fall asleep, increased stage 4 (deep) sleep and reduced the duration of rapid eye movement (REM) sleep.[46] Indeed, in a recent study of nabilone® efficacy in multiple sclerosis (MS), the authors noted that improved sleep architecture was one of the ancillary benefits of treatment.[11]

Professor David Baker, also working on MS, but using a mouse model of chronic relapsing experimental autoimmune encephalomyelitis (CREAE) that develops spasticity, found that cannabinoid administration ameliorated the spasticity in CREAE without affecting muscle tone in normal mice.[47]

This work set the scene for further studies on the modulation of motor function by ECs (see below).[6] More recent studies by Professor Baker's team showed

Table 2. Cannabinoid receptor distribution and physiological implications[6,35,49]

Cannabinoid CB1 receptors are found in high densities in specific brain regions. They are also present in peripheral neurons and in non-neuronal tissues, such as adipose, skeletal muscle, liver, gastrointestinal tract and pancreas.[6,35]

High levels	**Physiological implications**
hippocampus	learnng, memory, stress responses
basal ganglia (globus pallidus/substantia nigra)	motor control
hypothalamus	feeding, restoring homeostasis after stress
cerebellum	coordination and motor control
areas of the cerebral cortex	higher cognitive functioning
areas of the nucleus accumbens	reward — part of the reward pathway
Medium levels	
periaqueductal gray (PAG) of midbrain	pain modulation
rostral ventrolateral medulla (RVM)	pain modulation
superficial layers of spinal cord	spinal processing and modulation of pain
dorsal horn and dorsal root ganglion	peripheral pain perception and modulation
hypothalamus	temperature regulation
pituitary gland	endocrine and reproductive function
amygdala	emotional response and fear
brainstem	arousal
nucleus of the solitary tract	nausea and vomiting
Low levels	
brainstem	
cardiopulmonary centres	

Cannabinoid CB2 receptors are found in bone and throughout the immune (tonsils, spleen, mast cells, lymphocytes) and reproductive systems. However, recent studies have shown low levels of CB2 receptor-like immunoreactivity in rat brain neurons in the brainstem and cerebellum, and in microglia.[6,35,49] They are upregulated in brain pathology, presumably as a protective response to damage [Professor Mechoulam *pers comm*]. *Information taken from speakers' presentations and literature*[35,49]

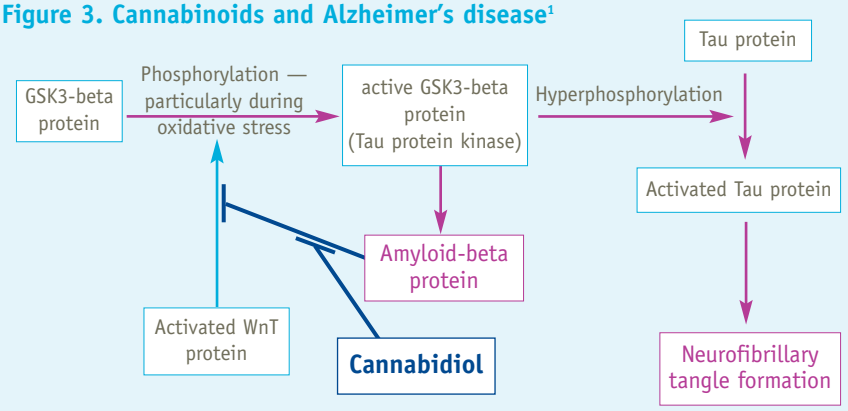
that selective inhibition of EC uptake in CREAE mice reduced spasticity, perhaps highlighting a potential route for new therapeutic agents to control spasticity.[6,48]

■ ECs mediate neuroprotective and anti-inflammatory actions. This is a very broad area of research, and various aspects were considered by several speakers.[1,2,3,6]

Brain trauma: Professor Mechoulam noted that after brain trauma from a range of sources, such as experimentally-induced hepatic encephalopathy in mice, 2-AG concentrations rise in the CNS.[1] He described experiments where 2-AG was given to animals after a CNS injury, and this reduced brain oedema, improved behavioural indices such as cognitive, motor and neurological function and shortened recovery time.[1] He noted that ARA-S produces the same neuroprotective actions, but by acting through different receptors. Interestingly, when the mice with hepatic encephalopahy were given 2-AG in combination with SR141716A (rimonabant▼) Professor Mechoulam said the greatest improvements were seen, suggesting that CB1 blockade combined with CB2 activation might be the most effective treatment option. However, peripheral CB2 actions, such as immune suppression, might make this unfeasible in some individuals.[49] Neuronal damage has been shown to increase production of ECs in other studies and neurons lacking CB1 receptors are thought to be more vulnerable to damage.[49]

Epilepsy: Dr Di Marzo presented data showing that anandamide is formed in the rodent hippocampus within five minutes during kainic acid (KA)-mediated seizures.[2] This is consistent with findings that endocannabinoid levels are elevated following CNS trauma or glutamate release described by Professor Mechoulam[1] and with the role of CB1 receptors in modulating glutamatergic neurotransmission.[2] Recent studies have shown that functional CB1 receptors co-localise with the glutamate transporter-1 on hippocampal pyramidal nerve terminals.[50] These researchers have also shown that KA-mediated excitation of hippocampal pyramidal neurons is increased in mice whose CB1 receptor expression has been selectively abolished, supporting a role for 'on demand' protection by ECs against excitotoxicity.[50] High levels of CB1 receptors are also found on cortical GABAergic interneurons, and to establish their role in modulating KA-mediated seizures the researchers generated knock-out mice with CB1 receptor deletions in specific sub-populations of neurons. In contrast to the *enhanced* susceptibility to KA-induced seizures seen in mice lacking CB1 receptors on *glutamatergic neurons*, the researchers found *similar levels* of KA-mediated seizures in mice lacking CB1 receptors on *GABAergic interneurons* compared with their wild type littermates.[50] The authors conceded that the apparent lack of effect of deleting CB1 receptors on GABAergic interneurons might be related to other researchers' findings that ECs are less effective at suppressing GABA release from interneurons at very high firing rates. The authors hypothesised that KA-induced seizures might activate the EC system to inhibit 'harmful' glutamatergic transmission but not the 'protective' GABAergic transmission.[50] Clearly, further work is needed in this complex area, but such data are adding to our understanding of the multifaceted role of ECs in maintaining normal CNS functioning, and in the modulation of excitatory currents in the hippocampus.

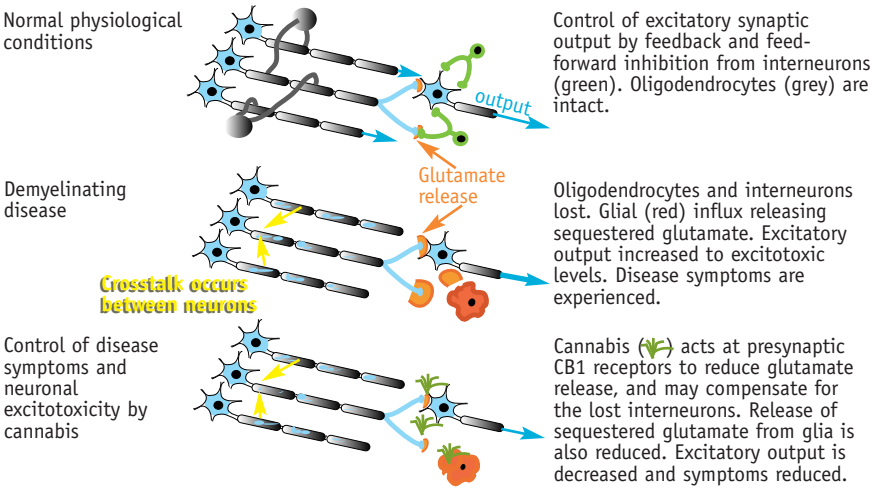
Figure 3. Cannabinoids and Alzheimer's disease[1]



Alzheimer's disease: Recent work, described by Professor Mechoulam, has added understanding to potential therapeutic targets for the treatment of Alzheimer's disease (AD). AD is characterised by two consistent pathological hallmarks: (i) The presence of extracellular beta-amyloid deposits (senile plaques) in the CNS — particularly hippocampus, cerebral cortex and amgydala — resulting in memory loss and behavioural changes, (ii) The presence of intracellular neurofibrillary tangles (NFTs) resulting from hyperphosphorylation of the microtubule-associated tau protein, which impair neuronal communication (Figure 3, constructed by Professor Mechoulam from the literature). A further consistent finding is reactive astrogliosis and activated microglia accumulation around senile plaques,[51] which are known to release a range of inflammatory mediators and could potentially contribute to increased oxidative stress and neurodegeneration locally (reviewed by Campbell and Gowran, 2007).[49]

The particular vulnerability of cholinergic neurons provides the rationale for anticholinesterase inhibition therapies.[49] Cannabinoid-based approaches to AD stem from reports of symptom improvement in AD sufferers who have used cannabis or dronabinol, and from evidence for anti-inflammatory actions of cannabinoids. This has prompted research to assess the effects of enhancing ECs in models of AD. Data from animal studies suggest that EC enhancement early in the course of the disease could improve symptoms.[52] With more severe disease memory impairment appears to worsen, suggesting that timing is all-important with regard to CB1 or CB2 effects in AD.[49,52] Whether this could be

Figure 4. How cannabis may control MS symptoms[6]



circumvented with combined CB1 blockade and CB2 activation has not been studied. However, Professor Mechoulam described some very recent experimental findings using CBD, which does not act through either CB1 or CB2 receptors, but which has potent antioxidant actions — and this has been also found to reduce cerebral infarct size in an animal stroke model by 66%.[1]

Professor Mechoulam's AD studies were undertaken in collaboration with Dr Maria de Ceballos' team at the Cajal Institute in Madrid.[1] Briefly, adult mice were injected intracerebroventricularly (icv) with either beta-amyloid or a scrambled peptide sequence (control group) and then treated with CBD. The impact of this on spatial navigation using a water maze test was then assessed.

The researchers found that icv amyloid injection caused significant cognitive impairment and this deficit was prevented by CBD. Other experiments suggested that CBD acts to block amyloid promotion of tau protein activation and NFT formation (Figure 3) and may point the way to new non-CB1/CB2 approaches to the management of AD.[1]

In addition to the protective effects of ECs on neuronal activity, Professor Mechoulam presented evidence pointing to their anti-inflammatory roles based upon work undertaken with Dr Mark Feldman at Imperial College, Professor Ruth Gallily from The Hebrew University of Jerusalem and Dr Lola Weiss from the Hadassah University Hospital in Jerusalem. In *in vitro* experiments carried out with Dr Feldman CBD was shown to reduce TNF-alpha, nitric oxide and ROI levels, and in an animal model of rheumatoid arthritis CBD improved clinical scores at low doses, but this activity was lost at high doses.

Other experiments undertaken with Professor Ruth Gallily and Dr Lola Weiss used a model of autoimmune diabetes in which almost all the mice develop diabetes by 12 weeks of age. The researchers gave these mice CBD and found that only 30% went on to develop diabetes and of these 77% were found to have of their Islets intact compared with only 8% of the control group, which provides further support for a role for ECs in immune diseases. This work has now progressed to clinical trials.

Multiple sclerosis: The recognised anti-inflammatory properties of ECs and the presence of CB2 receptors on immune system cells have stimulated research to define an involvement of the EC system in neuroinflammation. Anecdotal reports of perceived benefit to MS patients from cannabis use made this condition an obvious target for such research (*see* Baker *et al*, 2007).[53]

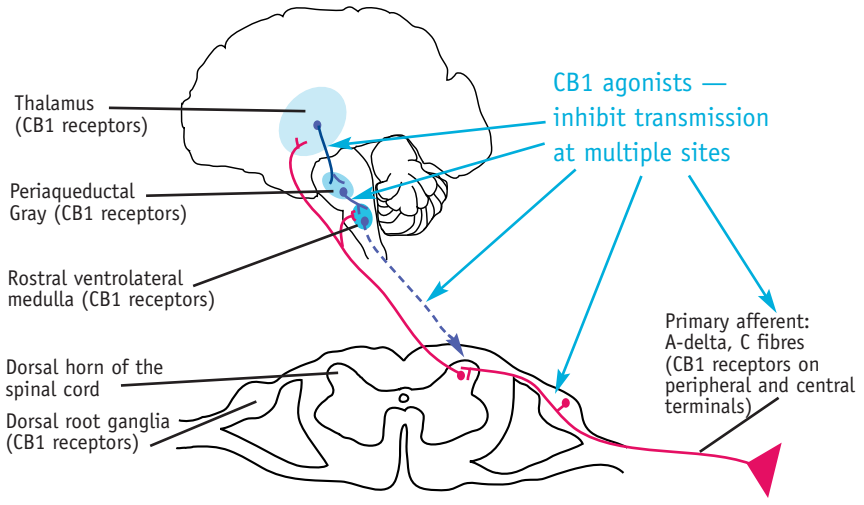
Professor Baker presented the rationale underlying his research strategy as illustrated in Figure 4 in which the possible effects of cannabis extracts on neuronal function in demyelinating disease is shown. In this scheme he suggests that cannabis [or exogenous cannabinoids, or elevated ECs] may be of greatest benefit in more severe disease when extensive demyelination exposes nerve axons allowing aberrant cross-transmission to occur. This, combined with a relative loss of inhibitory interneurons and glial influx leads to further excitation and excitotoxicity ensues. In this situation, he said, cannabinoids could limit excitation, and excitotoxic neuronal death in two ways: (i) by presynaptic CB1-mediated suppression of glutamate release — compensating for the lost inhibitory interneurons — and (ii) by ameliorating sequestered glutamate release from glia thereby lowering excitatory output.

The CB2 receptor is largely confined to glial cells in the brain and they are upregulated in activated microglia and astrocytes.[2,6,49,52,53] Because CB2 receptor activation *in vivo* reduces the production of inflammatory mediators it is thought that upregulation of CB2 receptors in pathological situations may be important in limiting neuroinflammation.[1,2,6]

Supporting this hypothesis using a pre-clinical animal model of virus-induced demyelinating disease that mimics MS, HU210 was found to reduce axonal damage and improve motor function (*see* Campbell and Gowran, 2007).[49] Since CB1 activation can cause unwanted psychoactive effects, Professor Baker focussed on possible ways to stimulate selectively localised populations of central CB1 receptors. This included targeted inhibition of FAAH using poorly CNS permeant compounds that would gain better access at sites of blood brain barrier breakdown where MS pathology is worse, thereby ensuring a local delivery to appropriate sites of action.

However, this still has the potential to elicit unwanted psychoactive effects. In an attempt to identify a means of avoiding these, more recent research from Professor

Figure 5. Cannabinoids and pain relief



Afferent pathway indicated in red; efferent pathway indicated in dark blue

Baker's team is aimed at evaluating the impact of selectively activating the GPR55 receptor, which is not associated with psychoactive events.[6]

Dr Di Marzo described situations in which EC brain levels are raised ***or*** lowered in neurodegenerative diseases. Increased CB1 signalling, he said, represents an adaptive response to neurodegeneration aimed at reducing the neurochemical imbalances, but it may also *contribute* to symptoms in conditions such as Parkinson's and Alzheimer's diseases.[2]

■ ECs mediate extinction of aversive memories and adaptive processes.

Pain: ECs have been shown to control pain at multiple levels through both CB1 and CB2 receptors. Dr Di Marzo presented a simplified pain circuit, which showed the main supraspinal sites of cannabinoids at the thalamus, the periaqueductal gray (PAG) and the rostral ventrolateral medulla (RVM).[2] Spinal sites of action include the dorsal horn of the spinal cord, where cannabinoids act on CB1 receptors to inhibit capsaicin-sensitive fibres, and to decrease noxious heat-evoked firing of wide dynamic range neurons[2] (reviewed by Lynch, 2005).[35] CB1 receptors are also thought to be present on the central and peripheral terminals of primary afferent neurons, providing yet further points of pain control (see Figure 5 adapted from Dr Di Marzo's presentation).

The literature suggests that cannabinoids can act synergistically with opioid agonists in the production of antinociception.[35] Pre-clinical studies evaluating cannabinoids suggest they are active in virtually every pain model tested.[35] In models of acute pain cannabinoids were found to act with comparable potency and efficacy to opioids, but they acted with greater potency and efficacy in chronic models of inflammatory and neuropathic pain.[35]

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The effects of chronic sciatic nerve constriction injury in rats were described by Dr Di Marzo.[2] This included rapid (within three days) elevation of anandamide levels in the spinal cord, followed by elevation at 7 days in the PAG. Levels of 2-AG were also raised in the PAG at 7 days.[2] Other researchers have reported an upregulation of CB1 receptors in the ipsilateral superficial dorsal horn of the spinal cord in rats after chronic sciatic nerve injury.[35] However, Dr Di Marzo also described actions of anandamide at the TRPV-1 receptor in dorsal root ganglia, where they cause thermal and inflammatory hyperalgesia. This could counteract the useful CB1-mediated analgesic effects of cannabinoid medicines. In search of a way round this problem, researchers have discovered that N-arachidonoyl-serotonin acts both as a FAAH inhibitor and as a TRPV-1 antagonist, and this has been found to have efficacy against neuropathic pain[54] and anxiety in animal experiments.[2]

The analgesic effect of THC in a battery of controlled clinical tests of acute pain — including heat, electrical, pressure and cold pain — has been found to be poor.[35,55] However, findings were better with chronic pain where improvements were seen after THC, CBD and whole plant extracts of THC (*see* Ware and Beaulieu, 2005).[55]

Dr Zajicek and colleagues reported their findings from a multicentre RCT of oral cannabinoids for the treatment of muscle spasticity associated with MS (the CAMS study) in 630 participants.[55,56] The aim of the study was to test the idea that cannabinoids have a beneficial effect on spasticity and other symptoms related to MS.[56] Patients received either whole cannabis extract, THC or placebo. In the 15-week study, using the Ashworth scale (which grades muscle rigidity from 0 to 4) to measure the primary outcome, no treatment effects of either oral cannabis extract or THC was found on muscle spasticity.[56] However, there was evidence of a beneficial treatment effect on secondary outcomes including mobility, general wellbeing and patient-reported perceptions of the impact of their treatment on spasticity, muscle spasms, pain and sleep quality.[56] In contrast to improvements seen

when walking time was measured, no improvements were seen using the Rivermead mobility index.[7,56]

In his presentation, Dr Zajicek explained that this illustrates one of the central problems in pain-evaluation studies — it is difficult to measure objective, clinically relevant outcomes that match patients' perceptions using standard methodology.[7] Dr Zajicek described patients in whom no improvement in pain scores, or even in perception of pain reduction, were seen. However, after cannabinoid medicines were stopped some of these patients reported feeling worse, suggesting there had been unmeasured benefits of the cannabinoid medicines.[7] The effects on pain relief are consistent with other reports of cannabinoid actions, and the patients' contrasting views with Ashworth scale ratings of the impact of cannabinoids on spasticity might suggest a clinical effect on the manifestations of spasticity rather than on muscle stiffness *per se*.[7,56]

Dr Zajicek is currently involved in the cannabis use in progressive inflammatory brain disease (CUPID) study. This is recruiting 500 participants, two thirds of whom will receive THC and one third placebo in a randomised, double blind process. This study includes better methods to monitor and measure the progress of the disease, including a primary outcome reporting scale for patients, and should help to better understand whether cannabinoids are effective in helping MS patients with symptom relief.[7]

Nabilone®, a derivative of cannabinol, was developed from pharmaceutical structure-evaluation studies for the management of severe nausea and vomiting associated with cancer chemotherapy use.[13] Clinical experience has led to it being trialled for its antihyperanalgesic efficacy. One such study reported the effect of 'off-label' nabilone® use in 20 adult patients with chronic non-cancer pain who were treated for an average of 18 months.[57] Three-quarters of the patients reported subjective overall improvement with nabilone®, 5 decreased nausea and vomiting, 10 improvement in quality or duration of sleep and nine reported reduced pain intensity. Three patients discontinued medication because of palpitations (1), dry mouth (1) or urinary retention (1), but the authors state that no serious side-effects were experienced by any patient.[57] In those patients who chose to continue receiving nabilone®, the authors state that the main reasons for continuation were beneficial effects on sleep architecture and nausea.[57]

Ko and colleagues[58] reported a case series of patients in different stages of MS with neuropathic pain who took nabilone® starting at 0.5mg nocte. Two patients needed a dose reduction to 0.2mg nocte and the remaining seven took 1mg nocte. The authors reported general improvements in most outcome measures, including the ability to reduce or stop taking opioid and psychotropic medicines.[58] These researchers also reported pain relief from nabilone® in four cases of fibromyalgia.[11]

The most recent clinical trial to be published concluded that dihydrocodeine provided better pain relief than nabilone® and had slightly fewer side-effects, although no major adverse effects occurred for either drug.[59] These conclusions were reached for a randomised, double blind, crossover trial of 14-week duration, comparing dihydrocodeine and nabilone® in 96 outpatients with chronic neuropathic pain, aged 23–84 years.[59] However, in an accompanying editorial, the paper was criticised for not being sufficiently powered to detect subgroups that might have benefited from nabilone® and for having a treatment period that was too short to measure treatment effects.[60] The high incidence of side-effects reported in the study led the editorial author to suggest that cannabinoids should not be used as a first-line treatment in patients who present with uncategorised pain.[60]

Actions on the cardiovascular system

Anandamide elicits hypotension *in vivo* — mediated exclusively by CB1 receptors and primarily caused by decreased cardiac contractility and output, with little change in vascular resistance.[30,61,62] Although CB1 receptor activation on coronary or cerebral vasculature can result in localised vasodilation, there is evidence to support additional receptor involvement in producing vasorelaxation.[30,61] The low levels of CB1 receptors in the brainstem cardiopulmonary centres probably accounts for their high safety margin[35] in producing cardiovascular or respiratory suppression.

Actions on the respiratory system

The main respiratory system concerns are on the potential health risks of inhaling cannabis smoke. This is qualitatively the same as that of tobacco smoke, containing carbon monoxide (which binds to haemo-

Box 2. *Echinacea* alkylamides are a new class of cannabinomimetics[3,64]

Dr Jurg Gertsch described his research with *Echinacea*, a herbal remedy used for the treatment of colds and upper respiratory tract infections, and reputed to be immunomodulatory. His team extracted N-alkylamides (NAAs) from *Echinacea* and other plants, and have found they are potent agonists at THC binding sites on CB2 receptors — with around 100-fold weaker binding at CB1 receptors and no activity (although some NAAs show binding affinity) at TRPV-1 receptors.[64] Noteably, depending on the stimulus applied and experimental system used, NAAs (i) modulate TNF-alpha expression in monocytes/macrophages, (ii) inhibit adenylyl cyclase, (iii) stimulate calcium release from intracellular stores and (iv) modulate certain cytokine profiles produced by stimulated and unstimulated (constitutive) T cells in human blood — effects that resemble those of 2-AG.[64]
Dr Gertsch has discovered that at least one of these NAAs, beta-caryophyllene, is found in almost *all* plants tested, including cinnamon, origanum, peppers and hops. The presence in many vegetable foods suggests that NAAs are likely to be ingested in quite high amounts by man. Beta-caryophyllene is a full agonist at CB2 receptors with anti-inflammatory actions. Research is ongoing to further characterise these anti-inflammatory effects, but they are seen at concentrations achieved in humans after *Echinacea* ingestion, and may therefore be therapeutically useful.[64]

© Christine Knott - *Echinacea purpurea*

globin at the expense of oxygen binding) but with qualitatively higher levels of polyaromatic hydrocarbons, which are known carcinogens.[63]

Actions on the immune system

Activation of CB2 receptors in lymphocytes, macrophages, neutrophils and mast cells in the periphery and in macroglia and astroglia in the CNS are thought to mediate anti-inflammatory effects.[1,6] These effects have been shown to involve changes in gene expresion[2,6] and they are mirrored by certain alkylamides extracted from *Echinacea* and other plants, as described by Dr Jurg Gertsch (*see* Box 2).[64]

Actions on the gastrointestinal system

Experiments in rodents show that satiation is associated with CB1 receptor activation and inhibition of gastric emptying.[2] In addition to their locations in discrete nuclei of the dorsovagal complex where they play a role in emesis, CB1 receptors are also found in efferents from the vagal ganglia and enteric nerve terminals (*see* Di Marzo and Izzo, 2006).[65] Enteric CB1 receptors are found on a sub-population of choline acetyltransferase positive neurons within the myenteric and submucosal plexii. *In vitro* anandamide reduces intestinal but not gastric emptying in a dose-dependent manner.[2] ECs can also activate TRPV-1 receptors detected in myenteric and submucosal neurons, resulting in increased acetylcholine release in animal experiments (although prolonged activation, such as may occur during chronic inflammation, desensitises the receptor). Under normal physiological conditions then, anandamide reduces intestinal motility and secretion through CB1 receptors.[65] Immunohistochemical data show CB2 receptors are particularly evident in colonic tissues from patients with inflammatory bowel disease, and CB2 activation inhibits intestinal motility in certain pathological states.[65]

Evidence is accumulating to suggest that during inflammatory conditions affecting the intestine, the tone of the EC system is increased. This is thought to result from either increased expression of EC receptors, or upregulation of EC levels, or both.[65] The enhanced EC tone is thought to afford protection against epithelial damage and increased motility by acting at several levels within the GiT as illustrated in Figure 6 (blue broken arrows indicate inhibition). In support of this, in experimental models of inflammation CB1 and CB2 agonists and genetic ablation of FAAH have been found to reduce colitis, suggesting possible therapeutic uses for cannabinoid medicines.[2]

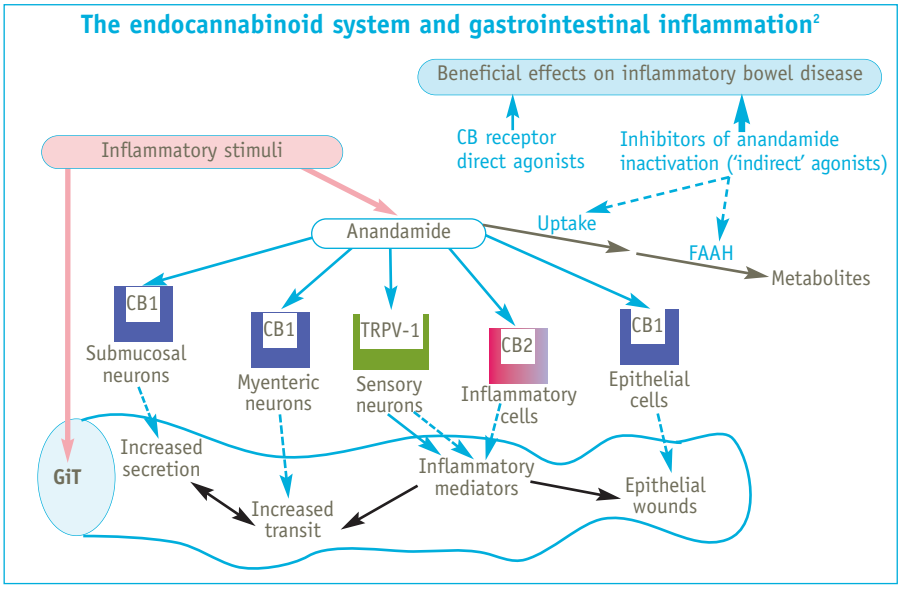

Figure 6. Inflammatory stimuli can drive the endocannabinoid system. Blue continuous arrows indicate stimulation and broken blue arrows indicate inhibition. In the presence of gastrointestinal tract (GiT) inflammation, such as seen in inflammatory bowel disease, anandamide release could activate CB2, CB2 and TRVP-1 receptors on a variety of cell types to reduce inflammation and diarrhoea. Cannabinoid medicines could act at selective cannabinoid (CB) receptor targets and/or could reduce anandamide uptake and/or metabolism. Figure taken from Di Marzo and Izzo, 2006.[2,65]

Experiments in rodents given a high fat diet for 14 weeks, showed reduced gastric emptying, and this was associated with the production of endogenous oleoylethanolamine (OEA), which is related to anandamide but not active at CB1 or CB2 receptors. OEA nevertheless inhibits food intake and explains the anorexic effects of endogenous cannabinoids. Rimonabant▼ acts at the hypothalamic and adipose tissue level, and CB1 receptor blockade in the GiT is consistent with reports of diarrhoea on starting treatment in some individuals, but it does not modify gastric emptying.[2]

Actions on the reproductive system

Activation of CB1 receptors leads to reduced sperm motility and reduced embryo implantation.[2]

Actions on the skeletal system

Regulation of the skeleton and maintaining bone mass appear to be the main physiological roles for CB2 receptors here. Recent studies have shown CB2 knockout (-/-) mice have a marked age-related trabecular bone loss.[66] These CB2 -/- mice are characterised by having increased activity of trabecular osteoblasts, increased osteoclast number and decreased osteoblast precursor number — the cell types that are known to express CB2 receptors.[66]

Giving wild type mice a CB2 agonist enhances osteoblast number and activity, and restrains osteoclastogenesis, apparently by direct and indirect inhibition of osteoclast precursor proliferation.[66] CB2 agonists also attenuate ovariectomy-induced bone loss and stimulate bone cortical thickness through suppressing osteoclast number and stimulating bone formation.[66]

Other researchers showed that both

CB1 and CB2 receptors are expressed on mouse osteoclasts and that CB1 receptors also play a role in regulating bone mineral density in mice.[67] Ofek's team have attempted to clarify the roles of CB1 receptors using two types of CB1 knock-out mouse with different phenotypes (*see* Tam *et al*, 2006).[68] They found that CB1 mRNA is weakly expressed in osteoclasts and CB1 immunoreactivity is present in sympathetic neurons close to osteoblasts.[68]

These data suggest that CB1 receptors may play a role in bone remodelling and bone mass partly through regulating noradrenaline release.[68] Interesting gender and mouse strain differences in relation to bone mass found in these CB1 knock-out mice can be exploited to learn further about the regulation of bone mass and remodelling.[68] Similarly, findings that anandamide attenuates experimentally induced bone loss led Professor Mechoulam to suggest that synthetic CB2 agonists might provide a promising strategy for novel antiosteoporosis drug development.[1]

Box 3. Metablomics approaches to unravelling cannabinoid clinical effects[4]

Much of the experimental research data presented by the speakers suggest that the already large endocannabinoid family and their associated receptors may have still further uncharacterised members to be discovered. Although this is an exciting prospect it can make interpretation of cannabinoid effects uncertain and clinical predictions correspondingly difficult, particularly for poorly selective compounds. Dr Arno Hazekamp described how metabolomics could help address this problem of multiple variables confounding the clinical picture in his presentation.[4]

Metabolomics is a statistical method of mapping metabolites that uses principal component analysis to group and correlate data from multiple variables into two, eventual, principal components that might influence a system. This is an iterative process whereby the variance of each of all data are used to separate out principal differences between groups. The data are then plotted on two axes in terms of primary and secondary principal component variables, and show clustering that allows visual identification of the resulting groupings. In this way, this method reduces large, unwieldy data sets into smaller sets and grouped variables that have similar outcomes can be correlated against other grouped variables to see how they interact and influence each other.

Dr Hazekamp described a wide range of applications of the technique, including how it could be used to analyse GC-MS data from plant extracts and define novel cannabinoid structures. He also described how it can be applied to clinical data to define genuine trends and relationships between cannabinoids and their putative effects, such as those seen in clinical trials in multiple sclerosis, and thereby help clarify the evidence-base. By grouping such data, and observing trends with time he explained that it may be possible to 'work backwards' from the metabolic effects observed to determine what effects the cannabinoid treatments had on the patients, despite not being able to measure these effects directly using traditional means such as visual analogue scales. In other words, these sort of analyses could show us what questions to ask to obtain meaningful answers!

Therapeutic opportunities for cannabinoid medicines

From the non-exhaustive list of EC actions given by the speakers a similarly extensive list of conditions in which ECs might be therapeutically useful were generated. This included nausea and vomiting associated with cancer chemotherapy; muscle spasticity; pain; anorexia; epilepsy; Parkinson's disease, Huntington's disease, Tourette's syndrome, Alzheimer's disease, glaucoma; bronchial asthma; mood disorders and psychiatric conditions; hypertension; osteoporosis and cancer. The breadth of data presented by the speakers suggest that there is considerable potential for drug development. However, there is a clear need for much fuller characterisation of the actions of cannabinoids, particularly of those in plant extracts.

Professor Michael Heinrich and Dr Jose Prieto-Garcia from the Centre for Pharmacognosy and Phytotherapy at the School of Pharmacy in London outlined the problems for users of cannabis or cannabis extracts. For instance, there is often significant variability in the supplied material, incomplete characterisation of the extract components and no legal supply. Professor Heinrich and Dr Prieto-Garcia are part of a European consortium, coordinated by Professor Heinrich — the CRAFT project — that aims to develop high quality, high value, standardised extracts (preferably low in THC content) that are orally active and suitable for clinical use in the treatment of migraine and rheumatoid arthritis.[5]

Their strategy involves growing selected plant cultivars, evaluating cannabinoid extracts from the cultivars in pharmacological screens to find the most active and then undertaking formulation and toxicology studies, applying metabolomics approaches (*see* Box 3) to their data.[5] To date they have isolated a series of extracts with reproducible *in vitro* antiinflammatory activity mediated through the inhibition of the NF-kappa B pathway including a novel orally active *Cannabis* extract that had previously been extracted from an orchid, denbinobin, which also showed potent anti-angiogenic activity.[5]

These researchers noted that methods of extraction can strongly influence the extract and subsequent experimental findings, but that extracts might result in a better therapeutic profile than single compounds.[5] However, alongside the need for a more complete characterisation of cannabinoid actions, which will help better predict or avoid unwanted effects, there is a lack of adequate data on cannabinoid drug handling ability in man.

Pharmacokinetic considerations

The major active components of the more than 60 cannabinoids are THC, CBD and cannabinol. The chemistry of cannabinoids is complex and information on pharmacokinetics is sparse except for THC. The bioavailability of THC from smoking depends upon factors such as smoking technique, the number of puffs inhaled, whether the butt is smoked (which increases the dose absorbed) and experience in smoking (*see* McGilveray, 2005).[69] Once inhaled, however, THC is rapidly — but highly variably — absorbed and it is detected in plasma within two minutes of inhalation. Elimination is biphasic with an initial (alpha) half-life of around four hours

and a beta half-life of around 25–36 hours — although there is no consensus on the terminal half-life and this is likely to be at least one week.[69]

Studies using orally administered synthetic THC (dronabinol) have shown that absorption is slower than from smoking but also highly variable. There is an extensive first-pass effect with only 10–20% of the encapsulated dose entering the systemic circulation.[69] Distribution is rapid and plasma protein binding is around 97%, mainly to low density lipoproteins (LDLs) with some binding to blood cells. The high lipid solubility gives rise to a large apparent volume of distribution at around 10L/Kg, with concentrations in fat being 1000-fold higher than in plasma and concentrations in heart tissue 10-fold higher than in plasma.[69] Animal studies indicate that fat and the spleen are the long-term storage sites for THC, from where it is very slowly released. Metabolism is complex, involving cytochrome P450 mixed function oxidases — in humans CYP 2CP appears to be the major hydroxylation route.[69] The major route of excretion of THC and its metabolites is biliary (because of enterohepatic recycling) with around 65% being recovered in faeces and around 20% in urine, although wide interindividual variations are reported.[69]

Pharmacokinetic studies using oral 14C-nabilone® showed rapid absorption of 95.8% of the dose, extensive distribution and rapid metabolism with a high first-pass effect and production of active metabolites. Nabilone® plasma elimination half-life was found to be 20.6 hours after oral administration compared with 35 hours obtained after intravenous administration and more than 90% of a dose was eliminated within seven days.[69] There also appears to be an early distribution phase with a half-life of around 10 minutes. Nabilone® metabolism is not fully characterised but metabolites are found in faeces and urine as seen with other cannabinoids.[69] For all cannabinoids further studies are needed to assess their potential for drug interactions.[8,69]

Medicines safety

Although cannabis has been used for millennia in traditional, empirical and folk medicine we require medicines to be validated through quality and quantity testing and by demonstrating efficacy and safety in RCTs. Not surprisingly, the known psychotropic and other potential unwanted effects, lack of quality and quantity control in cannabis and pharmacokinetic variability make cannabis a less than optimal candidate for RCTs. Studies undertaken with properly characterised cannabinoid medicines, such as nabilone® and rimonabant▼, have gained therapeutic ground in areas beyond their licenses, but studies with cannabis are limited.

The focus of Dr Rudolf Brenneisen's research at the University of Bern, Switzerland, is on characterising the profile of short-term cannabis metabolites in subjects who ingest

standardised amounts by smoking or oromucosal spray (Sativex®). The aim of their studies is to develop a doping test that would detect short-term rather than the long-term metabolites, which are currently measured. To help inform their studies they have collated currently available safety evaluation data from the literature, which suggest that with Sativex® use, mild-to-moderate adverse events occurred at a level of 3% or more. These were:[70–77]

- gastrointestinal: nausea, vomiting, diarrhoea, constipation, dry mouth, local mucosal irritations, plaques, ulcerations and burns
- general nervous system: dizziness, fatigue, somnolence, headache, psychotropic symptoms, feeling intoxicated and weakness

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- cardiovascular: tachycardia, hypotension, but only during initial dosing.

More severe adverse events also occurred, however, such as seizures, idiosyncratic reactions and cardiovascular problems.[73,75,78]

As part of the Switzerland adverse events monitoring pharmacovigilance programme the incidence of events after oral THC (dronabinol; Marinol®) have been evaluated. This study found the percent of the population taking dronabinol and experiencing either moderate, pronounced, strong or severe psychotropic and somatic adverse events were 26%, 19%, 5% and 5% respectively. No adverse event was experienced by 45% of the population.[5]

Clark and Lynch list the main adverse effects of cannabinoids that should be important to prescribing clinicians as those of drowsiness, impact on attention and cognition, the possibility of exaggerating existing psychosis or provoking others, postural hypotension and tachycardia.[79] In general, contraindications to the use of cannabinoids have been reported to include pregnancy, uncontrolled hypertension, active ischaemic heart disease, arrhythmias and schizophrenia.[79]

Future directions and conclusions

The speakers provided much food for thought in their presentations (more than I have been able to cover in this minor review). Clearly, the ECs appear to play a major modulatory role in a wide range of physiological systems. However, I felt that we are just beginning to understand the EC system and that more research is needed before cannabinoid medicines can realise their true potential. In particular, I felt research effort needs to be directed toward:

1. gaining a better characterisation of the EC system throughout the body. This will aid rational medicines design, more selective targeting and delivery and understanding of side-effect profiles, which will inform prescribers of contraindications and cautions. In addition, it is possible that further indications might be licensed for existing medicines if they are shown to be efficacious (through further clinical trials) and acting at selective targets.
2. characterising drug interactions. This is, so far, a rather neglected, albeit complex but very important area, which will provide much needed information when prescribing cannabinoids to people with comorbidities. Surveys indicate that a proportion of patients with a variety of medical problems have at some time tried cannabis to relieve symptoms.[55,63] Better understanding pharmacokinetic interactions might, therefore, occasionally assist in interpreting unexpected pharmacokinetic findings for prescribed drugs.
3. pharmacokinetcs studies. Careful, controlled studies are needed in patients undergoing trials, such as pain trials, and in patients with comorbid conditions, such as diabetes, hypertension, renal or hepatic impairment, compromised immune system and psychiatric illness. Some of this data could be obtained through existing (and future) clinical trials using licensed medicines outside of their licensed indications. Potentially, a metabolomics approach to data analysis might help by interpreting pooled data.
4. dependency potential. Investigations of the abuse potential of cannabinoids should accompany drug development research, as they have for nabilone® and dronabinol.[63] Clinical, legal and patient acceptance of cannabinoid medicines with proven lack of dependency are likely to be greater than of those with this adverse effect.

In addition to the licensed indications of the currently marketed cannabinoids, the 'off-label' studies suggest that pain relief is one area worth pursuing in further clinical trials. Better means of patient evaluation need to be devised to clarify any patient benefits and appropriate dosage strategies that cause the least side-effects. Clark and colleagues have drawn up guidelines for the use of cannabinoid medicines in people with chronic pain in whom other medicines have failed.[80] This includes a useful algorithm for evaluating patients and treating 'off-label' with nabilone® or dronabinol, or if applicable, cannabis,[63] which may help in devising suitable treatment strategies for testing established and new cannabinoid medicines.

Declaration of competing interests

The author declares she has no competing interests.

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Christine Knott, Editor, *Pharmacy in Practice*

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Note: Space constraints make it impossible to fully reference this review with original research material and readers are referred to more general reviews for further reading of the various subjects.

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