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### Endocannabinoid system and pain: an introduction

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The endocannabinoid (EC) system consists of two main receptors: cannabinoid type 1 receptor cannabinoid receptors are found in both the central nervous system (CNS) and periphery, whereas the cannabinoid type 2 receptor cannabinoid receptor is found principally in the immune system and to a lesser extent in the CNS. The EC family consists of two classes of well characterised ligands; the *N*-acyl ethanolamines, such as *N*-arachidonoyl ethanolamide or anandamide (AEA), and the monoacylglycerols, such as 2-arachidonoyl glycerol. The various synthetic and catabolic pathways for these enzymes have been (with the exception of AEA synthesis) elucidated. To date, much work has examined the role of EC in nociceptive processing and the potential of targeting the EC system to produce analgesia. Cannabinoid receptors and ligands are found at almost every level of the pain pathway from peripheral sites, such as peripheral nerves and immune cells, to central integration sites such as the spinal cord, and higher brain regions such as the periaqueductal grey and the rostral ventrolateral medulla associated with descending control of pain. EC have been shown to induce analgesia in preclinical models of acute nociception and chronic pain states. The purpose of this review is to critically evaluate the evidence for the role of EC in the pain pathway and the therapeutic potential of EC to produce analgesia. We also review the present clinical work conducted with EC, and examine whether targeting the EC system might offer a novel target for analgesics, and also potentially disease-modifying interventions for pathophysiological pain states.

#### Pain: Endocannabinoid: Analgesia

From an evolutionary standpoint, pain can be considered a necessary evil, providing a potent warning system to protect an individual from present and future harm. However, not all pain is part of this adaptive response, e.g. persistent pain after injury healing (chronic pain) or pain arising from damage to nerve tissue (neuropathic pain). Pain is the most common complaint in those seeking a physician, and a recent study suggests that pain represents the greatest economic burden of any pathological condition in the USA, with an estimated annual cost of \$565–635 billion<sup>(1)</sup>. Many chronic pain states are

refractory to standard analgesics, and even in those which do respond, pain control can be incomplete or only short-term in nature. Of the imperfect currently available analgesics, the most efficacious are the opioids which exploit an endogenous pain control pathway within the central nervous system. However, opioids have significant issues with tolerance, dependence, respiratory depression and opioid-induced hyperalgesia<sup>(2)</sup>. Over the past few decades, the existence of a second endogenous anti-nociceptive pathway has been revealed: the endocannabinoid (EC) system.

**Abbreviations:** 2-AG, 2-arachidonoylglycerol; ACC, anterior cingulate cortex; AEA, anandamide; CB<sub>1</sub>, cannabinoid type 1 receptor; CB<sub>2</sub>, cannabinoid type 2 receptor; COX, cyclooxygenase; EC, endocannabinoid; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase.

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## Cannabinoids and the endocannabinoid system

Extracts from *Cannabis sativa* have been used as analgesics for centuries, but paucity of knowledge of the molecular pharmacology, combined with moral reservations about the psychotropic effects of this drug and its abuse for recreational purposes, led to its prohibition in the early 20th century. However, the identification of  $\Delta^9$ -tetrahydrocannabinol as the major psychoactive component of cannabis extracts<sup>(3)</sup>, and the subsequent isolation of a cannabinoid receptor highly expressed in nervous tissue<sup>(4-6)</sup>, led to an explosion of research interest in this area. Since Devane's landmark papers, firstly identifying the cannabinoid receptor<sup>(4)</sup>, and subsequently anandamide (AEA) the first endogenous ligand<sup>(7)</sup> over 5000 articles featuring EC have been published. The extent of the literature signifies the breadth of pivotal roles this system plays in physiological and pathophysiological functioning, and it is now known that in neurones these are fundamentally predicated on the modulation of neuronal signalling via retrograde inhibition (for reviews, see<sup>(8,9)</sup>).

The EC system is composed of two G protein-coupled cannabinoid receptors (cannabinoid type 1 receptor (CB<sub>1</sub>) and cannabinoid type 2 receptor (CB<sub>2</sub>)), the EC ligands that activate them, and their synthetic and catabolic enzymes. The EC system possesses several unique properties when compared with other neurotransmitter systems, and it is these properties that underlie its role in analgesia: (i) EC act in a retrograde manner at neuronal synapses, being synthesised in the post-synaptic cell and travelling back across the synapse to interact with the receptors on the pre-synaptic cell<sup>(10,11)</sup>; (ii) EC are not stored in vesicles prior to release, but instead are produced through activity-driven 'on demand' synthesis following strong neuronal activation<sup>(12,13)</sup>; (iii) The EC system involves a multitude of ligands acting at just two major receptors, in contrast to the single ligand/multiple receptor paradigm present in other systems (e.g. glutamate,  $\gamma$ -aminobutyric acid, 5-hydroxytryptamine, etc.)<sup>(14)</sup>.

EC are thought to act as a brake on neuronal hyperactivity, being produced in response to high levels of stimulation and feeding back negatively on the circuit through interaction with pre-synaptic cannabinoid receptors. In pain pathways, these actions produce analgesia by inhibiting the transmission of pain signals.

### Cannabinoid receptors

The cannabinoid receptors have divergent expression patterns underlying their separate physiological roles. CB<sub>1</sub> is predominantly found on nerve cells, while CB<sub>2</sub> is mostly expressed on cells of the immune system, with some evidence of limited neuronal expression (see discussion and references in<sup>(15)</sup>).

The CB<sub>1</sub> receptor is predominantly found pre-synaptically on axon terminals<sup>(10)</sup>, and is coupled with adenylate cyclase via G<sub>i/o</sub> proteins. Activation leads to a reduction of pre-synaptic neurotransmitter release via

inhibition of N- and P/Q-type calcium channels, and activation of potassium channels<sup>(16)</sup>. The net result of these actions can be inhibition or excitation of neuronal circuits, depending on whether the pre-synaptic cell secretes excitatory or inhibitory neurotransmitters.

## Endocannabinoids

The EC are lipid signalling molecules, produced on-demand by the activity-dependent enzymatic cleavage of membrane phospholipids<sup>(17)</sup>. The most widely investigated are the arachidonic acid derivatives AEA and 2-arachidonoyl glycerol (2-AG; structures shown in Fig. 1). AEA was the first to be discovered, and is thus the most studied, although it is present at far lower levels in tissue than 2-AG (~170-fold lower in brain<sup>(18)</sup>). Both AEA and 2-AG activate cannabinoid receptors; 2-AG is a full agonist at both CB<sub>1</sub> and CB<sub>2</sub> receptors<sup>(19)</sup>, whereas AEA shows slight selectivity for CB<sub>1</sub> over CB<sub>2</sub>. AEA has also been shown to activate the ion channel receptor TRPV1<sup>(20)</sup> and as such can be considered an EC/endovanilloid substance (for review, see<sup>(21)</sup>).

## Endocannabinoids and nutrition

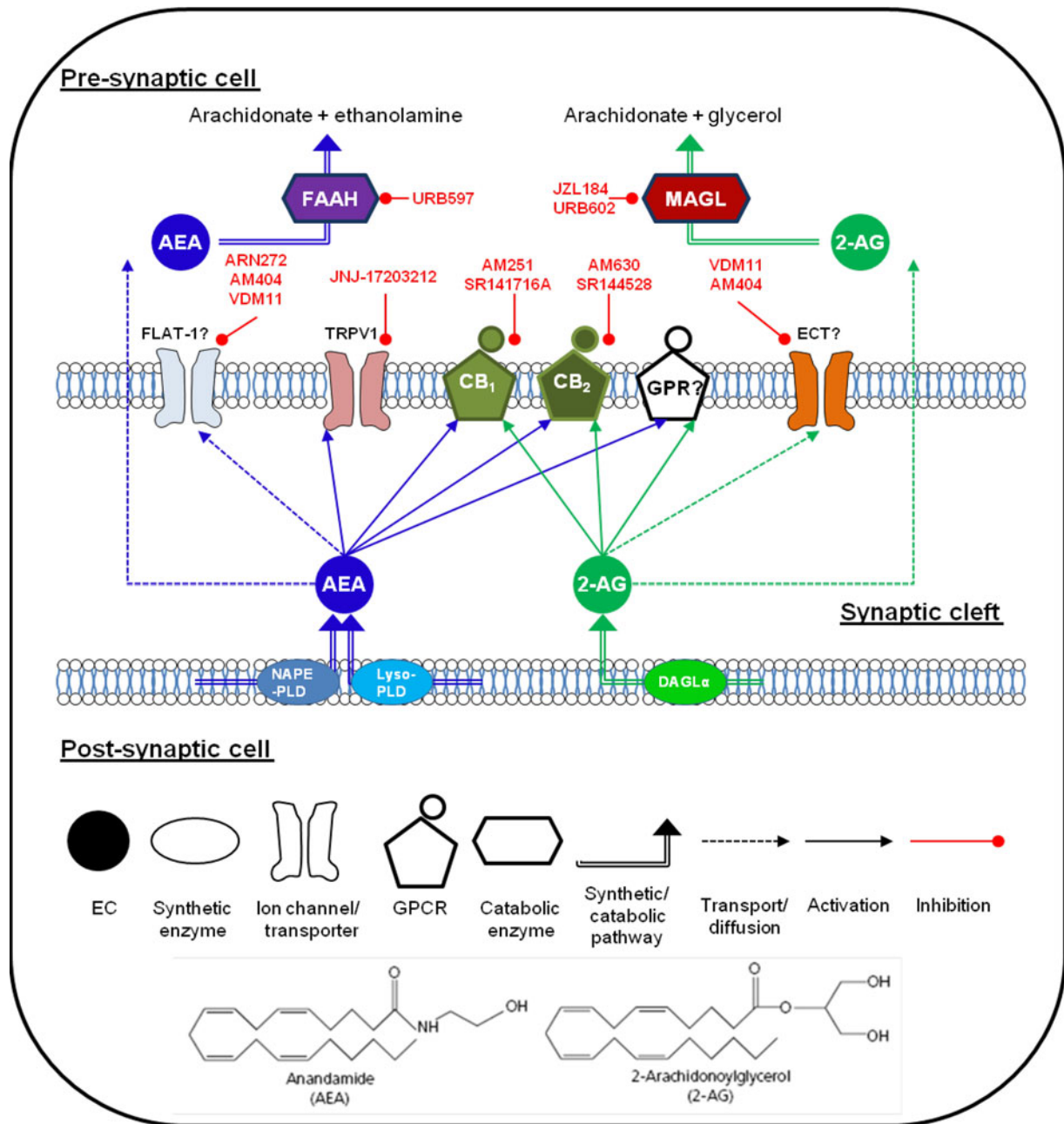
The EC are lipid-based, and as such are more susceptible to dietary-induced fluctuations in tissue levels than other transmitter substances<sup>(22,23)</sup>. Elevations in EC levels in epididymal fat and pancreas have been reported in a mouse model of diet-induced obesity<sup>(24)</sup>, and these diet-induced elevations may alter the EC system functionally, since female mice on a high-fat diet show decreased sensitivity to  $\Delta^9$ -tetrahydrocannabinol<sup>(25)</sup>. Recent clinical data suggests dietary modulation of EC also occurs in human subjects<sup>(26)</sup>, with an 84% decrease in plasma AEA observed following chronic exposure to dietary DHA and EPA. Although the functional relevance of circulating EC levels remains unclear, this robust effect of fatty acid intake suggests that nutritional factors can influence the EC system, and may offer a means to modulate the analgesic potential discussed here.

## Endocannabinoid synthesis and degradation

The major EC have distinct synthetic and degradative pathways, with the localisation of the synthetic and degradative machinery for each ligand determining its physiological effects (for review, see<sup>(27)</sup>). A schematic diagram of the actions of EC at a generic neuronal synapse can be seen in Fig. 1.

AEA was initially thought to be synthesised by a pathway involving the enzyme *N*-acyl-phosphatidylethanolamine-hydrolysing phospholipase D<sup>(28,29)</sup>. However, generation of an *N*-acylphosphatidylethanolamine-hydrolysing phospholipase D knock-out mouse revealed normal brain levels of AEA<sup>(30)</sup>, and additional synthetic pathways have since been identified<sup>(31,32)</sup>. It remains unclear which pathway predominates in the production





**Fig. 1.** (Colour online) Endocannabinoid (EC) signalling at a notional neuronal synapse. The major synthetic, signalling and catabolic pathways for anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are shown. Alongside cannabinoid receptors (CB<sub>1</sub>) and (CB<sub>2</sub>) the other G protein coupled receptors (GPCR) may be involved in cannabinoid signalling. FLAT-1, a truncated form of fatty acid amide hydrolase (FAAH), and ECT are the putative EC transporters. MAGL, monoacylglycerol lipase; DAGL, diacylglycerol lipase; GPR, specific G protein-coupled receptor such as GPR55. Compounds in red are recognised enzyme inhibitors/receptor antagonists, which can modulate EC signals.

of AEA in pain pathways. The major catabolic enzyme for AEA is fatty acid amide hydrolase (FAAH), although other catabolic pathways have been identified (for review see<sup>(33)</sup>).

2-AG is produced from cleavage of membrane phospholipids by phospholipase C-β to produce diacylglycerol species, and subsequent cleavage to form 2-AG by the enzymes diacylglycerol lipase-α and β<sup>(34)</sup>, and metabolism is primarily via monoacylglycerol lipase (MAGL)<sup>(35)</sup>.

Other enzymes participating in 2-AG metabolism have been identified<sup>(36)</sup>, although their roles in termination of 2-AG signalling have yet to be fully elucidated.

### Pain and nociception

Pain is an integrative experience, involving physiological, emotional and cognitive aspects (for a useful glossary of



pain terminology, see<sup>(37)</sup>). The subjective experience of pain varies significantly between individuals and cannot be reported by the non-human animals on which the majority of basic pain research is conducted. For the purposes of this review, we differentiate between the subjective experience of pain, and the measurable neuronal events which underlie it, hereafter referred to as nociception.

Nociceptive pathways begin with the transduction of a noxious stimulus, such as mechanical pressure, into action potentials by a specialised class of sensory afferent neurones in the periphery (e.g. mechanoreceptors in the skin). Action potentials travel via the axon of the primary afferent neurone, past the cell body located in a dorsal root ganglion, to a synapse in the superficial dorsal horn of the spinal cord. Following the integration of inputs from multiple cells types within the spinal cord, these action potentials will then pass up one of several ascending pathways to the brainstem, and subsequently to the thalamus, which then relays the signal to higher brain regions involved in the sensory (e.g. the somatosensory cortex) and emotional/affective (e.g. the amygdala and cingulate cortex) aspects of pain. There is significant cross-talk between supra-spinal nociceptive regions, and nociceptive signals can be amplified or dampened by descending modulatory pathways projecting from the brain to the spinal cord (pathways reviewed in<sup>(38–40)</sup>). Fig. 2 displays a schematic of a typical nociceptive pathway.

### Location of the endocannabinoid system in the pain pathway

The components of the EC system are expressed ubiquitously throughout the pain processing pathways, underlining its key modulatory role in nociception. Both ligands and receptors can be detected in the periphery, at the level of the spinal cord, and in nociceptive regions of the brain<sup>(6,41–71)</sup>. CB<sub>1</sub> is predominantly localised in neurones, while CB<sub>2</sub> is found in immune cells, although there is some evidence for non-neuronal CB<sub>1</sub> in B cells of the immune system<sup>(47–49)</sup> and astroglial cells of the central nervous system<sup>(59,64,68)</sup>. CB<sub>2</sub> expression has been reported in microglia of the central nervous system<sup>(62–64)</sup>, with some evidence suggesting neuronal CB<sub>2</sub> expression (see commentary in<sup>(69)</sup>), although this remains controversial.

### Peripheral mechanisms

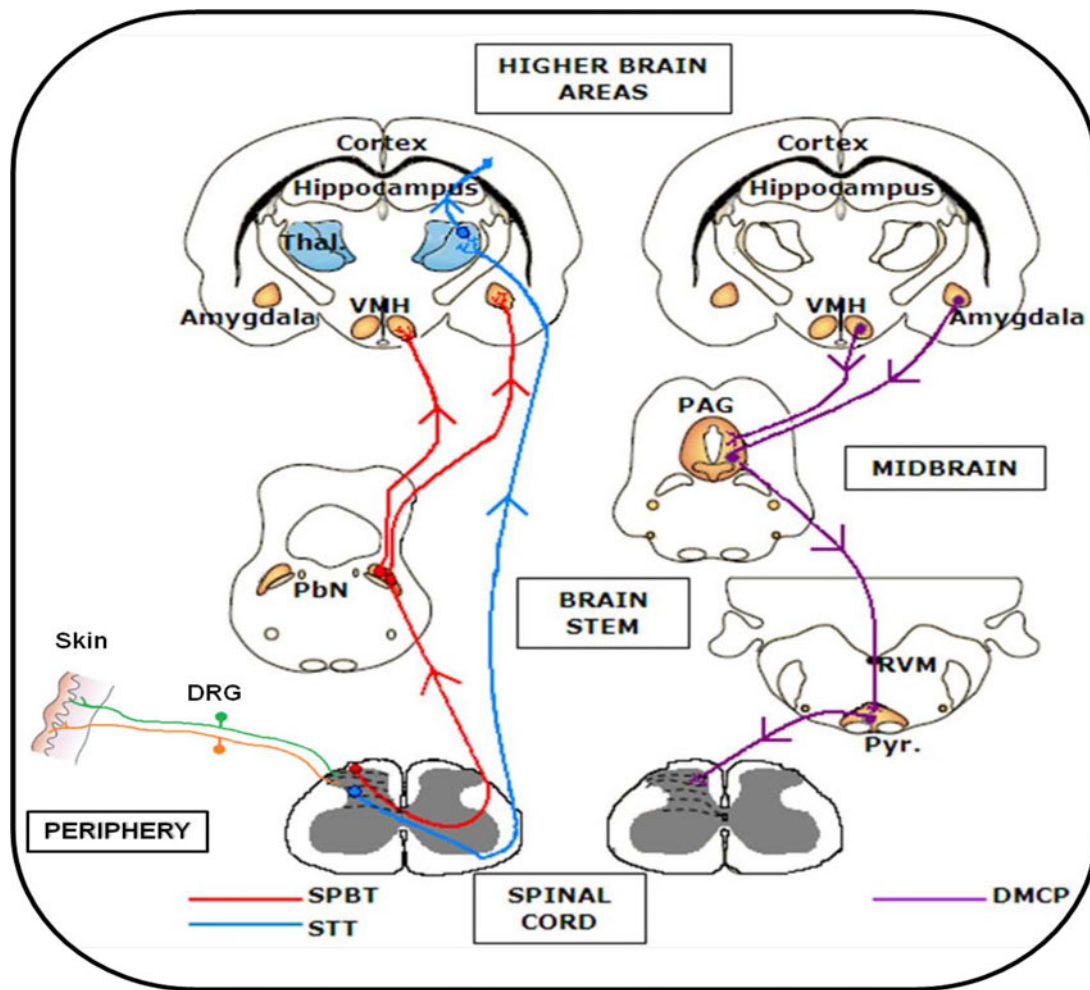
The peripheral compartment of the EC system plays a substantial role in cannabinoid-receptor-mediated anti-nociception, as demonstrated by the greatly reduced efficacy of locally and systemically administered cannabinoids following selective deletion of peripheral CB<sub>1</sub><sup>(70)</sup>. Evidence indicates that both neuronal and non-neuronal cannabinoid receptors contribute to the anti-nociceptive effects of peripheral EC, and the contributions of these and the ligands AEA, and 2-AG have been partially

revealed via use of specific enzyme inhibitors and receptor antagonists in animal models of pain.

The most studied paradigm is that of inflammatory pain, in which application of an inflammatory substance to the rodent hindpaw elicits an oedemic response and measurable nociceptive behaviour. Peripheral administration of AEA in the formalin model temporarily reduced the nociceptive behaviour in a CB<sub>1</sub>-sensitive manner<sup>(71)</sup>. Conversely, blocking CB<sub>1</sub> and/or CB<sub>2</sub> receptors prior to formalin administration increased nociceptive responses, suggesting an intrinsic role for EC in inflammatory pain. This may be restricted to early inflammatory pain states, since the hindpaw levels of EC are decreased at later time-points (see reviews in<sup>(17,72,73)</sup>). Similarly, intra-plantar administration of 2-AG blocked the second phase of formalin-evoked pain behaviour in rats<sup>(74)</sup>, via a CB<sub>2</sub>-mediated mechanism.

Exogenously administered AEA and 2-AG are rapidly metabolised by FAAH and MAGL, respectively. Further studies have therefore focused on the use of FAAH and MAGL inhibitors to prolong the effects of endogenous EC actions. Systemic inactivation of FAAH via compounds such as URB597, OL135 and PF-3845 has been shown to be anti-nociceptive in models of acute and inflammatory pain<sup>(75–80)</sup>. Elevations in both AEA and 2-AG have been shown, as well as reduced carrageenan-induced hyperalgesia<sup>(81)</sup> and expansion of peripheral receptive field size of wide dynamic range neurons (a marker of central sensitisation) following intra-plantar URB597<sup>(82)</sup>. Similarly, capsaicin-induced pain behaviour and thermal hypersensitivity were attenuated following blockade of peripheral MAGL via JZL184<sup>(83)</sup>, and peripheral administration of JZL184 produces local inhibition of MAGL activity, increased levels of 2-AG and anti-nociceptive effects in both phases of the formalin model through mechanisms involving both CB<sub>1</sub> and CB<sub>2</sub><sup>(84)</sup>. A peripherally restricted FAAH inhibitor URB937<sup>(85)</sup>, which cannot cross the blood-brain barrier, blocked the hypersensitivity induced by both inflammation and peripheral nerve injury via a CB<sub>1</sub>-mediated mechanism, without altering thermal nociceptive thresholds. These data reinforce the notion that peripheral EC tone is increased in pain states, and that actions of EC at peripheral CB<sub>1</sub> receptors can reduce the transmission of nociceptive information. Further enhancing EC signalling may therefore prove an effective analgesic strategy, although it should be noted that FAAH and MAGL are not the only enzymes that can degrade EC<sup>(33,36)</sup>, and thus, the effects of specific enzyme inhibitors may not present the entire story.

Alongside direct anti-nociceptive effects at CB<sub>1</sub> receptors on afferent neurones, EC can also act at peripheral CB<sub>2</sub> on immune cells such as macrophages<sup>(86)</sup>, lymphocytes<sup>(87)</sup> and mast cells<sup>(88)</sup>. CB<sub>2</sub> receptor activation inhibits the production and release of pro-inflammatory and pro-nociceptive mediators, such as reactive oxygen species<sup>(89)</sup> and cytokines<sup>(87)</sup>. In addition, metabolism of 2-AG produces arachidonic acid, a key precursor of pro-inflammatory PG, and disruption of 2-AG hydrolysis reduces the available pool of arachidonic acid and



**Fig. 2.** (Colour online) Nociceptive pathways. Schematic of nociceptive pathways. Nociceptive stimuli are conducted from the periphery to the dorsal horn of the spinal cord, and then transmitted to the supra-spinal regions via the spinothalamic tract (STT, blue) and spinoparabrachial tract (SPBT, red). The major descending modulatory control pathway (DMCP, purple) is displayed on the right. This pathway crosses the midline at the level of the medulla. Coloured areas indicate the position of synapses in each pathway. The positions of laminae I–VI in the dorsal horn are indicated by dotted lines, while the black region in the brain represents the lateral ventricles. Thal., thalamus; VMH, ventromedial hypothalamus; PbN, parabrachial nucleus; PAG, periaqueductal grey matter; RVM, rostromedial medulla; Pyr., pyramidal tract; DRG, dorsal root ganglion. Adapted from<sup>(89)</sup>.

thus reduces inflammation<sup>(90)</sup>. In summary, elevating levels of peripheral AEA produces anti-nociceptive effects in models of inflammatory and neuropathic pain, largely via the inhibitory actions of CB<sub>1</sub> receptors on the primary neurones which transmit nociceptive signals. Similarly, increased 2-AG signalling at peripheral CB<sub>1</sub> receptors is also anti-nociceptive, but there also appears to be a prominent CB<sub>2</sub>-mediated component, probably involving the inhibition of pro-nociceptive actions of immune cells.

### Spinal mechanisms

Exogenous application of EC is anti-nociceptive at the level of the spinal cord<sup>(91,92)</sup>, while intra-thecal administration of a CB<sub>1</sub> receptor antagonist produces

hyperalgesia in mice<sup>(93)</sup>, enhancing nociception-evoked firing of wide dynamic range neurones in the dorsal horn of the spinal cord<sup>(94)</sup>. In addition, spinal EC are elevated in animal models of acute and chronic pain<sup>(95–97)</sup>. These data indicate a role for the spinal EC system in nociceptive transmission. In the surgical incision model of acute resolving pain in rats, spinal levels of AEA are reduced at early time-points coinciding with maximal mechanical hypersensitivity, returning to baseline as nociceptive behaviour subsides<sup>(64)</sup>. In comparison, 2-AG levels were elevated at time-points coinciding with the appearance of glial cell activation and up-regulation of CB<sub>2</sub> receptors, suggesting a temporal segregation of AEA and 2-AG signalling. In agreement with these data, spinal levels of AEA are significantly elevated at early time-points in the chronic constriction injury (neuropathic pain model) model of



neuropathic pain in mice<sup>(98)</sup>. Spinal administration of URB597 reduced mechanically evoked responses of wide dynamic range neurones in rats that underwent spinal nerve ligation and this effect was blocked by pre-administration of a CB<sub>1</sub> selective receptor inverse agonist/antagonist<sup>(99)</sup>. Similarly, we have shown that spinal application of the MAGL selective inhibitor JZL184 produced a dose-related reduction in mechanically-evoked nociceptive neurotransmission in the spinal cord of naive rats, which was reversed in the presence of the CB<sub>1</sub> selective antagonist, a CB<sub>1</sub> selective receptor inverse agonist/antagonist<sup>(100)</sup>. Spinally administered JZL184 was also able to prevent intra-plantar carrageenan-induced receptive field expansion of dorsal horn wide dynamic ranges, indicating that the inhibition of MAGL can block mechanisms underlying the development of central sensitisation following peripheral inflammation.

Further evidence of the involvement of the spinal EC system is provided by alterations seen in receptor expression in established pain states. CB<sub>1</sub> expression is elevated in the spinal cord of neuropathic rats from 4d post-injury, with levels continuing to rise until day 14<sup>(101)</sup> while CB<sub>2</sub> receptor up-regulation also occurs by day 4<sup>(102)</sup>. Genetic deletion of CB<sub>2</sub> receptors results in enhanced microglia and astrocytic expression in the contralateral spinal horn following nerve injury, accompanied by profound contralateral mechanical and thermal allodynia<sup>(62)</sup>. Conversely, overexpression of CB<sub>2</sub> receptors protected against nerve injury-induced thermal and mechanical allodynia and prevented glial activation in the spinal cord. Numerous other studies have also implicated EC signalling in glial cell activation<sup>(64,103,104)</sup>.

Exogenous 2-AG has been shown to stimulate microglial migration, whereas the CB<sub>2</sub> receptor antagonist a CB<sub>2</sub> selective receptor antagonist inhibits basal microglial migration<sup>(105)</sup>. However, caution should be taken when interpreting these results as cell culture models might not reflect the *in vivo* situation. Nevertheless, these converging lines of research strongly suggest that the EC system, especially at the level of the spinal cord, is intimately involved in glial cell signalling. Further information regarding the links between the EC system and neuro-glial interactions can be found in the following review<sup>(106)</sup>. The analgesic actions of EC and cannabinoid receptor activation in the spinal cord suggest that targeting the EC system at this level could inhibit both neuronal hyper-excitability and glial cell activation. Thus, enhancing this endogenous pathway could prove to have a wide range of therapeutic applications in the treatment of multiple pain states, including the underlying central sensitisation.

### Supra-spinal mechanisms

The supra-spinal sites of cannabinoid anti-nociceptive action were first identified in rodents via microinjection of CB<sub>1</sub> ligands into pain-associated regions including the rostroventral medulla, the dorsal raphe nucleus,

the periaqueductal grey matter and the amygdala, prior to tests of acute nociception<sup>(107)</sup>. Later work revealed the role of endogenous ligands by demonstrating mobilisation of AEA<sup>(108)</sup>, and CB<sub>1</sub>-mediated anti-nociception, following either electrical stimulation of these regions or a peripheral administration of formalin<sup>(109)</sup>. Enhancing AEA signalling in these areas via inhibition of FAAH activity is anti-nociceptive in acute pain<sup>(110)</sup>, probably via disinhibition of descending inhibitory inputs from the brainstem to the spinal cord, inhibiting spinal nociceptive signalling (for a review of the pathways involved, see<sup>(38–40)</sup>).

Supra-spinal EC are also responsible for a phenomenon known as stress-induced analgesia, in which brief exposure to environmental stress (e.g. immersion in cold water, or an electric shock to the paw) produces reduced nociceptive responses in a subsequent pain test. Detailed study of this effect revealed the mobilisation of both AEA and 2-AG in the periaqueductal grey matter<sup>(111)</sup>, and suggested that 2-AG acting at CB<sub>1</sub> receptors was the predominant mechanism. Additional work indicated that further enhancing stress-induced EC signalling via FAAH or MAGL inhibition produces still greater anti-nociceptive effects<sup>(112,113)</sup>, and confirmed the pivotal role of 2-AG signalling in the periaqueductal grey matter<sup>(114)</sup>. The involvement of stress in human pain responses and the presence of an EC-mediated mechanism are now being studied clinically<sup>(115)</sup>. These data indicate a physiological role for supra-spinal EC in acute nociception, although agonism of supra-spinal CB<sub>1</sub> receptors is an unappealing prospect due to the psychotropic side-effects. Instead, research has focused on modulating the altered EC signalling seen in pain states.

The EC system is plastic, with changes in levels of receptor expression, ligand concentrations and synthetic and catabolic enzymatic activity occurring in pain states (reviewed in<sup>(116)</sup>). Elevated levels of EC in the periaqueductal grey matter and dorsal raphe nucleus have been observed at 3 and 7d in the chronic constriction injury model of neuropathic pain<sup>(98)</sup>. Interestingly, desensitisation of CB<sub>1</sub> receptors in a pain-related cortical brain region has been described in this model at 10d, when nociceptive behaviour is maximal<sup>(117)</sup>. CB<sub>1</sub> receptor desensitisation is known to occur following chronic exposure to ligands<sup>(118)</sup>, and thus may reflect the result of chronically elevated EC levels in the brain. It remains to be seen whether pharmacologically enhancing EC signalling in pain-related brain regions is advantageous or detrimental under these conditions.

Supra-spinal EC can also modulate the affective (or emotional) aspects of pain, which are mediated by frontal and limbic brain regions, and can be dissected from the somatosensory aspects. A recent neuroimaging study of the analgesic effects of  $\Delta^9$ -tetrahydrocannabinol on capsaicin-evoked cutaneous pain in human subjects revealed no change in the intensity of pain sensation<sup>(119)</sup>. Instead, test subjects reported that  $\Delta^9$ -tetrahydrocannabinol reduced the unpleasantness of capsaicin-evoked cutaneous ongoing pain, rather than reduced sensation, concurrent with reduced activity in the anterior cingulate cortex and enhanced activity in

the right amygdala compared with the control subjects. Whether modulation of the EC system can mimic these effects is as yet unknown. Ablation of the anterior cingulate cortex has been utilised as an effective last-resort treatment for intractable pain<sup>(120)</sup>, and thus unlocking the potential of the EC system in this region could address the great unmet clinical need in chronic pain states.

### Novel metabolic pathways

Modulating EC levels by FAAH and MAGL inhibition, as described earlier, can produce anti-nociceptive effects. However, bioactive lipids such as AEA and 2-AG are promiscuous, and can be metabolised by multiple enzymes<sup>(121)</sup>. Artificially elevating EC levels can unmask alternative metabolic routes, producing additional bioactive products. Interestingly, pathological conditions such as chronic pain states are associated with changes in levels of enzymes, such as cyclooxygenase (COX), lipoxygenase,  $\alpha\beta$  hydrolase and members of the cytochrome P450 family<sup>(122–125)</sup>, which can metabolise the EC to novel lipid signalling molecules. The physiological actions of these metabolic products are as yet unknown, but some preliminary investigations have been performed. The cytochrome P450 metabolite of AEA, 5,6-epoxyeicosatrienoic acid ethanolamide, has been shown to be a potent CB<sub>2</sub> receptor ligand<sup>(125)</sup>, whereas the 15-lipoxygenase metabolite of 2-AG, 15-hydroxyeicosatetraenoic acid glyceryl ester, acts as a PPAR $\alpha$  agonist<sup>(123)</sup>. COX-2 metabolites of AEA and 2-AG have been shown to have pro-nociceptive actions in the spinal cord. COX-2 metabolises AEA to prostamide F2 $\alpha$ , whose spinal application increases the firing of nociceptive neurons and reduces paw withdrawal latencies, and levels of prostamide F2 $\alpha$  are elevated in spinal cord tissue in mice with knee inflammation<sup>(124)</sup>. Similarly, the COX-2 metabolite of 2-AG, PGE2 glycerol ester, is endogenously generated in rat tissue, and induces mechanical allodynia and thermal hyperalgesia following intraplantar administration<sup>(126)</sup>. Based on these reports, it is clear that determining the levels of these potential ligands in pain states is of great interest, as many of these metabolites may have effects on pain processing. These findings may also limit the utility of FAAH and MAGL inhibitors as therapeutics in chronic pain states, as they may increase substrate levels for generation of alternative pro-nociceptive EC metabolites, and thus counteract the anti-nociceptive effects of AEA and 2-AG.

### Cannabinoids and endocannabinoids in clinical trials

Despite the growing use of medicinal marijuana, and the development of licensed cannabinoid drugs such as Sativex for multiple sclerosis<sup>(127)</sup>, concerns remain over dependence, tolerance and the cognitive side-effects produced by these medications. Despite the wealth of

pre-clinical data on alternative EC-mediated compounds, the only major clinical trial conducted utilising an EC-directed compound looked at the ability of the selective FAAH inhibitor PF-04457845 to produce analgesia in an osteoarthritic patient population<sup>(128)</sup>. Despite significant elevations in plasma AEA, no analgesic effect was observed. Although disappointing, perhaps, the negative outcome of this trial may indicate a limitation of elevating AEA to induce analgesia in pain states. Previous work has shown that, in addition to being a CB<sub>1</sub> receptor agonist, at higher concentrations AEA binds to and activates the pro-nociceptive TRPV1 channel<sup>(129)</sup>. Since AEA can be also converted into pro-nociceptive signalling molecules in the presence of COX-2 activity, then it is feasible that under pathological pain states where COX-2 activity is up-regulated (e.g. osteoarthritis), pro-nociceptive effects of AEA at TRPV1 may outweigh the anti-nociceptive actions. It has previously been suggested that the development of dual FAAH and COX-2 inhibitors<sup>(130)</sup> or substrate-selective inhibitors of COX-2<sup>(131)</sup> would be advantageous in terms of uncoupling the pro- and anti-nociceptive actions of AEA, and producing compounds with superior analgesic profiles. An alternative approach that has been explored pre-clinically utilises *N*-arachidonoyl-serotonin, a dual FAAH inhibitor/TRPV1 antagonist. This compound has enhanced anti-nociceptive effects *v.* a FAAH inhibitor alone, in several models of pain<sup>(72,132,133)</sup>. In addition, changes in AEA biotransformation in the aged patient population may contribute to the lack of analgesia following FAAH inhibition. A recent report describes greater susceptibility to chronic pain and decreased AEA-mediated anti-nociceptive effects in aged animals<sup>(134)</sup>.

An approach with potentially greater therapeutic appeal than FAAH inhibition involves the targeting of 2-AG signalling. The recent development of MAGL inhibitors such as JZL184 and KML 29<sup>(135,136)</sup>, and subsequent preclinical studies suggest that low doses of MAGL inhibitors are devoid of analgesic tolerance<sup>(137)</sup>, and also decrease arachidonic acid pools that are required for the generation of pro-nociceptive molecules such as PGE2<sup>(90)</sup>. This approach thus delivers a dual analgesic mechanism, elevating 2-AG and reducing pro-inflammatory PG levels. This may have particular utility in the treatment of inflammatory bowel diseases and the associated pain, especially as MAGL inhibitors have already been shown to attenuate non-steroidal anti-inflammatory drug-induced gastric haemorrhages in mice<sup>(138)</sup>. Inflammatory bowel diseases, such as irritable bowel syndrome, result in significant pain and distress. Recently, several converging lines of evidence suggest that targeting the EC system may provide much sought after disease modifying therapeutics for these conditions<sup>(139)</sup>. At the present stage, little clinical work has been conducted to evaluate the efficacy of FAAH and/or MAGL inhibition in treating inflammatory bowel diseases and associated pain. Given the large unmet clinical need in this area, the disease-modifying potential of an EC therapy with a dual mechanism involving both CB<sub>1</sub>- and CB<sub>2</sub>-mediated analgesia, as well as a reduction

of pathologically elevated levels of pro-inflammatory mediators, is very appealing.

### Concluding remarks

Owing to the breadth and depth of the literature cited here, we have only presented a fraction of the excellent studies conducted in this area, though we hope this information is sufficient to demonstrate the significant potential of targeting the EC system to analgesic effect. It is our belief that such an approach can produce novel efficacious analgesic agents required to help fill the unmet clinical need in chronic pain states. However, to reach this goal, the current gap between the wealth of pre-clinical data and the paucity of clinical trials must be bridged. Translation from the laboratory to the clinic is fraught with difficulties, as evidenced by the failure of the FAAH inhibitor PF-04457845 in osteoarthritic patients, despite a seemingly well-designed study. Future attempts in this area should perhaps utilise patient stratification based on aspects of disease aetiology or stratification based on neuroimaging of the pain matrix in human subjects. Clinical work is also clearly needed that focuses on whether targeting the EC system in highly inflammatory conditions such as irritable bowel syndrome, may offer new analgesic treatments.

It remains to be seen whether medications producing chronic elevations of EC will suffer from similar side-effects to those seen with phytocannabinoids, or the issues of tolerance highlighted in recent animal studies. However, the recent success of Sativex clearly highlights the potential of this area of research.

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### Conflicts of interest

None.

### Authorship

Both the authors contributed equally to the work.

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