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REVIEW

# Supraspinal modulation of pain by cannabinoids: the role of GABA and glutamate

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Recent physiological, pharmacological and anatomical studies provide evidence that one of the main roles of the endocannabinoid system in the brain is the regulation of γ-aminobutyric acid (GABA) and glutamate release. This article aims to review this evidence in the context of its implications for pain. We first provide a brief overview of supraspinal regulation of nociception, followed by a review of the evidence that the brain's endocannabinoid system modulates nociception. We look in detail at regulation of supraspinal GABAergic and glutamatergic neurons by the endocannabinoid system and by exogenously administered cannabinoids. Finally, we review the evidence that cannabinoid-mediated modulation of pain involves modulation of GABAergic and glutamatergic neurotransmission in key brain regions.
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**Keywords:** pain; brain; cannabinoids; GABA; glutamate; neurotransmission; nociception

**Abbreviations:** ↑, Increase; ↓, Decrease; ↔, no change; 2-AG, 2 arachidonylglycerol; BLA, basolateral amygdala; CCK, cholecystokinin; FAAH, fatty acid amide hydrolase; GABA, γ-aminobutyric acid; GAD, glutamic acid decarboxylase; mGlu, metabotropic glutamate; PAG, periaqueductal gray; RVM, rostral ventromedial medulla



## Introduction

Pain is a complex sensory and psychological experience, and although many of the critical loci involved in pain have been identified, the precise mechanisms underlying the perception and modulation of pain are poorly understood. Acute pain is a protective facility, warning the organism of possible or actual damage. Peripheral noxious stimuli trigger a cascade of physiological events, which propagate to the brain and are integrated and processed by limbic and cortical structures to coordinate the appropriate behavioural response.

Chronic pain is more complicated and is a major health problem. Forty-eight million Americans experience chronic pain-related health problems with the cost of treatment estimated at $100 billion a year (Holden and Pizzi, 2003). Approximately four billion workdays are lost annually at a cost of $65 billion in lost productivity due to chronic pain (Gentry, 1999). In Europe, one in five people suffer from chronic pain of moderate-to-severe intensity (Holden and Pizzi, 2003; Breivik *et al*., 2006).

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Cannabinoid drugs cited in this review: Cannabinoid agonists: $\Delta^9$-THC; WIN 55,212-2; CP55,940; HU210; $CB_1$ receptor antagonists: rimonabant (SR141716A), AM251; FAAH inhibitors: URB597, AA-5-HT, MAFP; MGL inhibitor: URB602, MAFP; anandamide transport inhibitor: AM404.
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Cannabis has been used for pain relief for centuries. With the discovery and isolation of its main psychoactive constituent, $\Delta^9$-tetrahydrocannabinol (Mechoulam and Gaoni, 1967), and receptor targets, a better understanding of the antinociceptive properties of this drug and related cannabinoid compounds has been possible. However, the precise mechanisms underlying the modulation of pain by cannabinoids are as yet unclear. Extensive experimental and clinical evidence suggests a presynaptic location of cannabinoid receptors on GABAergic (GABA: γ-aminobutyric acid) and glutamatergic neurons in brain areas associated with pain modulation. Moreover, a large body of evidence implicates supraspinal GABA and glutamate in the regulation of pain, and functional studies have demonstrated that the release of these amino-acid neurotransmitters is controlled by the brain's endogenous (endo) cannabinoid system. This review examines the role of the brain's endocannabinoid system in modulation of pain with an emphasis on the regulation of GABA and glutamate in animal models of acute, inflammatory and neuropathic pain.

## The pain pathways

The manifestation of pain, and its modulation, is mediated by ascending and descending pathways. Neurons in the

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ascending pain pathways receive input from peripheral primary afferent fibres and project from the dorsal horn of the spinal cord to a number of supraspinal sites. The two major ascending pain pathways in mammals are the spinothalamic and the spinoparabrachial tracts, which encode the sensory-discriminatory and affective aspects of pain respectively (for extensive reviews see Millan, 1999, 2002). The thalamus and parabrachial nucleus receive information from projection neurons in various laminae of the dorsal horn, and then relay this sensory information to cortical and amygdalar regions where the information is decoded as a 'painful stimulus'. The descending pathways, in turn, modulate neuronal activity in ascending pathways, and can exert an inhibitory or facilitatory effect on the sensation of pain. Interestingly, the anatomical regions involved in facilitation and inhibition of nociception often overlap. Differences in the mechanisms underlying facilitation and inhibition of nociception lie primarily in the receptor subtypes coupled to differing intracellular mechanisms (Millan, 1999, 2002). Neurons of the descending inhibitory pain pathway originate in the amygdala and hypothalamus and project to the lower brainstem (including the A5, A6/A7 noradrenergic neurons) and spinal cord, via the periaqueductal gray (PAG) and rostral ventromedial medulla (RVM) (see below). There is an accumulating body of neurochemical, pharmacological, electrophysiological and behavioural evidence for the role of GABA receptors ($GABA_A$ and $GABA_B$), and ionotropic (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid, *N*-methyl-D-aspartate and kainate) and metabotropic glutamate ($mGlu_{1-8}$) receptors in modulating supraspinal pain pathways (for recent reviews see Bleakman *et al*., 2006; Enna and McCarson, 2006; Neto *et al*., 2006). Indeed, GABAergic and glutamatergic neurons at most, if not all, supraspinal components of the descending pain pathways mediate facilitatory and/or inhibitory effects on pain perception.

## The endocannabinoid system

The endocannabinoid system is comprised of the cannabinoid$_1$ ($CB_1$) receptor, cannabinoid$_2$ ($CB_2$) receptor, endogenous cannabinoid ligands, their metabolizing enzymes and a putative anandamide uptake site (Figure 1). $CB_1$ receptors are expressed presynaptically on neurons in both the peripheral and central nervous systems as well as on a wide range of peripheral tissues. $CB_2$ receptors are expressed largely in non-neural tissues including immune cells, but now there is accumulating evidence that $CB_2$ receptor protein and mRNA is also expressed in the brain (Van Sickle *et al*., 2005; Gong *et al*., 2006; Onaivi *et al*., 2006) and spinal cord (Zhang *et al*., 2003; Wotherspoon *et al*., 2005; Beltramo *et al*., 2006). Splice variants of the $CB_1$ receptor have also been identified (Shire *et al*., 1995; Ryberg *et al*., 2005) and evidence suggests there may be additional, as yet undiscovered, cannabinoid receptor subtypes (Breivogel *et al*., 2001; Fride, 2002; Wenger *et al*., 2003; see review by Brown this issue). Within the central nervous system, the $CB_1$ receptor is found in high density and its distribution is heterogenous. Both $CB_1$ (Matsuda *et al*., 1990) and $CB_2$ receptors (Munro *et al*., 1993) are $G_{i/o}$ protein-coupled receptors that are negatively coupled to adenylyl cyclase (Howlett *et al*., 1999) and positively coupled to mitogen-activated protein kinase (Bouaboula *et al*., 1995). In addition, $CB_1$ receptors are coupled to ion channels through $G_{i/o}$ proteins, positively for A-type and inwardly rectifying potassium channels and negatively for N-type and P/Q-type calcium channels and D-type potassium channels (Pertwee, 1997, 1999; Mu *et al*., 1999). In this respect, $CB_1$ receptor activation can affect the release of neurotransmitters by modulating calcium and potassium conductance.

The endogenous cannabinoid ligands, or endocannabinoids, are polyunsaturated fatty acids and include the compounds, arachidonyl ethanolamine (anandamide), 2-arachidonylglycerol (2-AG), noladin ether, palmitoylethanolamine, homo-g-linolenylethanolamide, 7,10,13,16-docosatetranylethanolamine, virodhamine and *N*-arachidonoyldopamine. Most endocannabinoids are derived from arachidonic acid, which is a known precursor for an array of other biochemical mediators. It is believed that endocannabinoids are biosynthesized as required and immediately released from cells to exert their physiological effects. In the case of anandamide and 2-AG, this biosynthesis is catalysed by calcium-sensitive enzymes and seems to occur with calcium influx following cell depolarization, or mobilization of intracellular calcium stores. The metabolism of the endocannabinoids occurs intracellularly; however, the precise mechanism by which these compounds are taken up into the cell is, as yet, unclear. It has been postulated that re-uptake may occur via more than one mechanism, including endocytosis and the interaction of endocannabinoids with transporter proteins to carry them across the membranes (Beltramo *et al*., 1997; Beltramo and Piomelli, 2000; Hillard and Jarrahian, 2003; McFarland and Barker, 2004).

Once inside the cell, endocannabinoids are metabolized by fatty acid amide hydrolase (FAAH), which demonstrates selectivity for anandamide (Cravatt *et al*., 1996), and by monoacylglycerol lipase, which selectively degrades 2-AG (Dinh *et al*., 2002). Immunohistochemistry has demonstrated that in many brain regions, FAAH (Egertova *et al*., 2003; Gulyas *et al*., 2004) and monoacylglycerol lipase (Dinh *et al*., 2002; Gulyas *et al*., 2004) are expressed in a pattern corresponding to that of the $CB_1$ receptor (Egertova *et al*., 1998; Tsou *et al*., 1998; Ueda *et al*., 2000; Giuffrida *et al*., 2001). The neuroanatomy of the endocannabinoid system is, therefore, ideally organized to facilitate its role in retrograde signalling, the process by which endocannabinoids released postsynaptically modulate neurotransmission via an action at $CB_1$ receptors located presynaptically.

## Supraspinal regulation of pain by cannabinoids

The development of potent, selective pharmacological agonists and antagonists for the $CB_1$ and $CB_2$ receptors (Little *et al*., 1988; Rinaldi-Carmona *et al*., 1994; Hillard *et al*., 1999), $CB_1$ (Ledent *et al*., 1999; Zimmer *et al*., 1999; Marsicano *et al*., 2003; Domenici *et al*., 2006), $CB_2$ (Buckley *et al*., 2000) and FAAH (Cravatt *et al*., 2001) knockout mice, and selective FAAH (Boger *et al*., 2000; Kathuria *et al*., 2003;





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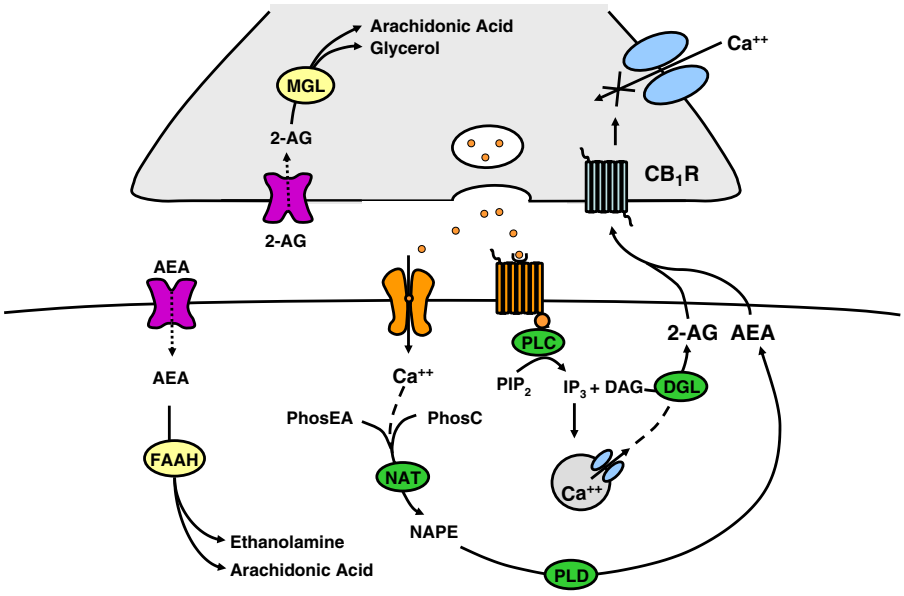


**Figure 1** Diagrammatical representation of an endocannabinoid synapse. Anandamide (AEA) and 2-arachidonylglycerol (2-AG) are synthesized following an increase in cytosolic calcium ($Ca^{++}$) resulting from activation of postsynaptic ion channels or G protein-coupled receptors. The activation of $G_q$ protein-coupled receptors results in the synthesis of inositol trisphosphate ($IP_3$) and diacylglycerol (DAG) from phosphoinositol bisphosphate ($PIP_2$). $IP_3$ mobilizes calcium release from intracellular stores triggering the formation of 2-AG from DAG by the enzyme diacylglycerol lipase (DGL). The activation of $Ca^{++}$ gating ion channels facilitates the influx of $Ca^{++}$, which leads to the formation of *N*-arachidonoyl-phosphatidylethanolamine (NAPE) from phosphatidylethanolamine (PhosEA) and phosphatidylcholine (PhosC) via the enzyme *N*-acyltransferase (NAT). NAPE is then hydrolized to anandamide by a phospholipase D-type enzyme (NAPE-PLD). The cannabinoids are released from the postsynaptic neuron and travel retrogradely to the presynaptic membrane to activate cannabinoid receptors (e.g. cannabinoid$_1$ receptor, $CB_1R$). The activation of the $CB_1$ receptor results in inhibition of $Ca^{++}$ channels in the presynaptic membrane and a number of other signal transduction-mediated events, which generally result in suppression of neuronal activity and neurotransmitter release. 2-AG is catabolized to arachidonyl acid and glycerol by monoacylglycerol lipase (MGL), while fatty acid amide hydrolase (FAAH) breaks down AEA to arachidonic acid and ethanolamine.

Deutsch, 2005) and monoacylglycerol lipase inhibitors (Saario *et al*., 2004, 2006; Makara *et al*., 2005) has proven indispensable in the advancement of the field of cannabinoid research. There are now a large number of studies providing evidence of a role for the endocannabinoid system in nociception and these have been reviewed extensively elsewhere (Pertwee, 2001; Finn and Chapman, 2004; Hohmann and Suplita, 2006; Jhaveri *et al*., this issue). Moreover, the promise of this research may soon be realized in the clinical setting with the recent launch of the cannabis-based drug Sativex in Canada for the adjunctive relief of neuropathic pain in multiple sclerosis patients. Subsequent considerations in this review will focus on the supraspinal neural substrates and neurochemical mechanisms mediating cannabinoid-induced antinociception with an emphasis on the role of the amino-acid neurotransmitters GABA and glutamate.

Direct evidence for the involvement of supraspinal cannabinoid receptors in the modulation of pain has been obtained from a number of studies employing intracerebral microinjection of cannabinoids or endocannabinoid system modulators in animal models of acute, inflammatory or neuropathic pain (Table 1). Early work demonstrated that intracerebroventricular administration of antisense oligonucleotides directed against $CB_1$ mRNA inhibited the antinociceptive effect of the cannabinoid receptor agonist CP55,940 in mice, suggesting a role for supraspinal $CB_1$ receptors in cannabinoid-mediated antinociception (Edsall *et al*., 1996). Further studies demonstrated that intracerebroventricular injection of non-selective cannabinoid receptor agonists suppressed nociception in the rat tail-flick test (Table 1), and these effects were reversed by the $CB_1$ receptor antagonist, rimonabant (Lichtman *et al*., 1996; Lichtman and Martin, 1997; Martin *et al*., 1998; Welch *et al*., 1998). Martin *et al*. (1999a) demonstrated that the cannabinoid receptor agonist WIN55,212-2 was antinociceptive in the tail-flick test when injected into a number of rat brain regions including subnuclei of the amygdala, thalamus, PAG and RVM (Table 1). Additional evidence supporting a role for the amygdala as an important site mediating cannabinoid-induced antinociception comes from work demonstrating that bilateral lesions to the amygdala abolish the antinociceptive effects of systemically administered WIN55,212-2 in the tail-flick test in rhesus monkeys (Manning *et al*., 2001).

*In vivo* electrophysiological studies have enabled the activity of ON and OFF cells in the RVM to be assessed in lightly anaesthetized rats during the tail-flick test. Microinjection of the cannabinoid receptor agonists WIN55,212-2 and HU210 into the RVM increased the rat tail-flick latency (Martin *et al*., 1998). WIN55,212-2 also decreased the firing of the ON cells while decreasing the duration of the OFF-cell pause and increasing ongoing OFF-cell activity (Meng and Johansen, 2004). Similarly, the local administration of WIN55,212-2 into the nucleus reticularis gigantocellularis pars-$\alpha$, an area in the RVM, also increased latency to withdrawal in the rat tail-flick test and reduced nociceptive





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**Table 1** The effects of supraspinal injection of cannabinoids in rat models of pain

| *Cannabinoid* | *Injection location* | *Model* | *Effect* | *Reference* |
|---|---|---|---|---|
| *Cannabinoid receptor agonists* | | | | |
| WIN55,212-2 | ICV | TFT | Antinociceptive | Martin *et al.* (1993) |
| | GiA | | | Monhemius *et al.* (2001) |
| | dlPAG | | | de Novellis *et al.* (2005) |
| | BLA | | | Hasanein *et al.* (2007) |
| | RVM | | | Martin *et al.* (1998); Meng and Johansen (2004) |
| | ICV, RVM, GiA, dPAG, BLA, CeA, thalamus, A5 NEergic group, DRN | | | Martin *et al.* (1999a) |
| | dlPAG | PWT | Antinociceptive | Palazzo *et al.* (2001) |
| | vlPAG | PWT | Antinociceptive/ pronociceptive | Maione *et al.* (2006) |
| | GiA | FT | Antinociceptive | Monhemius *et al.* (2001) |
| | BLA | | | Hasanein *et al.* (2007) |
| $\Delta^9$-THC | ICV | TFT | Antinociceptive | Lichtman *et al.* (1996) |
| HU210 | RVM | TFT | Antinociceptive | Martin *et al.* (1998) |
| | dPAG | FT | Antinociceptive | Finn *et al.* (2003) |
| CP55,940 | ICV | TFT | Antinociceptive | Martin *et al.* (1993); Lichtman *et al.* (1996) |
| *$CB_1$ receptor antagonists* | | | | |
| Rimonabant | dlPAG | PWT | Pronociceptive | Palazzo *et al.* (2001) |
| | BLA | SIA | Pronociceptive | Connell *et al.* (2006) |
| | RVM | | | Suplita *et al.* (2005) |
| | dlPAG | | | Hohmann *et al.* (2005); Suplita *et al.* (2005) |
| *Inhibitors of endocannabinoid degradation* | | | | |
| URB597 | vlPAG | PWT | Antinociceptive/ pronociceptive | Maione *et al.* (2006) |
| | dlPAG | SIA | Antinociceptive | Hohmann *et al.* (2005) |
| | BLA | | No effect | Connell *et al.* (2006) |
| URB602 | dlPAG | SIA | Antinociceptive | Hohmann *et al.* (2005) |
| | BLA | | No effect | Connell *et al.* (2006) |
| MAFP | dlPAG | SIA | Antinociceptive | Hohmann *et al.* (2005) |
| AA-5-HT | RVM, dlPAG | SIA | Antinociceptive | Suplita *et al.* (2005) |

Abbreviations: BLA, basolateral amygdala; CeA, central nucleus of the amygdala; DRN, dorsal raphe nucleus; FT, formalin test; GiA, gigantocellularis pars-$\alpha$; ICV, intracerebroventricular; PAG, periaqueductal gray; PWT, paw withdrawal test; RVM, rostral ventromedial medulla; SIA, stress-induced analgesia model; TFT, tail-flick test; $\Delta^9$-THC, $\Delta^9$-tetrahydrocannabinol.

This table reports the effects of cannabinoid compounds on nociception in a number of animal models including the TFT, PWT, FT and SIA. The TFT and PWT are models of acute thermal nociception measuring the latency to withdrawal of the animal's paw or tail from the heat source. The formalin test is a model of tonic persistent inflammatory pain, where formalin is injected into the plantar surface of the hind paw, and nociceptive behaviours are then observed and scored. The stress-induced analgesia model employs continuous footshocks and subsequent scoring of rat tail-flick responses with footshock stress increasing the latency to tail withdrawal.

responses to subcutaneous formalin administration (Monhemius *et al.*, 2001). Intra-RVM administration of rimonabant reversed the antinociceptive effects observed in all the above studies (Martin *et al.*, 1998; Monhemius *et al.*, 2001; Meng and Johansen, 2004) suggesting a modulatory role for RVM $CB_1$ receptors in the descending pain pathway (Table 1).

In the rat thermal plantar test, the microinjection of WIN55,212-2 into the dorsolateral (Palazzo *et al.*, 2001) and ventrolateral (Maione *et al.*, 2006) PAG increased the latency of the nociceptive response; an effect which was reversed by rimonabant (Palazzo *et al.*, 2001). The effects of microinjection of the FAAH inhibitor URB597 into the ventrolateral PAG were shown to depend on the dose administered. Low doses resulted in an immediate and prolonged hyperalgesic response to the rat thermal plantar test, while medium doses resulted in a bi-phasic analgesic/hyperalgesic response and high doses produced an immediate analgesic response (Maione *et al.*, 2006). URB597 was shown to dose-dependently increase anandamide levels, while 2-AG levels were maximal with the lowest dose of URB597 administered. The antinociceptive responses coincided with changes in the activity of RVM ON- and OFF neurons. The differences between endocannabinoid concentrations and consequent nociceptive and electrophysiological responses were attributed to selective activation of $CB_1$ and/or transient receptor potential vanilloid receptor type-1 receptors (Maione *et al.*, 2006). These findings support the involvement of the endocannabinoid system in the descending pain pathway in animal models of acute pain (Table 1).

Evidence for a role of supraspinal cannabinoid receptors in the modulation of inflammatory pain comes from work demonstrating that microinjection of HU210 into the dorsal PAG decreased the second phase of formalin-evoked nociceptive behaviour in rats, an effect which was blocked by rimonabant and accompanied by an attenuation of formalin-evoked c-Fos expression in the caudal lateral PAG (Finn *et al.*, 2003). Similarly, the intra-PAG microinjection of WIN55,212-2 delayed the response of formalin-treated rats to the tail-flick test, as well as the formalin-induced increase





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in activity of ON cells and decrease in OFF-cell pause in the rat RVM (de Novellis *et al*., 2005). Both these responses were blocked by rimonabant. A more recent study determined that intra-basolateral amygdala (BLA) microinjection of WIN55,212-2 dose-dependently increased the latency to withdrawal in the tail-flick test and decreased pain behaviours in both phases of the formalin test, effects reversed by the $CB_1$ receptor antagonist AM251 (Hasanein *et al*., 2007). Further support for the involvement of the brain's endocannabinoid system in inflammatory pain was provided by the observation that electrical stimulation of the rat PAG, as well as formalin injection into the hindpaw, increased anandamide release in the PAG as determined by microdialysis coupled to liquid chromatography/mass spectrometry (Walker *et al*., 1999).

Additional evidence for an endogenous cannabinoid pain-suppressing system comes from work using an animal model of unconditioned stress-induced analgesia employing continuous footshocks with subsequent scoring of rat tail-flick responses (Table 1). It was demonstrated that intra-dorsolateral PAG, intra-RVM or intra-BLA microinjection of rimonabant suppressed stress-induced analgesia relative to control animals (Hohmann *et al*., 2005; Suplita *et al*., 2005; Connell *et al*., 2006). 2-AG levels in the dorsal midbrain were markedly increased 2 min post-footshock and returned to baseline after 15 min, while anandamide displayed an increased concentration which peaked at 7–15 min post-footshock (Hohmann *et al*., 2005). Further work demonstrated that intra-dorsal PAG, intra-RVM but not intra-BLA microinjection of inhibitors of endocannabinoid degradation enhanced stress-induced antinociception, while there was no effect on basal nociceptive thresholds in non-shocked rats (Hohmann *et al*., 2005; Suplita *et al*., 2005; Connell *et al*., 2006). The enhancement of stress-induced analgesia by these enzyme inhibitors was blocked by coadministration of rimonabant. Meanwhile, in a model of conditioned fear-induced analgesia which involves assessment of formalin-evoked nociceptive behaviour in an aversively conditioned context, Finn *et al*. (2004) demonstrated that this form of psychological stress-induced analgesia is attenuated by systemic administration of rimonabant. Despite good evidence for a role of the brain's endocannabinoid system in conditioned fear (Marsicano *et al*., 2002; Cannich *et al*., 2004), the neural substrates and neurochemical mechanisms involved in endocannabinoid-mediated fear-induced analgesia remain to be elucidated.

Studies employing animal models of nerve injury have been carried out to determine the potential role of the brain's endocannabinoid system in modulation of neuropathic pain. An increase in $CB_1$ receptor mRNA in the contralateral thalamus in rats with sciatic nerve ligation was reported (Siegling *et al*., 2001), suggesting that $CB_1$ receptor upregulation may account for the increased analgesic efficacy of cannabinoids in chronic pain conditions. Microinjection of rimonabant into the nucleus reticularis gigantocellularis pars-$\alpha$ reversed the inhibitory effects of nerve ligation on formalin-evoked nociceptive behaviour (Monhemius *et al*., 2001), suggesting that increased endocannabinoid signalling through $CB_1$ receptors in the nucleus reticularis gigantocellularis pars-$\alpha$ following nerve ligation acts to reduce nociception. A recent study evaluating changes in rat supraspinal endocannabinoid levels 3 or 7 days following chronic constriction injury of the sciatic nerve has yielded some interesting results (Petrosino *et al*., 2007). An increase in the levels of anandamide and 2-AG was reported in the PAG 3 days after chronic constriction injury, while after 7 days, anandamide levels were increased in the dorsal raphe nucleus, PAG and RVM, and levels of 2-AG were increased in the PAG and RVM. There were also decreases in palmitoylethanolamine in the dorsal raphe nucleus and RVM 7 days post-ligation. Similarly, Palazzo *et al*. (2006) demonstrated an increase in levels of anandamide, but not 2-AG, in the dorsal raphe nucleus 7 days after chronic constriction injury, effects accompanied by an increase in serotonergic firing and release. The effects of chronic constriction injury on serotonergic firing and release were reversed by either single or 7-day systemic administration of the anandamide reuptake inhibitor, AM404. The effects of AM404 were reversed by rimonabant. In further electrophysiological and microdialysis experiments, 7 days treatment with WIN55,212-2 also produced similar effects to AM404 (Palazzo *et al*., 2006). These results suggest that endocannabinoid-mediated modulation of central serotonergic function may facilitate antinociception, although further studies are necessary to confirm this hypothesis.

There is good evidence for localization of $CB_1$ receptors on serotonergic (Haring *et al*., 2007), noradrenergic (Oropeza *et al*., 2007), dopaminergic (Rodriguez De Fonseca *et al*., 2001) and cholinergic (Nyiri *et al*., 2005b) neurons. In addition, cannabinoid compounds have been shown to impact on neuronal activity and/or neurotransmitter release from cholinergic (Table 2) and monoaminergic (Table 3) neurons. Despite this evidence, there are surprisingly few studies investigating the direct involvement of these neurotransmitters in supraspinally mediated cannabinoid-induced antinociception. In addition to the study by Palazzo *et al*. (2006) discussed above, it has been demonstrated that the antinociceptive effects of the cannabinoid receptor agonist, WIN55,212-2, in the rat tail-flick test are attenuated following lesion of the descending noradrenergic spinal pathways (Gutierrez *et al*., 2003). Thus, while the central serotonergic and noradrenergic systems may be involved in cannabinoid-induced antinociception, there is at present an insufficient body of data and a need for further research in this area. Cannabinoid-mediated modulation of central GABA and glutamate and its implications for pain is, however, better understood and is, therefore, the focus of the remainder of this review.

## Anatomical and functional evidence for modulation of supraspinal GABAergic and glutamatergic neurotransmission by the endocannabinoid system: implications for pain

Studies of $CB_1$ receptor localization in the brain have been carried out using a number of techniques including retrograde/anterograde labelling, immunohistochemistry, *in situ* hybridization and autoradiography. Using the aforementioned techniques it has been determined that the expres-





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**Table 2** The effect of cannabinoid compounds on supraspinal acetylcholine release

| *Cannabinoid* | *Effect* | *Brain area* | *Species* | *Reference* |
|---|---|---|---|---|
| *Cannabinoid receptor agonists* | | | | |
| *WIN55,212-2* | | | | |
| *In vitro* release | ↓[$^3$H]ACh | Hippocampal neurons | Rat | Gifford *et al.* (2000) |
| | | Cortical neurons | | |
| | | Hippocampal slices | Rat | Gifford and Ashby (1996); Kathmann *et al.* (2001b) |
| | | | Mouse | Kathmann *et al.* (2001a, b) |
| | | Cortical slices | Mouse | Kathmann *et al.* (2001b) |
| | ↔[$^3$H]ACh | Striatal slices | Mouse | Kathmann *et al.* (2001a, b) |
| Microdialysis | ↓ACh | Hippocampus | Rat | Gessa *et al.* (1997, 1998) |
| | | Prefrontal cortex | Rat | Gessa *et al.* (1998); Verrico *et al.* (2003) |
| | ↓↑ACh | Hippocampus | Rat | Tzavara *et al.* (2003) |
| | ↑ACh | Hippocampus | Rat | Acquas *et al.* (2000) |
| | | Prefrontal cortex | | Acquas *et al.* (2001) |
| *CP55,940* | | | | |
| *In vitro* release | ↓[$^3$H]ACh | Hippocampal slices | Rat | Gifford *et al.* (1997); Kathmann *et al.* (2001b) |
| | | | Mouse | Kathmann *et al.* (2001b) |
| Microdialysis | ↓ACh | Hippocampus | Rat | Gessa *et al.* (1997) |
| *$\Delta^9$-THC* | | | | |
| Microdialysis | ↓ACh | Hippocampus | Rat | Carta *et al.* (1998); Gessa *et al.* (1998); Nava *et al.* (2001) |
| | ↑ACh | Hippocampus | Rat | Pisanu *et al.* (2006) |
| | | Prefrontal cortex | Rat | Verrico *et al.* (2003); Pisanu *et al.* (2006) |
| *HU210* | | | | |
| Microdialysis | ↑ACh | Hippocampus | Rat | Acquas *et al.* (2000) |
| | | Prefrontal cortex | | Acquas *et al.* (2001) |
| *$CB_1$ receptor antagonists* | | | | |
| *Rimonabant* | | | | |
| *In vitro* release | ↑[$^3$H]ACh | Hippocampal slices | Rat | Gifford *et al.* (1997, 2000); Gifford and Ashby (1996) |
| | ↔[$^3$H]ACh | Cortical, striatal slices | Rat | Gifford and Ashby (1996); Gifford *et al.* (2000) |
| | | Striatal slices | Mouse | Kathmann *et al.* (2001a) |
| Microdialysis | ↑ACh | Prefrontal cortex | Rat | Gessa *et al.* (1998); Tzavara *et al.* (2003) |
| | | Hippocampus | Rat | Gessa *et al.* (1997, 1998) |
| | | | Mouse | Degroot *et al.* (2006) |
| *AM251* | | | | |
| Microdialysis | ↑ACh | Hippocampus | Rat; mouse | Degroot *et al.* (2006) |

Abbreviation: Ach, acetylcholine; $\Delta^9$-THC, $\Delta^9$-tetrahydrocannabinol.
Upwards or downwards arrows indicate increases or decreases, respectively, in Ach release, whereas no change is indicated by a '↔'.



sion of the $CB_1$ receptor gene is restricted to specific cell types, which serve distinct functional roles in a variety of neurological processes (Freund and Hajos, 2003; Freund *et al.*, 2003). There are a large number of studies demonstrating a role for supraspinal GABA and glutamate in animal models of pain (for review see Bleakman *et al.*, 2006; Enna and McCarson, 2006; Neto *et al.*, 2006). Here, we provide a summary of the distribution of $CB_1$ binding sites on GABAergic and glutamatergic neurons in brain regions known to play an important role in nociception, review the evidence for cannabinoid-mediated modulation of GABAergic and glutamatergic transmission (Table 4) and discuss its importance in the context of pain (Table 5).

## Cortical and limbic areas

A number of cortical and limbic areas play an important role in the affective-motivational dimension of pain. Using *in situ* hybridization and immunohistochemistry, it has been shown that the $CB_1$-positive cells in cortical areas represent a small percentage of the total cell population in rat brain and reside on heterogenous GABAergic interneurons (Mailleux and Vanderhaeghen, 1992; Matsuda *et al.*, 1993; Tsou *et al.*, 1998). Further double-labelling studies have shown that mice cortical cells expressing the $CB_1$ receptor also co-express glutamic acid decarboxylase (GAD65), the GABA synthesizing enzyme that characterizes GABAergic cells (Marsicano and Lutz, 1999). These GABAergic interneurons can be further subdivided into separate groups based on the expression of cell type-specific neurochemical markers. Double immunostaining determined that the majority of $CB_1$-positive GABAergic neurons also stained positive for cholecystokinin (CCK) in rat somatosensory cortex (Bodor *et al.*, 2005), rat hippocampus (Katona *et al.*, 1999; Nyiri *et al.*, 2005a), rat septum (Nyiri *et al.*, 2005b), rat BLA (Katona *et al.*, 2001; McDonald and Mascagni, 2001) and mouse forebrain (Marsicano and Lutz, 1999). In addition to the



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**Table 3** The effect of cannabinoid compounds on supraspinal monoaminergic neurotransmitter release and the firing of supraspinal monoaminergic neurons

| *Cannabinoid* | *Effect* | *Brain area* | *Species* | *Reference* |
|---|---|---|---|---|
| *Cannabinoid receptor agonists* | | | | |
| *WIN55,212-2* | | | | |
| *In vitro* release | ↔[$^3$H]DA | N. accumbens; C. striatum slices | Rat | Szabo *et al.* (1999) |
| | ↓[$^3$H]NE | HC slices | Human | Schlicker *et al.* (1997) |
| | | HC, cerebellar, hypothalamic, cortical slices | G. pig | Schlicker *et al.* (1997) |
| | ↔[$^3$H]NE | HC slices | Rat; Mouse | Schlicker *et al.* (1997) |
| | ↓[$^3$H]5-HT | Cortical membranes | Mouse | Nakazi *et al.* (2000) |
| Tissue levels | ↔DA | N. accumbens, C. striatum | Rat | Verrico *et al.* (2003) |
| | ↓DA | Prefrontal cortex | | |
| Electrophysiology | ↑DA firing | Substantia nigra | Rat | French *et al.* (1997) |
| | | VTA | Rat | French *et al.* (1997); Diana *et al.* (1998); Gessa *et al.* (1998); Pistis *et al.* (2001) |
| | ↓DA firing | VTA | Rat | Pillolla *et al.* (2007) |
| | ↔NE firing | L. coeruleus | Rat | Mendiguren and Pineda (2006) |
| | ↑NE firing | L. coeruleus | Rat | Mendiguren and Pineda (2006); Muntoni *et al.* (2006) |
| Microdialysis | ↑DA | N. accumbens | Rat | Tanda *et al.* (1997); Lecca *et al.* (2006) |
| | ↑NE | Frontal cortex | Rat | Oropeza *et al.* (2005) |
| *CP55,940* | | | | |
| *In vitro* release | ↔[$^3$H]DA | N. accumbens, C. striatum slices | Rat | Szabo *et al.* (1999) |
| | ↓[$^3$H]5-HT | Cortical membranes | Mouse | Nakazi *et al.* (2000) |
| Electrophysiology | ↔NE firing | L. coeruleus | Rat | Mendiguren and Pineda (2006) |
| | ↑NE firing | L. coeruleus | | Mendiguren and Pineda (2006) |
| *$\Delta^9$-THC* | | | | |
| *In vitro* release | ↓↑[$^3$H]DA; ↓↑[$^3$H]NE | Hypothalamic, striatal neurons | Rat | Poddar and Dewey (1980) |
| Tissue levels | ↔DA | N. accumbens, C. striatum | Rat | Jentsch *et al.* (1998); Verrico *et al.* (2003) |
| | ↓DA | Prefrontal cortex | | Jentsch *et al.* (1998); Verrico *et al.* (2003) |
| Electrophysiology | ↑DA firing mesolimbic | VTA | Rat | French (1997); French *et al.* (1997); Diana *et al.* (1998); Gessa *et al.* (1998); Ng Cheong Ton *et al.* (1988); Malone and Taylor (1999); Melis *et al.* (2000); Wu and French (2000) |
| | ↑DA firing Nigrostriatal | Substantia nigra | Rat | Tanda *et al.* (1997) French *et al.* (1997); Wu and French (2000) |
| | ↑NE firing | L. coeruleus | Rat | Melis *et al.* (2000); Muntoni *et al.* (2006) |
| Microdialysis | ↑DA | N. accumbens | Rat | Tanda *et al.* (1999) |
| *Anandamide* | | | | |
| Electrophysiology | ↑DA firing | N. accumbens | Rat | Solinas *et al.* (2006) |
| *$CB_1$ receptor antagonists* | | | | |
| *Rimonabant* | | | | |
| *In vitro* release | ↔[$^3$H]DA | N. accumbens; C. striatum slices | Rat | Szabo *et al.* (1999) |
| | ↑[$^3$H]NE | HC slices | Human | Schlicker *et al.* (1997) |
| | ↔[$^3$H]NE | HC, cerebellar, hypothalamic, cortical slices | G. pig | Schlicker *et al.* (1997) |
| | ↔[$^3$H]5-HT | Cortical membranes | Mouse | Nakazi *et al.* (2000) |
| Electrophysiology | ↓DA firing Mesolimbic | VTA | Rat | Pistis *et al.* (2001); Pillolla *et al.* (2007) |
| | ↓NE firing | L. coeruleus | Rat | Muntoni *et al.* (2006) |
| Microdialysis | ↑DA; ↑NE | Prefrontal cortex | Rat | Tzavara *et al.* (2003) |
| | ↔DA; ↔NE | N. accumbens; | Rat | Tzavara *et al.* (2003) |
| | ↑5-HT | Striatum prefrontal cortex, N. accumbens | Rat | Tzavara *et al.* (2003) |
| *Inhibitors of degradation* | | | | |
| *URB597* | | | | |
| Electrophysiology | ↑5-HT firing | Dorsal raphe | Rat | Gobbi *et al.* (2005) |

Abbreviations: C. striatum, corpus striatum; DA, dopamine; G. pig, guinea pig; HC, hippocampus; L. coeruleus, locus coeruleus; N. accumbens, nucleus accumbens; NE, noradrenaline; 5-HT, serotonin; $\Delta^9$-THC, $\Delta^9$-tetrahydrocannabinol; VTA, ventral tegmental area.
Upwards or downwards arrows indicate increases or decreases, respectively, in monoaminergic neurotransmitter release or firing of monoaminergic neurons as measured by electrophysiology, whereas no change is indicated by a '↔'.





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**Table 4** Studies investigating the functional effects of cannabinoid compounds on supraspinal release of GABA and glutamate, and on the firing of supraspinal GABAergic and glutamatergic neurons

| *Cannabinoid* | *Effect* | *Brain Area* | *Species* | *Reference* |
|---|---|---|---|---|
| *WIN55,212-2* | | | | |
| *In vitro* electrophysiology | ↓IPSPs | HC neurons | Rat | Irving *et al.* (2000) |
| | | BLA | Rat | Katona *et al.* (2001) |
| | | Neocortex | Rat | Bodor *et al.* (2005) |
| | | PAG | Rat | Vaughan *et al.* (2000) |
| | | RVM | Rat | Vaughan *et al.* (1999) |
| | | HC slices | Rat | Hajos *et al.* (2000, 2001); Hoffman and Lupica (2000); Hajos and Freund (2002); Foldy *et al.* (2006) |
| | | Lateral amygdala | Mouse | Trettel and Levine (2002); Azad *et al.* (2003) |
| *In vitro* electrophysiology | ↓EPSPs | Prefrontal cortex | Rat | Auclair *et al.* (2000) |
| | | PAG | Rat | Vaughan *et al.* (2000) |
| | | HC slices | Rat | Hajos *et al.* (2001); Hajos and Freund (2002) |
| | | BLA, cortex | Mouse | Domenici *et al.* (2006) |
| | | Lateral amygdala | Mouse | Azad *et al.* (2003) |
| | | HC slices | Mouse | Misner and Sullivan (1999); Domenici *et al.* (2006) |
| *In vitro* release | ↓[$^3$H]GABA | HC neurons | Rat | D'Amico *et al.* (2004) |
| | | HC slices | Rat | Katona *et al.* (1999) |
| | | | Human | Katona *et al.* (2000) |
| | ↑[$^3$H]Glut | PFC neurons | Rat | Ferraro *et al.* (2001) |
| Microdialysis | ↓GABA | PFC | Rat | Ferraro *et al.* (2001) |
| | ↑Glutamate | PFC | Rat | Ferraro *et al.* (2001) |
| *CP55, 940* | | | | |
| *In vitro* electrophysiology | ↓IPSPs | HC slices | Rat | Hajos *et al.* (2000) |
| | | BLA | Rat | Katona *et al.* (2001) |
| *In vitro* release | ↓[$^3$H]GABA | HC neurons | Rat | D'Amico *et al.* (2004) |
| ***AM251*** | | | | |
| *In vitro* release | ↓[$^3$H]GABA | HC slices | Rat | Neu *et al.* (2007) |

Abbreviations: BLA, basolateral amygdala; EPSPs, excitatory postsynaptic potentials; GABA, γ-aminobutyric acid; HC, hippocampus; IPSPs, inhibitory postsynaptic potentials; PAG, periaqueductal gray; PFC, prefrontal cortex; RVM, rostral ventromedial medulla.
Upwards or downwards arrows indicate increases or decreases, respectively, in the release of GABA and glutamate neurotransmitters, or in the firing of GABAergic and glutamatergic neurons, whereas no change is indicated by a '↔'. IPSPs and EPSPs are temporary changes in postsynaptic membrane potential caused by the flow of ions into or out of the cell. IPSPs are generally initiated by the activation of GABA receptors on the postsynaptic neuron and suppress the firing of the postsynaptic neuron, while glutamate receptor activation generally instigates EPSPs, which enhance the firing of the postsynaptic neuron.

**Table 5** The role of supraspinal GABA and glutamate in the antinociceptive effects of the cannabinoid receptor agonist, WIN55,212-2

| *WIN55,212-2 injection location* | *Antinociception reversed by:* | *Model* | *Reference* |
|---|---|---|---|
| dlPAG | Rimonabant -$CB_1$ R antagonist<br>MSOP-group III mGlu antagonist<br>MPEP-$mGlu_5$ antagonist<br>EGlu-group II mGlu antagonist<br>APV-NMDA R antagonist | PWT | Palazzo *et al.* (2001) |
| dlPAG | Rimonabant ($CB_1$ R antagonist) MPEP ($mGlu_5$ antagonist) | FT, EPhys | de Novellis *et al.* (2005) |
| I.v. | Intra-CeA muscimol ($GABA_A$ receptor agonist) | TFT, FT | Manning *et al.* (2003) |
| S.c. | Intra-RVM muscimol ($GABA_A$ receptor agonist) | TFT, EPhys | Meng *et al.* (1998) |

Abbreviations: $CB_1$ R, cannabinoid$_1$ receptor; CeA, central nucleus of the amygdala; Ephys, electrophysiology; GABA, γ-aminobutyric acid; FT, formalin test; i.v., intravenous; mGlu, metabotropic glutamate; PWT, paw withdrawal test; RVM, rostral ventromedial medulla; s.c., subcutaneous; TFT, tail-flick test.
This table reports the effects of GABAergic and glutamatergic compounds on the antinociceptive effects of the cannabinoid receptor agonist, WIN55,212-2, in a number of rat models including the TFT, PWT, FT and changes in the firing of various neurons as measured by EPhys. The TFT and PWT are models of acute thermal nociception measuring the latency to withdrawal of the animal's paw or tail from the heat source. The formalin test is a model of tonic persistent inflammatory pain, where formalin is injected into the plantar surface of the hind paw, and nociceptive behaviours are then observed and scored. The stress-induced analgesia model employs continuous footshocks with subsequent scoring of rat tail-flick responses with footshock stress increasing the latency to tail withdrawal.

large CCK-positive cells, a much smaller subset of $CB_1$-positive GABAergic interneurons were reported to contain calcium-binding proteins in somatosensory cortex (Bodor *et al.*, 2005), hippocampus (Katona *et al.*, 1999; Marsicano and Lutz, 1999; Tsou *et al.*, 1999) and BLA (Marsicano and Lutz, 1999; McDonald and Mascagni, 2001). It has been suggested that because CCK and calcium-binding proteins are expressed in separate populations of $CB_1$-positive



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GABAergic interneurons, endocannabinoids could modulate population synchrony as well as individual neuronal plasticity (Bodor *et al*., 2005).

A more recent study provides evidence for $CB_1$ receptors on presynaptic glutamatergic terminals (Katona *et al*., 2006). It was shown that principal cell populations of the hippocampus contain high levels of diacylglycerol lipase-α (an enzyme involved in 2-AG formation) concentrated in heads of dendritic spines. Electron microscopy observations revealed that these specialized postsynaptic dendritic spine domains receive glutamatergic inputs. These dendritic spine domains release 2-AG by retrograde neurotransmission to activate $CB_1$ receptors on presynaptic glutamatergic axon terminals. The colocalization of $CB_1$ receptors with hippocampal vesicular glutamate transporter type 1 has also been demonstrated (Monory *et al*., 2006), suggesting that cannabinoids can impact on glutamate neurotransmission. We have recently demonstrated that $CB_1$ receptors in the BLA colocalize with GAD67, a marker for GABAergic neurons, fibres and terminals (Figure 2).

In immunohistochemistry studies on primate brain slices, $CB_1$ receptors were reported on putative glutamatergic pyramidal projection neurons as well as on GABAergic neurons in the cortex, hippocampus and amygdala (Ong and Mackie, 1999). However, in another study, $CB_1$ immunoreactivity was found exclusively in GABAergic neurons and axon terminals in these regions (Eggan and Lewis, 2007). The authors suggest that the differences observed may be due to differential ability of antibodies to recognize different phosphorylated forms of the $CB_1$ receptor.

Consistent with the anatomical localization studies, electrophysiological and neurotransmitter release studies have demonstrated a functional role of $CB_1$ receptors in the modulation of GABA and glutamate release and firing (Table 4; for review see Doherty and Dingledine, 2003). In rat hippocampal brain slices, endocannabinoid and $CB_1$ receptor agonist application decreased the amplitude of evoked inhibitory postsynaptic potentials of GABAergic neurons and this decrease was reversed by $CB_1$ receptor antagonist application (Hajos *et al*., 2000, 2001; Hoffman and Lupica, 2000; Irving *et al*., 2000; Hajos and Freund, 2002). Furthermore, it was determined that cannabinoid-mediated inhibition of inhibitory postsynaptic potentials was dependent on the firing rates of the presynaptic interneurons, as an increase in the frequency of action potentials reversed WIN55,212-2-mediated inhibition of GABA release from hippocampal slices (Foldy *et al*., 2006). Further studies demonstrated that GABA release from CCK-positive CA1 hippocampal slices is under tonic inhibitory control by endocannabinoids, whose release can, in turn, be regulated by G protein-coupled receptors on the postsynaptic neuron (Neu *et al*., 2007). The inhibitory effects of CB agonists on IPSCs were absent in $CB_1$ receptor knockout mice and were reversed with the coapplication of rimonabant in wild-type mice, confirming that cannabinoid-mediated modulation of GABA action potentials is $CB_1$ receptor-dependent (Hajos *et al*., 2000, 2001). Similarly, endocannabinoid-mediated suppression of GABA currents was shown both in slices from the rat amygdala (Katona *et al*., 2001) and mouse neocortex (Galarreta *et al*., 2004; Trettel *et al*., 2004; Bodor *et al*., 2005). Furthermore, the extracellular release of GABA from rat cerebral cortex (Ferraro *et al*., 2001) and human and rat hippocampal brain slices (Katona *et al*., 2000; D'Amico *et al*., 2004) was decreased with the application of endocannabinoids and CB receptor agonists. Evidence for the direct involvement of the endocannabinoid system in GABA-mediated antinociception is provided by the observation that microinjection of the $GABA_A$ receptor agonist muscimol into the central nucleus of the amygdala, but not the BLA, prevented the antinociceptive effects of intravenous administration of WIN55,212-2 in the rat tail-flick and formalin tests (Manning *et al*., 2003) (Table 5).

Cannabinoid receptor agonists have also been shown to reduce the amplitude of glutamatergic excitatory postsynaptic potentials in slices from mouse hippocampus (Misner and Sullivan, 1999), rat prefrontal cortex (Auclair *et al*., 2000), mouse lateral amygdala (Azad *et al*., 2003) as well as other cortical and non-cortical areas such as the ventral tegmental area (Melis *et al*., 2004; Riegel and Lupica, 2004), substantia nigra (Szabo *et al*., 2000; Freiman and Szabo, 2005; Marinelli *et al*., 2007), nucleus accumbens (Robbe *et al*., 2001) and striatum (Huang *et al*., 2001; Kofalvi *et al*., 2005) (Table 4). However, the role of the $CB_1$ receptor in cannabinoid-mediated release of glutamate is not yet clear, although the aforementioned studies would suggest that a reduction in firing would suppress glutamate release. In studies where rimonabant was administered, there was a reversal of these reductions in firing and presumably, glutamate release. The

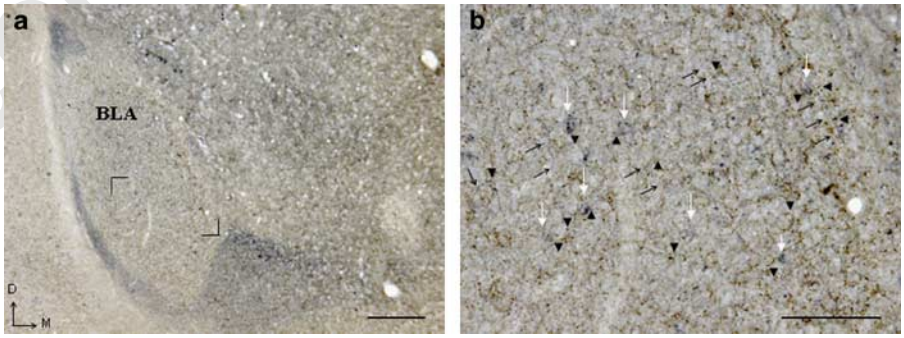


**Figure 2** $CB_1$ receptor immunoreactivity on GABAergic neurones in the rat basolateral amygdala (BLA). Dual immunolabelling for GAD67 (blue) and $CB_1$ receptor (brown) demonstrates that the $CB_1$ receptor (arrow head) is expressed in close proximity to GAD67-immunoreactive cells (white arrows) and fibres (black arrows). (**b**) High magnification of boxed area in (**a**). Scale bar (**a**) = 200 μm, (**b**) = 100 μm. D = dorsal; M = medial.





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involvement of $CB_1$ receptors in the regulation of glutamate release was complicated by the finding that in $CB_1^{-/-}$ knockout mice, WIN55,212-2 no longer reduced GABAergic transmission, but it still affected glutamate transmission (Hajos *et al*., 2001).

These findings, together with the limited evidence for $CB_1$ receptor localization on glutamatergic neurons in various regions of the brain, led to the hypothesis that the effects of cannabinoids on glutamate transmission were mediated by a novel cannabinoid receptor, distinct from $CB_1$, which has not yet been identified. However, a recent study using conditional mutant mice lacking $CB_1$ receptors in all the principal forebrain neurons, but not in GABAergic interneurons, reported that WIN 55,212-2 did not reduce excitatory responses in glutamatergic neurons in the forebrain areas as it did in wild-type mice and mice lacking $CB_1$ receptors exclusively in GABAergic neurons (Domenici *et al*., 2006). While these results do not preclude the existence of a novel $CB_1$-like receptor, they provide strong evidence for the control of glutamatergic neurotransmission by $CB_1$ receptors.

## Thalamus

The thalamus, with its numerous subnuclei, plays a critical role in the sensory-discriminatory dimension of pain. *In situ* hybridization studies have reported low $CB_1$ mRNA expression in the thalamus (Mailleux *et al*., 1992; Mailleux and Vanderhaeghen, 1992) and subsequent studies have shown that there is $CB_1$ receptor protein expression in certain nuclei within the thalamus (Cristino *et al*., 2006), for example the anterior dorsal thalamic nuclei, the habenular nucleus and the reticular thalamic nucleus (Tsou *et al*., 1998; Moldrich and Wenger, 2000). The precise identity of neurotransmitters involved in conveying nociceptive information to and from the thalamus remains unclear. A substantial proportion of thalamic neurons is GABAergic inhibitory interneurons (Ralston, 1991; Ulrich and Huguenard, 1997). Interestingly, the majority of neurons in the thalamus are output neurons and it is believed that they often target *N*-methyl-D-aspartate, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid and mGlu receptors in target areas, suggesting a role for glutamatergic neurons originating in the thalamus. Yet, there is no direct anatomical evidence for expression of cannabinoid receptors on these neurons in the thalamus.

## Hypothalamus

The hypothalamus is a brain area involved in the modulation of neuroendocrine function and is a component of the descending inhibitory pain pathway. It is also involved in coordinating the stress response and in mediating anxiety. Studies have shown that $CB_1$ receptors in the hypothalamus are colocalized with calretinin, a marker for glutamatergic nuclei, but not with GAD65 or CCK (Marsicano and Lutz, 1999). This suggests that cannabinoid receptor activation in this area may alter the activity of glutamatergic neurons. Although there has been no direct evidence for the localization of $CB_1$ receptors on GABAergic neurons in the hypothalamus, de Miguel *et al*. (1998) observed a parallel between hormone levels and GABA levels with cannabinoid receptor agonism and antagonism. It was also demonstrated that hypothalamic neuroendocrine cells can release endocannabinoids, which then suppresses glutamate release and postsynaptic spiking in the hypothalamus (Di *et al*., 2005). However, as with other regions of the brain including the midbrain and thalamus, there is still some uncertainty with respect to the precise identity and localization of $CB_1$ receptor-containing neurons.

## Periaqueductal gray and rostroventral medulla

The PAG is a longitudinally orientated tubular structure organized functionally into four columnar regions. Activation of the individual columns results in specific behavioural effects including confrontational defence, flight, quiescence, hypoactivity and analgesia. While GABAergic and glutamatergic neurons, as well as $CB_1$ receptors, are known to exist in the PAG, there exists only functional evidence to suggest the localization of $CB_1$ receptors on the respective neuron types. Studies on rat brain PAG slices demonstrated that the amplitude of GABAergic and glutamatergic postsynaptic currents was reduced by the cannabinoid receptor agonists WIN55,212-2, anandamide and methanandamide, effects blocked by rimonabant (Vaughan *et al*., 2000).

In the rat thermal plantar test, the microinjection of WIN55,212-2 into the dorsolateral PAG increased the latency of the nociceptive response (Palazzo *et al*., 2001). These antinociceptive effects were prevented by intra-PAG administrations of rimonabant, as well as MPEP, EGlu, MSOP and APV ($mGlu_5$, group II mGlu, group III mGlu and *N*-methyl-D-aspartate receptor antagonists respectively), but not CPCOOEt ($mGlu_1$ receptor antagonist) (Palazzo *et al*., 2001). In another study, intra-dorsolateral PAG microinjection of WIN55,212-2 resulted in a delayed tail-flick response in formalin-treated animals compared with controls (de Novellis *et al*., 2005). Intra-PAG WIN55,212-2 microinjection also prevented the formalin-induced increase in basal activity of ON cells and decreased the OFF-cell pause in the rat RVM. Interestingly, both the behavioural and electrophysiological responses were blocked by intra-PAG administrations of rimonabant, as well as MPEP but not CPCOOEt (de Novellis *et al*., 2005). Overall, these data suggest that endogenous glutamate acts via mGlu and *N*-methyl-D-aspartate receptors in the PAG to mediate cannabinoid-induced antinociception. However, the analgesic effect of intra-PAG CHPG ($mGlu_5$ receptor agonist) as seen in the plantar test, was blocked by MPEP but not rimonabant (Palazzo *et al*., 2001), suggesting that while glutamate may mediate the antinociceptive effects of cannabinoids, the reverse (i.e. endocannabinoid mediation of glutamate-induced analgesia) does not appear to be the case.

As discussed earlier, the RVM is a critical component of the descending inhibitory pain pathway. Evidence for localization of $CB_1$ receptors in the RVM has been provided by autoradiography (Herkenham *et al*., 1991) and *in situ* hybridization (Matsuda *et al*., 1993), although the expression





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of $CB_1$ receptors on GABAergic or glutamatergic neurons in the RVM is yet to be confirmed anatomically. Application of submicromolar concentrations of WIN55,212-2, anandamide and methanandamide reduced the amplitude of postsynaptic GABAergic currents in the rat brain slices, an effect which was blocked by rimonabant (Vaughan *et al.*, 1999). The antinociceptive effect of systemic $CB_1$ receptor activation was prevented by preinjection of muscimol into the RVM (Meng *et al.*, 1998), suggesting a role for RVM GABAergic receptors in the mediation of cannabinoid-induced antinociception.

## Spinal cord

The spinal cord is a projection target for neurons descending as part of the inhibitory pain pathway. An interaction between cannabinoid and mGlu receptors at the spinal level has been demonstrated with evidence that the antihyperalgesic effect of WIN55,212-2, administered intrathecally to rats with loose ligation of the sciatic nerve, was reversed by intrathecal administration of the $mGlu_5$ receptor antagonist, MPEP (Hama and Urban, 2004). In the rat formalin test, intrathecal pretreatment with rimonabant attenuated the antinociceptive effect of the $GABA_B$ receptor agonist baclofen administered intrathecally suggesting a role for endocannabinoids in mediating the antinociceptive effects of GABA agonists at the spinal level (Naderi *et al.*, 2005).

## Summary and general discussion

It is now clear from work in animal models that activation of supraspinal cannabinoid receptors or elevation of brain endocannabinoid levels is sufficient to induce antinociception. Moreover, anatomical and functional evidence points

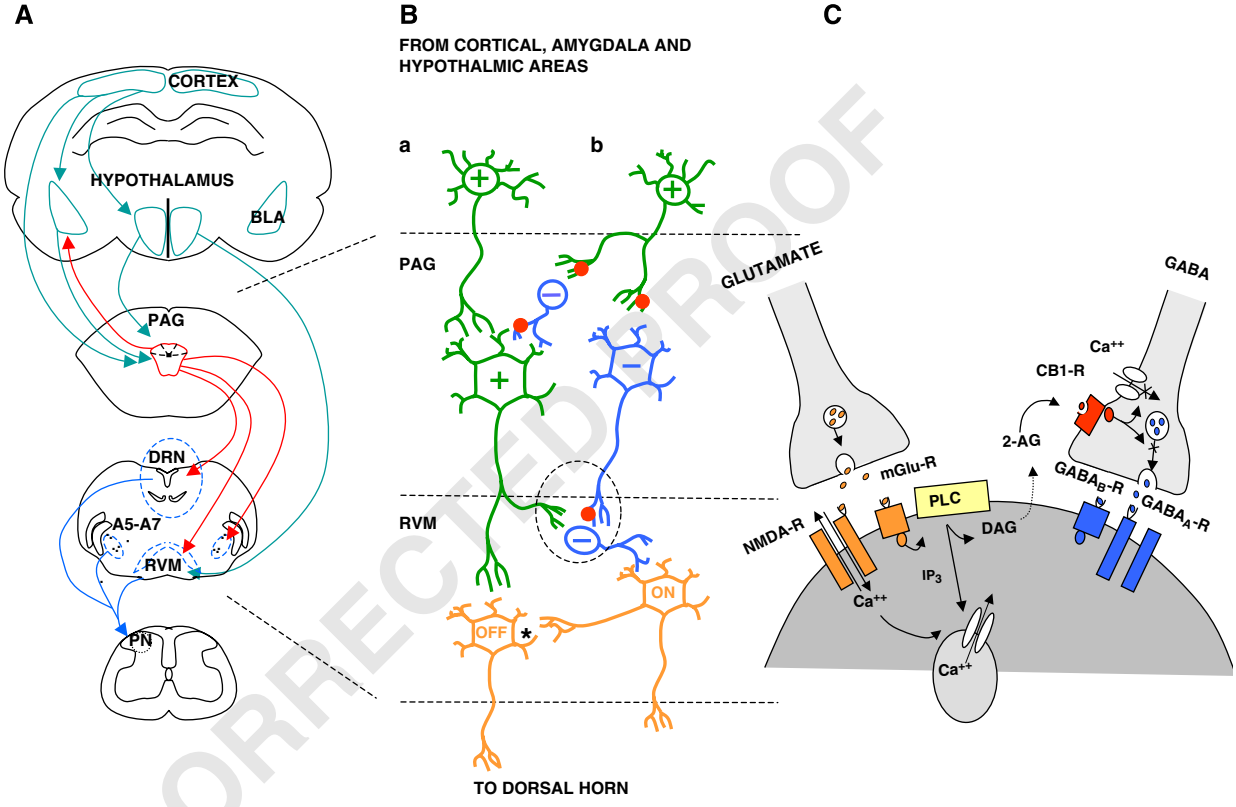


**Figure 3** Possible mechanism for endocannabinoid-mediated control of nociception. (**A**) Diagrammatical representation of some of the interactions between various brain regions of the descending pain pathway. The PAG receives critical input from various cortical as well as from the hypothalamus and amygdala. The net input of afferent neurons to the PAG determines the firing of the various PAG cell types. (**B**) Two possible outcomes of this net input. In resting conditions (no pain) the sum effect on the input of ON and OFF cells to the dorsal horn is neutral. Painful stimuli are proposed to selectively activate pathway (b), where these excitatory neurons from pathways upstream of the PAG project onto inhibitory projection neurons (possibly GABAergic) as well as inhibitory GABAergic interneurons. This activation of inhibitory interneurons in the PAG prevents firing of excitatory projection neurons (possibly glutamate) and negatively impacts on OFF cells in the RVM. Simultaneously, GABAergic projection neurons from the PAG synapse on GABAergic interneurons in the RVM and disinhibit their suppression of firing of ON neurons to result in nociception. The mediation of antinociception is achieved through pathway (a), when excitatory neurons from pathways upstream of the PAG activate excitatory neurons in the PAG. These excitatory neurons in turn activate the firing of OFF cells, and inhibit the firing of ON cells through GABAergic interneurons. It is also proposed that the activity of OFF cells negatively impacts on the firing of ON cells through an inhibitory mechanism and possibly impacts on OFF-cell duration (represented by an asterix). (**C**) The circled section of (**B**), and illustrates the possible mechanism behind cannabinoid-mediated antinociception. The activation of various receptor subtypes leads to an increase in intracellular calcium by various pathways. This increase in calcium concentration initiates endocannabinoid synthesis and release. The released endocannabinoids can then prevent the presynaptic release of neurotransmitters possibly by inhibiting calcium influx or vesicular release of neurotransmitters. See abbreviations list.





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towards an involvement of supraspinal GABA and glutamate in mediating the antinociceptive effects of cannabinoids (Figure 3). However, further studies are needed to fully elucidate the mechanisms involved and their potential clinical importance. An integrative approach employing powerful techniques such as *in vivo* electrophysiological recording from GABAergic and glutamatergic neurons and microdialysis to assess GABA and glutamate release in discrete brain regions may afford the best opportunity to study the mechanisms underlying cannabinoid-induced antinocieption in clinically relevant animal models of pain. In this respect, there is a paucity of these studies in models of inflammatory and neuropathic pain. Small animal functional and/or pharmacological magnetic resonance imaging also provide an opportunity to explore the effects of modulators of the endocannabinoid, glutamatergic and GABAergic systems, and their interactions, in discrete brain regions in the presence or absence of nociceptive tone.

Work to date has focused largely on the role of supraspinal $CB_1$ receptors. However, accumulating evidence for the presence of the $CB_2$ receptor in the brain (Van Sickle *et al*., 2005; Gong *et al*., 2006; Onaivi *et al*., 2006) now justifies the need for studies to address the gap in knowledge regarding the potential role of supraspinal $CB_2$ receptors in nociception and modulation of neurotransmission. Our understanding of the endocannabinoid system and its complexity is expanding rapidly. The implications of the recent discovery that many cannabinoids also target and mediate biological effects through an action at peroxisome proliferator-activated receptors for the pain field remain unknown (Burstein, 2005; LoVerme *et al*., 2005; Sun *et al*., 2006). Studies are required to examine the extent to which these nuclear receptors may mediate the antinociceptive effects of cannabinoids.

The goal of much of this work is the development of therapies relevant to the clinical setting. To this end, clinical trials, which combine pain outcome measures with functional magnetic resonance imaging and/or positron emission tomography, would be very informative with respect to identifying supraspinal sites of action of novel cannabinoid-based analgesics. Targeted, site-specific intracerebral delivery of cannabinoids or coadministration of cannabinoids with drugs modulating GABAergic and glutamatergic activity in pain pathways may one day be used as a therapeutic strategy to treat some types of intractable pain.

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## Conflict of interest

The authors state no conflict of interest.

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