The endocannabinoid signalling system: Implications for anaesthesia and the pain clinic

Antonios Dougalis, George Lees

School of Health, Natural and Social Sciences, University of Sunderland, Wharncliffe street, SR1 3SD, Sunderland, UK

Department of Pharmacology & Toxicology, Otago School of Medical Sciences, University of Otago, PO Box 913, Dunedin, New Zealand

Summary

$\Delta^9$-Tetrahydrocannabinol ($\Delta^9$THC) is the major psychoactive ingredient of the plant Cannabis sativa that has been extensively used by humans for over 4000 years for both therapeutics and economic growth. Until a decade ago, the effects of $\Delta^9$THC were frequently attributed to non-specific alterations in membrane structure, based on its high lipophilicity, a theory similar to that proposed a century ago (by Meyer and Overton) for anaesthetics. Recent evidence identified a distinct protein receptor site (cannabinoid receptor, CB1) in the human brain activated not only by $\Delta^9$THC, but also by two endogenously produced lipid compounds, anandamide and 2-arachidonylethanolamide. The cannabinoid has been subsequently tested in a plethora of in vitro, in vivo and clinical (to lesser extent) assays that revealed interesting features of their pharmacology and physiological roles.

Cannabinoids have the potential to interact with a number of drugs that are used in clinical anaesthetic practice, while in turn anaesthetic agents may produce their effects through modulation of the endocannabinoid signalling system. Herein, we briefly review the discovery, pharmacological actions and putative physiological functions of endocannabinoids. We then discuss some of these finding in relation to anaesthesia in order to identify therapeutic potentials, highlight specific problems and stimulate further debate.

© 2005 Elsevier Ltd. All rights reserved.

The discovery

The plant Cannabis Sativa has been used as a recreational and therapeutic agent for over 4000 years, but its wide therapeutic potential has only
emerged in the last decade, since hard evidence has materialized for the existence of a central endocannabinoid signalling system modulating central processing.\(^1\)\(^2\) Currently two agents are licensed clinically (drobabinol and nabilone) in the UK and US Pharmacopoeias, respectively, for inhibition of emesis and appetite stimulation. These are two of the widely acknowledged affects of cannabis, while a lot of other cannabinoids are under pre-clinical or clinical assessment as potential treatments of pain, epilepsy, stroke and other disorders.\(^3\)\(^-\)\(^5\)

Chemical analysis of plant extracts revealed at least 60 different cannabinoid moiities, amongst them the major psychoactive and therapeutic ingredient of the preparation, \(\Delta^9\)-tetrahydrocannabinol (\(\Delta^9\)THC).\(^6\) Several hundred other compounds are generated by pyrolysis (when the preparation is smoked), although they have been less well characterized pharmacologically, but may also contribute to the effects of the plant by interacting or synergizing with bioactive components in the mixture.\(^7\) \(\Delta^9\)THC is highly lipophilic and readily enters the brain by crossing the blood brain barrier, while some of its metabolites are even more potent than the parent compound.\(^7\) It has been suggested that at least some of the effects of cannabinoids are due to non-specific disruption of the cellular membrane bilayer (fluidity alterations), which may stimulate or inhibit the actions of membrane bound proteins (enzymes or ion channels).\(^8\)\(^,\)\(^9\) This proposal is in analogus with the early theory of actions of anaesthetics based on the correlation between lipid solubility and potency first noted by Meyer and Overton (reviewed by Franks and Lieb\(^10\)). However, anaesthetics, irrespective of their putative mode of action, exhibit marked stereoselectivity in their actions.\(^10\)\(^,\)\(^11\) In the same manner, \(\Delta^9\)THC exhibited stereoselectivity in its actions; \([-\)] trans-isomer is much more active than \([+\]). The speculation on non-selective lipid perturbation abated somewhat when the criteria for a high affinity, stereoselective and pharmacologically distinct cannabinoid receptor, coupling to adenylyl cyclase in brain tissue, had been fulfilled.\(^12\)\(^,\)\(^13\) To date \(\Delta^9\)THC has been shown to bind at submicromolar concentrations at two cannabinoid receptors, CB1 and CB2, which have been cloned and described with respect to their localization, structure, mRNA and protein sequence, ligand binding properties and signal transduction pathways.\(^14\)\(^,\)\(^21\) More recently a putative CB3 receptor has been described in CNS, which may underpin the residual pharmacological effects of cannabinoids in CB1 knock-out animals.\(^22\)

### The endocannabinoids

Two endogenous brain lipids (endocannabinoids), anandamide (ANA) and 2-arachidonyleglycerol (2-AG), have been identified by extractions from porcine brain as activators of the CB1 receptor in various in vitro and in vivo assays.\(^23\)\(^,\)\(^24\)\(^,\)\(^25\) In the same manner other fatty acid lipids, such as oleamide, noladin ether and virodhamine have also been reported to have cannabinoid-like activities and to bind the cannabinoid receptor,\(^26\)\(^,\)\(^27\) although they have been much less extensively studied than ANA and 2-AG.\(^28\)\(^,\)\(^29\). Their cannabinomimetic effects are in some cases controversial (e.g. oleamide, see\(^30\)\(^,\)\(^31\)). Endocannabinoids, ANA and 2-AG, are synthesized via hydrolysis of phospholipid membrane precursors in neurons and glial cells and through distinctly different biosynthetic routes. Their synthesis/release can be initiated by intracellular calcium rise or through neurotransmitter receptor activation (e.g. Metabotropic glutamate receptors, muscarinic receptors).\(^28\) Interestingly, although their release does not seem to involve vesicular fusion or rely on dedicated release machinery,\(^2\) their termination of action involves coordination of two proteins, an active transport system back into cells (endocannabinoid transporter, ET) and a cleavage system via an intracellularly bound enzyme (fatty acid amide hydrolase, FAAH\(^25\)\(^,\)\(^2\)\(^,\)\(^32\)\(^-\)\(^34\)). The FAAH enzyme responsible for their degradation has a remarkable similarity in localization with the CB1 receptor, which is now considered as a principal modulatory site for the endocannabinoid signalling pathway.\(^35\)\(^,\)\(^36\) Localization studies have shown that the CB1 receptor is developmentally regulated\(^37\)\(^,\)\(^14\) and is predominately expressed in the CNS over the periphery (highest density in basal ganglia, hippocampus, amygdala, cerebellum, periquadactal grey of spinal cord and cortex), while CB2 receptors are not expressed centrally, but rather in cells of the immune system. According to behavioural and pharmacological similarities to the prototype \(\Delta^9\)THC, cannabinoid agonists can be subdivided into four groups: the classical natural (\(\Delta^9\)THC), the non-classical synthetic (CP-55940), the synthetic aminoalkylindole (R[+]WIN-55212-2) and the endogenous eicosanoids (ANA and 2-AG) (Fig. 1). Different profiles in certain bioassays for the cannabinoid receptors have been identified for these compounds,\(^7\)\(^,\)\(^29\), which might reflect their differential intrinsic potencies and functional coupling, lipophilicity, additional target(s) activation, existence of multiple types of cannabinoid receptors and differential synthesis, uptake or metabolism. For example, \(\Delta^9\)THC and ANA, but not
R(+)-WIN 55212-2, CP 55940 or 2-AG behave like partial agonists for CB1, rather than full agonists. A further complication is that ANA, but not any other members of the synthetic cannabinoid agonists, has been shown to activate the vanilloid receptors (VR1), which may be important in its antinociceptive effects.

Cannabinoid receptor physiology and pharmacology

The CB1 receptor belongs to the G protein coupled receptor superfamily; it is a pertussis toxin (PTX) sensitive G<sub>i/0</sub> seven transmembrane domain protein structure that couples negatively to adenylate cyclase and has an apparent molecular weight of 64 kDa. The CB1 receptor has been cloned from rat, mouse and human tissues and exhibits 97–99% amino acid sequence identity across species. As with other inhibitory receptors coupled to G proteins (e.g. adenosine 1 receptor, A1), CB1 receptor activation leads to modulation of ion channel function either directly or indirectly by cAMP mediated inhibition of protein kinase A (PKA) ion channel phosphorylation. A-type potassium transient currents (K<sub>A</sub>) and M-currents are stimulated, while N and P/Q-type voltage-gated Ca<sup>2+</sup> channels are inhibited (e.g. 45–49) (Fig. 2). CB1 receptors have been shown to interfere with various other biochemical transduction pathways in a PTX sensitive manner. These include regulation of focal adhesion kinase (FAK) secondary to cAMP dependent PKA inhibition, mitogen-activated protein kinase (MAPK), phosphatidylinositol-3-kinase (PI3K) as well as immediate early gene expression and protein synthesis.

CB1 receptors appear to be presynaptically expressed specifically on terminals of neurons containing GABA as neurotransmitter, co-localized with the neuropeptide cholecystokinin (CCK)50–56, although they also appear postsynaptically, but only in the spinal cord.22 The existence of another putative CB3 receptor (capsaicin sensitive) located presynaptically on glutamatergic terminals has recently been proposed based on functional electrophysiological data, although its molecular identity and subcellular localization is largely unknown. In functional terms, cannabinoids cause presynaptic inhibition of neurotransmitter release.58 This can lead to depression of synaptic transmission (e.g. 59,60), depression of EPSCs (e.g. 61) and IPSCs,50,62 inhibition of spontaneous epileptiform activity,63,64 blockade of LTP/LTD...
Cannabinoids inhibit GABAergic transmission (and possibly glutamatergic) in many nuclei including the hippocampus, cerebellum, amygdala, nucleus accumbens, substantia nigra, pars reticulata/striatum and the dorsal horn of spinal cord via a presynaptic mechanism (e.g. see 28). The in vivo effects of cannabinoids in both man and animals include impairment of memory and cognition (in certain, but not all tests), antinociception, catalepsy and euphoria 7,40,67–70, while the long-term effects from chronic cannabinoid abuse has not been yet fully clarified and might indeed be controversial 7,51,52,70.

Physiological functions and implications for anaesthesia

Memory

The disruptive effects of cannabinoids on memory and cognition are well known from behavioural studies 68,70, and from neurophysiological paradigms such as LTP (a widely applied in vitro model), which constitutes a putative cellular correlate for cognitive processing (e.g. 31,59). However, interesting results from in vitro neurophysiological studies in the hippocampus and cerebellum 2,71,72 reported that locally released endocannabinoids (within 20–25 μm of release site) are the messengers underpinning depolarization-induced suppression of inhibition (DSI), a type of short-term plasticity phenomenon 73, which leads to retrograde inhibition of GABAergic transmission on briefly depolarized neurons. Interestingly, DSI does not exist in CB1 (+/−) knock-out mice 1,74 and is only utilized by pyramidal cells, but not GABAergic interneurons 51,52. By this retrograde mechanism cannabinoids have been shown to facilitate homosynaptic hippocampal LTP by disinhibition in single hippocampal cells 75, which is in contrast to the blockade of population LTP. This global versus local modulation of the cannabinoid signalling may lead to differential effects on the behaviour of native circuits and might explain the memory deficits induced by recreational use of cannabis which are in contrast with the physiological roles of the

Figure 2 Signal transduction mechanisms for the cannabinoid receptor. Cannabinoids are released from the postsynaptic neurons via depolarization and calcium influx and activate CB1 receptors in the presynaptic terminal to reduce the probability of neurotransmitter release via a G protein dependent inhibition of N and P/Q-type voltage-gated calcium channels. Also CB1 receptor activation positively modulates the mitogen-activated protein kinase (MAPK) pathway and an inwardly rectifying potassium channel (Kir), while through a negative coupling to adenylate cyclase and consequently cAMP and protein kinase A (PKA), it negatively modulates the phosphorylation of the A-type outward potassium current (KA) that controls the closure of the channels and ultimately stimulates this hyperpolarizing current.
endogenous compounds. To complement these in vitro physiological data, an in vivo behavioural study identified endocannabinoids as essential components of the mnemonic circuit in rodents. CB1 receptor knock-out mice (–/–) lack the capacity for fear extinction and endocannabinoids facilitate such processes in mammals through their selective inhibitory effects on local inhibitory networks in the amygdala. These two major findings together defined the first possible networks in the amygdala. These two major findings together defined the first possible networks in the amygdala. These two major findings together defined the first possible networks in the amygdala.

Animal studies also suggest that cannabinoids might share common analgesic pathways with opioids and exhibit synergistic actions to increase their therapeutic benefit without the need for dose escalation. A viable hypothesis for a physiological role for endocannabinoids has been proposed by Wallace et al., who suggested that endocannabinoids might both limit epileptogenesis and also contribute to the chronic pathology in the in vivo pilocarpine model of epilepsy (see also in vitro study by Dougalis et al. ). In parallel with this, endocannabinoids can lead to pain suppression in mammals and the hypothesis that they can act as endogenous painkillers has been tested successfully, implying that the endogenous system tonically regulates nociception. Both pain and epilepsy are directly important in clinical anaesthetic practice and clearly more work is required to clarify the conditions and the mechanisms under which cannabinoids may produce therapeutic benefits, bearing in mind that not all people may respond in the same way to cannabinoids.

**Cardiorespiratory dynamics**

Although the centrally mediated effects of cannabinoids are of great importance for their psychoactive properties, their regional haemodynamic and respiratory effects seem of equal importance in clinical practice. Cannabinoids produce in vitro vasodilation in a number of vascular beds, although the mechanisms for that are not clear and may be controversial. ANA can elicit a concentration dependent bradycardia and a triphasic blood pressure response in anaesthetized rats: a transient hypotension secondary to a vagally mediated bradycardia is followed by a brief pressor (CB1 - independent) and a prolonged depressor response (CB1 - dependent). The latter two effects being similar to those of 9THC (also probably to 2-AG, ). In contrast, in conscious animals, a bradycardic effect is followed by a pressor response and a less prolonged depressor response (small or even absent) probably due to the dependence of

---

**The endocannabinoid signalling system: Implications for anaesthesia and the pain clinic**

Nociception and epilepsy

The potential involvement of endocannabinoids in suppression of pain and epilepsy emerged from an accumulating body of evidence suggesting that they can modify both neuronal hyperexcitability and pain threshold in a variety of in vitro and in vivo models as well as in clinical settings in human subjects. Antiepileptic effects of cannabinoids have been reported in various animal models and humans (e.g. reviewed by Gordon and Devinsky ), while antinociceptive actions in humans were clinically useful in certain kinds of pain (e.g. chronic neuropathic), but not others [e.g. postoperative] . The mechanisms behind those actions are still under investigation and may involve activation of more than one target than originally envisaged (e.g. CB1, VR1 and opioid receptors, while they may also be dependent upon actions in both the periphery and CNS (spinal and supraspinal levels, e.g. ). The anti-inflammatory drugs indomethacin and flurbiprofen, may also mediate (in part) their analgesic effects through modulation of endocannabinoid signalling pathways. Animal studies also suggest that cannabinoids might share common analgesic pathways with opioids and exhibit synergistic actions to increase their therapeutic benefit without the need for dose escalation. A viable hypothesis for a physiological role for endocannabinoids has been proposed by Wallace et al., who suggested that endocannabinoids might both limit epileptogenesis and also contribute to the chronic pathology in the in vivo pilocarpine model of epilepsy (see also in vitro study by Dougalis et al. ). In parallel with this, endocannabinoids can lead to pain suppression in mammals and the hypothesis that they can act as endogenous painkillers has been tested successfully, implying that the endogenous system tonically regulates nociception. Both pain and epilepsy are directly important in clinical anaesthetic practice and clearly more work is required to clarify the conditions and the mechanisms under which cannabinoids may produce therapeutic benefits, bearing in mind that not all people may respond in the same way to cannabinoids.
cannabinoid actions on preexisting sympathetic tone. Analysis of cardiorespiratory actions in anaesthetized rats has shown that activation of CB1 receptors markedly impairs ventilation, which in turn amplifies cardiovascular depression. Other animal studies (e.g.,) highlight the importance of experimental procedures in governing the depressant cardiovascular effects of cannabinoids, while the subtly different pharmacology between natural, endogenous and synthetic agents may also account for their differential effects. 

In humans, administration of THC leads to acute tachycardia, orthostatic hypotension, prolongation of left ventricular ejection time (LVET), shortening of pre-ejection period (PEP) and impairment of sympathetic reflexes (also increased sympathetic and decreased parasympathetic tone). Chronic administration is followed by bradycardia and hypotension, decreased sympathetic activity, increased parasympathetic activity and a substantial increase in blood volume, although tolerance develops to some effects especially in the young population. The cardiorespiratory consequences and interactions with sympathetic (e.g., propranolol) and parasympathetic (e.g., atropine) drugs have been described in healthy populations suggesting that caution should be exercised in cardiovascular patients using these agents when undergoing anaesthesia. Importantly, safe therapeutic use of cannabinoid drugs (choosing patients with a short history of usage, in particular) requires an appreciation of their pharmacological actions under both acute and repetitive administration in humans and the subsequent pharmacodynamic and pharmacokinetic changes and interactions with other agents.

Summary and conclusions

Basic research into the effects of cannabinoids has progressed rapidly, since the discovery of the central CB1 receptor. In the past decade, numerous reports have highlighted and revealed interesting features of cannabinoid–brain interactions and established the endocannabinoid signalling system as a source of tonic modulation in central and peripheral circuits. Recent evidence has highlighted the potential involvement of the endocannabinoid signalling system (through FAAH enzyme) in carrying forward (in part) the anaesthetic effects of propofol.

Unfortunately, there is a lack of evidence from well controlled, double blind, randomized clinical trials on the effectiveness of cannabinoid preparations in certain therapeutic arenas. Similarly, there may be a lack of awareness, or even dangerous misconceptions about the use of herbal recreational drugs amongst the public. The wide availability of cannabis combined with both low liability for dependence and toxicological potential has made the preparations very popular worldwide especially amongst younger people (9% of population). Also the increasing medical use and assessment of such drugs has given rise to sociological liberalization and a reduction in forensic/legal regulation and highlights the fact that physicians may be more likely to see intoxicated patients in their clinics.

Evidence suggests that cannabinoids may interact with a range of anaesthetic and depressant drugs used in clinical practice and further studies should be conducted to characterize these interactions (both pharmacokinetic and pharmacodynamic) in the CNS and periphery. Synergistic interactions have the potential to increase therapeutic benefit, but awareness of such issues can also help to prevent hazardous adverse drug interactions in cannabis (ab)using patients undergoing general anaesthesia. We finally stress here the importance of obtaining accurate information about the patient’s recreational drug habits. Not all patients would be keen to share this information due to lingering fears of legal consequences, potential information sharing with insurance companies and stigmatization. It is vital that these matters are discussed candidly with the patient with the understanding that confidentiality will be maintained.

Acknowledgement

The authors would like to thank the Royal Pharmaceutical Society of Great Britain for providing funding for research on endocannabinoid signalling and its physiological and pathophysiological roles.

References


60. Dougalis A, Lees G. Oleamide attenuates the frequency of 4-aminopyridine (4AP)-induced spontaneous epileptiform activity in the CA3 pyramidal neurons of the rat hippocampal slice via a CB1 receptor-independent mechanism. J Physiol (London) 543P, 25P [Ref Type: Generic, 2002].


76. Frizza J, Chesper GB, Jackson DM, Malor R, Starmer GA. The effect of delta 9-tetrahydrocannabinol, cannabidiol,


