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Cannabinoid receptors and pain

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Abstract

Mammalian tissues contain at least two types of cannabinoid receptor, CB_1 and CB_2, both coupled to G proteins. CB_1 receptors are expressed mainly by neurones of the central and peripheral nervous system whereas CB_2 receptors occur centrally and peripherally in certain non-neuronal tissues, particularly in immune cells. The existence of endogenous ligands for cannabinoid receptors has also been demonstrated. The discovery of this 'endocannabinoid system' has prompted the development of a range of novel cannabinoid receptor agonists and antagonists, including several that show marked selectivity for CB_1 or CB_2 receptors. It has also been paralleled by a renewed interest in cannabinoid-induced antinociception. This review summarizes current knowledge about the ability of cannabinoids to produce antinociception in animal models of acute pain as well as about the ability of these drugs to suppress signs of tonic pain induced in animals by nerve damage or by the injection of an inflammatory agent. Particular attention is paid to the types of pain against which cannabinoids may be effective, the distribution pattern of cannabinoid receptors in central and peripheral pain pathways and the part that these receptors play in cannabinoid-induced antinociception. The possibility that antinociception can be mediated by cannabinoid receptors other than CB_1 and CB_2 receptors, for example CB_2-like receptors, is also discussed as is the evidence firstly that one endogenous cannabinoid, anandamide, produces antinociception through mechanisms that differ from those of other types of cannabinoid, for example by acting on vanilloid receptors, and secondly that the endocannabinoid system has physiological and/or pathophysiological roles in the modulation of pain.

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Nomenclature

AM381	stearylsulphonyl fluoride
AM404	*N*-(4-hydroxyphenyl)arachidonylamide
cyclic AMP	cyclic 3′,5′-adenosine monophosphate
CGRP	calcitonin gene-related peptide
COX	cyclo-oxygenase
DAMGO	[D-Ala^2, N-Me-Phe^4, Gly-ol^5]-enkephalin
DMH	dimethylheptyl
DPDPE	[D-Pen^2, D-Pen^5]-enkephalin
EC_{50}	concentration eliciting a half-maximal response
ED_{50}	dose eliciting a half-maximal response
GABA	γ-aminobutyric acid
HEK	human embryonic kidney
5-HT	5-Hydroxytryptamine (serotonin)
HU-210	11-Hydroxy-dimethylheptyl-Δ^8-tetrahydrocannabinol
i.a.	intra-arterial
i.c.	intracerebral
i.c.v.	intracerebroventricular
i.pl.	intraplantar (injection into plantar surface of paw)
i.t.	intrathecal
K_B	equilibrium dissociation constant of a competitive reversible antagonist
K_i	dissociation constant of an unlabelled ligand as determined in a radioligand competitive binding assay
NMDA	*N*-methyl-D-aspartate
THC	tetrahydrocannabinol

1. Introduction

1.1. The endocannabinoid system

Two types of cannabinoid receptor have so far been identified, CB_1, cloned in 1990, and CB_2, cloned in 1993 (Pertwee, 1997, 1998). Both receptor types are coupled through $G_{i/o}$ proteins, negatively to adenylate cyclase and positively to mitogen-activated protein kinase. In addition, CB_1 receptors are coupled to ion channels through $G_{i/o}$ proteins, positively to A-type and inwardly rectifying potassium channels and negatively to N-type and P/Q-type calcium channels and to D-type potassium channels (Mu et al., 1999; Pertwee, 1997,

1998). CB_1 coupling to A-type and D-type potassium channels is thought to be through adenylate cyclase (Mu et al., 1999). CB_1 receptors may also mobilize arachidonic acid and close 5-HT_3 receptor ion channels (Pertwee, 1997) and, under certain conditions, activate adenylate cyclase through G_s proteins (Calandra et al., 1999; Glass and Felder, 1997). In addition, there are reports that CB_1 receptors are negatively coupled to voltage-gated L-type calcium channels both in cat cerebral arterial smooth muscle cells (Gebremedhin et al., 1999) and in retinal bipolar cell axon terminals of larval tiger salamanders (Straiker et al., 1999). Ho et al. (1999) have obtained evidence that inwardly rectifying potassium channels can serve as a signalling mechanism for CB_2 as well as CB_1 receptors, at least in *Xenopus* oocytes that have been transfected with such channels together with CB_1 or CB_2 receptors. In other experiments, in which *Xenopus* oocytes were co-transfected with cannabinoid receptors and certain G protein subunits, this research group also obtained evidence that CB_1 but not CB_2 receptors can activate phospholipase C through G protein containing $G_{\alpha 14}$, $G_{\alpha 15}$ or $G_{\alpha 16}$ subunits. Other recent findings are that CB_1 receptors on hippocampal CA1 pyramidal neurones are negatively coupled to M-type potassium channels (Schweitzer, 2000) and that CB_1 receptors on cultured cerebellar granule neurones can operate through a phospholipase C-sensitive mechanism to enhance NMDA-elicited calcium release from inositol 1,4,5-triphosphate-gated intracellular stores (Netzeband et al., 1999). There is also a recent report that one consequence of the reduction in calcium influx that arises from CB_1-receptor mediated inhibition of N-type or P/Q-type calcium channels, is an attenuation of depolarization-induced activation of neuronal nitric oxide synthase (Hillard et al., 1999).

CB_1 receptors are found in particularly high concentrations within the central nervous system. However, they are also present on some peripheral neurones as well as in certain nonneuronal tissues (Pertwee, 1997, 1998 and section 5.3 of this review). Although the concentration of CB_1 receptors is considerably less in peripheral tissues than in the central nervous system, this does not imply that peripheral CB_1 receptors are unimportant. This is because some peripheral tissues may contain high concentrations of CB_1 receptors, localized in discrete regions such as nerve terminals that form only a small part of the total tissue mass. Within the central nervous system, the distribution pattern of CB_1 receptors is heterogeneous and can account for several prominent pharmacological properties of CB_1 receptor agonists, for example their ability to impair cognition and memory and to alter the control of motor function. Thus the cerebral cortex, hippocampus, caudate-putamen, substantia nigra pars reticulata, globus pallidus, entopeduncular nucleus and cerebellum all contain significant numbers of CB_1 receptors (Pertwee, 1997, 1998). As discussed in section 5 of this review, CB_1 receptors are also found in brain areas that process or modulate nociceptive information.

Some CB_1 receptors are located at central and peripheral nerve terminals (Ong and Mackie, 1999a; Pertwee, 1997) where they probably modulate the release of both excitatory and inhibitory neurotransmitters when activated (Table 1; Kim and Thayer, 2000). It would seem then that presynaptic CB_1 receptors mediate mixed inhibitory-disinhibitory effects on neurotransmission through suppression of transmitter release whilst postsynaptic CB_1 receptors, at least on hippocampal CA1 pyramidal neurones, are likely to have an excitatory effect on neurotransmission through their ability to close M-type potassium channels (Schweitzer, 2000).

CB_2 receptors occur mainly in immune cells where they may mediate an immunosuppressant effect (Pertwee, 1997, 1998). Although CB_2 mRNA has not been detected on neuronal tissue from human or rat brain (Munro et al., 1993; Galiègue et al., 1995), there is evidence for its presence in rat brain microglia (Kearn and Hillard, 1999). There is also one report of the presence of CB_2 mRNA together with CB_1 mRNA in mouse cerebellar tissue (Skaper et al., 1996). Evidence that CB_2 or CB_2-like receptors contribute to antinociceptive effects of some cannabinoids is discussed in section 4 as is the possibility that yet other types of cannabinoid receptor may exist and that these too can mediate antinociception.

The discovery of cannabinoid receptors was followed in 1992 and 1995 by the demonstration of the existence of endogenous cannabinoid receptor agonists (Pertwee, 1997, 1998; Di Marzo et al., 1998b). The most important of these are arachidonylethanolamide (anandamide) and 2-arachidonyl glycerol (Fig. 1) and there is evidence that both these compounds can serve as neuromodulators or neurotransmitters. This comes from demonstrations that they are synthesized by neurones, that they can undergo depolarization-induced release from neurones and that once released they are rapidly removed from the extracellular space (Di Marzo et al., 1998b). For anandamide and 2-arachidonyl glycerol, such removal seems to depend on a carrier-mediated, saturable uptake process present in neurones and astrocytes (anandamide transporter; Di Marzo et al., 1998b; Piomelli et al., 1999). Once within the cell, anandamide is presumably hydrolysed to arachidonic acid and ethanolamine by fatty acid amide hydrolase (Di Marzo et al., 1998b). This is a microsomal enzyme, found both in neurones and in some non-neuronal tissues, that seems to serve as a general fatty acid amide hydrolase.

Table 1
In vitro inhibition of transmitter release by cannabinoid receptor agonists through CB_1 receptors in mammalian tissues[a]

Tissue preparation	Transmitter-releasing stimulus	Transmitter	References
Rat hippocampal slices	ES	Ach	[c]
Guinea-pig intestinal tissue (MPLM)	ES	Ach	[d]
Guinea-pig cerebrocortical slices	ES	NA	Schlicker et al. (1997)
Human and guinea-pig hippocampal slices	ES	NA	Schlicker et al. (1997)
Guinea-pig hippocampal slices	NMDA or kainate	NA	Kathmann et al. (1999a)
Guinea-pig hypothalamic slices	ES	NA	Schlicker et al. (1997)
Guinea-pig cerebellar slices	ES	NA	Schlicker et al. (1997)
Guinea-pig retinal discs	ES	NA	Schlicker et al. (1996)
Human atrial appendage segments	ES	NA	Molderings et al. (1999)
Rat atria	ES	NA	Ishac et al. (1996)
Rat vas deferens	ES	NA	Ishac et al. (1996)
Rat striatal slices	NMDA	DA	Kathmann et al. (1999a)
Rat striatal slices	ES	DA	Cadogan et al. (1997)
Guinea-pig retinal discs	ES	DA	Schlicker et al. (1996)
Mouse cerebrocortical slices	ES or Ca^{2+}	5-HT	Nakazi et al. (2000)
Mouse hypothalamic slices	ES	5-HT	Kathmann et al. (1999b)
Rat hippocampal slices	ES	GABA	Katona et al. (1999)
Rat striatal slices	ES	GABA[b]	Szabo et al. (1998)
Rat midbrain slices (SNR)	ES or none	GABA[b]	[e]
Rat brain slices (PAG)	ES	GABA[b]	Vaughan et al. (2000)
Rat brain slices (RVM)	ES	GABA[b]	Vaughan et al. (1999)
Rat primary cultured hippocampal cells	Low $[Mg^{2+}]_o$	Glu[b]	[f]
Rat brain slices (PAG)	ES	Glu[b]	Vaughan et al. (2000)
Rat cerebellar slices	ES	Glu[b]	Lévénès et al. (1998)
Rat primary cultured hippocampal cells	ES	Glu[b]	Sullivan (1999)
Rat primary cultured cerebellar granule cells	High KCl	D-Asp	Breivogel et al. (1999)

[a] ES, Electrical stimulation; NMDA, *N*-methyl-D-asparate; PAG, Periaqueductal grey; RVM, Rostral ventromedial medulla; SNR, Substantia nigra pars reticulata; MPLM, Myenteric plexus-longitudinal muscle preparation; $[Mg^{2+}]_o$, Extracellular magnesium concentration; Ach, Acetylcholine; NA, Noradrenaline; DA, Dopamine; 5-HT, 5-hydroxytryptamine; GABA, γ-aminobutyric acid; D-Asp, D-asparate.
[b] Indirect electrophysiological evidence for decreased transmitter release.
[c] Gifford and Ashby, 1996; Gifford et al., 1997a,b, 1999.
[d] Coutts and Pertwee, 1997; Pertwee et al., 1996.
[e] Chan and Yung, 1998; Chan et al., 1998.
[f] Shen et al., 1996; Shen and Thayer, 1998a,b, 1999.

The same enzyme can also catalyse the hydrolysis of 2-arachidonyl glycerol (Di Marzo et al., 1998b). Cannabinoid receptors and their endogenous ligands together constitute what is now often referred to as the 'endocannabinoid system'.

1.2. Cannabinoid receptor ligands

Much of the research on the antinociceptive properties of cannabinoids has been conducted with prototypic compounds of the four main chemical classes of cannabinoid receptor agonist (Pertwee, 1999a). These are the 'classical' cannabinoid, Δ^9-THC, the 'nonclassical' cannabinoid, CP55940, the aminoalkylindole, WIN55212, and the 'eicosanoid' cannabinoid, anandamide (Figs. 1 and 2). Δ^9-THC, CP55940, WIN55212 and many of their analogues contain chiral centres and exhibit marked stereoselectivity in both binding assays and functional tests (Pertwee, 1997, 1999a). For classical and nonclassical cannabinoids, those with the same absolute stereochemistry as (−)-Δ^9-THC at 6a and 10a (6a*R*, 10a*R*) have the greater activity [the (−)-enantiomers]. Thus, for example, the nonclassical cannabinoid CP55940 is a (−)-enantiomer and has higher affinity for CB_1 or CB_2 receptors than its (+)-enan-

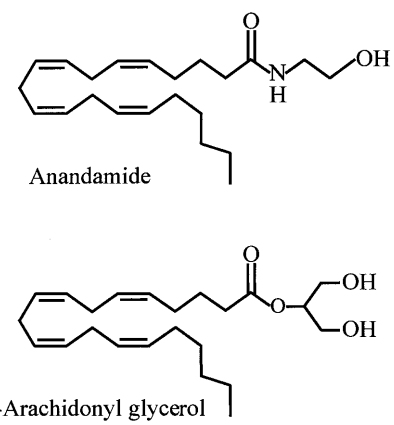


Fig. 1. Structures of two endogenous cannabinoid receptor ligands, arachidonylethanolamide (anandamide) and 2-arachidonyl glycerol.

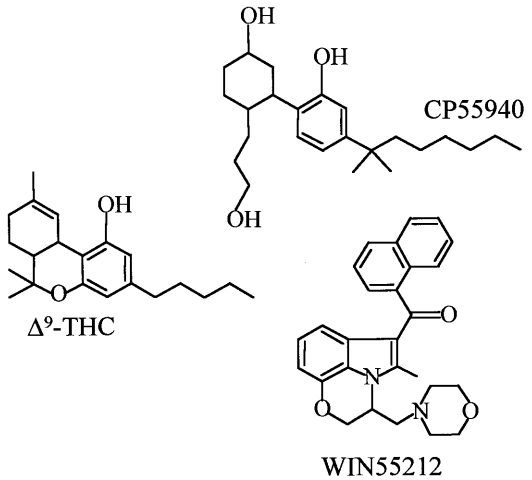


Fig. 2. Structures of three cannabinoid receptor agonists, the 'classical' cannabinoid, Δ^9-tetrahydrocannabinol (Δ^9-THC), the 'non-classical' cannabinoid, CP55940 and the aminoalkylindole, WIN55212.

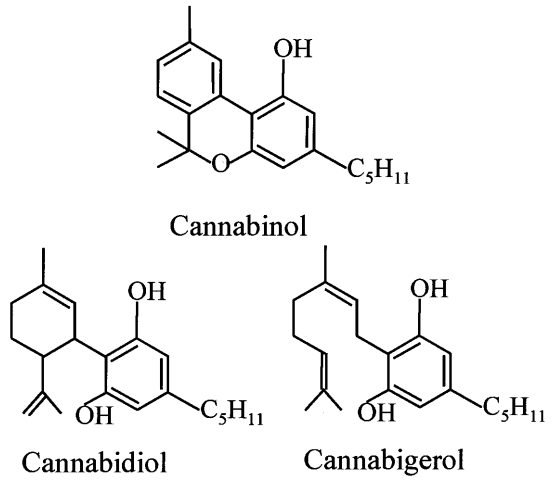


Fig. 4. Structures of cannabinol, cannabidiol and cannabigerol.

tiomer, CP56667. However, for WIN55212, the *R* -(+) enantiomer is the more active. Although anandamide itself does not contain any chiral centres, some of its synthetic analogues do. (−)-Δ^9-THC and CP55940 exhibit little difference in their affinities for CB_1 and CB_2 receptors whereas anandamide exhibits marginal selectivity for CB_1 receptors, and (+)-WIN55212 modest selectivity for CB_2 receptors. Unlike CP55940 and (+)-WIN55212, both (−)-Δ^9-THC and anandamide seem to have significantly less efficacy at CB_2 than at CB_1 receptors. The antinociceptive properties of a number of different analogues of THC, CP55940, WIN55212 and anandamide have been investigated and the structures of some of these are shown in Figs. 3–7. The ability of these compounds to interact with CB_1 and CB_2 receptors has been detailed elsewhere (Pertwee, 1999a). It is noteworthy, however, that cannabinol behaves as a partial agonist at CB_1 receptors with even less CB_1 affinity than (−)-Δ^9-THC, whilst cannabidiol and cannabigerol lack significant agonist activity at these receptors. Methanandamide (Fig. 6) differs from anandamide in being less susceptible to enzymic hydrolysis, in showing greater selectivity and affinity for CB_1 receptors and in possessing a chiral centre, its (*R*)-isomer possessing nine-fold higher CB_1 receptor affinity than its (*S*)-isomer. Two other cannabinoid receptor ligands often mentioned in this review are SR141716A, a potent and selective CB_1 receptor antagonist, and SR144528, a potent and selective CB_2 receptor antagonist (Fig. 8) (Pertwee, 1999a).

2. Antinociceptive activity of cannabinoid receptor agonists

This has been investigated in a wide range of animal pain models that fall essentially into the following categories: acute (phasic) pain models in which a short

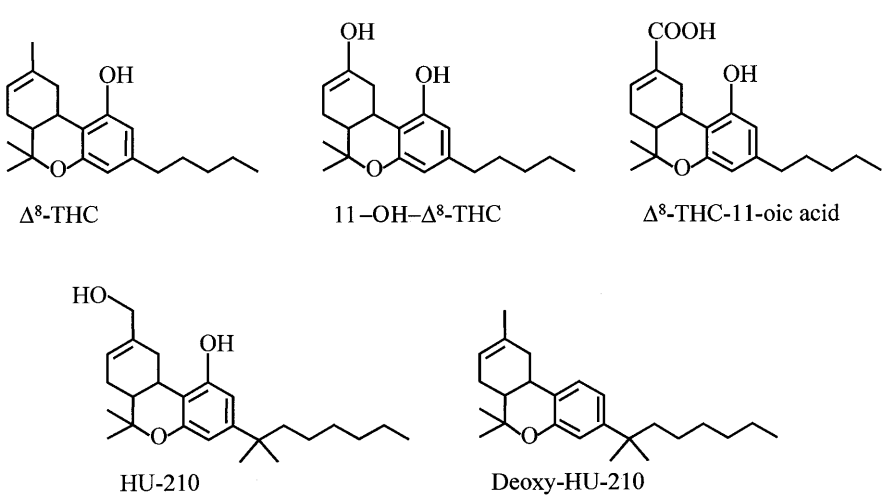


Fig. 3. Structures of Δ^8-tetrahydrocannabinol (Δ^8-THC) and some of its analogues.

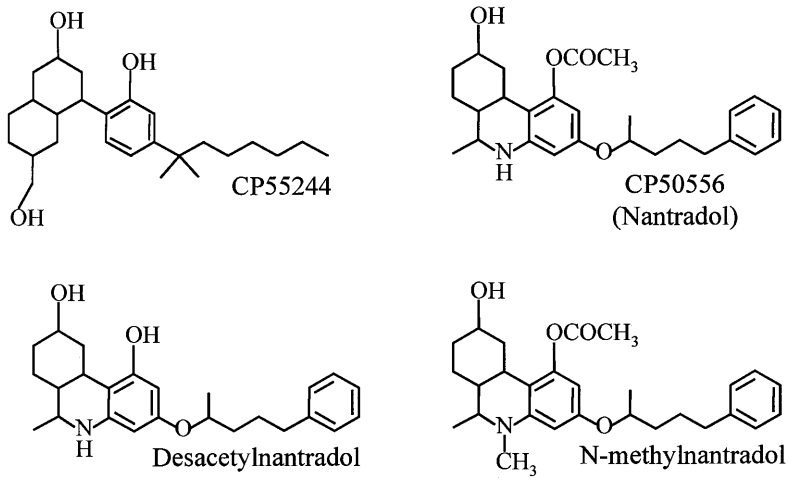


Fig. 5. Structures of some 'non-classical' cannabinoids.

lasting noxious stimulus is abruptly applied and tonic/chronic pain models in which longer lasting signs of pain are induced either by nerve injury or by tissue injury elicited by injection of an inflammatory agent (Tables 2 and 3). Among the most widely used acute pain models have been those in which noxious heat stimuli are applied to rodent paws, as in the hot plate test, or to rodent tails, either as radiant heat or by placing the tails of the animals into warm water. Another commonly used test has been one in which rodent paws are compressed by noxious mechanical pressure. In all these tests various kinds of motor response serve as indicators of nociception (tail flicking/withdrawing, paw licking, jumping, escape behaviour, etc.) Commonly used tissue injury models have been those in which a noxious agent is injected subcutaneously, usually into the plantar surface of a rodent hind paw and sometimes into monkey tails. The noxious agents so injected have been yeast, formalin, carrageenan, capsaicin or Freund's adjuvant. All of these treatments produce inflammation together with signs of local hyperalgesia that can be detected by monitoring limping, paw licking and paw lifting behaviour or increased sensitivity of the injected paw or tail to thermal or mechanical stimuli. Injection of formalin into rodent hind paws induces a biphasic response: an early-phase of nociceptive behaviour that begins immediately, peaks within 5 min and then fades rapidly and a late-phase of longer-lasting nociceptive behaviour that is observed 15–60 min after formalin injection. Another tissue injury model sometimes used is the mouse abdominal stretch test in which a noxious agent, usually phenylbenzoquinone, is injected into the peritoneal cavity to induce a series of distinct abdominal stretching or writhing movements that provide a quantitative measure of nociception. To date, the only nerve injury model to have been used in cannabinoid experiments is one in which painful unilateral mononeuropathy is induced in rats. This is achieved by placing four loose ligatures around the trunk of the right sciatic nerve to induce chronic constriction injury of the nerve and so render the hind paw on the lesioned limb hypersensitive to both thermal and mechanical stimuli. In all these models afferent nociceptive input to the spinal cord is thought to be through C-fibres (small diameter, unmyelinated, slow conducting) and Aδ-fibres (medium diameter, myelinated, faster conducting). Where mechanical allodynia (touch-evoked pain) is induced, for example after nerve injury, afferent nociceptive input may also be through Aβ-fibres (large diameter, myelinated, fast-conducting) that normally only transfer non-nociceptive information to the spinal cord.

2.1. Antinociception in acute pain models

Cannabinoid receptor agonists have long been known to exhibit antinociceptive activity in animal models of acute pain. The most widely studied of these cannabinoids has been Δ^9-THC and Table 2 shows the wide range of animal models of acute pain in which this agent exhibits antinociceptive activity when adminis-

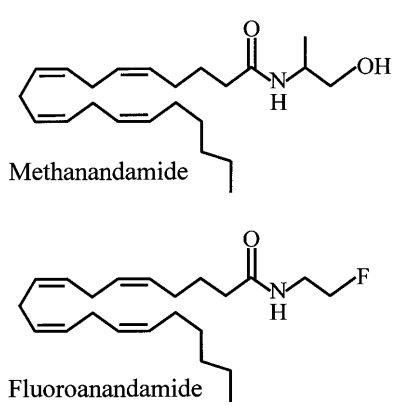
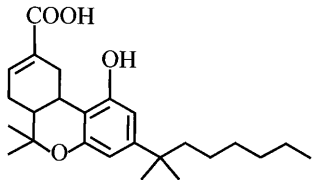


Fig. 6. Structures of two synthetic analogues of anandamide.



1',1'-dimethylheptyl-Δ^8-THC-11-oic acid

Fig. 7. Structure of 1′,1′-dimethylheptyl-Δ^8-THC-11-oic acid.

tered orally, systemically or directly into brain or spinal cord. Other cannabinoid receptor agonists that show activity in rats or mice in acute pain models include:

1. Analogues of THC administered peripherally (Bicher and Mechoulam, 1968; Weissman et al., 1982; Melvin et al., 1984; Koe et al., 1985; Burstein et al., 1988; Little et al., 1989; Shook and Burks, 1989; Compton et al., 1990; Doyle et al., 1990; Reggio et al., 1990, 1991; Burstein et al., 1992; Martin et al., 1993a; Yan et al., 1994; Martin et al., 1995a,b; Huffman et al., 1996; Huffman and Lainton, 1996; Showalter et al., 1996; Wiley et al., 1996; Reggio et al., 1997; Singer et al., 1997; Burstein et al., 1998; Singer et al., 1998 Martin et al., 1999a; Seltzman, 1999; Zimmer et al., 1999; Pertwee et al., 2000), intrathecally (Welch and Stevens, 1992; Welch, 1994; Welch et al., 1995b, 1998; Pertwee et al., 2000), intracerebroventricularly (Welch, 1994; Welch et al., 1995b, 1998; Pertwee et al., 2000) or directly into rat rostral ventromedial medulla (Martin et al., 1998);
2. CP55940 and related compounds administered peripherally (Weissman et al., 1982; Melvin et al., 1984; Koe et al., 1985; Little et al., 1988; Lichtman and Martin, 1991b; Compton et al., 1992b; Welch, 1993; Martin et al., 1995b; Showalter et al., 1996; Seltzman, 1999), intrathecally (Yaksh, 1981; Lichtman and Martin, 1991b; Welch and Stevens, 1992; Welch, 1993, 1994; Welch et al., 1995b; Pugh et al., 1997; Welch et al., 1998; Mason et al., 1999; Welch and Eads, 1999) intracerebroventricularly (Martin et al., 1993b; Welch, 1994; Welch et al., 1995b; Lichtman et al., 1996; Lichtman and Martin, 1997; Welch et al., 1998) or directly into rat posterior ventrolateral periaqueductal grey (Lichtman et al., 1996);
3. (+)-WIN55212 and related compounds administered peripherally (Haubrich et al., 1990; Compton et al., 1992a; Rinaldi-Carmona et al., 1994; Martin et al., 1995b; Huffman and Lainton, 1996; Showalter et al., 1996; Dutta et al., 1997; Meng et al., 1998; Wiley et al., 1998; Huffman, 1999), intrathecally (Hohmann et al., 1998) intracerebroventricularly (Martin et al., 1993b; Raffa et al., 1999) or directly into rat rostral ventromedial medulla (Martin et al., 1998) or into certain other brain areas (Martin et al., 1999c; see section 5.1);
4. Anandamide administered peripherally (Fride and Mechoulam, 1993; Smith et al., 1994a; Adams et al., 1995; Barg et al., 1995; Mechoulam et al., 1995; Showalter et al., 1996; Ryan et al., 1997; Adams et al., 1998; Smith et al., 1998b; Martin et al., 1999b), intrathecally (Smith et al., 1994a; Welch et al., 1995a, 1998; Mason et al., 1999) or intracerebroventricularly (Raffa et al., 1999);
5. Analogues of anandamide administered peripherally (Abadji et al., 1994; Adams et al., 1995, 1998; Barg et al., 1995; Martin et al., 1999b; Ryan et al., 1997; Seltzman et al., 1997; Showalter et al., 1996) or intrathecally (Welch et al., 1995a); and
6. 2-arachidonyl glycerol administered peripherally (Mechoulam et al., 1995).

One analogue of THC with antinociceptive activity that merits special mention is 3-(5′-cyano-1′,1′-dimethylpentyl)-1-(4-*N*-morpholinobutyryloxy)-Δ^8-tetrahydrocannabinol hydrochloride (Pertwee et al., 2000). This compound (O-1057) differs from Δ^9-THC as well as from most other cannabinoids with antinociceptive activity in being readily soluble in water. O-1057 shows markedly greater antinociceptive potency than Δ^9-THC in the mouse tail flick test when administered orally or intravenously and is an agonist at both CB_1 and CB_2 receptors (Fig. 9). By removing the need for a solubilizing agent, the availability of a water-soluble cannabinoid will facilitate cannabinoid delivery not only in the laboratory but also in the clinic, particularly where administration to patients is to be by injection or aerosol inhalation.

When administered peripherally, the majority of cannabinoids so far investigated in animal models of acute pain can act through CB_1 receptors to depress motor activity and induce hypothermia. This they do at doses similar to those at which they show activity in acute pain tests (Kosersky et al., 1973; Martin et al., 1995b; Weissman et al., 1982). Even so, there is good evidence that the production of antinociception by CB_1 receptor

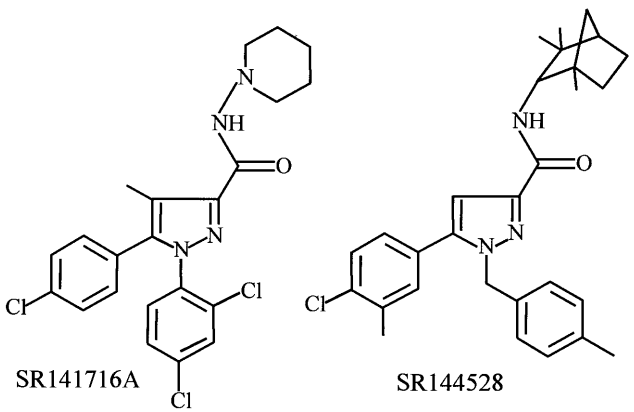


Fig. 8. Structures of the CB_1-selective antagonist, SR141716A, and the CB_2-selective antagonist, SR144528.

Table 2
Animal models of acute pain in which Δ^9-THC shows antinociceptive activity

Noxious stimulus	Response	Species	Effective routes	Reference
Clamp or clip on tail	Biting at clip, etc.	Mouse	p.o.	[a]
		Rat	s.c.	[b]
Clamp on tail	Minimum alveolar concentration (MAC) of cyclopropane required to prevent motor response	Rat	i.p.	[c]
Clamp on tail	Minimum alveolar concentration (MAC) of halothane required to prevent motor response	Dog	i.v.	[d]
Thermal to tail	Tail flick	Mouse	i.v., i.p., s.c., p.o.	[e]
			i.t.	[f]
			i.c.v.	[g]
		Rat	i.v., i.p., i.t., i.c.v.	[h]
Thermal to feet (hot plate)	Paw lick, jumping or escape	Mouse	i.v., i.p., s.c., p.o.	[i]
		Rat	i.p., s.c., i.v., i.c.v.	[j]
Thermal to an area of back	Skin twitch reflex	Dog (chronic spinal)	i.v.	[k]
Pressure to toe	Flexor reflex	Dog (chronic spinal)	i.v.	[k]
Pressure to hind paw	Motor response	Rat	i.p.	[l]
Electrical to feet	Motor response	Rat	s.c.	[m]
Electrical to feet or brain sites	Vocalization or motor responses	Rat	i.p.	[n]
Electrical to feet	Lever pressing	Monkey	i.p.	[o]
Electrical to sciatic nerve	Vocalization or motor responses	Rabbit	Not specified	[p]
Electrical to tooth (dentine)	Vocalization or motor responses	Dog	i.v.	[q]

[a] Sofia et al. (1973).
[b] Melvin et al., 1984; Weissman et al., 1982.
[c] Vitez et al., 1973.
[d] Stoelting et al., 1973.
[e] Bhargava and Matwyshyn, 1980; Bloom and Dewey, 1978; Buxbaum, 1972; Cichewicz et al., 1999; Compton et al., 1996, 1992b; Davis and Hatoum, 1983; Dewey et al., 1970, 1972; Koe et al., 1985; Lichtman et al., 1993; Little et al., 1988; Martin, 1985a,b; Martin et al., 1993a, 1999a; Melvin et al., 1984; Pertwee et al., 1991; Reche et al., 1996a,b; Smith et al., 1994a; Smith and Martin, 1992; Smith et al., 1994b; Sofia and Barry, 1983; Spaulding et al., 1972; Thorat and Bhargava, 1994a,b; Weissman et al., 1982; Welch, 1993.
[f] Lichtman et al., 1992; Pugh et al., 1995, 1997, 1996; Rowen et al., 1998; Smith et al., 1994a; Smith and Martin, 1992; Smith et al., 1994b; Welch, 1994; Welch et al., 1995a, 1998; Welch and Stevens, 1992; Welch et al., 1995b.
[g] Raffa et al., 1999; Welch, 1994; Welch et al., 1998, 1995b.
[h] Bhattacharya et al., 1989; Buxbaum, 1972; Carta et al., 1999; Gallager et al., 1972; Hine, 1985; Lichtman et al., 1996; Lichtman and Martin, 1991a,b, 1997; Mason et al., 1999; Novelli et al., 1983; Sofia and Barry, 1983.
[i] Burstein et al., 1988; Buxbaum, 1972; Dewey et al., 1970, 1972; Doyle et al., 1990; Shook and Burks, 1989; Kaymakcalan and Deneau, 1972; Martin, 1985a; Melvin et al., 1984; Reche et al., 1996a,b; Segelman et al., 1974; Sofia and Barry, 1983; Sofia et al., 1973, 1975; Spaulding et al., 1972; Takahashi and Karniol, 1975; Wilson and May, 1975.
[j] Bhattacharya et al., 1989; Buxbaum, 1972; Carta et al., 1999; Gallager et al., 1972; Kaymakcalan and Deneau, 1972; Novelli et al., 1983; Tulunay et al., 1981.
[k] Gilbert, 1981.
[l] Smith et al., 1998b.
[m] Melvin et al., 1984; Weissman et al., 1982.
[n] Parker and Dubas, 1973.
[o] Scheckel et al., 1968.
[p] Bicher and Mechoulam, 1968.
[q] Kaymakcalan et al., 1974.

Table 3
Animal models of inflammatory (tissue-injury) or neuropathic (nerve-injury) tonic pain in which cannabinoid receptor agonists show antinociceptive activity[a]

Noxious stimulus	Nociceptive response	Species	Antinociceptive agonist	Route of administration	Reference
Phenylbenzoquinone into peritoneum	Abdominal stretching	Mouse	Δ^9-THC	i.v., s.c., p.o.	[b]
Phenylbenzoquinone into peritoneum	Abdominal stretching	Mouse	Other cannabinoids[b]	i.v., s.c., p.o.	[c]
Phenylbenzoquinone into peritoneum	Abdominal stretching	Mouse	Δ^9-THC, anandamide fluoroanandamide	i.t.	Welch et al., 1995a
Phenylbenzoquinone into peritoneum	Abdominal stretching (rat) or vocalization (cat)	Rat	Δ^9-THC	s.c.	Kaymakcalan and Deneau, 1972
		Cat	Δ^9-THC	s.c.	Kaymakcalan and Deneau, 1972
Acetic or formic acid into peritoneum	Abdominal stretching	Mouse	Δ^8 and Δ^9-THC, 11-OH-Δ^8- and 11-OH-Δ^9-THC, CBN	i.v., s.c., p.o.[h]	[d]
Other noxious agent into peritoneum[i]	Abdominal stretching	Mouse	Δ^9-THC, CBN, 11-OH-Δ^9-THC	p.o.	[e]
Yeast into hind paw (i.pl.) 1 h before agonist	Reaction to paw pressure (threshold pressure)	Rat	Δ^9-THC (at 0.5–64 mg/kg)	p.o.	[f]
Yeast into hind paw (i.pl.) 1 h before agonist	Reaction to paw pressure (threshold pressure)	Rat	Δ^9-THC (only at 100 mg/kg)	p.o.	Kosersky et al., 1973
Freund's adjuvant into hind paw (i.pl.) 18 days before agonist	Reaction to paw pressure (threshold pressure)	Rat	Δ^9-THC, AEA	i.p.	Smith et al., 1998b
Freund's adjuvant into hind paw (i.pl.) 24 h before agonist	Paw withdrawal from von Frey filament	Rat	WIN	i.t.	Martin et al., 1999d
Formalin into hind paw (i.pl.) 4 h after agonist	Early- and late-phase limping, paw lifting, etc. using a scoring system	Rat	Δ^9-THC	p.o.	Moss and Johnson, 1980
Formalin into hind paw (i.pl.) just after agonist	Late-phase nociception using a scoring system	Rat	AEA, PEA (no effect on early-phase)	i.p.	Jaggar et al., 1998a
Formalin into hind paw (i.pl.) 10 min after agonist	Early- and late-phase paw lifting and licking using a scoring system	Rat	WIN, CP55940	i.p.	[g]
Formalin into hind paw (i.pl.) together with agonist	Early- and late-phase paw licking duration	Mouse	WIN, HU-210, PEA, MethAEA	i.pl.	Calignano et al., 1998
Formalin into hind paw (i.pl.) together with agonist	Early-phase paw licking duration	Mouse	AEA (no effect on late-phase)	i.pl.	Calignano et al., 1998
Formalin into hind paw (i.pl.) together with agonist	Paw licking duration	Mouse	AEA	i.v.	Calignano et al., 1998
Formalin into hind paw (i.pl.) 5 min after agonist	Early- and late-phase paw lifting and licking using a scoring system	Mouse	Dimethylheptyl-Δ^8-THC-11-oic acid	i.v.	Burstein et al., 1998
Formalin into hind paw (i.pl.) 90 min after agonist	Number of late-phase paw licks	Mouse	HU-308 (no effect on early-phase)	i.p.	Hanus et al., 1999
Capsaicin into hind paw (i.pl.) 5 min after agonist	Paw withdrawal from radiant heat. Paw withdrawal from von Frey filament	Rat	WIN	i.v.	Li et al., 1999
Capsaicin (s.c.) into terminal 1–4 cm of tail together with agonist	Tail withdrawal from warm-water stimulus (46°C)	Rhesus monkey	Δ^9-THC	s.c. (to tail)	Ko and Woods, 1999
Carrageenan into hind paw (i.pl.) just before agonist	Jumping or paw licking when on hot plate (54–55°C)	Mouse	AEA	i.t.	Richardson et al., 1998b
Carrageenan into hind paw (i.pl.) just before or ~2 h before agonist	Paw withdrawal from radiant heat	Rat	AEA	i.pl.	Richardson et al., 1998c
Turpentine into bladder after agonist	Signs of bladder wall inflammation	Rat	AEA (PEA inactive)	i.a.	Jaggar et al., 1998b

Table 3 (*Continued*)

Noxious stimulus	Nociceptive response	Species	Antinociceptive agonist	Route of administration	Reference
Turpentine into bladder before agonist	Signs of bladder wall inflammation	Rat	AEA, PEA	i.a.	Jaggar et al., 1998a
Loose ligation of right sciatic nerve to induce unilateral painful mononeuropathy	Paw withdrawal from radiant heat	Rat	WIN	i.p.	Herzberg et al., 1997
			Δ^9-THC	i.t.	Mao et al., 2000
	Paw withdrawal from (a) acetone (cold); (b) pin prick; (c) von Frey filament	Rat	WIN	i.p.	Herzberg et al., 1997

[a] AEA, arachidonylethanolamide (anandamide); CBN, cannabinol; MethAEA, methanandamide; PEA, palmitylethanolamide; WIN, (+)-WIN55212; HU-210, 11-hydroxy-dimethylheptyl-Δ^8-tetrahydrocannabinol; HU-308, see Fig. 10; i.pl., intraplantar injection; i.a., intra-arterial injection.

[b] Compton et al., 1996; Dewey et al., 1970, 1972; Formukong et al., 1988; Johnson and Melvin, 1986; Kaymakcalan and Deneau, 1972; Koe et al., 1985; Martin, 1985a; Melvin et al., 1984; Osgood et al., 1978; Sanders et al., 1979; Weissman et al., 1982.

[c] Δ^8-THC (Dewey et al., 1970, 1972), nabilone (Koe et al., 1985), hexahydrocannabinol (Johnson and Melvin, 1986; Koe et al., 1985; Melvin et al., 1984; Weissman et al., 1982), CBN (Formukong et al., 1988; Sanders et al., 1979), cannabigerol (Formukong et al., 1988), Δ^8-THC-11-oic acid (Doyle et al., 1990), 1′,1′-dimethylheptyl-Δ^8-THC-11-oic acid (Burstein et al., 1998), L-nantradol (Johnson and Melvin, 1986; Koe et al., 1985), CP55940 (Johnson and Melvin, 1986; Koe et al., 1985; Melvin et al., 1993) and certain analogues of CP55940 (Johnson and Melvin, 1986; Koe et al., 1985; Melvin et al., 1984, 1993; Weissman et al., 1982) or THC (Osgood et al., 1978). Cannabidiol has been reported to be active p.o. (Formukong et al., 1988) but inactive s.c. (Koe et al., 1985).

[d] Bicher and Mechoulam, 1968; Kaymakcalan and Deneau, 1972; Sanders et al., 1979; Sofia et al., 1973, 1975; Watanabe et al., 1990; Welburn et al., 1976.

[e] Jackson et al., 1976; Sanders et al., 1979.

[f] Sofia et al., 1973, 1975.

[g] Tsou et al., 1996; Hohmann et al., 1999b.

[h] Cannabidiol inactive at doses of up to 400 mg/kg p.o. (Sofia et al., 1975; Welburn et al., 1976).

[i] PGE_1, 5-HT or bradykinin.

agonists in these tests is not a consequence of motor impairment or hypothermia. Thus,

1. when administered intrathecally, some pharmacological agents (yohimbine, nor-binaltorphimine, and antisera to dynorphin A) can attenuate cannabinoid-induced antinociception in the rat or mouse tail flick test without affecting cannabinoid-induced hypomotility, catalepsy or hypothermia (Lichtman and Martin, 1991a; Pugh et al., 1996; Smith et al., Smith et al., 1994a,b). Similarly, injection of muscimol directly into rat rostral ventromedial medulla attenuates (+)-WIN55212-induced antinociception in the tail flick test without affecting (+)-WIN55212-induced motor impairments (Meng et al., 1998).
2. Intrathecal administration of L-nantradol or desacetyl-L-nantradol to rats has been reported to induce antinociception in the tail flick and hot plate tests at doses that do not produce detectable impairments of motor function (Yaksh, 1981).
3. Bilateral injection of CP55940 into rat caudate nucleus produces catalepsy but no antinociception in the tail flick test (Lichtman et al., 1996).
4. Mice rendered tolerant to the antinociceptive effect of intrathecal Δ^9-THC in the tail flick test show no sign of tolerance to the motor depressant effects of intrathecal Δ^9-THC (Rowen et al., 1998). It has also been reported that mice can be made tolerant to Δ^9-THC-induced antinociception in the tail flick test (by pretreatment with an opioid) without also making them tolerant to Δ^9-THC-induced hypomotility, catalepsy or hypothermia (Bloom and Dewey, 1978; Smith et al., 1994b).
5. Intraperitoneal administration of the NMDA antagonist, MK-801, can attenuate Δ^9-THC-induced antinociception in the mouse tail flick test without affecting Δ^9-THC-induced hypothermia (Thorat and Bhargava, 1994b).

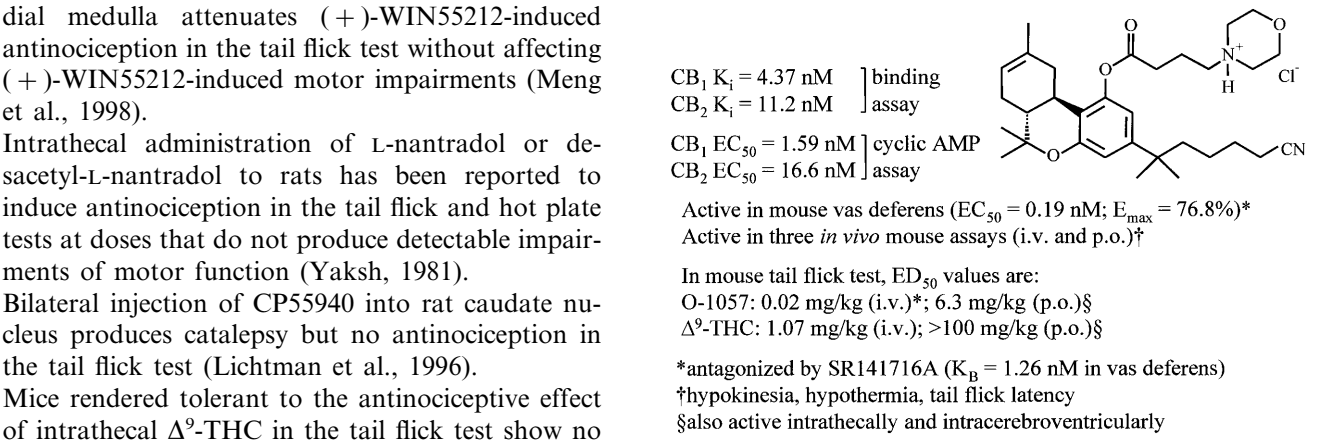


Fig. 9. Structure and some pharmacological properties of the water-soluble cannabinoid receptor agonist, O-1057 (This figure summarizes results obtained by Pertwee et al., 2000).

6. Lichtman et al. (1993) have reported that Δ^9-THC-induced antinociception in the mouse tail flick test is still detectable when hypothermia is prevented by elevating ambient temperature to about 38°C. They have also obtained evidence that Δ^9-THC-induced depression of the tail flick response does not arise from changes in tail skin temperature.

2.2. Antinociception in tonic pain models

As shown in Table 3, cannabinoid receptor agonists, including Δ^9-THC, (+)-WIN55212, anandamide and methanandamide, also exhibit antinociceptive activity in animal models of tonic pain/hyperalgesia. Similar properties have been reported for the WIN55212 analogue, pravadoline, a compound that lacks significant affinity for cannabinoid CB_1 receptors (Haubrich et al., 1990; Kumar et al., 1995). In some of the experiments listed in Table 3, a noxious agent was injected into the urinary bladder to induce viscero–visceral hyperreflexia (turpentine) or into the peritoneal cavity to induce abdominal stretch and other behavioural signs of nociception (usually phenylbenzoquinone). In the other experiments, animals were subjected to repetitive noxious transcutaneous electrical stimuli or were rendered hyperalgesic by injection of a noxious agent or by loose ligation of the sciatic nerve. When a noxious agent was used to induce hyperalgesia, this was administered intradermally to increase the sensitivity of the paws or tails of animals to noxious pressure or temperature. There is evidence from several of these investigations that cannabinoids have greater potency in suppressing responses to noxious pressure or thermal stimuli when these stimuli are applied to paws or tails that have been inflamed or made hyperalgesic than when they are applied to uninflamed/non-hyperalgesic paws or tails (Table 4). There are also several reports that Δ^9-THC and certain other cannabinoids show greater potency as antinociceptive agents in the abdominal stretch test than in models of acute non-tonic pain (tail flick or hot plate test) (Burstein et al., 1998; Cowan et al., 1985; Johnson and Melvin, 1986; Koe et al., 1985; Martin, 1985a; Melvin et al., 1984; Osgood et al., 1978; Weissman et al., 1982; Welch et al., 1995a). In line with the evidence that cannabinoids are effective against inflammatory pain, is the finding that these agents also show anti-inflammatory properties in several experimental models (Table 5). 1′,1′-dimethylheptyl-Δ^8-THC-11-oic acid is particularly potent (section 4.4). Anti-inflammatory effects of Δ^9-THC may stem, at least in part, from its ability to stimulate glucocorticoid release (Paton and Pertwee, 1973) as there is a report that the ability of this cannabinoid to oppose carrageenan-induced paw oedema in rats can be markedly attenuated by hypophysectomy or bilateral adrenalectomy (Sofia et al., 1973).

There are one or two instances in which no significant differences have been detected between potency in the abdominal stretch test and potency in the hot plate test (Sofia et al., 1973, 1975). There have also been two investigations in which the ability of Δ^9-THC or anandamide to suppress responses to pressure appeared to be no greater in hyperalgesic paws than in non-hyperalgesic paws. In one of these (Kosersky et al., 1973), Δ^9-THC exhibited much lower antinociceptive potency (and also lower anti-inflammatory potency; Table 5) than that observed in other similar studies (Sofia et al., 1973, 1975). One possibility, is that this reflects the use of different vehicles (Dewey, 1986): propylene glycol by Sofia et al. (1973, 1975) and bovine serum albumin by Kosersky et al. (1973). In the other investigation (Smith et al., 1998b), too few doses of Δ^9-THC (one dose) or anandamide (two doses) were used to allow any firm conclusion to be drawn as to whether either of these cannabinoids was more potent as an antinociceptive agent in the hyperalgesic rats than in the non-hyperalgesic animals.

It seems unlikely that there is any cause and effect relationship between the ability of cannabinoids to depress motor activity through CB_1 receptors in the central nervous system and their ability to induce antinociception in animals models of tonic pain. Thus, when applied directly to hyperalgesic paws or tails, CB_1 receptor agonists can induce antinociception at doses that are too low to produce effective concentrations within the central nervous system (Ko and Woods, 1999; Richardson et al., 1998b and Table 4). Moreover, when given to mice, Δ^9-THC (i.v.) appears to be several times more potent as an antinociceptive agent in the phenylbenzoquinone abdominal stretch test (Martin, 1985a) than as a depressant of locomotor activity or inducer of catalepsy in the ring test (Martin et al., 1995b). In addition, there are several reports that cannabinoids can suppress motor responses to noxious stimuli applied to paws or tails that have been made hyperalgesic when they are administered at doses that have no detectable effect on motor responses to noxious stimuli of non-hyperalgesic paws or tails (Table 4). There is also a report that (+)-WIN55212 is effective against hyperalgesia in rats at intravenous doses that do not significantly impair motor coordination, as measured in a rotarod treadmill test (Li et al., 1999).

3. Antinociception and CB_1 receptors

There is no doubt that cannabinoids induce antinociception in animal models of both acute and tonic pain, at least in part, through the activation of CB_1 receptors. Thus, many of the criteria for a response that is mediated by receptors in general or by CB_1 receptors in particular are satisfied for cannabinoid-induced anti-

Table 4
Evidence for and against the hypothesis that hyperalgesia/allodynia increases sensitivity to cannabinoid-induced antinociception

Compound	Species	Noxious stimulus	Cause of hyperalgesia	Lowest active dose against hyperalgesia	Non-hyperalgesic criterion or tissue	Lowest active dose against hyperalgesic nociception	Dose units and route	Reference
Evidence for hypothesis								
Cannabinol	Rat	Pressure to paw	Injection of yeast into hind paw[a]	40	Uninflamed contraleteral hind paw	>320	mg/kg (p.o.)	Sofia et al., 1975
Δ^9-THC				2		8		Sofia et al., 1975
Δ^9-THC				0.5		8		Sofia et al., 1973
(+)-WIN55212	Rat	Paw pressure	Injection of Freund's adjuvant into hind paw	10	Uninflamed contralateral hind paw	30	μg (i.t.)	Martin et al., 1999d
(+)-WIN55212	Rat	Paw pressure	Injection of capsaicin into hind paw[a]	0.1	Uninflamed contralateral hind paw	>0.2	mg/kg (i.v.)	Li et al., 1999
(+)-WIN55212	Rat	Radiant heat	Injection of capsaicin into hind paw[a]	0.01	No capsaicin injection into either hind paw	>0.2	mg/kg (i.v.)	Li et al., 1999
Anandamide	Mouse	Heat (hot plate)	Injection of carrageenan into hind paw	0.024	No carrageenan injection	>24 318	pg (i.t.)	Richardson et al., 1998b
Δ^9-THC	Rhesus monkey	Tail heat (warm water)	Injection of capsaicin into tail tip	100	No capsaicin injection	>320	μg (s.c.)	Ko and Woods, 1999
(+)-WIN55212	Rat	Radiant heat to hind paw	Loose ligation of right sciatic nerve	0.43[b]	Unlesioned contralateral hind paw or sham surgery	>4.3	mg/kg (i.p.)	Herzberg et al., 1997
		Cold to hind paw[d]		2.14[b]		>2.14		
		Pressure to hind paw[e]		2.14[b]		>2.14		
		Pin prick to hind paw		2.14[b]		>2.14		
Evidence against hypothesis[f]								
Δ^9-THC	Rat	Paw pressure[a]	Injection of yeast into hind paw[a]	100	Uninflamed contralateral hind paw	100	mg/kg (p.o.)	Kosersky et al., 1973
Δ^9-THC Anandamide	Rat	Paw pressure	Injection of Freund's adjuvant into hind paw	5[c]	No injection of Freund's adjuvant	5[c]	mg/kg (i.p.)	Smith et al., 1998b
				40, >10[b]		40, >10[b]		

[a] Unilateral injection.
[b] Lowest dose tested.
[c] No other doses tested.
[d] Application of acetone to plantar surface of hind paw using a blunted needle.
[e] von Frey filament.
[f] See section 2.2 for discussion of this evidence.

Table 5
Anti-inflammatory properties of cannabinoids[a]

Compound	Route	Species	Bioassay	Reference
Δ^9-THC	p.o.	Rat	Carrageenan-induced paw oedema[c]	Sofia et al., 1973
Δ^9-THC	p.o.[b]	Rat	Adjuvant-induced paw oedema[d]	Sofia et al., 1973
Δ^9-THC, Δ^9-THC-11-oic acid	p.o.	Mouse	Platelet activating factor-induced paw oedema	Burstein et al., 1989
Δ^9-THC, Δ^9-THC-11-oic acid	Topical	Mouse	Arachidonic acid-induced ear oedema	Burstein et al., 1989
Δ^9-THC, CBN, CBD, CBG	Topical	Mouse	TPA-induced erythema of ear	Formukong et al., 1988
Δ^8-THC-11-oic acid	p.o.	Mouse	Platelet activating factor-induced paw oedema	Doyle et al., 1990
DMH-Δ^8-THC-11-oic acid	p.o.	Mouse	Platelet activating factor-induced paw oedema	Burstein et al., 1992
DMH-Δ^8-THC-11-oic acid	p.o.	Mouse	Arachidonic acid-induced paw oedema	Burstein et al., 1992
DMH-Δ^8-THC-11-oic acid	p.o.[b]	Rat	Freund's adjuvant-induced paw oedema and redness	Zurier et al., 1998
DMH-Δ^8-THC-11-oic acid	p.o.[b]	Mouse	Inflammation in subcutaneous air pouch[e]	Zurier et al., 1998
HU-308	i.p.	Mouse	Arachidonic acid-induced ear oedema	Hanus et al., 1999
Methanandamide[h]	i.pl.	Rat	Capsaicin-induced plasma extravasation[f]	Richardson et al., 1998c
Anandamide	i.pl.	Rat	Carrageenan-induced paw oedema	Richardson et al., 1998c
Anandamide[i]	i.a.	Rat	Prevention of signs of bladder wall inflammation[g]	Jaggar et al., 1998b
Anandamide[j]	i.a.	Rat	Reversal of signs of bladder wall inflammation[g]	Jaggar et al., 1998a

[a] CBN, cannabinol; CBD, cannabidiol, CBG, cannabigerol; DMH-Δ^8-THC-11-oic acid, 1′1′-dimethylheptyl-Δ^8-THC-11-oic acid; THC, tetrahydrocannabinol; TPA, tetradecanoyl phorbol acetate; methanandamide, (*R*)-(+)-arachidonyl-1′-hydroxy-2′ propylamide; i.a., intra-arterial; i.pl., intraplantar; see Fig. 10 for the structure of HU-308.
[b] Repeated administration.
[c] Δ^9-THC was inactive when bovine serum albumin was used as the vehicle (Kosersky et al., 1973) rather than propylene glycol (Sofia et al., 1973).
[d] *Mycobacterium tuberculosis* into right hind paw.
[e] Inflammation induced by injection of interleukin-1β and tumour necrosis factor α into subcutaneous air pouch.
[f] Increased vascular permeability.
[g] Induced by injection of turpentine into the urinary bladder.
[h] Methanandamide (175 ng i.pl.) antagonized by SR141716A (250 ng i.pl.).
[i] Palmitylethanolamide not active.
[j] Palmitylethanolamide also active.

nociception. These criteria are discussed below and further details appear in Table 6.

1. The antinociceptive potency of Δ^9-THC is no less than that of morphine, an agent already known to induce receptor-mediated analgesia. A number of cannabinoids show even greater potency than Δ^9-THC, for example in the mouse tail flick test after intravenous administration (Martin et al., 1995b). It is also noteworthy that after subcutaneous injection, Δ^9-THC appears to have significantly less antinociceptive potency or efficacy than morphine in both rats and mice (Bloom and Dewey, 1978; Martin, 1985a; Melvin et al., 1984; Smith et al., 1998a; Wilson and May, 1975). However, it is possible that in these experiments, the antinociceptive tests were performed too soon after Δ^9-THC administration, there being evidence that Δ^9-THC is absorbed particularly slowly when injected subcutaneously (Kaymakcalan and Deneau, 1972).
2. A graded relationship exists between dose of cannabinoid (often Δ^9-THC or CP55940) and the degree of antinociception produced. This has been demonstrated in animal models of both acute and tonic pain.
3. The antinociceptive potencies of cannabinoid receptor agonists are structure-dependent and those of non-eicosanoids correlate well with their binding affinities for CB_1 receptors. Cannabinoids that have been used in such investigations bind more or less equally well to CB_1 and CB_2 receptors (see references in Table 6 and Pertwee, 1999a). Hence the resulting data do not rule out the possibility that cannabinoids can induce antinociception through CB_2 or CB_2-like receptors (section 4.4).
4. The antinociceptive potencies of spinally injected cannabinoid receptor agonists correlate negatively with their lipid solubility. This observation suggests that in spite of their high lipophilicity, cannabinoids do not induce antinociception by interacting with membrane phospholipids through non-receptor mediated mechanisms.
5. Enantiomeric pairs of chiral cannabinoid receptor agonists exhibit marked stereoselectivity as antinociceptive agents, the more active enantiomer having the higher CB_1 receptor affinity. Stereoselectivity of a range of cannabinoid receptor agonists has been observed in both acute and tonic pain models (Table 7). It is noteworthy that in some experiments it has been found that the antinociceptive potency of CP56667, a (+)-isomer, matches or even exceeds that of its (−)-isomer, CP55940, even though CP56667 has significantly

Table 6
Evidence that cannabinoid-induced antinociception is mediated by CB_1 receptors[a]

Criterion	Pain model	Species	Reference
The antinociceptive potency of Δ^9-THC is no less than that of morphine, an agent already known to induce receptor-mediated analgesia	Tail flick, hot plate, tail pinch, tail clamp or flinch jump test (i.v., i.p., p.o.)	Mouse or rat	[h]
	Rat paw hyperalgesia (yeast-treated paw) or mouse PBQ abdominal stretch test (i.v., p.o.)	Mouse or rat	[i]
A graded relationship exists between dose of cannabinoid (often Δ^9-THC or CP55940) and the degree of antinociception produced	Tail flick or hot plate test (i.v., i.p., s.c., p.o.)	Mouse or rat	[j]
	Tail flick or hot plate test (i.t., i.c.v.)	Mouse or rat	[k]
	Rodent paw or monkey tail hyperalgesia or mouse acetic acid or PBQ abdominal stretch test (i.v., i.p., p.o., s.c., i.pl., i.t.)	Mouse, rat or rhesus monkey	[l]
The antinociceptive potencies of cannabinoid receptor agonists are structure-dependent and those of non-eicosanoids correlate well with their binding affinities for CB_1 receptors	Tail flick test (i.v., i.t.)	Mouse	[m]
	PBQ abdominal stretch test (s.c.)	Mouse	[n]
The antinociceptive potencies of spinally injected cannabinoid receptor agonists correlate negatively with their lipid solubility	Tail flick (i.t.)	Mouse	[o]
Enantiomeric pairs of chiral cannabinoid receptor agonists exhibit marked stereoselectivity, the more active enantiomer having the higher CB_1 receptor affinity[b]	Tail flick or hot plate test (i.v., s.c., i.t., i.c.v., i.c.[c])	Mouse or rat	[p]
	Rat paw hyperalgesia, mouse PBQ abdominal stretch test or noxious heat-evoked firing in rat WDR neurones (i.v., i.p., s.c., i.c.v.)	Mouse or rat	[q]
Cannabinoids induce antinociception when injected directly into areas of the central nervous system that are known to contain CB_1 receptors	Tail flick or hot plate test (i.t., i.c.v. or i.c.[d])	Mouse or rat	[r]
	Mouse paw hyperalgesia or noxious heat-evoked firing in rat WDR neurones (i.c.v., i.t.)	Mouse or rat	[s]
The selective CB_1 receptor antagonist, SR141716A (i.p., i.v., s.c., i.pl., i.t., i.c.v., i.c.[e]) can prevent the antinociceptive effects of cannabinoid receptor agonists (i.v., i.p., s.c., i.pl., i.t., i.c.v., i.c.[e]) at an appropriately high potency	Tail flick or hot plate test	Mouse or rat	[t]
	Reflex activity of RVM neurones in tail flick test	Rat	[u]
	Paw pressure test	Rat	[v]
	Rodent paw or monkey tail hyperalgesia/allodynia or mouse PBQ abdominal stretch test	Mouse, rat or rhesus monkey	[w]
Antisense oligodeoxynucleotide (i.c.v.) directed against the CB_1 receptor attenuates antinociception induced by CP55940	Warm water tail withdrawal test (i.c.v.)	Mouse	[x]

Table 6 (*Continued*)

Criterion	Pain model	Species	Reference
Antinociceptive responses to Δ^9-THC and HU-210 are absent or markedly attenuated in CB_1 knockout mice[f]	Hot plate, tail flick or warm water tail withdrawal test (i.p.)	Mouse	[y]
Antinociception induced by cannabinoids can be attenuated by pertussis toxin and certain other agents expected to impair CB_1 receptor signalling	Tail flick test (i.t., i.c.v., i.c.[g])	Mouse or rat	[z]

[a] PBQ, phenylbenzoquinone; RVM, rostral ventromedial medulla; WDR, wide dynamic range neurones.

[b] (−)-Δ^8-tetrahydrocannabinol (Wilson and May, 1975), (−)-Δ^9-tetrahydrocannabinol (Martin, 1985b), CP55940 (Compton et al., 1992b; Koe et al., 1985; Lichtman et al., 1996; Lichtman and Martin, 1991b; Little et al., 1988), CP55244 (Koe et al., 1985; Little et al., 1988), L-nantradol (Koe et al., 1985; Little et al., 1988; Welch and Stevens, 1992; Welch et al., 1995b), L-methylnantradol (Lichtman et al., 1992), (−)-11-hydroxy-dimethylheptyl-Δ^8-tetrahydrocannabinol (Little et al., 1989), (+)-WIN55212 (Compton et al., 1992a; Hohmann et al., 1999c; Li et al., 1999; Martin et al., 1998; Tsou et al., 1996), (−)-11-hydroxy-dimethylheptylhexahydrocannabinol (Yan et al., 1994).

[c] Rat posterior ventrolateral periaqueductal grey (Lichtman et al., 1996) and rat rostral ventromedial medulla (Martin et al., 1998).

[d] Rat posterior ventrolateral periaqueductal grey (Lichtman et al., 1996; Martin et al., 1999c), rat rostral ventromedial medulla (Martin et al., 1999c, 1998), central and basolateral nuclei of the amygdala, submedius, lateral and posterior nuclei of the thalamus, superior colliculus, and A5 noradrenergic group in the brain stem (Martin et al., 1999c).

[e] Rat rostral ventromedial medulla (Martin et al., 1998).

[f] Antinociceptive response abolished {Δ^9-THC in hot plate test (Ledent et al., 1999; Zimmer et al., 1999) and HU-210 in tail flick test (Zimmer et al., 1999)}, markedly attenuated {Δ9-THC in warm water tail withdrawal test (Ledent et al., 1999)} or unaffected {Δ^9-THC in tail flick test, (Zimmer et al., 1999)}.

[g] Rat posterior ventrolateral periaqueductal grey (Lichtman et al., 1996).

[h] Buxbaum, 1972; Martin, 1985a; Smith et al., 1998a; Sofia et al., 1975; Thorat and Bhargava, 1994a.

[i] Martin, 1985a; Sofia et al., 1975.

[j] Bloom and Dewey, 1978; Buxbaum, 1972; Lichtman and Martin, 1991b, 1997; Lichtman et al., 1993; Martin, 1985b; Reche et al., 1996a,b, 1998; Smith et al., 1998a; Smith and Martin, 1992; Sofia and Barry, 1983; Sofia et al., 1975; Thorat and Bhargava, 1994a.

[k] Edsall et al., 1996; Gallager et al., 1972; Lichtman and Martin, 1991b, 1997; Pugh et al., 1997; Raffa et al., 1999; Smith and Martin, 1992; Welch, 1993; Welch et al., 1995a, 1998; Welch and Stevens, 1992; Welch et al., 1995b; Yaksh, 1981.

[l] Calignano et al., 1998; Formukong et al., 1988; Ko and Woods, 1999; Richardson et al., 1998b,c; Sofia et al., 1973, 1975; Tsou et al., 1996; Welch et al., 1995a.

[m] Compton et al., 1993; Martin et al., 1991; Thomas et al., 1991; Welch and Stevens, 1992.

[n] Melvin et al., 1993.

[o] Lichtman et al., 1992.

[p] Compton et al., 1992a,b; Koe et al., 1985; Lichtman et al., 1996; Lichtman and Martin, 1991b; Lichtman et al., 1992; Little et al., 1988, 1989; Martin, 1985b; Martin et al., 1998; Welch and Stevens, 1992; Welch et al., 1995b; Welch and Eads, 1999; Wilson and May, 1975; Yan et al., 1994.

[q] Hohmann et al., 1999c; Koe et al., 1985; Li et al., 1999; Tsou et al., 1996.

[r] Gallager et al., 1972; Lichtman et al., 1996; Lichtman and Martin, 1991b; Martin et al., 1999c, 1993b, 1998; Mason et al., 1999; Raffa et al., 1999; Smith and Martin, 1992; Welch, 1993, 1994; Welch and Stevens, 1992; Welch et al., 1995b; Yaksh, 1981.

[s] Hohmann et al., 1999c; Richardson et al., 1998b.

[t] Adams et al., 1998; Carta et al., 1999; Compton et al., 1996; Lichtman and Martin, 1997; Martin et al., 1998; Mason et al., 1999; Reche et al., 1996a; Rinaldi-Carmona et al., 1994; Welch et al., 1998.

[u] Meng et al., 1998.

[v] Smith et al., 1998b.

[w] Calignano et al., 1998; Compton et al., 1996; Herzberg et al., 1997; Ko and Woods, 1999; Mao et al., 2000; Martin et al., 1999d; Richardson et al., 1998c.

[x] Edsall et al., 1996.

[y] Ledent et al., 1999; Zimmer et al., 1999.

[z] Lichtman et al., 1996; Welch et al., 1995a,b.

less affinity than CP55940 for CB_1 receptors (section 4.1). The basis for this apparent anomaly remains to be established.

6. Cannabinoids induce antinociception when injected directly into areas of the central nervous system that are known to contain CB_1 receptors. These are brain areas that are thought to have a role in nociception, for example the posterior ventrolateral periaqueductal grey and rostral ventromedial medulla, and exclude areas such as the caudate nucleus and substantia nigra at which cannabinoids probably act through CB_1 receptors to impair motor function (sections 2.1. and 5.1).
7. The selective CB_1 receptor antagonist, SR141716A, can prevent the antinociceptive effects of cannabinoid receptor agonists at an appropriately high potency. It is important to bear in mind that although SR141716A is CB_1-selective it is not CB_1-specific and will, at sufficiently high doses, block CB_2 as well as CB_1 receptors (Pertwee, 1999a).

However, many of these experiments have been performed with appropriately low doses of SR141716A. Moreover, the CB_2-selective agonist, HU-308 (Fig. 10), has been found to lack activity in the mouse hot plate test (Hanus et al., 1999 and section 4.4) and Calignano et al. (1998) have reported that the degree of antinociception induced by intraplantar anandamide in mice in the formalin paw test is attenuated by SR141716A but not by SR144528 when these antagonists are administered intravenously at 0.1 mg/kg. In a few experiments in which a tonic pain model was used, SR141716A administered by itself has been found to exhibit hyperalgesic activity, raising the possibility that in these studies SR141716A attenuated cannabinoid-induced antinociception through the production of hyperalgesia rather than through competitive CB_1 receptor antagonism (section 9). In many other experiments, however, SR141716A alone had no effect on nociception. Consequently, results obtained from most SR141716A experiments do constitute good evidence that cannabinoid-induced antinociception can be mediated by CB_1 receptors. The finding that SR141716A blocks the inhibitory effect of methanandamide on capsaicin-evoked paw-skin plasma extravasation in rats (Table 5) supports a role for CB_1 receptors in at least this anti-inflammatory effect of cannabinoids. It is noteworthy that SR141716A has been reported to antagonize cannabinoids in rat or mouse tail flick tests, at doses that

Table 7
Animal pain models in which stereoselectivity by cannabinoid receptor agonists has been observed[a]

Compound	Route	Species	Test	ED_{50} or MPE_{50} (mg/kg)[b]		References
				(−)-isomer	(+)-isomer	
Δ^8-THC	sc	Mouse	Hot plate	8.8	>50	Wilson and May, 1975
Δ^9-THC	i.v.	Mouse	Tail flick	1	>60	Martin, 1985b
HU-210/211	i.v.	Mouse	Tail flick	0.009	>30	Little et al., 1989
HHC analogue	i.v.	Mouse	Tail flick	0.002	0.58	Yan et al., 1994
CP55244/3	i.v.	Mouse	Tail flick	0.01	>10	Little et al., 1988
CP55940/56667	i.v.	Mouse	Tail flick	0.09	6	Little et al., 1988
Nantradol	i.v.	Mouse	Tail flick	0.29	>10	Little et al., 1988
Nantradol	s.c.	Mouse	Tail flick	0.21	>56	Koe et al., 1985
WIN55212	i.v.	Mouse	Tail flick	>30	0.43	Compton et al., 1992a
CP55244/3	s.c.	Mouse	PBQ	0.01	>100	Koe et al., 1985
CP55940/56667	s.c.	Mouse	PBQ	0.06	14.6	Koe et al., 1985
Nantradol	s.c.	Mouse	PBQ	0.1	6.5	Koe et al., 1985
WIN55212	i.p.	Rat	Formalin into hind paw[c]	>10	5[d]	Tsou et al., 1996
WIN55212	i.v.	Rat	Capsaicin into hind paw	>0.1	~0.01	Li et al., 1999
WIN55212	i.v.	Rat	Radiant heat to capsaicin-injected hind paw	>0.1	<0.01	Li et al., 1999
WIN55212	i.v.	Rat	Von Frey filament to capsaicin-injected hind paw	>0.2	<0.1	Li et al., 1999
				μg per animal[b]		
CP55940/56667[e]	i.c.v.	Mouse	Tail flick	2.9	0.2	Welch et al., 1995b
CP55940/56667[e]	i.t.	Mouse	Tail flick	2.3	4.2	[f]
CP55940/56667	i.t.	Rat	Tail flick	100[d]	>1000	Lichtman and Martin, 1991b
CP55940/56667	PAG	Rat	Tail flick	15[d]	>15	Lichtman et al., 1996
Nantradol	i.c.v.	Mouse	Tail flick	0.02	>25	Welch et al., 1995b
Nantradol	i.t.	Mouse	Tail flick	0.04	>25	[f]
N-methylnantradol	i.t.	Mouse	Tail flick	0.5	>100	Lichtman et al., 1992
WIN55212	RVM	Rat	Tail flick	>5	5[d]	Martin et al., 1998

[a] HHC analogue, 11-hydroxy-dimethylheptyl-hexahydrocannabinol; HU-210/211, (−)/(+)-11-hydroxy-dimethylheptyl-Δ^8-tetrahydrocannabinol; i.c.v., intracerebroventricular; i.t., intrathecal; MPE_{50}, dose producing 50% of maximum possible effect; PAG, periaqueductal grey; PBQ, phenylbenzoquinone abdominal stretch test; RVM, rostral ventromedial medulla.

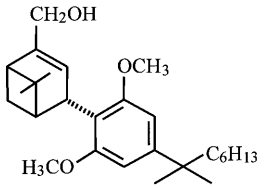
[b] For WIN55212, the (+)-isomer has greater affinity for CB_1 receptors than the (−)-isomer. For the other compounds, it is the (−)-isomer that has the greater CB_1 affinity (section 1.2).

[c] Early- and late-phase paw lifting and licking using a scoring system.

[d] Effective dose: no other dose used.

[e] In these experiments, the potency of the (+)-isomer, CP56667 matched or exceeded that of the (−)-isomer, CP55940, even though CP56667 has significantly less affinity than CP55940 for CB_1 receptors (section 1.2).

[f] Welch and Stevens, 1992; Welch et al., 1995b.



CB_2 K_i = 22.7 nM] binding
CB_1 K_i > 10 μM] assay

CB_2 EC_{50} = 5.6 nM] cyclic AMP
CB_1 EC_{50} > 1 μM] assay

*At 40 mg/kg i.p., inactive in mouse tetrad assay
†At 30 mg/kg i.p., reduces rat blood pressure
†At 50 mg/kg i.p., reduces arachidonic acid-induced mouse ear oedema
†At 50 mg/kg i.p., reduces late-phase response to i.pl. formalin#

*hypokinesia, catalepsy, hypothermia, hot plate response
†antagonized by SR144528 (0.5 or 1 mg/kg i.p.)
†not antagonized by SR141716A (3 or 5 mg/kg i.p.)
#number of licks of formalin-injected paw (mouse)

Fig. 10. Structure and some pharmacological properties of the CB_2-selective agonist, HU-308 (This figure summarises results obtained by Hanus et al., 1999).

do not to attenuate morphine-induced antinociception (Compton et al., 1996; Lichtman and Martin, 1997).

8. Antisense oligodeoxynucleotide directed against the CB_1 receptor attenuates antinociception induced by CP55940. Edsall et al. (1996), who made this observation, also found that mice pretreated with a mismatch oligonucleotide were no less susceptible to the antinociceptive effect of CP55940 than unpretreated control animals.
9. Antinociceptive responses to Δ^9-THC and HU-210 are absent or markedly attenuated in CB_1 knockout mice. Ledent et al. (1999) and Zimmer et al. (1999) have reported that in the hot plate test, Δ^9-THC-induced antinociception is detectable in wild-type but not in CB_1 receptor knockout mice. They have also found that in CB_1 knockout mice, Δ^9-THC-induced antinociception in the warm water tail withdrawal test is strongly reduced (Ledent et al., 1999) and HU-210-induced antinociception in the tail flick test is abolished (Zimmer et al., 1999). Interestingly, Zimmer et al. (1999) also found that in their knockout mice, Δ^9-THC fully retained its ability to induce antinociception in the tail flick test, a possible indication that this effect of Δ^9-THC is not mediated by CB_1 receptors alone (section 4).
10. Antinociception induced by cannabinoids can be attenuated by pertussis toxin and certain other agents expected to impair CB_1 receptor signalling. Lichtman et al. (1996) have found that pretreatment with pertussis toxin can attenuate antinociception induced by CP55940 in the rat tail flick test when the injection site for both agents is the posterior ventrolateral periaqueductal grey area, supporting the involvement of a $G_{i/o}$-coupled receptor such as CB_1. Similarly, Raffa et al. (1999) have reported that intracerebroventricular pretreatment of mice with pertussis toxin attenuates antinociceptive responses in the warm water tail withdrawal test to intracerebroventricular Δ^9-THC, (+)-WIN55212 or anandamide. Welch et al. (1995b) have shown that antinociception induced in the mouse tail flick test by intrathecal or intracerebroventricular administration of Δ^9-THC, Δ^8-THC or CP55940 can be reduced or abolished by intrathecal pretreatment with pertussis toxin. In addition, they found that antinociception induced in this bioassay by intracerebroventricular administration of the same cannabinoids could be reduced or abolished by intrathecal pretreatment with calcium or thapsigargin (increases intracellular calcium) although not by forskolin, 8-(4-chlorophenylthio)-cyclic AMP, dibutyryl-cyclic AMP or apamin (blocks low-conductance, calcium-gated potassium channels). Welch's group have also reported that when all injections are made intrathecally, antinociception induced in the mouse tail flick test by Δ^9-THC, Δ^8-THC or CP55940 can be attenuated by forskolin, 8-(4-chlorophenylthio)-cyclic AMP and apamin but not by dibutyryl-cyclic AMP, calcium or thapsigargin (Welch et al., 1995a,b). These findings point to signalling differences between spinal and supraspinal cannabinoid receptors (section 4.2). Dibutyryl-cyclic AMP has also been reported to be without effect on CP55940-induced antinociception in the tail flick test when both drugs are injected directly into rat posterior ventrolateral periaqueductal grey area (Lichtman et al., 1996).

4. Antinociception and non-CB_1 cannabinoid receptors

4.1. Non-CB_1 cannabinoid receptors in the spinal cord

There are indications in the literature that the spinal cord may contain mixed populations of CB_1 and non-CB_1 cannabinoid receptors and possibly also other types of mechanism capable of mediating cannabinoid-induced antinociception in the tail flick or hot plate test. More specifically,

1. Welch et al. (1998) have reported that the potency of SR141716A (i.p.) against antinociception induced in the mouse tail flick test by intrathecal administration of certain cannabinoids is agonist-dependent. SR141716A was highly potent against CP55940, showed intermediate potency against Δ^9-THC, Δ^8-THC and deoxy-HU-210 and even lower potency against anandamide (Table 8). The potency of other agents capable of attenuating cannabinoid-induced antinociception in the spinal cord has also been reported to be agonist-dependent. Thus, it has been found that although intrathecal pretreatment with

8-(4-chlorophenylthio)-cyclic AMP, forskolin or apamin attenuated the antinociceptive effect of Δ^9-THC (i.t.) in the mouse tail flick test, these agents had no effect on antinociception induced by intrathecal injection of anandamide or of its more potent analogue, fluoroanandamide (Welch et al., 1995a). These data suggest that in contrast to Δ^9-THC, both anandamide and fluoroanandamide produce antinociception in the spinal cord through mechanisms that are independent of cyclic AMP or of apamin-sensitive potassium channel modulation (Welch et al., 1995a). It is noteworthy that the antinociceptive effect of all three cannabinoids was blocked by pertussis toxin (i.t.) (Welch et al., 1995a). There are also reports that when injected intrathecally, the selective κ opioid receptor antagonist, nor-binaltorphimine, attenuates the antinociceptive effects of intrathecal Δ^9-THC, Δ^8-THC, CP55940 and L-nantradol in the mouse tail flick test but does not affect antinociception induced by intrathecal anandamide or fluoroanandamide (Smith et al., 1994a; Welch, 1993; Welch et al., 1995a). In addition, Pugh et al. (1997) have found that whereas the antinociceptive effect of intrathecal Δ^9-THC in the mouse tail flick test is attenuated by intrathecal pretreatment with antisera to dynorphin A(1–17), dynorphin A(1–8) or α-neoendorphin, that of CP55940 (i.t.) is not.

2. There are reports that whilst intrathecal Δ^9-THC, 11-OH-Δ^9-THC, Δ^8-THC and L-nantradol potenti-

Table 8
Some anomalous antinociceptive properties of anandamide[a]

Observation	Reference
For anandamide and 16 structural analogues, the correlation coefficient for a plot of log ED_{50} in the mouse tail flick test against log K_i for displacement of [^{3}H]CP55940 from specific cannabinoid binding sites on rat brain membranes is not statistically significant ($r = 0.46$).	Adams et al., 1995
The potency of intrathecal anandamide matches that of intrathecal Δ^9-THC in the mouse tail flick test but is less than that of intrathecal Δ^9-THC in the mouse phenylbenzoquinone abdominal stretch test.	Welch et al., 1995a
The ED_{50} of SR141716A (mg/kg i.p.) for antagonism of intrathecal anandamide in the mouse tail flick test (15.4) is greater than that for antagonism of intrathecal Δ^9-THC (0.76), Δ^8-THC (0.9), CP55940 (0.1) or deoxy-HU-210 (0.4).	Welch et al., 1998
SR141716A (3 or 30 mg/kg i.v.) does not antagonize anandamide (i.v.) in the mouse tail flick test.	Adams et al., 1998
SR141716A (10 mg/kg i.p.) does not antagonize anandamide (40 mg/kg i.p.) in the rat paw pressure test.	Smith et al., 1998b
Intrathecal 8-(4-chlorophenylthio)-cyclic AMP, forskolin and apamin attenuate the antinociceptive effect of intrathecal Δ^9-THC in the mouse tail flick test but do not affect antinociception in this test induced by intrathecal anandamide or fluoroanandamide.	Welch et al., 1995a
Intracerebroventricular cholera toxin decreases the antinociceptive effect in the mouse warm water tail withdrawal test of intracerebroventricular (+)-WIN55212, has a minimal effect on the antinociceptive effect of intracerebroventricular Δ^9-THC, and increases the antinociceptive effect of intracerebroventricular anandamide.	Raffa et al., 1999
Mice made tolerant to intrathecal Δ^9-THC in the mouse tail flick test by subcutaneous Δ^9-THC pretreatment show tolerance to intrathecal U50488H, CI-977, dynorphin A(1–8), dynorphin A(1–13) and dynorphin A(1–17) in this bioassay. No such cross tolerance is observed in mice made tolerant by intraperitoneal anandamide pretreatment to the antinociceptive effects of intrathecal anandamide, Δ^9-THC and CP55940.	Smith et al., 1994b; Welch, 1997
Intrathecal nor-binaltorphimine attenuates the antinociceptive effects of intrathecal Δ^9-THC, Δ^8-THC, CP55940 and L-nantradol in the mouse tail flick test but does not affect antinociception in this test induced by intrathecal anandamide or fluoroanandamide.	Welch et al., 1995a; Welch, 1993; Smith et al., 1994a
Intrathecal nor-binaltorphimine attenuates the antinociceptive effect of intrathecal Δ^9-THC in the rat tail flick test but does not affect antinociception in this test induced by intrathecal anandamide (or intrathecal CP55940).	Mason et al., 1999
The antinociceptive effect of intrathecal morphine in the mouse tail flick test is potentiated by intrathecal Δ^9-THC, 11-hydroxy-Δ^9-THC, Δ^8-THC and L-nantradol but not by intrathecal anandamide (or intrathecal CP55940).	Welch et al., 1995a; Welch and Stevens, 1992; Welch et al., 1995b

[a] Deoxy-HU-210, (−)-1-deoxy-11-hydroxy-dimethylheptyl-Δ^8-tetrahydrocannabinol; fluoroanandamide, arachidonyl-(2′-fluoroethyl)amide.

ate intrathecal morphine in the mouse tail flick test, intrathecal anandamide and CP55940 do not (Welch et al., 1995a; Welch and Stevens, 1992; Welch et al., 1995b and section 8).

3. Welch's group has found that a subcutaneous pretreatment regimen with Δ^9-THC that induces tolerance to this cannabinoid in the mouse tail flick test also induces tolerance in this bioassay to the κ opioid receptor agonists, U50488H and CI-977, administered intrathecally, as well as to intrathecal dynorphin A(1–8), dynorphin A(1–13) and dynorphin A(1–17) (Smith et al., 1994b; Welch, 1997). However, they found that anandamide (i.p.) could render mice tolerant to its own antinociceptive effect in the tail flick test (i.t. or i.p.) and to that of intrathecal Δ^9-THC and CP55940 without also inducing tolerance to intrathecal CI-977 or to intrathecally administered dynorphins A(1–8), (1–13) or (1–17).
4. In some experiments the relative antinociceptive potency of CP55940 and its (+)-isomer, CP56667, does not correspond to the relative affinity of this enantiomeric pair for CB_1 receptors. Thus there is one report that after intrathecal injection, CP55940 is no more potent in the mouse tail flick test than CP56667 (Welch and Stevens, 1992 and Table 7), even though as expected for a CB_1-mediated effect, the (−)-enantiomer of nantradol was found to exhibit greater potency than its (+)-enantiomer by the same route and using the same bioassay. More remarkable still is an observation that after intracerebroventricular injection, CP55940 was 16 times less potent than CP56667 in the mouse tail flick test (Welch et al., 1995b and Table 7). In other experiments, however, CP55940 has been found to show greater antinociceptive potency than CP56667. This was in the rat tail flick test after intrathecal administration or after direct injection into the periaqueductal grey area and in the mouse tail flick or abdominal stretch test after intravenous or subcutaneous injections (Table 7).

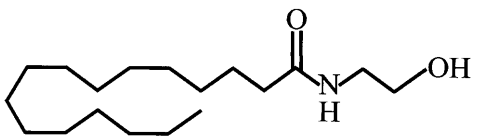
4.2. Differences between cannabinoid receptor populations in brain and spinal cord

Evidence that brain and spinal cord may contain different cannabinoid receptor types/subtypes that are capable of mediating cannabinoid-induced antinociception in the tail flick test is summarized below.

1. Welch et al. (1998) have reported that SR141716A shows greater efficacy and potency in attenuating antinociception induced in the mouse tail flick test by Δ^9-THC, Δ^8-THC or CP55940, when both antagonist and agonist are given intracerebroventricularly than when both agents are given intrathecally. This finding raises the possibility that when administered systemically, SR141716A acts mainly at supraspinal sites to antagonize cannabinoid-induced antinociception.
2. Results from experiments in which agents expected to modulate cannabinoid receptor signalling mechanisms were injected intrathecally, intracerebroventricularly or intracerebrally support the existence of signalling differences between brain and spinal cannabinoid receptors that mediate cannabinoid-induced antinociception (section 3). In particular, the data suggest that cyclic AMP has a more important effector role in cannabinoid-induced antinociception in mouse spinal cord than brain whilst intracellular calcium and apamin-sensitive potassium channels have more important effector roles in cannabinoid-induced antinociception in mouse brain than spinal cord.
3. Welch et al. (1995b) have found that when all injections are made intrathecally, antinociception induced by morphine in the mouse tail flick test can be potentiated by Δ^9-THC, Δ^8-THC and L-nantradol but not by CP55940 but that when all injections are made intracerebroventricularly, morphine-induced antinociception can be potentiated by all these cannabinoids.

4.3. Different antinociceptive tests, different cannabinoid receptors?

Not all types of antinociception induced by cannabinoids seem to be mediated by the same cannabinoid receptor types/subtypes. Compton et al. (1996) have found that SR141716A (i.p.) exhibits approximately seven times greater potency against Δ^9-THC (i.v.) in the mouse tail flick test than in the mouse phenylbenzoquinone abdominal stretch test. There are also some reports that the relative potencies of some cannabinoid receptor agonists varies with the antinociceptive test used. For example, Welch et al. (1995a) found that whilst the potency of intrathecal anandamide matched that of intrathecal Δ^9-THC in the mouse tail flick test, it was less than that of intrathecal Δ^9-THC in the mouse phenylbenzoquinone abdominal stretch test (Welch et al., 1995a). Their data also indicated the antinociceptive potencies of intrathecal Δ^9-THC, anandamide and fluoroanandamide to be higher in the abdominal stretch test than in the tail flick test. In a separate investigation, they found that intrathecal L-nantradol differed from intrathecal Δ^9-THC, 11-OH-Δ^9-THC and Δ^8-THC by failing to show antinociceptive activity in the mouse hot plate test at doses that were effective in the mouse tail flick test. Zimmer et al. (1999) have reported that, in CB_1 knockout mice, Δ^9-THC loses the ability to induce antinociception in the hot plate test but retains this ability in the tail flick test (section 3). Most recently, Hanus et al. (1999) have



Palmitylethanolamide

Fig. 11. Structure of palmitylethanolamide.

shown that the CB_2-selective agonist, HU-308 (Fig. 10), produces antinociception in the mouse formalin paw test but not in the mouse hot plate test (section 4.4).

4.4. CB_2 or CB_2-like receptors

Results from an investigation into mechanisms underlying the ability of anandamide and palmitylethanolamide (Fig. 11) to suppress behavioural responses induced by injection of formalin into the hind paws of mice (licking and flexing of the treated limb) have led Calignano et al. (1998) to postulate that antinociception in this animal model of tonic inflammatory pain can be mediated by CB_2-like receptors. Their experiments showed that when administered by intraplantar injection together with formalin, palmitylethanolamide was active at a low dose (50 μg), as were anandamide (50 μg), methanandamide (50 μg) and (+)-WIN55212 (500 μg). Palmitylethanolamide suppressed early- and late-phase responses to formalin, and both these effects could be prevented by SR144528 (CB_2-selective), although not by SR141716A (CB_1-selective). Anandamide suppressed early (but not late) phase responses to formalin, and this effect was prevented by SR141716A but not by SR144528. By themselves, both antagonists were hyperalgesic. Even so, the production of hyperalgesia by SR144528 is unlikely to have accounted for its ability to attenuate palmitylethanolamide-induced suppression of the late-phase response to formalin as this antagonist was found to enhance only the early-phase response to formalin. Combined injection of subeffective doses of anandamide and palmitylethanolamide into the hind paws (0.1 μg) induced a marked degree of antinociception that could be blocked by pretreatment with either SR141716A or SR144528. These results suggest that nociception in the formalin paw test is suppressed by palmitylethanolamide through peripheral non-CB_1 receptors. These are unlikely to be cannabinoid CB_2 receptors as palmitylethanolamide has little affinity for this receptor type, or indeed for CB_1 receptors (Lambert and Di Marzo, 1999; Lambert et al., 1999; Pertwee, 1999a). Consequently, since antinociception induced by palmitylethanolamide was found to be attenuated by SR144528, Calignano et al. (1998) have suggested that both these agents are ligands for putative CB_2-like receptors. In other experiments, Facci et al. (1995) have found that at sub-micromolar concentrations, palmitylethanolamide inhibits 5-HT release from RBL-2H3 cells (a rat mast cell line) and also readily displaces [^{3}H](+)-WIN55212 from specific binding sites on membranes obtained from these cells. Results from reverse transcription-PCR experiments with RBL-2H3 cells yielded positive results when CB_2-specific primers were used but negative results with CB_1-specific primers (Facci et al., 1995). Since palmitylethanolamide has little affinity for CB_2 receptors and since the cannabinoid receptor mRNA in RBL-2H3 cells was not completely sequenced by Facci et al. (1995), it may be that as for cannabinoid receptors in mouse paw, receptors on RBL-2H3 cells may be CB_2-like rather than CB_2. It is noteworthy that in more recent experiments, Lambert et al. (1999) were unable to confirm the presence of cannabinoid binding sites on RBL-2H3 cell membranes.

Further evidence that CB_2-like (or CB_2) receptors mediate antinociception in the formalin paw test comes from results obtained by Hanus et al. (1999) with a CB_2-selective agonist, HU-308 (Fig. 10). They found that the late-phase response in this test could be suppressed in mice by intraperitoneal injection of HU-308, and that this effect was blocked by SR144528, but not by SR141716A. Similar results were obtained when the measured response was arachidonic acid-induced ear oedema, pointing to a role for CB_2 or CB_2-like receptors in the suppression of inflammation. On the other hand, HU-308 lacked activity in the mouse hot plate test and also differed from CB_1 receptor agonists in not inducing hypokinesia, hypothermia or catalepsy in mice. Unlike palmitylethanolamide, HU-308 is a potent CB_2 receptor agonist. However, whether it produces antinociception in the formalin paw test through CB_2 or through CB_2-like receptors remains to be established.

Although results obtained with palmitylethanolamide and HU-308 suggest that antinociception is mediated by CB_2 or CB_2-like receptors in the formalin paw test but not in the hot plate test, the extent to which these receptors mediate antinociception in other pain models is unknown. It is noteworthy, however, that experiments with rats have shown that palmitylethanolamide not only produces antinociception in the formalin paw test (late-phase), but also reverses signs of persistent pain resulting from turpentine-induced inflammation of the urinary bladder (Jaggar et al., 1998a). Similar results were obtained with anandamide. Palmitylethanolamide induces antinociceptive responses in mice or rats both after i.pl. injection and when administered i.p., i.v. or i.a. (Calignano et al., 1998; Jaggar et al., 1998a). However, the overall distribution pattern of CB_2/CB_2-like receptors in pain pathways remains to be established as does the precise role of these receptors in antinociception. Although it remains possible that

palmitylethanolamide is an agonist for the putative CB_2-like receptor, it could also be that it is an indirect agonist that acts by enhancing the extracellular concentration of an endogenous cannabinoid through an inhibitory effect on tissue uptake or metabolism (Mechoulam et al., 1998).

Another cannabinoid that may induce anti-inflammatory and analgesic effects by acting through CB_2 or CB_2-like receptors is 1′,1′-dimethylheptyl-Δ^8-THC-11-oic acid (DMH-Δ^8-THC-11-oic acid). This agent has been reported to decrease adhesion of mouse peritoneal leukocytes and to suppress mouse paw oedema induced by intraplantar platelet activating factor or arachidonic acid at oral doses as low as 0.01 or 0.05 mg/kg (Burstein et al., 1992). On repeated oral administration, DMH-Δ^8-THC-11-oic acid (0.1 or 0.2 mg/kg) has also been found to reduce signs of inflammation in rodent models of both acute inflammation (elevated leukocyte counts induced by injection of interleukin-1β and tumour necrosis factor α into subcutaneous air pouches formed on the backs of mice) and chronic inflammation (paw redness, oedema, hyperalgesia and ankylosis induced in rats by intraplantar injection of Freund's complete adjuvant) (Zurier et al., 1998). There are also reports that orally or intravenously administered DMH-Δ^8-THC-11-oic acid produces antinociception in mouse hot plate and phenylbenzoquinone abdominal stretch tests, with intravenous ED_{50} values of 4.31 mg/kg and 1.24 mg/kg respectively and that, at 4.6 mg/kg i.v., the 11-oic acid suppresses both early- and late-phase nociceptive responses in the mouse formalin paw test (Burstein et al., 1992, 1998). Evidence that these effects may be mediated by CB_2 (or CB_2-like) receptors rather than CB_1 receptors comes from the observations firstly, that DMH-Δ^8-THC-11-oic acid does not induce any sign of CB_1 receptor agonism (catalepsy) in mice at anti-inflammatory/antinociceptive doses (Burstein et al., 1992; Burstein, 1999) and secondly, that this agent is more potent as an inhibitor of forskolin-stimulated cyclic AMP production in cultured cells transfected with CB_2 receptors ($EC_{50} = 116.2$ nM) than in cells transfected with CB_1 receptors ($EC_{50} = 927$ nM) (Rhee et al., 1997). It is noteworthy, however, that the affinity of DMH-Δ^8-THC-11-oic acid for CB_2 receptors is approximately five times less than that for CB_1 receptors (Rhee et al., 1997). Also, DMH-Δ^8-THC-11-oic acid differs from the CB_2-selective agonist, HU-308 (Hanus et al., 1999), in showing activity in the mouse hot plate test. Whether any of the in vitro or in vivo effects of DMH-Δ^8-THC-11-oic acid can be antagonized by SR144528 (or SR141716A) has yet to be established. In the meantime, it remains possible that this agent produces its anti-inflammatory effects by suppressing eicosanoid synthesis in inflamed tissue through inhibition of COX-2, an enzyme that is thought to be activated during inflammation and to be responsible for the production of eicosanoids that act as inflammatory mediators. Thus there are reports that, at concentrations of 1 μM or above, DMH-Δ^8-THC-11-oic acid can inhibit eicosanoid synthesis in various in vitro preparations (Burstein et al., 1992; Zurier et al., 1998) and that it can inhibit COX-2 at concentrations having no effect on COX-1 activity (1–25 μM) (Zurier et al., 1998). Because of its selectivity for COX-2, it is possible that DMH-Δ^8-THC-11-oic acid may be able to relieve inflammation without producing unwanted effects such as gastrointestinal and kidney toxicity that limit the clinical usefulness of less selective non-steroidal anti-inflammatory drugs (Burstein et al., 1998; Burstein, 1999).

4.5. Novel 'anandamide' receptors

Differences that they have observed between some of the pharmacological properties of eicosanoid and non-eicosanoid cannabinoids have led Wagner et al. (1999) to propose the existence of an SR141716A-sensitive anandamide receptor that is distinct from the CB_1 receptor. This proposal was based on the observation firstly, that in rat isolated buffer-perfused precontracted mesenteric arterial bed, vasodilation could be readily induced by anandamide and methanandamide but not by the established CB_1/CB_2 agonists, Δ^9-THC, HU-210 and (+)-WIN55212, and secondly, that the effect of anandamide was antagonized by 0.5 or 5 μM SR141716A. The dextral shift induced by 0.5 μM SR141716A in the log concentration response curve of anandamide was 8.3-fold. Insertion of this value into the Schild equation (Tallarida et al., 1979) yields a K_B value for SR141716A of 68.5 nM which is somewhat greater than expected for a CB_1 receptor-mediated effect (Rinaldi-Carmona et al., 1994; Pertwee, 1999a). However, this K_B value should be viewed with caution as Wagner et al. (1999) also obtained evidence that the anandamide-induced vasodilation they observed was mediated not only by an SR141716A-sensitive mechanism (on the endothelium) but also by an SR141716A-resistant, endothelium-independent mechanism (in vascular smooth muscle). It is possible that similar mechanisms contribute to anandamide-induced antinociception. Thus, although there is no doubt that anandamide is a CB_1 receptor agonist, there is growing evidence that the mechanisms that underlie its antinociceptive effects differ from those of non-eicosanoid cannabinoids. Some of this evidence is summarized in section 4.1. In addition, Adams et al. (1995) have shown that for anandamide and 16 of its structural analogues, the correlation coefficient for a plot of log

ED_{50} in the mouse tail flick test against log K_i for displacement of [^{3}H]CP55940 from CB_1 binding sites on rat brain membranes is not statistically significant ($r = 0.46$). This finding contrasts with an earlier report by Compton et al. (1993) that a significant correlation does exist between these two measures for 26 non-eicosanoid cannabinoids ($r = 0.9$). Whilst this discrepancy may be attributable to pharmacokinetic factors another possibility is that it reflects the presence in pain pathways of different cannabinoid receptor subtypes. Under some conditions SR141716A is significantly less potent against anandamide-induced antinociception than against non-eicosanoid CB_1 receptor agonists, whilst under other conditions it fails to antagonize anandamide altogether (Table 8). Thus Adams et al. (1998) have found that SR141716A does not antagonize anandamide in the mouse tail flick test when administered at intravenous doses even greater than those reported by Compton et al. (1996) to attenuate antinociception induced by Δ^9-THC. Similarly, Smith et al. (1998b) have reported that SR141716A (10 mg/kg i.p.) attenuates antinociception in the rat paw pressure test induced by Δ^9-THC (5 mg/kg i.p.) without affecting that induced by anandamide (40 mg/kg i.p.).

Whether anandamide does indeed act through a novel type of SR141716A-resistant receptor remains to be established. If it does, this receptor is probably not CB_2 (or CB_2-like). Thus anandamide has much lower efficacy at CB_2 than CB_1 receptors, lacking detectable CB_2 activity altogether in some bioassay systems (Pertwee, 1999a). It is possible, however, that anandamide modulates nociception by acting on vanilloid receptors.

4.6. Anandamide and vanilloid receptors

Results obtained from an investigation into the mechanism underlying the ability anandamide and its synthetic analogue, methanandamide, to produce endothelium-independent relaxation of precontracted strips of rat hepatic artery, rat small mesenteric artery and guinea-pig basilar artery have led Zygmunt et al. (1999) to conclude that both these fatty acid amides produce their vasorelaxant effect in these preparations by acting on vanilloid receptors. The evidence for this conclusion comes from the observations firstly, that the competitive vanilloid receptor antagonist, capsazepine, attenuated the vasodilator effects of capsaicin, anandamide and methanandamide, secondly, that capsazepine was equipotent against capsaicin- and methanandamide-induced relaxation of precontracted rat hepatic artery, thirdly, that capsaicin desensitized arterial strips to the relaxant effect of anandamide but not to that of the non-cannabinoids, acetylcholine and calcitonin gene-related peptide (CGRP) and, fourthly, that anandamide and methanandamide are structurally related to capsaicin.

The vasorelaxant effect of anandamide and methanandamide in the arterial preparations used by Zygmunt et al. (1999) does not seem to be mediated by CB_1, CB_2 or CB_2-like receptors as the ability to produce this effect did not extend to the potent CB_1/CB_2 receptor agonists, HU-210 and (+)-WIN55212, or to palmitylethanolamide at concentrations of 1 or 10 μM, and as anandamide- and methanandamide-induced vasorelaxation was not antagonized by 0.3 μM SR141716A. As the vasodilator response to capsaicin was found to be attenuated by 10 μM SR141716A (Zygmunt et al., 1999), it is important to note that results from binding experiments with guinea-pig brain membranes have shown that capsaicin does not have significant affinity for CB_1 receptors (Di Marzo et al., 1998a). The metabolites of anandamide are arachidonic acid and ethanolamine. However, results from control experiments and the inclusion of indomethacin and N^G-nitro-L-arginine in all the experiments with arterial strips make it unlikely that the vasorelaxant effects of anandamide were mediated by ethanolamine or arachidonic acid, or by cyclooxygenase products or nitric oxide.

Further support for the hypothesis that anandamide is a vanilloid receptor agonist comes from the demonstration that it induces vanilloid-characteristic outwardly rectifying cationic currents in HEK293 cells or *Xenopus* oocytes transfected with rat vanilloid receptors but not in untransfected cells and that its effect in transfected cells can be completely reversed by capsazepine (Zygmunt et al., 1999). In addition, Zygmunt et al. (1999) have found that in strips of rat hepatic artery, anandamide shares the ability of capsaicin to elicit release of CGRP, a presumed mediator of the vasodilation that both these agents produce. There has also been a report that anandamide induces a capsazepine-sensitive activation of human vanilloid receptors expressed by HEK293 cells (Smart et al., 2000). As in the experiments with rat vanilloid receptors, the human receptor was also activated by methanandamide and was unresponsive to non-eicosanoid cannabinoid receptor agonists (CP55940 and (+)-WIN55212). Unlike the rat vanilloid receptor, however, the human receptor responded to palmitylethanolamide, albeit more weakly than to anandamide. Smart et al. (2000) have also detected signs of anandamide-induced activation of vanilloid receptors in neonatal rat cultured dorsal root ganglia.

These investigations provide evidence for a non-CB_1/non-CB_2 receptor for anandamide in primary afferent neurones and arterial tissue and support the hypothesis that this receptor is a vanilloid receptor located on primary sensory neurones. It follows that anandamide

may serve as a physiological mediator not only at cannabinoid receptors but also at vanilloid receptors which, if true, will require a rethinking of cannabinoid and vanilloid receptor nomenclature. In some preparations, exogenously administered anandamide only produced detectable activation of vanilloid receptors at concentrations approaching 1 μM, and in other preparations was even less potent. It will now be important to establish whether endogenously produced anandamide ever reaches vanilloid receptors in sufficiently high concentrations to activate these receptors under physiological or pathophysiological conditions. Methanandamide, is more resistant than anandamide to enzymic hydrolysis (Martin et al., 1999b; Pertwee, 1999a) and yet was found to be no more potent or effective than anandamide as a vanilloid receptor agonist (Zygmunt et al., 1999; Smart et al., 2000). However, this may be because methanandamide has less affinity or efficacy at vanilloid receptors than anandamide. This possibility remains be investigated as indeed do the structure–activity relationships of anandamide analogues in general at vanilloid receptors. In the meantime the possibility that anandamide has rather low potency at vanilloid receptors in the preparations that have so far been used because it is being metabolized or removed from the extracellular space by a transporter mechanism cannot be excluded. It will also be important to investigate whether anandamide can modulate nociception by acting through vanilloid receptors.

5. Sites of action

5.1. Central sites of action

There is good evidence that cannabinoids produce antinociception by acting on sites located within both brain and spinal cord. Thus Δ^9-THC, CP55940, (+)-WIN55212, anandamide and certain analogues of these cannabinoid receptor agonists have been reported to produce antinociception in mice or rats when injected intrathecally or intracerebroventricularly (Tables 2 and 3, Section 2). Although some of these studies were conducted using the hot plate test, most made use of the tail flick test. There are also reports that after intrathecal administration, Δ^9-THC, CP55940, anandamide and fluoroanandamide show activity in the mouse phenylbenzoquinone abdominal stretch test (Welch et al., 1995a) or rat formalin paw test (Hohmann et al., 1999b) and that intrathecal injection of an antinociceptive dose of CP55940 (80 μg) can suppress noxious stimulus-evoked Fos protein-like immunoreactivity in some areas of the spinal cord including the neck region of the dorsal horn (Hohmann et al., 1999b). It seems likely that the predominant site of action of cannabinoids is within the brain when they are injected intracerebroventricularly as Martin et al. (1993b) have found that following intracerebroventricular administration of [^{3}H](+)-WIN55212 to rats, 99.9% of recovered radioactivity was in the brain and only 0.06% in the spinal cord. It also seems likely that cannabinoids act within the spinal cord when they are injected intrathecally. Thus Lichtman and Martin (1991b) have found intrathecal CP55940 to produce detectable antinociception in the tail flick test not only in rats with intact spinal cords but also in animals that have received a laminectomy between the sixth and seventh thoracic vertebrae. These data support a spinal site of action as spinal transection is expected to abolish descending inhibitory neural input from the brain. Similarly, it has been reported that transection of the spinal cord of mice fails to prevent the production of antinociception by intrathecal Δ^9-THC in the tail flick test (Smith and Martin, 1992). Some rostral diffusion of cannabinoids from spinal cord to pain centres in the brain does seem to occur after intrathecal administration. Thus Smith and Martin (1992) detected significant levels of radioactivity in the brains of mice that had been injected intrathecally with [^{3}H]Δ^9-THC and Lichtman and Martin (1991b) found that spinal transection reduces the degree of antinociception produced in rats by intrathecal CP55940.

Experiments with rats have revealed several sites within the brain at which cannabinoids can produce signs of antinociception in the tail flick test when injected through stereotaxically implanted cannulae (Fig. 12). More specifically, antinociceptive effects have been observed after injection of CP55940 into the ven-

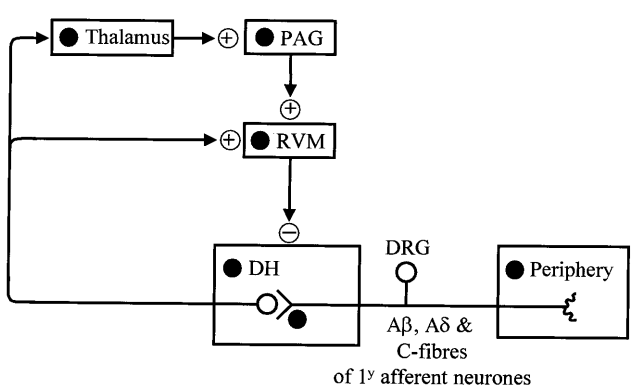


Fig. 12. Sites at which cannabinoids act through CB_1 receptors to induce antinociception in rats or mice. Other types of cannabinoid receptor may also mediate antinociception at some of these sites. Other brain areas that contain CB_1 receptors through which cannabinoids act to induce antinociception are the amygdala and the superior colliculus.

trolateral aspect of the periaqueductal grey in the region of the dorsal raphé (Lichtman et al., 1996), of HU-210 into the rostral ventromedial medulla (Martin et al., 1998) and of (+)-WIN55212 into the submedius and lateral posterior nuclei of the thalamus, the superior colliculus, the central and basolateral nuclei of the amygdala, the periaqueductal grey, the A5 noradrenergic group in the brainstem and the rostral ventromedial medulla (Martin et al., 1999c, 1998). Several rat brain areas have also been identified in which cannabinoids do not elicit detectable activity in the tail flick test. For CP55940, these are anterior ventrolateral and posterior dorsolateral areas of the periaqueductal grey and the caudate putamen (Lichtman et al., 1996) and for (+)-WIN55212, the cingulate cortex, nucleus accumbens, lateral hypothalamus, anterior pretectal, substantia nigra and cuneiform nucleus, several thalamic nuclei (ventral lateral, ventral medial, posterior, parafascicular and intralaminar) and brain stem sites dorsal to the rostral ventromedial medulla (Martin et al., 1999c, 1998).

There is good evidence that the antinociception observed after injection of cannabinoids into the cerebral ventricles or spinal cord or into discrete brain areas is mediated by CB_1 receptors. In particular, when injected by one or other of these routes, cannabinoids can readily be antagonized in the mouse or rat tail flick test by centrally or peripherally administered SR141716A (Table 6), by antisense oligodeoxynucleotide directed against the CB_1 receptor (section 3) or by drugs expected to impair CB_1 receptor signalling, including pertussis toxin (sections 3, 4.1 and 4.2). There are also several reports that when injected centrally, enantiomeric pairs of chiral cannabinoid receptor agonists exhibit marked stereoselectivity in the mouse or rat tail flick test, and that the enantiomer with the higher CB_1 receptor affinity has the greater antinociceptive activity (Table 7). It is noteworthy, however, that for one enantiomeric pair, CP55940 and CP56667, a correlation between antinociceptive potency and CB_1 affinity has not been observed in all investigations (sections 3 and 4.1).

Results both from autoradiographic experiments with [^{3}H]CP55940 or [^{3}H]11-OH-Δ^9-THC-dimethylheptyl and from immunocytochemical experiments with CB_1 receptor antibodies point to the presence of cannabinoid receptors in several areas of the brain at which cannabinoids have been demonstrated to induce antinociception (see above). In particular, the autoradiographic data support the presence of cannabinoid receptors in rat or human thalamus, superior colliculus, amygdala and periaqueductal grey (Glass et al., 1997; Herkenham et al., 1991; Mailleux and Vanderhaeghen, 1992; Thomas et al., 1992), whilst rat brain areas shown in immunocytochemical experiments to express CB_1 receptors include superior colliculus, central and basolateral nuclei of the amygdala, and rostral and caudal periaqueductal grey (Tsou et al., 1998). Some investigations have also provided evidence for the presence of cannabinoid receptors in a nociceptive area of the spinal cord, the dorsal horn/substantia gelatinosa/lamina X (Glass et al., 1997; Herkenham et al., 1991; Mailleux and Vanderhaeghen, 1992; Ong and Mackie, 1999b; Tsou et al., 1998; Sañudo-Peña et al., 1999). This evidence is in line with reports that membrane preparations of mouse and rat spinal cord contain high-affinity specific binding sites for [^{3}H]SR141716A (Richardson et al., 1998a) or [^{3}H]CP55940 (Richardson et al., 1998b; Welch et al., 1995a). Hohmann et al. (1998) have obtained further evidence that cannabinoid receptors that can mediate an antinociceptive effect are present on neurones in the dorsal horn of the spinal cord. They found that direct administration of (+)-WIN55212 to the dorsal surface of the spinal cord of urethane-anaesthetized rats suppressed responses evoked in wide dynamic range neurones in the lumbar dorsal horn by the application of noxious heat to the ipsilateral hind paw. This effect appeared to be CB_1-mediated as it could be attenuated by SR141716A (1 mg/kg i.v.) and as it was not produced by the inactive (−)-enantiomer of WIN55212. Whilst data that have been obtained from immunocytochemical experiments with CB_1 receptor antibodies or from binding experiments with [^{3}H]SR141716A point to the presence of CB_1 receptors on pain pathways, those that have been obtained from binding experiments using [^{3}H]CP55940 are less informative as this ligand has similar affinities for CB_1 and CB_2 binding sites (Pertwee, 1999a).

Two other investigations have provided further evidence that cannabinoids can induce a selective modulation of the processing of nociceptive stimuli in brain and spinal cord. Tsou et al. (1996) found intraperitoneal (+)-WIN55212 to decrease c-*fos* expression that had been evoked in the spinal ventral horn and in the superficial and neck regions of the spinal dorsal horn by injection of formalin into the plantar surface of the rat hind paw. No such changes in c-*fos* expression were detected after (−)-WIN55212 administration and neither enantiomer affected c-*fos* expression in the nucleus proprius. In the other study, Martin et al. (1996) found that when injected intravenously, (+)- but not (−)-WIN55212 could suppress neuronal activity in the ventroposterolateral nucleus of the thalamus that had been induced in urethane-anaesthetized rats by application of a noxious pressure stimulus to the contralateral hind paw. That this was part of a non-specific depressant effect on neuronal activity is unlikely as the activation of non-nociceptive low-threshold mechanosensitive neurones of the ventroposterolateral nucleus was not suppressed by (+)-WIN55212.

Recent data obtained by Strangman and Walker (1999) suggest one possible cause for the higher anti-

nociceptive potency of cannabinoids in animals sensitized to noxious stimuli (section 2.2). They investigated the effect of (+)-WIN55212 on 'wind-up', a centrally mediated increase in both the frequency and duration of spinal nociceptive responses that is induced by repetitive low-frequency noxious electrical stimulation of C-fibres and that may possibly contribute to the development of hyperalgesia and allodynia associated with chronic pain. They found that intravenous (+)-WIN55212 inhibited the 'wind-up' of wide dynamic range and nociceptive-specific neurones of the lumbar dorsal horn of anaesthetized, laminectomized rats that had been induced by repeated noxious transcutaneous electrical stimulation. This it could do in a stereoselective manner and at a dose that was subeffective for the inhibition of responses of C-fibres to single stimuli. In this respect (+)-WIN55212 differs from morphine which induces wind-up inhibition only at doses that also suppress the acute C-fibre response (Strangman and Walker, 1999).

5.2. Effects on descending control of spinal nociceptive neurones

Meng et al. (1998) have investigated the effect of intravenous (+)-WIN55212 on the activity of subpopulations of neurones in the rat rostral ventromedial medulla. These are neurones that exhibit either a pause in activity ('off' cells) or a burst of activity ('on' cells) just before the tail is withdrawn in response to a noxious radiant heat stimulus. Both the activity pause of 'off' neurones and the activity burst of 'on' neurones were eliminated by (+)-WIN55212 in anaesthetized rats and this effect could be reversed by intravenous injection of the CB_1-selective antagonist, SR141716A. Spinal nociceptive neurotransmission is facilitated by the 'on' cells and inhibited by the 'off' cells that project to the dorsal horn. Consequently, these results suggest that (+)-WIN55212 may induce antinociception by acting through CB_1 receptors to modulate descending control exerted on spinal nociceptive neurones by the rostral ventromedial medulla. A similar mechanism has been proposed for opioids. However, results from experiments with naloxone suggest that the effects of (+)-WIN55212 on the activity of neurones of the rostral ventromedial medulla are not mediated by endogenous opioids (Meng et al., 1998). By itself, intravenous SR141716A produced a hyperalgesic response in the tail flick test and prolonged the activity pause of 'off' neurones (sections 9.1 and 9.5).

Other evidence that cannabinoids modulate the activity of neurones projecting from brain to spinal cord comes from an investigation in which the measured response was the activity of wide dynamic range neurones of the lumbar dorsal horn of urethane-anaesthetized rats (Hohmann et al., 1999c). This investigation showed that (+)-WIN55212 could suppress activity evoked in these neurones by the application of noxious heat to the hind paw not only when this cannabinoid was given intravenously but also when it was injected directly into the cerebral ventricular system. Moreover, it was found that the ability of intravenous (+)-WIN55212 to suppress noxious heat-evoked activity of these neurones could be abolished by spinal transection. The suppressant effect of (+)-WIN55212 observed in intact rats was CB_1-receptor mediated as it was not produced by (−)-WIN55212 and could be prevented by SR141716A. CP55940 also suppressed noxious heat-evoked activity of wide dynamic range neurones. However, it did not affect pressure-evoked activity in nonnociceptive mechanosensitive neurones of the lumbar dorsal horn, suggesting that it was acting selectively on pain-sensitive neurones.

5.3. Effects on primary afferent neurones

High affinity specific binding sites for [^{3}H]CP55940 have been detected not only within the spinal cord (section 5.1) but also in rat trigeminal ganglia (Richardson et al., 1998b), pointing to the presence of cannabinoid receptors on primary afferent neurones. It is likely that these are CB_1 receptors. Thus, using in situ hybridization histochemistry, Hohmann and Herkenham (1999a,b) have demonstrated the presence of CB_1 but not CB_2 receptor mRNA in adult rat dorsal root ganglia. The results they obtained suggest that CB_1 receptor mRNA is present in 10–15% of all neurones in these ganglia. Immunocytochemical experiments with CB_1 receptor antibodies have also revealed the presence of cannabinoid receptors in rat dorsal root ganglia as well as in rat dorsal roots (Sañudo-Peña et al., 1999). Additional evidence for the presence of cannabinoid receptors on primary afferent neurones has been obtained by Ross et al. (1999). Using rabbit polyclonal antibodies raised against the first 77 amino acids of rat CB_1 receptors, they were able to detect cannabinoid receptors on the cell bodies and processes of neonatal rat cultured dorsal root ganglia by confocal microscopy. The presence of CB_1 receptors in this preparation was confirmed by fluorescence-activated cell sorting (FACS) analysis, performed with the same antibodies. Evidence that these receptors are functional comes from data obtained in electrophysiological experiments. These showed that (+)-WIN55212 (10 and 100 nM) could inhibit high voltage-activated Ca^{2+} currents in neonatal rat cultured dorsal root ganglia and that this effect was attenuated by SR141716A, albeit at a concentration that by itself enhanced Ca^{2+} currents (100 nM).

Using in vitro cannabinoid receptor binding and quantitative autoradiographic techniques, Hohmann

and Herkenham (1998) have found that only a minority of cannabinoid receptors within the spinal cord seem to be localized on small-diameter primary afferent fibres. They detected no more than a 16% decrease in cannabinoid receptor density in the superficial lumbar dorsal horn of 66 or 67 day old rats that had been treated with capsaicin 65 days previously so as to produce a selective depletion of sensory C-fibres. This observation prompted the proposal that most primary afferent neurones that express CB_1 mRNA are those with larger diameter fibres. Support for this hypothesis comes from another finding, by Hohmann and Herkenham (1999a) that CB_1 mRNA does not co-localize extensively in rat dorsal root ganglia with neuropeptides that are known to populate a significant proportion of small-diameter primary afferents. These neuropeptides are: (a) substance P which is thought to be expressed in $\sim 50\%$ of C-fibre neurones and 20% of Aδ fibre neurones and to be absent from Aα/Aβ-fibre neurones; (b) calcitonin gene-related peptide (CGRP) which is thought to be expressed in $\sim 50\%$ of C-fibre neurones, 33% of Aδ fibre neurones and 20% Aα/Aβ-fibre neurones; and (c) somatostatin, all of which is likely to be present in C-fibre neurones (Hohmann and Herkenham, 1999a). Only 24% of dorsal root ganglion neurones that express mRNA for CB_1 co-express substance P mRNA (Hohmann and Herkenham, 1999a). The same incidence of co-expression (24%) was observed for CB_1 and CGRP mRNA. This raises the possibility that some CB_1 receptors are present in neurones that synthesize both neuropeptides as almost all substance P-containing neurones co-localize CGRP (Hohmann and Herkenham, 1999a). Co-localization of CB_1 and somatostatin mRNA was minimal. Thus the majority of CB_1 mRNA was detected in dorsal root ganglion neurones that do not contain substance P, CGRP or somatostatin. These could be larger diameter Aβ- and/or Aδ-fibre neurones and may include large-diameter neurones that are known to express glutamate (Hohmann and Herkenham, 1999a). CB_1 receptors are also expressed by nerve growth factor-dependent neurones of chicken embryo dorsal root ganglia (Friedel et al., 1997). The presence of CB_1 receptors on Aβ- or Aδ-fibre afferent neurones would support a role for CB_1 receptors in neuropathic pain (Hohmann and Herkenham, 1999a). It is noteworthy that Strangman and Walker (1999) have obtained evidence that cannabinoids may be more potent in inhibiting C-fibre activity than Aβ- or Aδ-fibre activity in rat lumbar spinal dorsal horn. They found that responses of C-fibres to single noxious transcutaneous electrical stimuli could be suppressed by intravenous doses of (+)-WIN55212 that did not affect responses of Aβ- or Aδ-fibres to these stimuli.

An observation that unilateral dorsal rhizotomy across successive spinal segments from C3 to T1 or T2 induces marked ipsilateral loss of cannabinoid binding sites in the superficial dorsal horn provides evidence for the presence of cannabinoid receptors at the central terminals of primary afferent neurones (Hohmann et al., 1999a). A considerable number of cannabinoid binding sites remained in the dorsal horn after this rhizotomy-induced deafferentation, suggesting there to be a large population of postsynaptic cannabinoid receptors in this region of the spinal cord. These receptors may be present on the terminals of neurones that project from the brain and/or on intrinsic spinal neurones (Hohmann et al., 1999a).

Cannabinoid receptors also seem to be present on peripheral terminals of primary afferent neurones. Hohmann and Herkenham (1999b) have obtained evidence that cannabinoid receptors undergo anterograde axonal transport from dorsal root ganglia towards the peripheral terminals of sensory nerves. Their experiments showed that tight ligation of rat sciatic nerves caused a large build-up of cannabinoid receptors near the ligatures, on the proximal (central) side, as measured by in vitro receptor binding assays and by high-resolution emulsion autoradiography using [^{3}H]CP55940. A smaller accumulation of cannabinoid receptors was also detected on the distal (peripheral) side of the ligatures, indicating that some retrograde transport of cannabinoid receptors from peripheral terminals to the dorsal root ganglia for enzymic breakdown may have been occurring. When two ligatures were applied to the same nerve, only low levels of binding were detected between these ligatures, suggesting that cannabinoid receptors are not synthesized locally in the sciatic nerve. This is in line with results from experiments indicating the presence of cannabinoid (CB_1) receptor mRNA in dorsal root ganglia (see above).

Further support for the presence of CB_1 receptors at the peripheral terminals of primary afferent neurones comes from data suggesting that cannabinoids can induce signs of antinociception by acting through CB_1 receptors located in the skin. Richardson et al. (1998b,c) found that thermal hyperalgesia induced in rats by intraplantar injection of carrageenan into the hind paw could be suppressed by anandamide administered directly into carrageenan-treated paws (0.024 pg and above). Because of the rather low doses used in these experiments, it is likely that anandamide was acting close to the injection site and, consequently, that it can suppress thermal hyperalgesia by acting at sites outside the central nervous system. The antihyperalgesic effect of intraplantar anandamide (10 ng) was significantly attenuated by intraplantar SR141716A, co-administered at a reasonably low dose (100 ng) that by itself did not affect the nociceptive response, suggesting that this effect of anandamide is mediated by CB_1 receptors located in the hind paw (Richardson et al.,

1998c). Calignano et al. (1998) have reported that injection of low doses of (+)-WIN55212, anandamide or methanandamide directly into the hind paws of mice can suppress behavioural nociceptive responses induced by concomitant intraplantar injection of formalin (licking and flexing of the injected limb) (section 4.4). By themselves both SR141716A and SR144528 enhanced the early-phase response to formalin when injected intravenously and yet only SR141716A prevented the antinociceptive effects of (+)-WIN55212 and anandamide. Unlike anandamide, (+)-WIN55212 also inhibited the late-phase response to formalin and this effect too was prevented by SR141716A but not by SR144528. Finally, in experiments with rhesus monkeys subjected to a warm water tail withdrawal test, Ko and Woods (1999) found that thermal hyperalgesia induced by injection of capsaicin into the end of the tail could be suppressed by injection of low doses of Δ^9-THC into the same site. This effect of Δ^9-THC could be prevented by SR141716A when this was also injected into the tail at a dose that by itself did not affect nociception (100 μg).

6. Cannabinoids and endogenous mediators of nociception or inflammation

6.1. Excitatory and inhibitory amino acid transmitters

Vaughan et al. (1999, 2000) have postulated that, like opioids, cannabinoids may induce antinociception by reducing inhibitory GABAergic influences on output neurones that project from the periaqueductal grey and rostral ventromedial medulla. This hypothesis is based on evidence that cannabinoids act on presynaptic CB_1 receptors in these brain areas to inhibit the release of γ-aminobutyric acid (GABA). More specifically, they found that application of submicromolar concentrations of (+)-WIN55212, anandamide or methanandamide onto rat brain slices containing neurones from the periaqueductal grey or rostral ventromedial medulla reduced the amplitude of electrically-evoked GABAergic inhibitory postsynaptic currents ($EC_{50} = 520$ and 630 nM, respectively for (+)-WIN55212). This effect could be reversed by SR141716A, albeit at the rather high concentration of 3 μM. In contrast to μ-opioids, cannabinoids had no direct postsynaptic actions on neurons of the rostral ventromedial medulla or periaqueductal grey. In line with the hypothesis that cannabinoids may induce antinociception by inhibiting GABA release in the rostral ventromedial medulla, is a finding by Meng et al. (1998) that the ability of intravenous (+)-WIN55212 to induce antinociception in the rat tail flick test can be prevented by the $GABA_A$ receptor agonist, muscimol, injected directly into the rostral ventromedial medulla. It is unlikely that this interaction stems from any ability of this $GABA_A$ receptor agonist to prevent (+)-WIN55212 from impairing motor function as the same pretreatment with muscimol did not attenuate (+)-WIN55212-induced impairment of performance on a rotarod treadmill. That glutamate may also be involved in cannabinoid-induced antinociception is suggested by an observation that hyperalgesia induced by intrathecal SR141716A in the mouse hot plate test can be attenuated by intrathecal co-administration of either of two NMDA receptor antagonists, D-AP-5 and MK-801 (Richardson et al., 1998a). This finding is consistent with the hypothesis that increases in nociceptive thresholds induced by endogenously released (or exogenously administered) cannabinoids are due at least in part to a CB_1 receptor-mediated suppressant effect on glutamate release within the spinal cord and that SR141716A-induced hyperalgesia results from disinhibition of such release. It is noteworthy, however, that Thorat and Bhargava (1994b) have found intraperitoneal administration of MK-801 to attenuate Δ^9-THC-induced antinociception in the mouse tail flick test.

6.2. Peptides

There is evidence that cannabinoids may modulate neurogenic inflammation by inhibiting neurosecretion of the pro-inflammatory peptide, calcitonin gene-related peptide (CGRP), from central and peripheral terminals of capsaicin-sensitive primary afferent fibres. Thus Richardson et al. (1998b,c) have demonstrated that anandamide can inhibit potassium- and capsaicin-evoked release of immunoreactive calcitonin gene-related peptide (iCGRP) both from tissue obtained from the dorsal half of the lumbar enlargement of rat spinal cord (isolated rat spinal cord) and from an in vitro preparation of the dorsal skin of rat hind paw. No effect on basal release of iCGRP was detected in these experiments. It is likely that the effect observed in the spinal tissue was CB_1 receptor-mediated as anandamide inhibited capsaicin-evoked iCGRP release at a concentration as low as 1 nM and as this effect was readily abolished by SR141716A at just 10 nM (Richardson et al., 1998b). Anandamide may also have acted through receptors in the skin preparation as it was also active in this tissue at 1 nM. Although the identity of these receptors in the skin has yet to be explored, it is noteworthy that Zygmunt et al. (1999) have already obtained convincing evidence that anandamide can modulate release of CGRP in rat hepatic artery by acting through vanilloid receptors that are presumably located on the peripheral terminals of primary sensory neurones. In these experiments, however, anandamide was applied at a concentration of 10 μM and its effect on CGRP release was stimulatory rather than inhibitory. Non-eicosanoid cannabinoids do not seem to

act on vanilloid receptors to any significant extent (Smart et al., 2000; Zygmunt et al., 1999). However, whether cannabinoids other than anandamide can inhibit evoked CGRP release in the experimental models used by Richardson et al. (1998b,c) has yet to be investigated. Also still to be addressed is the question of whether cannabinoids modulate the neurosecretion of other pro-inflammatory agents. Candidates for further investigation include substance P, nerve growth factor and nitric oxide. Indeed, it has already been proposed by Jaggar et al. (1998b) that cannabinoids may act through neuronal CB_1 receptors to oppose the pro-inflammatory effects of nerve growth factor and there is evidence that CB_1 receptors mediate inhibition of nitric oxide production by rat microglial cells (Waksman et al., 1999). The possibility also remains that cannabinoids act through CB_2 or CB_2-like receptors on mast cells to prevent degranulation of these cells, thereby suppressing the release of peptide and non-peptide pro-inflammatory agents (Calignano et al., 1998; Facci et al., 1995; Jaggar et al., 1998b; Lambert and Di Marzo, 1999; Pertwee, 1999a).

7. Mediators of cannabinoid-induced antinociception

7.1. Monoamine neurotransmitters

Lichtman and Martin (1991a) have shown that the ability of intravenously administered Δ^9-THC to elevate tail flick latency can be attenuated by injection of yohimbine into the lumbar region of the rat spinal cord, suggesting that antinociception induced by cannabinoid receptor activation depends at least in part on the release from descending neurones of noradrenaline, acting on spinal α_2-adrenoceptors. In contrast, cannabinoid-induced antinociception does not seem to depend on 5-HT release in the spinal cord as Lichtman and Martin (1991a) also found antinociception induced by intravenous Δ^9-THC in the rat tail flick test was not significantly attenuated by intrathecal methysergide. Carta et al. (1999) have obtained evidence that Δ^9-THC-induced antinociception is mediated in rats by dopamine acting on dopamine D_2 receptors. Using the intraperitoneal route for all injections, they found that the antinociceptive effect of Δ^9-THC in hot plate and tail flick tests could be potentiated by selective D_2 receptor agonists and attenuated by selective D_2 receptor antagonists.

7.2. Opioid peptides

There is some evidence that cannabinoids may induce antinociception by acting through CB_1 receptors to enhance the release of opioids onto μ opioid receptors in the brain. Thus Reche et al. (1998) have found that combined intracerebroventricular administration of two inhibitors of opioid-degrading enzymes (thiorphan and bestatin) could potentiate antinociception in mouse tail flick and hot plate tests induced by intravenous Δ^9-THC. The potentiation in the hot plate test was prevented by pretreatment with SR141716A (2 mg/kg i.p.), indicating it to be CB_1-receptor mediated. It was also prevented by pretreatment with antagonists possessing selectivity for μ opioid receptors, naloxone (0.1 or 1 mg/kg s.c.) and β-funaltrexamine (2 nmol i.c.v.). No antagonism was observed following intracerebroventricular pretreatment with nor-binaltorphimine or subcutaneous pretreatment with the δ-selective opioid receptor antagonist, naltrindole (0.1 mg/kg). These findings are in line with an earlier report by Tulunay et al. (1981) that Δ^9-THC can be antagonized in the rat hot plate test by intracerebroventricular β-chlornaltrexamine.

There is also evidence that cannabinoid-induced antinociception may depend to some extent on the release of opioid peptides onto spinal κ opioid receptors and this is summarized below.

1. It has been reported that the ability of certain cannabinoid receptor agonists to produce antinociception in the mouse or rat tail flick test or mouse hot plate test following their systemic or intrathecal administration can be attenuated by intrathecal injection of the selective κ receptor antagonist, nor-binaltorphimine (Mason et al., 1999; Reche et al., 1996a; Smith et al., 1994a; Welch, 1993; Welch et al., 1995b), of antiserum for the endogenous selective κ-receptor agonists dynorphin A(1–8) and dynorphin A(1–17), or of α-neoendorphin antiserum (Pugh et al., 1997, 1996; Reche et al., 1996a). In contrast, no such attenuation has been detected after intrathecal injections of the selective δ-receptor antagonist, ICI174864, or of naloxone, which has some selectivity for μ-opioid receptors (Welch and Stevens, 1992; Welch et al., 1995b).
2. Pugh et al. (1995) have found that intrathecal injection of an antisense oligodeoxynucleotide directed against the κ_1 opioid receptor reduces antinociception induced in the mouse tail flick test by intrathecal Δ^9-THC. The same oligodeoxynucleotide also blocked the antinociceptive effect of a κ-receptor agonist (U50488H) but not that of selective μ- (DAMGO) or δ- (DPDPE) receptor agonists.
3. Mason et al. (1999) have found that an intrathecal dose of Δ^9-THC that induces antinociception in the rat tail flick test elevates levels of immunoreactive dynorphin A in cerebrospinal fluid collected from the spinal cords of the same animals. Both effects appeared to be CB_1 receptor mediated as they were attenuated by SR141716A (10 mg/kg i.p.). Whilst Δ^9-THC elevated spinal concentrations of dynorphin A, which is known to act on κ opioid receptors, it did not affect spinal concentrations of

leu-enkephalin, which is a dynorphin A metabolite and has selectivity for δ opioid receptors. Interestingly Mason et al. (1999) have found spinal concentrations of immunoreactive dynorphin A to be unaffected by antinociceptive intrathecal doses of two other cannabinoid receptor agonists, CP55940 and anandamide. Why this should be remains to be established. It is noteworthy, however, that although spinal κ opioid receptors may not mediate CP55940-induced antinociception in rats they may do so in mice. Thus, Pugh et al. (1997) have found that in mice, spinal concentrations of dynorphin B are increased by intrathecal CP55940, albeit at a dose 40 times higher than that at which it induces antinociception in the tail flick test. This opioid peptide shares the ability of dynorphin A to act on κ opioid receptors. In addition, Welch's group have found intrathecal nor-binaltorphimine to attenuate antinociception induced by intrathecal CP55940 in mice (Welch, 1993) but to be inactive against antinociception induced by intrathecal CP55940 (or anandamide) in rats (Mason et al., 1999).

4. There is one report that repeated subcutaneous treatment with a selective κ opioid receptor agonist (U50488H or CI-977) renders mice tolerant to the antinociceptive effects in the tail flick test of intrathecal challenges not only with U50488H or CI-977 but also with Δ^9-THC (Smith et al., 1994b). It has also been found that repeated subcutaneous treatment of mice with Δ^9-THC can induce tolerance to the antinociceptive effects in the tail flick test both of intrathecal Δ^9-THC and of intrathecal U50488H, CI-977 or dynorphins A(1–8), A(1–13) or A(1–17) (Smith et al., 1994b; Welch, 1997). Such pretreatment with Δ^9-THC did not induce tolerance to the antinociceptive effects of intrathecally injected agonists for μ (DAMGO) or δ opioid receptors (DPDPE) (Smith et al., 1994b). Pretreatment with an opioid (morphine) can also render mice and rats tolerant to the antinociceptive effect of Δ^9-THC when this is administered systemically instead of intrathecally (Bloom and Dewey, 1978; Hine, 1985; Thorat and Bhargava, 1994a). Similarly, Δ^9-THC can induce tolerance to the antinociceptive effect in mice and rats of systemically administered morphine as well as to its own antinociceptive effect (Hine, 1985; Kaymakcalan and Deneau, 1972; Pertwee, 1991; Thorat and Bhargava, 1994a). Tolerance to the antinociceptive effect of systemically administered morphine is not always observed in Δ^9-THC-tolerant mice (Martin, 1985a). Nor is tolerance to the antinociceptive effect of systemically or intrathecally administered Δ^9-THC always observed in morphine-tolerant mice (Martin, 1985a). There is also a more recent report that cross tolerance does not develop between the antinociceptive effects of intrathecal Δ^9-THC and morphine in a rat model of neuropathic pain (Mao et al., 2000).

5. Corchero et al. (1997) have detected increases in prodynorphin and proenkephalin gene expression in the spinal cords of rats that had received daily injections of Δ^9-THC (5 mg/kg i.p.) for 5 days. Repeated cannabinoid administration to rats also elevates proenkephalin gene expression in certain regions of the brain, including the periaqueductal grey area (Manzanares et al., 1998).

To explain their findings with Δ^9-THC, Mason et al. (1999) have proposed that when administered acutely into the spinal subarachnoid space, this cannabinoid enhances dynorphin A peptide release by acting through cannabinoid receptors in the substantia gelatinosa to inhibit tonically active neurones that exert an inhibitory effect on dynorphinergic neurones located within the outer laminae of the cord. They further propose that the dynorphin A peptides that are released in this way act on κ opioid receptors to suppress the release of neuropeptides such as substance P that are associated with nociceptive transmission so as to diminish the intensity of perceived noxious stimuli by retarding amplification of noxious transmission in the ascending pathways.

The extent to which opioid receptors are involved in antinociception induced by cannabinoids may vary with the type of noxious stimulus applied. Thus, Martin (1985a) reported that at 1 mg/kg s.c., naloxone antagonized Δ^9-THC in the mouse hot plate test. On the other hand, in mouse tail flick and phenylbenzoquinone abdominal stretch tests, he found naloxone to be effective against Δ^9-THC only at 10 or 20 mg/kg s.c. and to produce no detectable antagonism in the tail flick test at 5 mg/kg s.c. Similarly, Bhargava and Matwyshyn (1980) have found that naloxone at 1 mg/kg s.c. fails to antagonize Δ^9-THC in the mouse tail flick test whilst Meng et al. (1998) have reported that naloxone does not reduce the ability of (+)-WIN55212 to alter the firing rates of neurones of rat rostral ventromedial medulla that exhibit a pause in activity or an activity burst just before the tail is withdrawn in response to a noxious radiant heat stimulus. It has also been found that quadazocine, another opioid receptor antagonist with some selectivity for μ-opioid receptors, does not attenuate antinociception induced by Δ^9-THC in rhesus monkeys subjected to a warm water tail withdrawal test (Ko and Woods, 1999; Vivian et al., 1998). In these experiments all drugs were administered either directly into the tail together with capsaicin, or intramuscularly without capsaicin. These findings contrast with reports that at 1 mg/kg s.c., naloxone antagonizes 11-OH-Δ^8-THC in the mouse hot plate test (Wilson and May, 1975) and Δ^9-THC in mouse tail flick and hot plate tests (Reche et al., 1996a) and that at 5 mg/kg s.c. it antagonizes both Δ^9-THC and anandamide in a rat paw pressure test (Smith et al., 1998b).

8. Synergism between cannabinoids and opioids

Experiments with mice or rats using tail flick, hot plate or radiant heat paw withdrawal tests have shown that cannabinoids can interact synergistically with opioid receptor agonists in the production of antinociception. This synergism seems to be receptor-mediated since it can be blocked by both cannabinoid and opioid receptor antagonists (Cichewicz et al., 1999; Reche et al., 1996b; Smith et al., 1998a; Welch and Stevens, 1992). Although such synergism can occur when a cannabinoid and an opioid are coadministered peripherally (Cichewicz et al., 1999; Ghosh and Bhattacharya, 1979; Kaymakcalan and Deneau, 1972; Reche et al., 1996b; Smith et al., 1998a), it can also occur when the cannabinoid and opioid are both given intrathecally (Pugh et al., 1996; Welch, 1993; Welch and Stevens, 1992) or when both drugs or just the opioid are given intracerebroventricularly (Reche et al., 1996b; Welch et al., 1995b). This would suggest that cannabinoid-opioid synergism has both spinal and supraspinal components. The putative spinal component may stem from the combined abilities of opioids to activate spinal μ- or δ-receptors directly and of cannabinoids to release κ-opioids onto spinal κ-receptors (section 7.2) (Pugh et al., 1996, 1997). The evidence for this hypothesis comes from two sets of observations. First, it has been found that whilst opioid receptor agonists with μ- (morphine) or δ-selectivity (DPDPE), interact synergistically with cannabinoids in the mouse tail flick test when injected intrathecally, an opioid receptor agonist with κ-selectivity (U50488H) does not (Pugh et al., 1996; Welch, 1993; Welch and Stevens, 1992). Second, it has been shown that synergism between Δ^9-THC and morphine in the mouse tail flick or hot plate test can be blocked both by intrathecal injections of the κ-selective antagonist, nor-binaltorphimine and the δ receptor antagonist, naltrindole, and by intrathecal injections of antisera for the κ-selective endogenous opioid peptides, dynorphin A(1–8), dynorphin A(1–13) and dynorphin A(1–17) (Pugh et al., 1996; Reche et al., 1996b). Cichewicz et al. (1999) have also found that potentiation of orally administered morphine or codeine in the mouse tail flick test by a sub-effective oral dose of Δ^9-THC could be prevented by subcutaneous pretreatment with naloxone or naltrindole. However, subcutaneous pretreatment with nor-binaltorphimine did not prevent potentiation of morphine or codeine by Δ^9-THC. This finding suggests that although Δ^9-THC may trigger the release of opioid peptides onto κ opioid receptors when it is injected intrathecally (see above), this is not the only mechanism through which Δ^9-THC potentiates opioid-induced antinociception when it is administered orally. Indeed, there is already evidence that within the brain, cannabinoid-opioid synergism may be mediated to some extent by μ opioid receptors. Thus Reche et al. (1996b) have found that synergistic interactions observed between systemically administered Δ^9-THC and morphine in mouse tail flick and hot plate tests can be attenuated by intracerebroventricular injection of an opioid receptor antagonist with μ-selectivity (β-funaltrexamine). They also found that this interaction was not attenuated by intracerebroventricular injection of nor-binaltorphimine.

One apparent anomaly is that unlike several other cannabinoid receptor agonists, CP55940 does not enhance morphine-induced antinociception in the mouse tail flick test when it is injected intrathecally (Welch and Stevens, 1992). This finding lends further support to the hypothesis that mouse spinal cord contains a novel type or subtype of cannabinoid receptor (Pugh et al., 1997 and sections 4.1–4.2). It should be noted, however, that synergism between CP55940 and morphine has been detected in the mouse tail flick test when both drugs are injected intracerebroventricularly (Welch et al., 1995b). Also noteworthy is a recent report that an intrathecal dose of L-nantradol that induces antinociception in the rat tail flick test elevates levels of immunoreactive dynorphin B in cerebrospinal fluid without affecting levels of immunoreactive dynorphin A, leu-enkephalin or met-enkephalin (Welch and Eads, 1999). This effect, which was not produced by D-nantradol, is unlikely to account for the ability of L-nantradol to interact synergistically with morphine in the rat tail flick test as unlike dynorphin A, dynorphin B does not enhance morphine-induced antinociception (Welch and Eads, 1999).

9. Role of the endocannabinoid system in nociception

The evidence that cannabinoid receptors can mediate antinociceptive effects raises the possibility that the endocannabinoid system has a physiological and/or pathophysiological role in nociception. The availability of the selective CB_1 and CB_2 receptor antagonists, SR141716A and SR144528, and of oligonucleotides directed against CB_1 or CB_2 receptors, has made it possible to test this hypothesis by establishing whether any of these agents can induce signs of hyperalgesia when administered by themselves. A number of such experiments have already been performed using a wide range of pain models (sections 9.1–9.4). Other possible strategies for exploring the role of the endocannabinoid system in nociception are to look for endogenous cannabinoid release in the vicinity of nociceptive neurones, to investigate nociceptive responses of CB_1 or CB_2 knockout mice and to establish whether nociception is affected by agents that delay removal of endogenous cannabinoids from their sites of action through inhibitory effects on tissue uptake or metabolism. Results from experiments that have adopted one or other of these strategies are discussed in sections 9.6–9.7.

There are at least two possible mechanisms by which SR141716A or SR144528 might modulate the activity of the endocannabinoid system to alter nociception. One of these assumes that nociception is modulated by endogenous cannabinoid(s) released onto cannabinoid receptors and relies on the ability of SR141716A or SR144528 to antagonize this modulatory effect by competing for these receptors. Whether such antagonism would be observed for SR141716A against anandamide in all pain models is unlikely. Thus although SR141716A has been found block antinociceptive responses to intrathecal anandamide in the mouse tail flick test and to intraplantar anandamide in rat carrageenan and mouse formalin paw tests (Calignano et al., 1998; Richardson et al., 1998c; Welch et al., 1998) there are also reports that SR141716A does not attenuate antinociceptive responses to intravenous or intraperitoneal anandamide in the mouse tail flick test or in a rat paw pressure test (section 4.5). SR144528 is also of questionable value as an experimental tool for revealing responses induced by endogenously release anandamide. Thus there is no evidence that this fatty acid amide acts through CB_2 (or CB_2-like) receptors to produce antinociception and, in any case, anandamide has relatively low CB_2 efficacy (Pertwee, 1999a). The second mechanism springs from evidence that SR141716A and SR144528 may be inverse agonists rather than pure antagonists (Bouaboula et al., 1997; Coutts et al., 2000; Kearn et al., 1999; Pan et al., 1998; Pertwee, 1999a). This mechanism is based on the assumptions that cannabinoid receptors responsible for modulating nociception can exist in at least two interchangeable states, one precoupled to and the other uncoupled from the effector system and that inverse agonists bind preferentially to the uncoupled receptors so as to shift the equilibrium away from the receptors in the precoupled state (Fig. 13). Most experiments with antagonists have so far been carried out with SR141716A and as detailed in Table 9 and sections 9.1–9.5, these have yielded somewhat inconsistent results. Why this should be remains to be established.

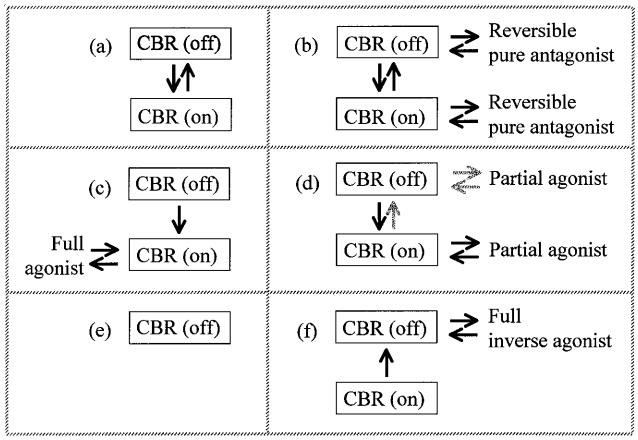


Fig. 13. The two-state model. According to this model: (a) a proportion of cannabinoid receptors can exist in two interchangeable states, a constitutively active 'on' state in which they can activate the effector system even in the absence of an agonist and an 'off' state which is not constitutively active; (b) a pure antagonist has equal affinity for both receptor states, so leaving the equilibrium between 'on' and 'off' states unchanged; (c) a full agonist has markedly higher affinity for the 'on' state so shifting the equilibrium towards this state; (d) a partial agonist also has higher affinity for the 'on' state but produces less of a shift towards this state than a full agonist as it also has significant affinity for the 'off' state; (e) under some conditions or at some locations cannabinoid receptors may exist entirely in the 'off' state; and (f) an inverse agonist has markedly higher affinity for the 'off' state so shifting the equilibrium towards this state. A more complex model to explain the actions of cannabinoid receptor inverse agonists has been proposed by Bouaboula et al. (1997).

9.1. Effects of SR141716A on radiant heat or warm water tail flick responses in rats, mice and monkeys

There have been two investigations, both with rats, in which SR141716A has been found to elicit a hyperalgesic response in the tail flick test. In one of these SR141716A was administered at a dose of 0.5 mg/kg i.v. (Meng et al., 1998) and in the other at a dose of 5 mg/kg i.p. (Costa and Colleoni, 1999). However, hyperalgesic responses to SR141716A have not been noted in other tail flick experiments with rats, mice or monkeys. Compton et al. (1996) found that SR141716A had no effect on the tail flick response when given to mice at a dose that blocked Δ^9-THC-induced antinociception in this bioassay (3 mg/kg i.v.). Indeed, at a higher dose (30 mg/kg i.v.), SR141716A exhibited antinociceptive activity, possibly reflecting non-specific interactions with phospholipids in neuronal membranes at this high dose. Similarly, Rinaldi-Carmona et al. (1994) reported SR141716A to have no effect on the mouse tail flick response at doses that blocked antinociception induced by (+)-WIN55212. There are also reports that SR141716A, given intrathecally to mice or intraperitoneally to rats, has no effect on the tail flick response at doses that block antinociceptive responses to intrathecally administered Δ^9-THC, CP55940 or other cannabinoids (Mason et al., 1999; Welch et al., 1998). SR141716A has also been found to be without effect in the rat tail flick test when injected directly into the rostral ventromedial medulla at a dose that blocked antinociception induced by HU-210 injected into the same brain site (Martin et al., 1998). Finally, Vivian et al. (1998) have reported SR141716A to have no effect on nociception in the warm water tail withdrawal test when given to rhesus monkeys at a dose that blocked antinociception induced in this bioassay by Δ^9-THC or (+)-WIN55212.

9.2. Effects of blocking agents on responses of unlesioned rodent paws to heat or mechanical pressure stimuli

Richardson et al. (1997, 1998a) found that hyperalgesia could be induced in the mouse hot plate test by intrathecal injection of either SR141716A or an oligonucleotide directed against the CB_1 receptor, raising the possibility that the underlying cause of some chronic pain states may be hypoactivity of the endocannabinoid system. The effect of SR141716A was dose-related ($ED_{50} = 0.0006$ pg). In contrast, Martin et al. (1999d) found that intrathecal SR141716A (1–30 μg) did not affect the sensitivity of uninflamed rat hind paws to mechanical stimuli (see also section 9.3). Other investigators have also failed to detect effects of SR141716A on nociception by uninjured rodent paws. Herzberg et al. (1997) found that at 0.5 mg/kg i.p., SR141716A had no effect on the sensitivity of rat hind paws to heat stimuli or mechanical pressure. This dose of SR141716A was sufficient to oppose the ability of (+)-WIN55212 to suppress responses to the same stimuli of paws that had been rendered hypersensitive to noxious stimuli by unilateral sciatic nerve ligation (see also section 9.4). Smith et al. (1998b) found SR141716A to have no effect on the sensitivity of uninflamed hind paws of rats to mechanical pressure when it was administered at a dose (10 mg/kg i.p.) that blocked the antinociceptive effect of Δ^9-THC in the same test.

9.3. Effects of SR141716A and SR144528 in models of inflammatory pain

Calignano et al. (1998) have found SR141716A (0.1 mg/kg i.v. or 10 μg i.pl.) and SR144528 (0.1 mg/kg i.v.) to enhance nociceptive responses of mouse hind paws (paw licking/flexing) that have been rendered hyperalgesic by intraplantar injection of formalin. Both early- and late-phase responses to formalin were affected by SR141716A whereas SR144528 affected only the early-phase response. Similarly, Strangman et al. (1998) have found that SR141716A (1 mg/kg i.p.) can induce signs

Table 9
Ability of SR141716A to induce signs of inflammation/hyperalgesia in certain animal models of inflammation or of inflammatory or neuropathic pain[a]

Species	Noxious stimulus	SR141716A		Hyperalgesic or pro-inflammatory effect observed	Reference
		Dose	Route		
Rat	Unilateral sciatic nerve ligation + mechanical stimulus to hind paw	0.5 mg/kg[b]	i.p.	Yes	Herzberg et al., 1997
Rat	Unilateral sciatic nerve ligation + radiant heat stimulus to hind paw	0.5 mg/kg[b]	i.p.	Yes	Herzberg et al., 1997
Mouse	Formalin into hind paw (early- and late-phase responses)	0.1 mg/kg[b,d]	i.v.	Yes	Calignano et al., 1998
Mouse	Formalin into hind paw (early-phase response)	10 μg[b]	i.pl.	Yes	Calignano et al., 1998
Rat	Formalin into hind paw (late-phase response)	1 mg/kg[b]	i.p.	Yes	Strangman et al., 1998
Rat	Carrageenan into hind paw + radiant heat stimulus to hind paw	0.1 μg[b]	i.pl.	No[e]	Richardson et al., 1998c
Rhesus monkey	Capsaicin into tail + warm water heat stimulus to tail	100 μg[b]	into tail	No[e]	Ko and Woods, 1999
Rat	Freund's adjuvant into hind paw + mechanical stimulus to hind paw	10 mg/kg[b]	i.p.	No[e]	Smith et al., 1998b
Rat	Freund's adjuvant into hind paw + mechanical stimulus to hind paw	1–30 μg	i.t.	No[c,e]	Martin et al., 1999d
Mouse	Arachidonic acid onto ear (to induce ear oedema)	5 mg/kg[b]	i.p.	No	Hanus et al., 1999

[a] i.pl., intraplantar; i.t., intrathecal.
[b] Only dose used.
[c] Freund's adjuvant injected unilaterally: signs of SR141716A-induced hyperalgesia observed in uninflamed contralateral paw (see section 9.3).
[d] SR144528 (i.v.) also induced signs of hyperalgesia (early-phase response only).
[e] SR141716A treatments used in these experiments attenuated antinociceptive responses to cannabinoid receptor agonists.

of increased nociception in rat hind paws that have been injected with formalin. In other investigations, however, SR141716A has been found not to affect nociception in animal models of inflammatory pain. Richardson et al. (1998c) reported that SR141716A (100 ng i.pl.) did not affect the sensitivity to radiant heat of rat hind paws that had been injected with carrageenan. This dose of SR141716A was sufficient to attenuate the antinociceptive effect of intraplantar anandamide, measured in the same bioassay. Smith et al. (1998b) investigated the effect of SR141716A on nociceptive responses of rat hind paws that had been made hyperalgesic by intraplantar injection of Freund's adjuvant. They found that SR141716A had no effect on the sensitivity of the hyperalgesic hind paws to mechanical pressure when it was administered at a dose (10 mg/kg i.p.) sufficient to prevent Δ^9-THC from suppressing paw responses to the same stimulus in rats that had not been injected with Freund's adjuvant. In experiments in which the tails of rhesus monkeys were made hyperalgesic by local injection of capsaicin, Ko and Woods (1999) found that SR141716A (100 μg injected subcutaneously into the tail tip) did not affect tail sensitivity to temperature as measured using a warm water tail withdrawal procedure. The same SR141716A treatment was effective against Δ^9-THC-induced delay of warm water tail withdrawal. Finally, Martin et al. (1999d) investigated the effect of intrathecal SR141716A (1–30 μg) on tactile allodynia, measured by applying calibrated von Frey filaments to the plantar surface of rat hind paws that had been inflamed by injection of Freund's adjuvant. SR141716A did not further increase the mechanical sensitivity of inflamed paws. However, it did increase the mechanical sensitivity of untreated (contralateral) hind paws in the same animals, suggesting that a paw contralateral to one that is inflamed is not equivalent to the paw of a normal animal and that peripheral tissue injury can modify activity of the endocannabinoid system in the spinal cord. In the same investigation, Martin et al. (1999d) detected marked increases in the number of spinal neurones expressing Fos in response both to intraplantar injection of Freund's adjuvant and to intrathecal SR141716A. The increased Fos expression induced by SR141716A occurred in laminae I and II (inflamed rats) laminae V and VI (inflamed and uninflamed rats) and the ventral horn (uninflamed rats) and was found to be greater in inflamed than uninflamed animals. These findings support the hypothesis that the CB_1 component of the endocannabinoid system modulates nociception not only under conditions of injury but also under basal conditions and that the spinal locus of this modulation is affected by tissue injury (Martin et al., 1999d).

9.4. Effect of SR141716A in a model of neuropathic pain

Herzberg et al. (1997) have obtained evidence that SR141716A enhances thermal hyperalgesia and mechanical allodynia in rats by showing it to increase the sensitivity to thermal and mechanical stimuli of hind paws that have been rendered hyperalgesic by unilateral sciatic nerve ligation. As the dose of SR141716A used (0.5 mg/kg i.p.) did not alter the sensitivity of unlesioned paws to these stimuli, the data from this investigation provide support for a role for the endocannabinoid system in the regulation of nociceptive thresholds for hyperalgesic but not non-hyperalgesic tissue.

9.5. Effects of SR141716A on nociceptive neurones

Hohmann et al. (1999c) found SR141716A to have no effect on noxious heat-evoked activity of wide dynamic range neurones of the lumbar dorsal horn of the rat at a dose (1 mg/kg i.v.) that did attenuate the suppressant effects of (+)-WIN55212 and CP55940 on noxious heat-evoked activity of these neurones. In other investigations, however, effects of SR141716A on neurones of nociceptive pathways have been noted. Meng et al. (1998) investigated the effect of SR141716A on the activity of a subpopulation of neurones in the rat rostral ventromedial medulla that exhibit a pause in activity just before the tail is withdrawn in response to noxious radiant heat. They found that at 0.5 mg/kg i.v., SR141716A prolonged the activity pause of these 'off' neurones, evidence that the endocannabinoid system may modulate descending control exerted on spinal nociceptive neurones by the rostral ventromedial medulla. Chapman (1999) reported that intrathecal administration of SR141716A (0.01–1 ng) to anaesthetized rats produced a dose-related facilitation of the non-potentiated component of C-fibre evoked responses of dorsal horn neurones to repetitive transcutaneous electrical stimulation. The same SR141716A treatments did not affect Aβ-fibre evoked responses. Nor did spinal administration of SR144528 (0.001–1 ng) affect C-fibre or Aβ-fibre evoked responses. Ross et al. (1999) found that whereas high voltage-activated Ca^{2+} currents of neonatal rat cultured dorsal root ganglia were inhibited by (+)-WIN55212, they were enhanced by SR141716A (100 nM).

9.6. Experiments with CB_1 knockout mice

CB_1 knockout mice developed by Ledent et al. (1999) exhibited normal nociception in tail immersion, tail pressure, hot plate and acetic acid abdominal stretch tests suggesting that 'the endogenous activa-

tion of the CB_1 receptor is not crucial for the control of pain or that other endogenous systems might compensate for the absence of this receptor (or both)'. Unexpectedly, Zimmer et al. (1999) found another strain of CB_1 knockout mouse to show normal nociception in the tail flick test and *reduced* sensitivity to noxious stimuli in the hot plate test and the early phase of the formalin paw test. Further experiments are required to establish the bases both for the unexpected observation that the CB_1 knockouts developed by Zimmer et al. (1999) exhibit signs of hypoalgesia rather than hyperalgesia in some tests and for the reported differences between this CB_1 knockout strain and the one developed by Ledent et al. (1999). In the meantime, it is noteworthy that whereas Zimmer et al. (1999) crossed their CB_1 knockouts to an inbred C57BL/6J genetic background, Ledent et al. (1999) crossed their knockouts to an outbred CD_1 strain of mouse.

9.7. Endogenous cannabinoid production and nociception

Walker et al. (1999) have obtained evidence from experiments with rats that anandamide release increases in inflammatory pain in at least one area of the brain, the periaqueductal grey, and that the anandamide released at this brain site may be antinociceptive. Anandamide was collected in these experiments by in vivo microdialysis and then assayed by mass spectrometry. Their experiments showed firstly, that electrical stimulation of the periaqueductal grey induces antinociception in the tail flick test, secondly, that this antinociceptive effect can be attenuated by intracerebroventricular SR141716A (100 μg) and thirdly, that it is possible to trigger anandamide release into the periaqueductal grey both by applying electrical stimuli to this part of the brain and by injecting formalin subcutaneously into the hind paw. Additional experiments are now required to establish whether exogenous application of anandamide to the periaqueductal grey produces antinociception and, if it does, whether this effect is antagonized by SR141716A. Further evidence for the production of antinociception by endogenously-released anandamide comes from an earlier observation that phenylmethylsulphonyl fluoride, an inhibitor of anandamide metabolism, shows antinociceptive activity in the mouse tail flick test (Compton and Martin, 1997). It remains to be established whether more selective inhibitors of anandamide metabolism or indeed, of anandamide uptake, are also antinociceptive when administered by themselves. Further experiments are also required to establish the role in nociception of 2-arachidonyl glycerol (sections 1.1 and 2.1) and, indeed, of any other endogenous cannabinoids that may prove to exist. In the meantime, it is noteworthy that Huang et al. (1999) have obtained evidence for the presence of 2-arachidonyl glycerol in rat primary afferent neurones. Concentrations of this compound were seven-fold lower in sciatic nerve than in dorsal root ganglia or in tissue from lumbar spinal cord suggesting that it is present mainly in cell bodies.

10. Summary and general discussion

Cannabinoid receptor agonists are active in animal models of acute pain both when administered peripherally and when injected directly into the brain or spinal cord (section 2.1). They also show activity in models of tonic pain and allodynia, when administered systemically or by intrathecal injection or when applied directly to an inflamed tissue (monkey tail or rodent hind paw, urinary bladder or peritoneum) (section 2.2). The suppression of motor responses to noxious stimuli induced by CB_1 receptor agonists in animal pain models does not seem to stem from the known ability of these agents to impair motor function or to induce hypothermia (sections 2.1 and 2.2).

There is strong evidence that cannabinoids can induce signs of antinociception in models of acute or tonic pain through CB_1 receptors located on neurones both within and outside the brain and spinal cord (sections 3 and 5). Within the brain, these receptors are present in the thalamus, periaqueductal grey, amygdala, superior colliculus and in the A5 noradrenergic group of the brainstem. They are also present in the rostral ventromedial medulla, where they seem to modulate the activity of neurones projecting to nociceptive regions of the spinal cord. Spinal CB_1 receptors that modulate nociception are thought to be located at the terminals of neurones that project from the brain and/or on intrinsic spinal neurones. CB_1 receptors are also present at the central and peripheral terminals of primary afferent neurones, both on C-fibres and on larger diameter Aβ/Aδ-fibres. These receptors co-localize significantly with substance P and calcitonin gene-related peptide but only minimally with somatostatin. They may also be present on nerve growth factor-dependent primary afferent neurones. The presence of significant numbers of CB_1 receptors on large diameter primary afferent fibres helps to explain the efficacy shown by cannabinoid receptors agonists against signs of neuropathic pain induced in rats by chronic constriction injury of the sciatic nerve (Table 3), since this kind of pain is thought to be elicited in part by abnormal spontaneous discharges of myelinated Aβ- and Aδ-fibres (Hohmann and Herkenham, 1999a). As large diameter primary afferent fibres are far less densely populated with μ-opioid receptors than with cannabinoid receptors whilst small diameter primary afferent neurones are more densely populated with μ-opioid receptors than with cannabinoid receptors, it is likely

that CB_1 receptor agonists will prove to be more effective than opioids in suppressing pain of this kind (Hohmann et al., 1999a; Hohmann and Herkenham, 1998, 1999a). Further support for this hypothesis comes from the observation that (+)-WIN55212 differs from morphine in having less potency as an inhibitor of the acute response to C-fibre activation than as an inhibitor of 'wind-up' of spinal wide dynamic range and nociceptive-specific neurones, a process that may contribute to the development of hyperalgesia and allodynia associated with chronic pain (Strangman and Walker, 1999 and section 5.1).

Antinociception resulting from CB_1 receptor activation may be due in part to direct inhibition of GABA release within the periaqueductal grey and rostral ventromedial medulla and of glutamate release within the spinal cord (section 6.1). It may also depend to some extent on an indirect disinhibition of dopamine release onto D_2 receptors, of noradrenaline release onto spinal α_2-adrenoceptors and of opioid peptide release onto brain μ-receptors and spinal κ-receptors (section 7). One expected consequence of such an increase in spinal κ-receptor activation is suppression of any ongoing release of nociceptive neuropeptides such as substance P. CB_1 receptor-mediated disinhibition of opioid peptides release within brain and spinal cord may also underlie the ability of certain cannabinoids to potentiate opioid-induced antinociception in animal models of acute pain (section 8). Other mechanisms through which some cannabinoids can or may act to induce antinociception include the inhibition of inflammatory eicosanoid production, for example by 1′,1′-dimethylheptyl-Δ^8-THC-11-oic acid, the activation of CB_2 receptors, for example by the CB_2-selective agonist HU-308, and the activation of putative CB_2-like receptors, for example by HU-308 and/or palmitylethanolamide (section 4). Whilst CB_1 receptors are known to be present mainly on central or peripheral neurones, the cell types in pain pathways that express CB_2 or CB_2-like receptors remain to be identified. These are unlikely to be neuronal but could be cells that produce and release inflammatory or pro-inflammatory agents, for example mast cells (section 6.2). Thus it is possible that whilst CB_1 receptors modulate transmission in neuronal pain pathways, CB_2 and/or CB_2-like receptors modulate the release of endogenous inflammatory/pro-inflammatory and anti-inflammatory agents from non-neuronal cells located in the vicinity of nociceptive neurones (Molina-Holgado et al., 1999). CB_1 receptors may also modulate release of endogenous inflammatory/pro-inflammatory and anti-inflammatory agents and indeed there is evidence that anandamide can act through CB_1 receptors to inhibit the release of calcitonin gene-related peptide in both skin and spinal cord (section 6.2) and to increase interleukin-6 production by mouse brain cortical astrocytes infected with Theiler's murine encephalomyelitis virus (Molina-Holgado et al., 1998). For anandamide and certain other eicosanoid cannabinoids, there is also some evidence that antinociceptive responses can be elicited through the activation of non-CB_1/non-CB_2/non-CB_2-like cannabinoid receptors that are possibly but not necessarily vanilloid receptors (sections 4.5 and 4.6). The existence of such a mechanism has not yet been conclusively demonstrated. If they do exist, 'anandamide receptors' presumably play a dominant role in the antinociception induced by systemic administration of anandamide as this is not attenuated by SR141716A, at least in mice.

Cannabinoids appear to be more potent against tonic pain and allodynia than against acute pain induced by the application of a noxious stimulus to uninjured tissue (section 2.2). Why this should be remains to be established. However, one possible explanation is that since cannabinoids seem to act by inhibiting the release of nociceptive agents from neuronal and/or non-neuronal tissues, they induce antinociception more readily in inflamed or injured tissues in which the background release of endogenous nociceptive agents is likely to be relatively high. It is also possible that cannabinoids act through more powerful or varied antinociceptive mechanisms in inflamed or injured tissue than in normal tissue, e.g. (1) enzymes that catalyse the production of inflammatory/pro-inflammatory agents; and/or (2) a greater number/variety of antinociceptive cannabinoid receptors.

One physiological role of the endocannabinoid system may be to suppress tonic pain (section 9). Signs of such modulation have been detected within the brain and also in inflamed and injured tissues outside the central nervous system. Whether the endocannabinoid system ever modulates acute pain also remains possible although less certain. The production of antinociception by the endocannabinoid system could depend on an increased release of endogenous cannabinoid(s) and/or on the presence of precoupled (constitutively active) cannabinoid receptors in pain pathways. Already there is evidence from rat experiments that anandamide release in the periaqueductal grey increases in response to inflammatory pain in the hind paw. However, the question of whether constitutively active cannabinoid receptors have a role in pain modulation is still unanswered. Such a mechanism might explain the hyperalgesic effects of SR141716A and SR144528 that have been observed in some experiments as both these agents are thought to be inverse agonists rather than pure antagonists (section 9). However, such hyperalgesia could also be an indication that these agents are antagonizing responses to endogenously released cannabinoid(s). Yet to be explained are reports that SR141716A induces detectable signs of hyperalgesia in some models of inflammatory pain but not in others. Such signs have been detected in rodent paws that have been inflamed

with formalin but not in rodent paws or monkey tails that have been inflamed using carrageenan, capsaicin or Freund's adjuvant (section 9.3). One factor contributing to this variability may be the apparent resistance of at least some actions of anandamide to antagonism by SR141716A. It is also possible that the degree of release of endogenous cannabinoids and the extent of cannabinoid receptor precoupling is not the same in all experimental models. One set of observations that does not support an antinociceptive role for the endocannabinoid system comes from experiments with CB_1 knockout mice which have been reported to exhibit normal nociception in some pain models and reduced sensitivity to noxious stimuli in others (section 9.6).

The ability of cannabinoids to produce signs of analgesia, particularly in animal models of tonic pain, does of course have important clinical implications. Indeed, there are already some human data to suggest that cannabinoids are effective against postoperative, cancer and phantom limb pain (Finnegan-Ling and Musty, 1994; Jain et al., 1981; Noyes et al., 1975b,a) as well as against pain associated with multiple sclerosis and spinal cord injury (Brenneisen et al., 1996; Consroe et al., 1997; Martyn et al., 1995; Maurer et al., 1990; Pertwee, 1999b). A major challenge that faces those who wish to exploit the analgesic properties of cannabinoids in the clinic is the need to devise strategies that will reduce or abolish the adverse central effects of cannabinoids without attenuating their sought-after clinical effects. As the unwanted central effects of cannabinoids are mediated largely or entirely by CB_1 receptors within the brain, one possibility would be to focus on cannabinoids that induce analgesia by acting on biological targets other than the CB_1 receptor, for example enzymes that catalyse the production of prostaglandins or other inflammatory agents, CB_2 or CB_2-like receptors, or perhaps vanilloid receptors (section 4). Another strategy for minimizing the unwanted central effects of cannabinoids would be to exploit increases in the tonic activity of the endocannabinoid system that seem to accompany inflammation or peripheral nerve damage, at least in animals. This might be achieved by developing therapeutic agents from compounds such as AM381 or AM404 (Pertwee, 1999a) that activate the endocannabinoid system indirectly by selectively inhibiting the tissue uptake or metabolism of endogenous cannabinoids so as to increase their levels at cannabinoid receptors. These drugs should be more selective than direct agonists as they are unlikely to affect all parts of the endocannabinoid system at one time, producing instead effects only at sites where ongoing production of endogenous cannabinoids is taking place. Hence, just as monoamine levels can be enhanced to combat depression, so too modulation of endogenous cannabinoid concentrations at their sites of action could prove to be clinically beneficial. A third strategy would be to exploit the synergistic interactions that occur between cannabinoids and opioids for antinociception (section 8). Whether such synergism would make it of therapeutic advantage to administer a cannabinoid in combination with an opioid will depend largely on the extent to which the unwanted effects of these drugs are also augmented after combined administration. However, there is already one report that oral administration of a cannabis extract reduced the requirement for morphine by a patient suffering from severe chronic abdominal pain (Holdcroft et al. 1997). In view of the evidence that CB_1 receptors are present on nociceptive neurones in the spinal cord (section 5) and that intrathecal administration of CB_1 receptor agonists induces antinociception in animals, another possibility would be to give CB_1 receptor agonists by the intrathecal route. This strategy is already sometimes adopted for baclofen, to reduce the incidence of its adverse effects in multiple sclerosis patients. As analgesia can be induced by the activation of CB_1 cannabinoid receptors present on sensory neurones outside the brain or spinal cord (section 5.3), it may also be worth designing cannabinoids that do not readily cross the blood brain barrier and yet retain the ability to activate CB_1 receptors located outside the central nervous system.

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