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Cannabinoid Mechanisms of Pain Suppression

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Abstract A large body of literature indicates that cannabinoids suppress behavioral responses to acute and persistent noxious stimulation in animals. This review examines neuroanatomical, behavioral, and neurophysiological evidence supporting a role for cannabinoids in suppressing pain at spinal, supraspinal, and peripheral levels. Localization studies employing receptor binding and quantitative autoradiography, immunocytochemistry, and in situ hybridization are reviewed to examine the distribution of cannabinoid receptors at these levels and provide a neuroanatomical framework with which to understand the roles of endogenous cannabinoids in sensory processing. Pharmacological and transgenic approaches that have been used to study cannabinoid antinociceptive mechanisms are described. These studies provide insight into the functional roles of cannabinoid CB_1 (CB1R) and CB_2 (CB2R) receptor subtypes in cannabinoid antinociceptive mechanisms, as revealed in animal models of acute and persistent pain. The role of endocannabinoids and related fatty acid amides that are implicated in endogenous mechanisms for pain suppression are discussed. Human studies evaluating therapeutic potential of cannabinoid pharmacotherapies in experimental and clinical pain syndromes are evaluated. The potential of exploiting cannabinoid antinociceptive mechanisms in novel pharmacotherapies for pain is discussed.

Keywords Endocannabinoid · Spinal cord · Periaqueductal gray · Supraspinal · Peripheral · CB1 · CB2 · THC · Hyperalgesia · Clinical pain

The study of the role of endocannabinoids in pain is founded in research on pain mechanisms, a vital field that is steadily evolving on many fronts. A brief overview of the current thinking on the neural basis of pain is thus provided as background

to discussing how cannabinoids and endocannabinoids modulate pain sensation. Extensive reviews of pain mechanisms may be found in a relatively recent volume edited by Wall and Melzack (1999). This review will focus on preclinical studies that evaluate evidence from neuroanatomical, behavioral, electrophysiological, and neurochemical approaches that provide insight into the roles of cannabinoids and endocannabinoids in suppressing pain. Peripheral, spinal, and supraspinal sites of cannabinoid actions are discussed, as well as the endogenous ligands implicated in endocannabinoid mechanisms of pain suppression. This review will also present results from clinical studies that provide insight into the therapeutic potential for cannabinoid pharmacotherapies for pain in man.

1
Brief Overview of Pain Mechanisms

Pain is a complex psychological phenomenon comprising sensory, emotional, and motivational components. The negative emotion and the motivation to escape from the stimulus are essential features of pain—without them the experience would be non-painful tactile stimulation. In the early twentieth century, "labeled-line" theory dominated thinking about pain. In this conception, specific nociceptors in the periphery transmit signals about noxious stimuli to the spinal cord, which relays the information to a pain center in the brain, which in turn gives rise to the sensation of pain. This notion has broken down, first with the realization that while there are specific nociceptors in the periphery, activity from incoming non-nociceptive fibers interacts with that from nociceptors, changing the spinal transmission properties of the nociceptive fibers. Hence, increased activity in larger, non-nociceptive fibers lessens the impact of activity in nociceptors (typically smaller unmyelinated C fibers and finely myelinated Aδ fibers). The second and perhaps even more significant finding was the discovery that the brain contains circuits that modulate the ability of nociceptors to excite ascending pain-transmission pathways. These circuits can either dampen or facilitate pain. The observations by Beecher (1959), who observed soldiers in World War II who felt no pain despite serious injuries, were important in rethinking the labeled line theory, leading to the more sophisticated view that the experience of pain is regulated by the relative activity in peripheral, spinal, and brain networks of pro- and anti-nociceptive circuits. These networks, described in more detail below, provide substrates for actions of cannabinoids on pain.

1.1
Nociceptors

The term nociceptor refers to sensory receptors that respond to noxious stimuli (see Kruger et al. 2003 for review). A variety of cutaneous primary afferent nociceptors have been described, primary among them are the unmyelinated C fibers that are characterized by free nerve endings. The C-polymodal nociceptor responds to

mechanical, heat, and chemical stimuli. Primary afferents have a unique morphology. The cell bodies, which are found in the dorsal root ganglion, lack dendrites and synapses and are encased in satellite cells that insulate them. The axon bifurcates, sending a branch to the spinal cord and branch to the periphery. Hence, the sensory apparatus is found on an axon terminal, and indeed action potentials in the peripheral nerve lead to secretion of neurotransmitter at both the peripheral and central terminals. The biochemical machinery of nociceptors includes a variety of molecular transduction elements such as transient receptor potential (TRP) channels, acid sensing channels, and P2X3 receptors, as well as particular neurotransmitters including glutamate, substance P, and calcitonin gene-related peptide (CGRP). On the central terminals are found presynaptic receptors that modulate neurotransmitter release.

1.2 Ascending Pain Pathways

Upon activation of spinal neurons by nociceptors, information about noxious stimuli is carried to the brain by ascending pathways. Multiple pathways have been described (for review see Millan 1999, especially Table 4 therein).

1.2.1 Spinothalamic Tract

The classical ascending pathway (Fig. 1) is the spinothalamic tract, a contralaterally projecting fiber bundle that ascends in the anterolateral aspect of the spinal white matter to the ventral posterolateral thalamus with extensive collateralization to brainstem structures prominent among these being the periaqueductal gray (PAG).

1.2.2 Dorsal Column Visceral Pain Pathway

The dorsal column pathway may be of major importance for visceral pain (Berkley and Hubscher 1995; Willis et al. 1995). This pathway originates from the visceral processing circuitry in the gray matter surrounding central canal of the spinal cord and ascends ipsilaterally in the dorsal columns, the white matter areas adjacent to the midline on the dorsal aspect of the spinal cord. The putative involvement of the dorsal column in visceral pain is noteworthy for two reasons, the first being that it supercedes the classical understanding of the dorsal columns as being the trajectory for discriminative touch sensations, and second that it provides a new understanding of complex neural pathways for visceral compared to somatosensory pain. Rather than operating in isolation, the dorsal column and spinal routes cooperate to produce the many perceptions of touch and pain (Berkley and Hubscher 1995). This ensemble view encourages the development of novel, integrative pharmacotherapies and treatments (Berkley and Hubscher 1995).

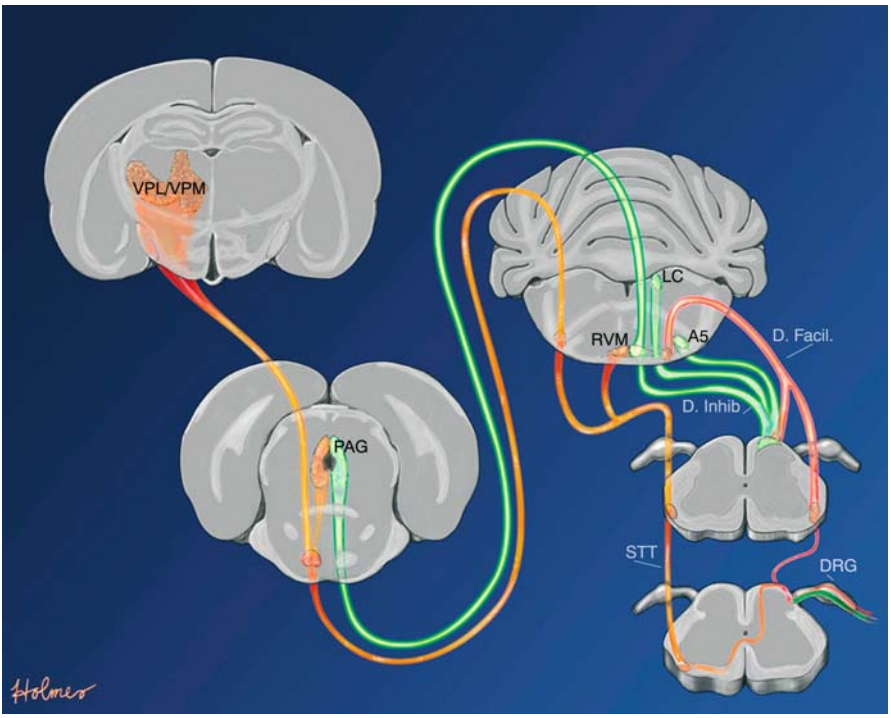

Fig. 1. Schematic of neural pathways that process and modulate the transmission of information about nociceptive signals. In *orange*, the spinothalamic tract is shown, with signals originating in the peripheral nerve, crossing the midline, and ascending the anterolateral white matter of the spinal cord with many collateral outputs to the brainstem shown for the RVM and PAG. This tract terminates in the VPL/VPM thalamus. In *green*, descending pain inhibitory pathways are shown, which connect the PAG to the RVM, and from there makes connections in the spinal cord. Other descending inhibitory pathways originating in the LC and noradrenergic nucleus A5 are also shown. In *red*, pathways that facilitate pain are shown originating in the RVM and descending to the spinal cord. Abbreviations: *A5*, noradrenergic nucleus A5; *D. Facil.*, descending facilitation pathway; *D. Inhib*, descending inhibitory pathways; *DRG*, dorsal root ganglion; *LC*, locus coeruleus; *PAG*, periaqueductal gray; *RVM*, rostral ventromedial medulla; *STT*, spinothalamic tract; *VPL*, ventroposterolateral nucleus; *VPM*, ventral posteromedial nucleus

1.3 Descending Modulation of Pain

1.3.1 Descending Pain Inhibition

With the observation by Kang Tsou (Tsou and Jang 1964) of the potent analgesic effects of morphine applied by microinjection to the periaqueductal gray came the early realization that the brain plays an active role in determining whether pain is felt following noxious stimulation. Later, it was observed by Reynolds (1969) that electrical stimulation of this region in the rat produced sufficient analgesia for a pain-free laparotomy without additional anesthesia. Akil et al. (1976) noted

that this analgesic phenomenon could be reversed by naloxone, suggesting that the electrical stimulation releases an endogenous opiate-like substance that led to analgesia. These observations set the stage for extensive studies of how the PAG can entirely block pain sensations (reviewed by Fields et al. 1991). It became clear that this occurs through projections from the PAG to the rostral ventromedial medulla (RVM), and from there to the spinal cord. Specific on- and off-cells in the RVM were found to control the excitability of ascending spinal pathways. On-cells fire just before a nocifensive flexion reflex and off-cells, which are spontaneously active, stop firing just before a nocifensive flexion reflex. This pathway is activated by certain forms of stress and appears to naturally serve to control the organism's response to noxious stimuli, being able to entirely suppress pain under certain conditions.

1.3.2
Descending Pain Facilitation

More recently, it has become clear that the RVM can facilitate as well as dampen pain (reviewed by Porreca et al. 2002). Stimulation of the RVM at relatively low current intensities increases the responses of spinal dorsal horn neurons to noxious stimuli. The role of this facilitation in chronic pain is suggested by studies showing that blockade of the RVM with lidocaine reduces abnormal tactile responses in rats with neuropathic pain (Pertovaara et al. 1996). Other studies of inflammatory and neuropathic pain converge in showing that descending facilitation is an important component of pathological pain.

1.4
Implications for Understanding Cannabinoid Actions in Pain

The above outline of current understanding of the neural processing that underlies pain provides a foundation for understanding the effects of exogenous and endogenous cannabinoids in pain. Cannabinoids act at all of the sites discussed above, i.e., the periphery, spinal cord, and central circuits for pain facilitation and pain modulation. In the following sections, we review the current understanding of the systemic effects of cannabinoids and their sites of action within pain processing circuits from anatomical, physiological, and behavioral perspectives.

2
Antinociception and Suppression of Pain Neurotransmission by Systemically Administered Cannabinoids

Cannabinoid antinociception is observed in preclinical behavioral studies employing different modalities of noxious stimulation including thermal, mechanical, and chemical (see Walker et al. 2001 for review). Perhaps the earliest recorded scientific demonstration of cannabinoid antinociception was provided by one of the

fathers of modern pharmacology, Ernest Dixon (1899). He observed that dogs that inhaled cannabis smoke failed to react to pin pricks. Early studies by Bicher and Mechoulam (1968) and Kosersky et al. (1973) provided a foundation for subsequent work that verified the ability of cannabinoids to profoundly suppress behavioral reactions to acute noxious stimuli and inflammatory and nerve injury-induced pain. In these early studies, it was noted that the potency and efficacy of cannabinoids rival that of morphine (Bloom et al. 1977; Buxbaum 1972). However, cannabinoids also produce profound motor effects [e.g., immobility, catalepsy; (Martin et al. 1991)], a potential confound for behavioral studies, which inevitably employ motor responses to noxious stimuli as a measure of pain sensitivity.

In part to address this potential confound, subsequent electrophysiological and neurochemical studies examined the question of whether cannabinoids suppress activity within pain circuits. These studies provided convincing evidence that cannabinoids suppress nociceptive transmission in vivo (see Hohmann 2002 for review). Walker's laboratory first demonstrated that cannabinoids suppress noxious stimulus-evoked neuronal activity in nociceptive neurons in the spinal cord (Fig. 2)

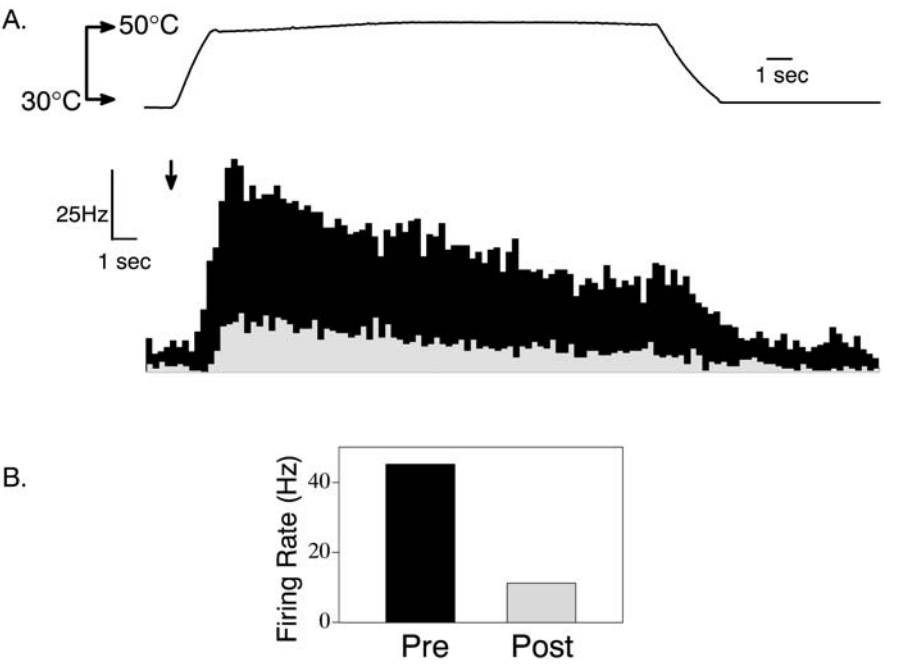

Fig. 2. Example of inhibition of noxious heat-evoked activity in a lumbar dorsal horn neuron by the cannabinoid WIN55,212-2. The responses of the neuron to a 50°C stimulus were examined during 16 stimulus trials. **A** The noxious stimulus, illustrated by the temperature waveform (*top center*), was administered at 2.5-min intervals. The *black peristimulus time histogram* represents baseline firing prior to injection of the synthetic CB1R/CB2R agonist WIN55,212-2 (125 μg/kg i.v.).The *gray peristimulus time histogram* represents the firing rate for the first five post-injection trials. **B** Comparison of the mean firing rate during the stimulus for the five baseline trials to the firing rate during the stimulus for the first five post-injection trials illustrating, approximately, a 75% decrease in responsiveness. (Redrawn from Hohmann et al. 1999b)

and thalamus (Hohmann et al. 1995, 1998, 1999b; Martin et al. 1996; Strangman and Walker 1999). This suppression is observed in nociceptive neurons, generalizes to different modalities of noxious stimulation (mechanical, thermal, chemical), is mediated by cannabinoid receptors (CBRs), and correlates with the antinociceptive effects of cannabinoids (Hohmann et al. 1995, 1998, 1999b,c; Martin et al. 1996). Cannabinoids suppress C fiber-evoked responses in spinal dorsal horn neurons recorded in normal and inflamed rats (Drew et al. 2000; Kelly and Chapman 2001; Strangman and Walker 1999). Spinal Fos protein expression, a neurochemical marker of sustained neuronal activation (Hunt et al. 1987), is also suppressed by cannabinoids in animal models of persistent pain (Farquhar-Smith et al. 2002; Hohmann et al. 1999c; Martin et al. 1999b; Nackley et al. 2003a, 2003b; Tsou et al. 1996). This suppression occurs through cannabinoid CB_1 receptor (CB1R)- and cannabinoid CB_2 receptor (CB2R)-selective mechanisms. These studies provided a foundation for subsequent work, which has identified the sites of action of cannabinoids within pain circuits and the actions of specific endocannabinoids within these circuits.

3
CB1R-Mediated Antinociception: Peripheral, Spinal, and Supraspinal Actions

3.1
Methodological Considerations

The distribution of CBRs in brain was first mapped by Herkenham et al. (1991) using receptor binding and autoradiographic methods. This approach permits quantitative evaluation of the density and distribution of receptors, but lacks cellular resolution. The development of specific antibodies for CBRs has permitted characterization of the cellular distribution of CBRs (Egertová et al. 2003; Egertová et al. 1998; Tsou et al. 1998a). Immunocytochemical approaches, however, are suited to qualitative rather than quantitative evaluation of CBR densities.

CBRs have been studied in rat spinal cord using autoradiographic (Herkenham et al. 1991; Hohmann et al. 1999a; Hohmann and Herkenham 1998) and immunocytochemical (Farquhar-Smith et al. 2000; Morisset et al. 2001; Salio et al. 2002b; Salio et al. 2001; Sanudo-Pena et al. 1999; Tsou et al. 1998a) techniques. It is important to note that localization studies employing antibodies raised against the N-terminal of the CB1R protein may reveal different patterns of immunostaining from antibodies raised against the C-terminal tail and support different conclusions regarding the anatomical localization of CBRs. Antibodies recognizing the intracellular C-terminal domain of CB1R might be expected to behave differently depending on the level of tissue fixation and receptor internalization. It is possible that N-terminal antibodies underestimate localization of CB1R to plasma membrane and primarily reflect synthesis, storage, or transport sites; detection of CB1R at the plasma membrane would require an antibody recognizing the N terminus to penetrate the extracellular space (Salio et al. 2002b). Moreover, N-terminal antibodies are unable to recognize a splice variant of CB_1, CB_{1A} (Shire et al. 1995),

because the splice variant bears a truncated N terminus (Salio et al. 2002b). However, it is unclear whether these isoforms are differentially distributed in the spinal dorsal horn. This review will compare the distribution of CB1R mRNA and CB1R immunoreactivity in rat dorsal root ganglion cells. The distribution of CBRs in rat spinal cord revealed by receptor binding and quantitative autoradiography will subsequently be compared with patterns of CB1R immunostaining revealed by immunocytochemistry using antibodies recognizing different epitopes of CB1R.

3.2 Antinociception Mediated by CB1Rs in the Periphery

The distribution of CBRs outside the central nervous system is consistent with behavioral and neurochemical data that implicate a role for peripheral CB1Rs in cannabinoid antinociception. The distribution of CB1Rs in dorsal root ganglia and peripheral nerve is therefore reviewed here. The role of CB2Rs in cannabinoid antinociceptive mechanisms is reviewed in Sect. 4.

Traditionally, the dorsal root ganglion (DRG) has been used as a model of the peripheral nerve because of its more convenient size, location, and the ability to correlate cell size and neurochemical phenotype with peripheral axon caliber. Hohmann and Herkenham (1999a,b) used in situ hybridization to test the hypothesis that dorsal root ganglion cells, the source of primary afferent input to the

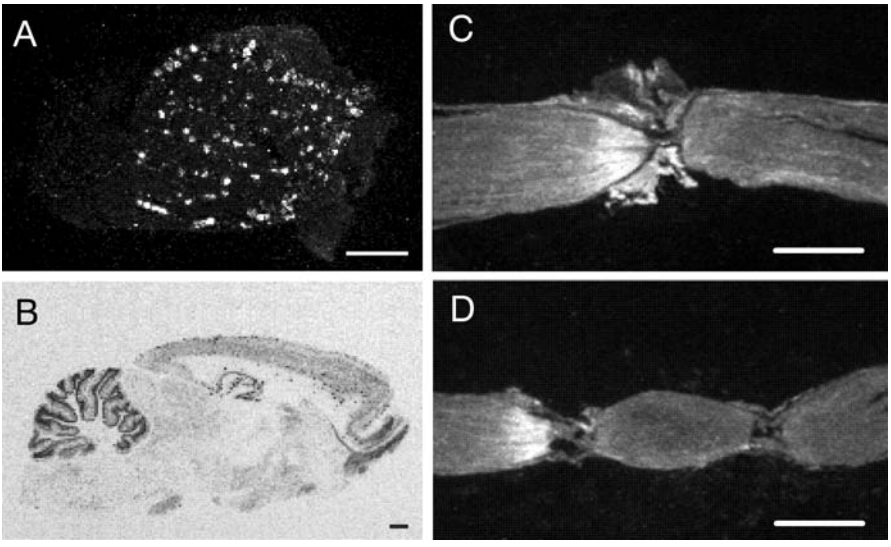

Fig. 3. Distribution of cannabinoid CB_1 receptor (CB1R) mRNA in rat (**A**) dorsal root ganglia and (**B**) brain. Cannabinoid binding sites accumulate proximal to a tight ligation of the sciatic nerve. [^{3}H]CP55,940 binding and high-resolution emulsion autoradiography was used to demonstrate flow of cannabinoid receptors to the periphery. Dark-field photomicrographs show damming of cannabinoid receptors proximal as opposed to distal to (**C**) a single tight ligation and (**D**) the more proximal of two separate ligatures applied to the sciatic nerve. *Scale bars* = 1 mm. (From Hohmann and Herkenham 1999a)

spinal cord, synthesize cannabinoid CB1Rs (Fig. 3A, B). CB1R mRNA was highly expressed in dorsal root ganglion cells of heterogeneous cell size, and predominant in intermediate-sized neurons. These data are consistent with immunocytochemical studies using an N-terminal antibody in native DRG that confirmed the presence of CB1Rs in small, medium, and large cells of rat dorsal root ganglia (Salio et al. 2002a).

Both CB1Rs and CB2Rs have been identified in primary cultures of dorsal root ganglion cells derived from neonatal rats (Ross et al. 2001a). The location and phenotypes of cells expressing CB2Rs in dorsal root ganglion likely represent an important topic of future investigation. It is unclear if CB2Rs are expressed in satellite glial cells, the main glial cells in sensory ganglia, that have recently been shown to be histologically altered in animal models of nociception (Hanani et al. 2002; Li and Zhou 2001). Neuronal expression of CB2R mRNA in native DRG (Hohmann and Herkenham 1999a) and trigeminal ganglia (Price et al. 2003) was similar to background under conditions in which CB1R mRNA was clearly demonstrated. These data suggest that: (1) a high-affinity low-capacity CB2R site may be synthesized in the DRG and contribute peripheral cannabinoid actions, (2) a CB_2-like receptor may mediate the observed effects, and/or (3) a CB2R mechanism exerts its actions indirectly (e.g., by inhibiting the release of inflammatory mediators that excite nociceptors).

3.2.1
Phenotypes of Dorsal Root Ganglion Cells Expressing CB1Rs and CB1R mRNA

To better understand the role of cannabinoids in sensory processing, phenotypes of dorsal root ganglion cells that synthesize CB1Rs have been investigated by several laboratories (Ahluwalia et al. 2000, 2002; Bridges et al. 2003; Hohmann and Herkenham 1999b; Price et al. 2003). Small-diameter cells in the dorsal root ganglia, in general, correspond to nociceptors and thermoreceptors, respond to high-threshold stimuli, and have unmyelinated or thinly myelinated axons. The small-diameter cells fall into two categories—the nerve growth factor-sensitive population of cells that synthesize neuropeptides and express trkA (Averill et al. 1995; Molliver et al. 1995) and those that are sensitive to glial cell-derived neurotrophic factor, contain the enzyme fluoride-resistant acid phosphatase (Nagy and Hunt 1982), bind isolectin B4 (IB4) (Silverman and Kruger 1990), and do not express trks. We evaluated localization of CB1Rs to dorsal root ganglion cells that synthesize preprotachykinin A (a precursor for substance P) and α-CGRP (Hohmann and Herkenham 1999b) using double-label in situ hybridization. In native dorsal root ganglia, only small subpopulations of cells expressing CB1R mRNA colocalized mRNAs for neuropeptide markers of primary afferents preprotachykinin A and α-CGRP (Hohmann and Herkenham 1999b). Neurons expressing mRNA for somatostatin were CB_1-mRNA negative (Hohmann and Herkenham 1999b).

Quantification of double-labeled cells in this study revealed that less than 9% and 13% of cells containing mRNA for precursors of CGRP or substance P mRNA, re-

spectively, expressed CB1R mRNA (Hohmann and Herkenham 1999b). Moreover, the vast majority of CB1R mRNA-expressing cells (75%) in the dorsal root ganglia of naive rats failed to colocalize these neuropeptides. Direct support for localization of CB1Rs to dorsal root ganglion cells bearing myelinated fibers has recently been demonstrated (Bridges et al. 2003; Price et al. 2003). These observations indicate that under normal conditions, CB1Rs are localized mainly to non-nociceptive primary afferent fibers. Inflammation and axotomy induce marked changes in peptide phenotypes of dorsal root ganglia cells (Calza et al. 1998; Donaldson et al. 1994; Galeazza et al. 1995; Hanesch et al. 1995; Ji et al. 1994, 1995; Leslie et al. 1995; Neumann et al. 1996), indicating that different coexpression levels may also exist in chronic pain states.

In native DRG, CB1R is largely associated with myelinated A fibers. Bridges et al. (2003) demonstrated that the majority (69%–80%) of CB1R-immunoreactive cells (labeled using an antibody directed against the C-terminal of CB1R) coexpress neurofilament 200 (Bridges et al. 2003). This marker is largely restricted to primary afferent A fibers. A modest degree of colocalization of CB1R immunoreactivity was observed with IB4 (17%–26%) and CGRP (10%) immunoreactive cells in DRG (Bridges et al. 2003), markers of nociceptors. In addition, 10% of mRNA expressing cells were immunoreactive for transient receptor potential vanilloid family ion channel 2 (TRPV2), the noxious heat-transducing channel found in medium and large lightly myelinated Aδ fibers. Moreover, this study demonstrated that only 11%–20% of CB1R mRNA expressing cells were immunoreactive for TRPV1, a marker of nociceptive C fibers. Similar results are observed in native trigeminal ganglia, where only minor colocalization of CB1R is observed with markers of nociceptors (TRPV1, substance P, CGRP, IB4) and high levels of colocalization (75%) of CB1R with N52, a maker of myelinated non-nociceptive fibers, were observed (Price et al. 2003).

The phenotypes of cells expressing CB1Rs in native DRG differs from that reported in cultured DRG, where colocalization of CB1Rs with markers of nociceptors is more prevalent. CB1Rs have been identified in small-diameter cells expressing capsaicin-sensitive TRPV1 (VR1) receptors in cultured DRG cells (Ahluwalia et al. 2000, 2002). In contrast to observations in native DRG, approximately 80% of the CB1R-like immunopositive cells showed TRPV1-like immunoreactivity, while 98% of the TRPV1-like immunolabeled neurons showed CB_1-like immunostaining (Ahluwalia et al. 2000). A further study demonstrated that CB1R-immunoreactive cells colocalized immunoreactivity for CGRP and IB4 (Ahluwalia et al. 2002). In this study, approximately 20% of CB1R immunostained neurons did not show either CGRP or IB4 immunoreactivity, indicating that they were non-nociceptive. These data support localization of CB1Rs to nociceptive neurons as well as non-nociceptive neurons in dorsal root ganglion cells raised in culture, in contrast with the modest colocalization of CB1Rs with markers of nociceptors observed in native dorsal root (Bridges et al. 2003; Hohmann and Herkenham 1999b) and trigeminal (Price et al. 2003) ganglia. However, in cultured DRG neurons, cannabinoids attenuate depolarization-dependent Ca^{++} influx in intermediate-sized (800–1500 μm^2) dorsal root ganglion cells raised in cultures derived from adult rats, but these effects were largely absent in small (< 800 μm^2) neurons (Khasabova et al. 2002).

The differences in colocalization reported here may be attributed to differences between native and cultured dorsal root ganglion cells and/or the use of different antibodies recognizing different epitopes of CB1R. Lower numbers of TRPV1 immunoreactive cells are observed in DRG cultures raised in the absence of neurotrophic factors, but no changes are observed in the number of CB_1-expressing cells under these same conditions (Ahluwalia et al. 2002). Elimination of neurotrophic factors from culture media is also associated with a modest but significant shift in the distribution of the size of CB1R-immunoreactive cells to larger diameters (Ahluwalia et al. 2002).

3.2.2
Axonal Transport of CBRs to the Periphery

We used [^{3}H]CP55,940 binding and high-resolution emulsion autoradiography to test the hypothesis that CBRs synthesized in dorsal root ganglion cells are transported to the periphery. Transport of CBRs to the periphery was occluded by tight ligation of the sciatic nerve (Hohmann and Herkenham 1999a). These data suggest that CBRs synthesized in the DRG are likely to undergo anterograde transport and be inserted on terminals in the peripheral direction (Fig. 3C, D). This observation is also consistent with the observation of CB1R immunoreactivity in rat peripheral nerve and in ventral roots (Sanudo-Pena et al. 1999). More work is necessary to determine if CBRs synthesized in the DRG are differentially transported to peripheral vs central terminals and whether transport of these receptors is modulated by persistent pain states.

3.2.3
Peripheral CB1R-Mediated Antinociception: Acute and Persistent Pain States

Behavioral and neurochemical studies implicate a role for peripheral CB1Rs in cannabinoid antinociception in models of acute, inflammatory, and neuropathic pain states.

Peripheral CB1R-Modulation of Inflammatory Nociception

Richardson and colleagues first demonstrated that activation of peripheral CB1Rs suppresses thermal hyperalgesia and edema in the carrageenan model of inflammation (Richardson et al. 1998c). Hyperalgesia refers to a lowering of the pain threshold or increase in sensitivity to a normally painful stimulus. Anandamide, administered to the site of injury, suppressed the development and maintenance of carrageenan-evoked thermal hyperalgesia (Richardson et al. 1998c). The same dose administered to the noninflamed contralateral paw was inactive, suggesting that antihyperalgesia occurred at low doses that do not produce antinociception. Antihyperalgesia induced by anandamide was blocked by the CB1R-competitive antagonist/inverse agonist SR141716A, demonstrating mediation by CB1R. Intraplantar administration of the mixed CB_1/CB_2 agonist WIN55,212-2 also attenuates

the development of carrageenan-evoked mechanical hyperalgesia, allodynia, and spinal Fos protein expression (Nackley et al. 2003b); these latter actions were completely blocked by coadministration of either a CB1R or CB2R antagonist.

Peripheral CB1R in Acute Antinociception and Antinociceptive Synergism

Cannabinoids induce a site-specific topical antinociception to thermal stimulation (Dogrul et al. 2003; Johanek and Simone 2004; Ko and Woods 1999; Yesilyurt et al. 2003). This local antinociceptive effect synergizes with spinal cannabinoid antinociception, as reflected by a 15-fold leftward shift in the dose–response curve (Dogrul et al. 2003), and also synergizes with topical morphine antinociception (Yesilyurt et al. 2003). The latter effects were blocked by a CB1R antagonist (Yesilyurt et al. 2003).

Peripheral CB1R Modulation of Formalin-Evoked Nocifensive Behavior

Intraplantar administration of formalin induces a biphasic pain response that is characterized by an early acute period (phase 1), a brief quiescent period, and a second phase of sustained "tonic" pain behavior (phase 2). The early phase reflects formalin-activation of Aβ, Aδ, and C-primary afferent fibers (McCall et al. 1996; Puig and Sorkin 1996). The late phase also activates Aδ and C fibers not activated during phase 1 (Puig and Sorkin 1996) and involves inflammation and long-term changes in the central nervous system (Coderre and Melzack 1992). Intraplantar administration of exogenous anandamide produces antinociception in the formalin test (Calignano et al. 1998), an effect blocked by systemic administration of the CB1R antagonist SR141716A. Anandamide produced antinociception only during phase 1, which likely reflects the short duration of action of anandamide, as the metabolically stable analog methanandamide suppressed pain behavior during both phase 1 and 2 (Calignano et al. 1998).

Peripheral CB1R Modulation of Capsaicin-Evoked Hyperalgesia

Intradermal administration of capsaicin to rats or humans induces hyperalgesia. Primary hyperalgesia, especially that elicited by noxious thermal stimulation, is mediated partly by sensitization of C-polymodal nociceptors (Baumann et al. 1991; Kenins 1982; LaMotte et al. 1992; Simone et al. 1987; Szolcsanyi et al. 1988; Torebjork et al. 1992). Secondary hyperalgesia is elicited in surrounding uninjured tissue and involves central nervous system sensitization rather than sensitization of peripheral nociceptors (Baumann et al. 1991; LaMotte et al. 1992; LaMotte et al. 1991; Simone et al. 1989) and requires conduction in primary afferent A fibers (Torebjork et al. 1992). Systemic administration of the mixed CB_1/CB_2 agonist WIN55,212-2, but not its receptor-inactive enantiomer, suppresses capsaicin-evoked thermal and mechanical hyperalgesia and nocifensive behavior (Li et al. 1999), demonstrating that the actions of WIN55,212-2 were receptor-mediated. A peripheral CB1R mechanism is implicated in the attenuation of capsaicin-evoked heat hyperalgesia by locally administered cannabinoids in nonhuman primates (Ko and

Woods 1999). Topical administration of the cannabinoid agonist HU210 to human skin also suppresses capsaicin-evoked thermal hyperalgesia and touch-evoked allodynia (Rukwied et al. 2003), although pharmacological specificity has not been assessed. Cannabinoid modulation of capsaicin-evoked hyperalgesia involves peripheral and central mechanisms. A CB1R mechanism is also implicated in the attenuation of hyperalgesia induced by locally administered cannabinoids following intradermal capsaicin (Johanek et al. 2001) or cutaneous heat injury (Johanek and Simone 2004).

The efficacy of peripheral cannabinoid mechanisms in suppressing neuronal activation evoked by corneal application of the small-fiber excitant mustard oil has been documented at the level of the lower brainstem. Corneal nociceptor activity, assessed using mustard oil-evoked Fos protein expression at the trigeminal interpolaris/caudalis (Vi/Vc) transition, was suppressed by direct corneal application of WIN55,212-2, and these effects were blocked by systemic administration of SR141716A (Bereiter et al. 2002), but CB2R mechanisms were not assessed. These suppressions occurred in the absence of changes in Fos at the subnucleus caudalis junction, thereby suggesting a role for CB1R mechanisms, at least in part, in regulating reflexive aspects of nociception and/or contributing to homeostasis of the anterior eye. More work is necessary to determine if CB2R mechanisms are implicated in regulation of corneal nociceptor activity.

Peripheral CB1R Modulation of Capsaicin-Evoked Neuropeptide Release

Anandamide suppressed capsaicin-evoked plasma extravasation in vivo through a peripheral CB1R mechanism (Richardson et al. 1998c) and inhibits capsaicin-evoked CGRP release in rat dorsal horn (Richardson et al. 1998a) and peripheral paw skin in vitro (Richardson et al. 1998c). Although pharmacological specificity was not assessed in the in vitro superfusion studies, these effects occurred at low concentrations [100 nM; (Richardson et al. 1998c)], consistent with mediation by CB1R.

Capsaicin-evoked CGRP release is enhanced in paw skin derived from rats with diabetic neuropathy induced by streptozotocin (Ellington et al. 2002). The mixed CB_1/CB_2 agonist CP55,940 attenuated capsaicin-evoked CGRP release in diabetic and nondiabetic animals, and these effects were blocked by a CB1R but not a CB2R antagonist (Ellington et al. 2002). Interestingly, anandamide inhibited capsaicin-evoked CGRP release in nondiabetic but not in diabetic rat skin, but neither the CB1R nor the CB2R antagonist attenuated these effects. Functional changes following diabetic neuropathy may have prevented these inhibitory effects of anandamide on capsaicin-evoked CGRP release. Anandamide also increased capsaicin-evoked CGRP release at high concentrations, possibly through a TRPV1 mechanism, although susceptibility to blockade by TRPV1 antagonists would be required to establish pharmacological specificity. Anandamide also inhibits in vivo release of CGRP and somatostatin induced by systemically administered resiniferatoxin, a potent TRPV1 ligand; the inhibitory effects of anandamide on plasma neuropeptide levels were blocked by a CB1R antagonist (Helyes et al. 2003).

Peripheral CB1R Modulation of Nerve Injury-Induced Nociception

A role for CB1Rs in suppressing hyperalgesia and allodynia induced by nerve injury has been demonstrated in multiple models of neuropathic pain (Bridges et al. 2001; Fox et al. 2001; Herzberg et al. 1997; Mao et al. 2000). Fox and colleagues demonstrated that intraplantar administration of WIN55,212-2 suppresses mechanical hyperalgesia following partial ligation of the sciatic nerve; these effects were blocked by the CB1R antagonist/inverse agonist SR141716A administered systemically (Fox et al. 2001). These data suggest a peripheral CB1R action in neuropathic pain, although CB2R mechanisms were not assessed. WIN55,212-2 also suppresses thermal hyperalgesia as well as mechanical and cold allodynia following spinal nerve ligation (Bridges et al. 2001). These latter effects were blocked by systemic administration of a CB1R but not a CB2R antagonist (Bridges et al. 2001), suggesting that the antihyperalgesic effects of systemically administered WIN55,212-2 were mediated by CB1R (Bridges et al. 2001; Herzberg et al. 1997).

3.3 Antinociception Mediated by CB1R in Spinal Cord

3.3.1 Distribution of CBRs on Central Terminals of Primary Afferents

Receptors are typically bidirectionally transported from the soma to central and peripheral terminals (Young et al. 1980). To identify afferents likely to contain CBRs, Hohmann and Herkenham assessed their pre- and postsynaptic distributions in the spinal cord using receptor binding and quantitative autoradiography (Hohmann et al. 1999a; Hohmann and Herkenham 1998). Destruction of sensory C fibers with neonatal capsaicin treatment produced only modest (16%) decreases in cannabinoid binding sites in the superficial dorsal horn, as measured by receptor binding and quantitative autoradiography (Hohmann and Herkenham 1998). These data suggest that a majority of spinal CBRs is not localized to central terminals of primary afferent C fibers. Multisegment unilateral cervical dorsal rhizotomy (C3-T1 or T2) produced time-dependent losses in cannabinoid binding densities in the dorsal horn (Hohmann et al. 1999a) of larger magnitude than that induced by neonatal capsaicin treatment. This observation is unsurprising because rhizotomy destroys the central terminals of both small- and large-diameter fibers. Rhizotomy suppressed [^{3}H]CP55,940 binding in the superficial and neck region of the dorsal horn as well as in the nucleus proprius without affecting binding in lamina X or the ventral horn. By contrast, massive losses in μ-opioid binding sites were observed in lamina I and II in adjacent sections following either neonatal capsaicin or rhizotomy (Hohmann et al. 1999a; Hohmann and Herkenham 1998), consistent with previous reports (Besse et al. 1990; Nagy et al. 1980). These data support the conclusion that CB1Rs occur both pre- and postsynaptically in the spinal dorsal horn, with the majority of receptors occurring postsynaptically. This conclusion is consistent with the observation of CB1R-immunoreactive fibers in dorsal roots (Sanudo-Pena et al. 1999) and in axons of Lissauer's tract (Salio et

al. 2002b), and immunocytochemical studies showing that CB1R and vanilloid receptor (TRPV1) immunostaining is reduced in parallel in the superficial dorsal horn following neonatal capsaicin treatment (Morisset et al. 2001). Of course, postsynaptic changes that occur subsequent to an extensive rhizotomy (Hohmann et al. 1999a) can also contribute to the pattern of receptor changes observed.

By contrast, Farquhar-Smith and colleagues, using an antibody directed against the C-terminal of CB1R, demonstrated that lumbar dorsal rhizotomy induced a minor, though significant, reduction in CB1R immunoreactivity (Farquhar-Smith et al. 2000). Consistent with these observations, CB1R immunoreactivity in the superficial dorsal horn showed a laminar overlap with markers of thin primary afferents, as identified by immunoreactivity for CGRP, substance P, isolectin B4 (IB4), and TRPV1, but very little colocalization of CB_1 was observed with any of these markers at the single-fiber level (Farquhar-Smith et al. 2000). Similarly, minimal colocalization of CB1Rs was observed with these markers in dorsal root ganglion cells, using the same antibody (Bridges et al. 2003). These data collectively suggest that the majority of CB1Rs are not localized to central terminals of nociceptive primary afferents, but rather are localized on postsynaptic sites, and provide indirect support for the hypothesis that CB1Rs in spinal cord are localized predominantly to fibers of intrinsic spinal neurons.

3.3.2 Distribution of CB1R mRNA and CB1R Immunoreactivity in Spinal Cord

The presence of CB1R mRNA in rat dorsal horn has been reported (Mailleux and Vanderhaeghen 1992). Hohmann (2002) characterized the laminar distribution of CB1R mRNA-expressing cells in rat lumbar spinal cord using a highly sensitive cRNA probe. CB1R mRNA was found in all spinal laminae except lamina IX; motoneurons in this region, which are immunoreactive for fatty acid amide hydrolase (FAAH) (Tsou et al. 1998b), were CB1R mRNA negative. Expression was dense in lamina X and sparsest in III and IV. CB1R mRNA was highly expressed in lamina V and VI and the medial part of IV. These laminae contained many large cells with high levels of expression. In general, primary afferents that project to deeper parts of the dorsal horn (here III–VI) include coarser caliber fibers than those projecting to the superficial laminae, although small diameter fibers are also observed. Small-diameter fibers from viscera also project to lamina V, VII, and X (see Grant 1995 for review). By contrast, the superficial dorsal horn (lamina I and II) had many small cells with low levels of expression compared to cells observed in deeper lamina. Lamina I and II neurons receive inputs from unmyelinated as well as finely myelinated primary afferents (see Grant 1995 for review). Thus, in situ hybridization studies demonstrate that spinal neurons synthesize CB1Rs, although they do not address putative localization of these receptors to spinal interneurons and/or terminals of supraspinally projecting efferents.

Immunocytochemical studies have provided information about the cellular elements expressing CB1Rs in the spinal cord. The C-terminal antibody employed by Farquhar-Smith et al. (2000) exclusively labeled fibers and terminals, whereas

the antibody employed by Tsou and colleagues (Sanudo-Pena et al. 1999) additionally labeled cell bodies. Tsou and colleagues, using an antibody raised against the first 77 residues of the N terminus of CB1R, identified beaded immunoreactive fibers throughout the spinal dorsal horn and in lamina X surrounding the central canal (Tsou et al. 1998a). Further work by this group also revealed the presence of lightly stained cells throughout the spinal cord gray matter (Sanudo-Pena et al. 1999). Farquhar-Smith and colleagues, using an antibody directed against the C-terminal 13 amino acids of CB1R, demonstrated immunoreactivity for CB1Rs in fibers and terminals with no consistent immunoreactivity observed in any cell bodies (Farquhar-Smith et al. 2000).

3.3.3 Evidence for CB1Rs on Spinal Interneurons

There is considerable support for localization of CBRs in rat spinal cord postsynaptic to primary afferents at both light and electron microscope levels. Direct evidence for postsynaptic localization of CB_1 in spinal dorsal horn is derived from the observation that intrinsic excitatory interneurons in lamina IIi that expressed protein kinase C isoform γ showed high levels of colocalization with CB_1 (Farquhar-Smith et al. 2000); this pattern may suggest an anatomical basis for the efficacy of cannabinoids in ameliorating inflammatory and neuropathic pain (Bridges et al. 2001; Fox et al. 2001; Herzberg et al. 1997; Malmberg et al. 1997; Mao et al. 2000).

CB1R immunoreactivity has also been localized to dorsal horn interneurons containing γ-aminobutyric acid (GABA) (Salio et al. 2002b). GABA presynaptically inhibits primary afferent input to the spinal cord. The observation of GABAergic dendrites postsynaptic to primary afferents also suggests that primary afferents are anatomically positioned to activate GABAergic inhibitory circuits. GABAergic interneurons can also synapse directly on dorsal horn neurons to reduce excitatory input. The demonstrated colocalization of CB1R with GABA is consistent with functional studies demonstrating a CB1R-mediated presynaptic inhibition of GABAergic and glycinergic transmission in recordings performed in rat medullary dorsal horn in vitro (Jennings et al. 2001). By contrast, postsynaptic effects on medullary substantia gelatinosa neurons were not observed (Jennings et al. 2001). These data suggest that cannabinoids act through a disinhibitory action on lamina II neurons by inhibiting GABAergic transmission.

Immunoreactivity for CB1R and μ-opioid receptors (MOR) is also colocalized on lamina II interneurons at the ultrastructural level (Salio et al. 2001). In this work, CB1R was predominantly localized postsynaptically in dendrites and cell bodies, but immunoreactive axons and axon terminals were also observed (Salio et al. 2001). Both species showed rare labeling of the plasma membrane. Since MOR1 is not colocalized with GABA (Gong et al. 1997; Kemp et al. 1996), these data support the presence of CB1R in distinct populations of intrinsic spinal neurons (Salio et al. 2001). By contrast, colocalization of CB1R with MOR1 in thin primary afferent terminals could not be convincingly demonstrated in this work (Salio et al. 2001).

3.3.4
Evidence for CB1Rs on Afferents Originating Supraspinally

CB1R immunoreactivity is highly expressed at all spinal levels in fibers of the dorsolateral funiculus (DLF) and in the intermediolateral nucleus (Farquhar-Smith et al. 2000). Interruption of descending pathways (and ascending pathways from lamina I) that course in the DLF produced only a 5% change in CB1R immunoreactivity (Farquhar-Smith et al. 2000). These data suggest that CB1R immunoreactivity, in general, is not localized on terminals of neurons originating supraspinally and suggest localization of CB1R to intrinsic spinal neurons and/or ascending projections (Farquhar-Smith et al. 2000). Because visceral primary afferents project to the nucleus of the DLF, CB1Rs are appropriately positioned to influence visceral afferent input as well as viscero-somatic integration (Farquhar-Smith et al. 2000). These observations are consistent with cannabinoid modulation of visceral hyperalgesia (see Hohmann 2002 for review). Ascending projections to the brainstem, hypothalamus, and thalamus have been shown to originate in lamina X (Molander and Grant 1995). The presence of CB1R immunoreactivity in lamina X and in the intermediolateral nucleus may also reflect interaction of CB1R with neurons of the sympathetic nervous system (Farquhar-Smith et al. 2000).

3.3.5
Evidence for CB1Rs on Nonneuronal Cells at the Spinal Level

CB1R has recently been demonstrated in astrocytes in laminae I and II of the spinal dorsal horn using multiple antibodies directed against the C-terminal tail of CB_1 (Salio et al. 2002a). By contrast, astrocytes were not labeled in rat spinal cord when an N-terminal-specific anti-CB1R antibody was employed (Salio et al. 2002b). The functional roles of putative CB1R subtypes in spinal glial cells require further investigation (Salio et al. 2002a).

3.3.6
Antinociceptive and Electrophysiological Effects of Spinally Administered Cannabinoids

Antinociceptive effects of cannabinoids are mediated, in part, at the spinal level. Spinal reflexive responses to noxious stimuli are inhibited by cannabinoids in spinally transected dogs (Gilbert 1981). Support for spinal mechanisms of cannabinoid analgesic action is also derived from the ability of intrathecally administered cannabinoids to produce antinociception (Smith and Martin 1992; Welch et al. 1995; Yaksh 1981). The behavioral data are consistent with the ability of spinally administered cannabinoids to suppress noxious heat-evoked and after-discharge firing (Hohmann et al. 1998) and noxious stimulus-evoked Fos protein expression in the spinal dorsal horn neurons (Hohmann et al. 1999c). Spinal administration of a CB1R-selective agonist also inhibits C fiber and Aδ fiber-evoked responses of wide dynamic range (WDR) neurons through a CB1R mechanism with only minor

effects on A-β fiber-evoked responses (Kelly and Chapman 2001). Systemic and intrathecally administered cannabinoids retain a weak but long-lasting antinociceptive effect in spinally transected rats (Lichtman and Martin 1991b; Smith and Martin 1992), providing compelling evidence for spinal mechanisms of cannabinoid antinociception.

Spinal administration of a cannabinoid (HU210) also suppresses C fiber-mediated post-discharge responses, a measure of neuronal hyperexcitability, in carrageenan-inflamed and noninflamed rats (Drew et al. 2000); these effects were blocked by a CB1R antagonist. Spinal administration of anandamide produced CB1R-mediated effects in carrageenan-inflamed rats that were similar to that reported for HU210, but only inconsistent effects were observed in noninflamed rats (Harris et al. 2000). Upregulation of CB1Rs is also observed in the spinal cord following nerve injury, suggesting that regulation of spinal CB1Rs may contribute to the therapeutic efficacy of cannabinoids in neuropathic pain states (Lim et al. 2003). These data implicate involvement of spinal CB1Rs in both acute and persistent pain states.

3.4 Antinociception Mediated by CB1Rs in Supraspinal Pain Circuits

Support for supraspinal sites of cannabinoid antinociceptive action is derived from the antinociceptive effects of cannabinoids following intracerebroventricular administration (Hohmann et al. 1999b; Martin et al. 1993) and the attenuation of cannabinoid antinociception following disruption of communication between brain and spinal cord. Both the antinociceptive (Lichtman and Martin 1991b) and electrophysiological (Hohmann et al. 1999b) effects of systemically administered cannabinoids are attenuated following spinal transection, suggesting the involvement of supraspinal sites of cannabinoid analgesic action. Intrathecal administration of the α_2 antagonist yohimbine but not the serotonin antagonist methysergide also blocks the antinociceptive effect of systemically administered Δ^9-tetrahydrocannabinol (Δ^9-THC) (Lichtman and Martin 1991a). Furthermore, the antinociceptive efficacy of systemically administered cannabinoids is markedly attenuated following neurotoxic destruction of descending noradrenergic projections to the spinal cord (Gutierrez et al. 2003). These data collectively implicate a role for descending noradrenergic systems in cannabinoid antinociceptive mechanisms.

Direct evidence for supraspinal sites of cannabinoid antinociception is derived from studies employing intracranial administration of cannabinoids. Site-specific injections of cannabinoid agonists to various brain regions have permitted the identification of brain loci implicated in cannabinoid antinociception. The active sites included the dorsolateral periaqueductal gray, dorsal raphe nucleus, RVM, amygdala, lateral posterior and submedius regions of the thalamus, superior colliculus, and noradrenergic A5 region (Martin et al. 1995, 1998, 1999a). These studies suggest that endocannabinoid actions at these sites are sufficient to produce antinociception.

3.4.1 Role of the Periaqueductal Gray

Studies of metabolically stable anandamide analogs and the effects of anandamide in FAAH knockout mice lead to the conclusion that anandamide would produce antinociceptive effects upon release in the appropriate brain, spinal, or peripheral sites. Electrical stimulation of the dorsal aspect of the periaqueductal gray (PAG) caused CB1R-mediated analgesia evidenced by a markedly reduced effect following administration of SR141716A (Walker et al. 1999). This work suggested that the dorsal PAG serves as a substrate for cannabinoid antinociception. Exogenously applied cannabinoids have been shown to inhibit GABAergic and glutamatergic neurons in rat PAG neurons through presynaptic mechanisms (Vaughan et al. 2000). These effects occurred in the absence of direct postsynaptic actions on PAG neurons, thus providing a neurophysiological basis for cannabinoid modulation of nociceptive transmission through presynaptic actions.

Metabotropic glutamate and *N*-methyl-D-aspartate (NMDA) receptors are required for cannabinoid antinociception at the level of the PAG. Infusion of the CBR agonist WIN55,212-2 into the PAG produced dose-dependent increases in paw withdrawal latencies to a noxious thermal stimulus (Palazzo et al. 2001). This effect was blocked by pretreatment with SR141716A. Blockade of $mGlu_5$ metabotropic glutamate receptors but not $mGlu_1$ receptors blocked the effects of WIN55,212-2. Both $mGlu_5$ and $mGlu_1$ receptors belong to group I class of metabotropic glutamate receptors that are G protein-coupled and positively coupled to phospholipase C. Pretreatment with antagonists for group II (which includes $mGlu_2$ and $mGlu_3$) and group III (which includes $mGlu_4$, $mGlu_6$, $mGlu_7$, and $mGlu_8$) metabotropic glutamate receptors also suppressed WIN55,212-2-induced analgesia. This latter class of receptors is negatively coupled to adenylate cyclase and preferentially localized to presynaptic active zones associated with autoreceptors. In addition to these metabotropic receptors, a selective antagonist for ionotropic glutamate (NMDA) receptors also blocked the antinociceptive effects of WIN55,212-2.

It has been postulated that the effect of antagonism of group II and III metabotropic receptors on cannabinoid antinociception is attributable to an increased release of GABA in the PAG (Palazzo et al. 2001). Because GABAergic interneurons within the PAG tonically inhibit descending antinociceptive pathways (Moreau and Fields 1986), an inhibition of PAG descending pathways may underlie the observed blockade of cannabinoid antinociception through modulation of GABAergic interneurons. In vitro studies demonstrate that cannabinoids inhibit GABA and glutamate release presynaptically in the PAG in the absence of direct postsynaptic effects on PAG neurons (Vaughan et al. 2000). By contrast, antagonists for $mGlu_5$ and NMDA, which are localized postsynaptically, could reduce the tonic excitatory control of glutamate on descending antinociceptive pathways with cells of origin in the PAG (Palazzo et al. 2001), thereby modulating cannabinoid antinociception through a distinct mechanism.

3.4.2 Role of Rostral Ventral Medulla

Researchers have targeted synthetic cannabinoids at other brainstem nuclei including the RVM (Martin et al. 1998; Monhemius et al. 2001; Vaughan et al. 1999) and the nucleus reticularis gigantocellularis (Monhemius et al. 2001) to better characterize sites of cannabinoid-mediated antinociception. Site-specific administration of cannabinoids (WIN55,212-2 and HU210) in the RVM produced significant antinociception in the tail-flick test (Martin et al. 1998). Mediation by CBRs was established because the antinociceptive effects of HU210 were blocked by the CB1R antagonist SR141716A, and the receptor-inactive enantiomer WIN55,212-3 failed to induce antinociception following microinjection to the same site (Martin et al. 1998).

Electrophysiological studies have provided insight into the mechanisms mediating these antinociceptive effects. Cannabinoids modulate on- and off-cells in the RVM (Meng et al. 1998), demonstrating their ability to control descending pain modulatory signaling in a manner similar to that of morphine. Pharmacological inactivation of the RVM with site-specific administration of the $GABA_A$ receptor agonist muscimol blocked the antinociceptive effects but not the motor deficits of systemically administered WIN55,212-2 (Meng et al. 1998). At the cellular level, it appears that cannabinoids exert their physiological effects in the RVM by presynaptic inhibition of GABAergic neurotransmission (Vaughan et al. 1999).

3.4.3 Role of the Basolateral Amygdala

The amygdala is a nuclear complex located in the limbic forebrain that plays a key role in the coordination of fear and defensive reactions. The amygdala is optimally positioned anatomically to receive and integrate sensory information from multiple modalities and, in turn, to mediate emotional, autonomic, and somatic motor reactions to salient stimuli (especially threatening stimuli) (Davis and Whalen 2001). Within the amygdala, CB1R immunoreactivity has been detected in a subset of GABAergic interneurons in the basolateral complex (Marsicano et al. 2002), a site implicated in the formation and storage of aversive memories (Medina et al. 2002). Endocannabinoids are elevated in the basolateral amygdala in a conditioned fear-aversion paradigm (Marsicano et al. 2002), supporting the hypothesis that endocannabinoids serve naturally to inhibit extinction of aversive memories. Presentation of the conditioned aversive stimulus during extinction trials elicited elevated levels of the endocannabinoids 2-arachidonoylglycerol (2-AG) and anandamide in the basolateral nucleus of the amygdala but not the medial prefrontal cortex (another brain area implicated in memory formation) of mice. Marsicano et al. (2002) reported that endocannabinoids and CB1Rs in the basolateral nucleus of the amygdala are crucial to the long-term depression of GABAergic inhibitory currents, positing that endocannabinoids regulate aversive memory extinction via selective inhibition of local inhibitory networks in the amygdala.

The amygdala also plays a critical role in modulating antinociception. Microinjection of cannabinoids into the basolateral nucleus of the amygdala produces antinociception in the tail-flick test (Martin et al. 1999a). Microinjection of μ-opioid agonists into the basolateral nucleus of the amygdala similarly results in marked antinociceptive responding in the radiant heat tail-flick (Helmstetter et al. 1993, 1995) and formalin tests (Manning and Mayer 1995). Moreover, bilateral lesions of the amygdala rendered nonhuman primates less sensitive to the antinociceptive effects of the potent synthetic cannabinoid WIN55,212-2 (Manning et al. 2001). In rodents, microinjection of the $GABA_A$ agonist muscimol into the central nucleus of the amygdala but not into the basolateral nucleus of the amygdala, reduced the antinociceptive effects of systemic WIN55,212-2 (Manning et al. 2003). Moreover, the endocannabinoid-degrading enzyme FAAH is localized in the basolateral and lateral amygdala (Egertová et al. 2003; Tsou et al. 1998b). These data indicate that a mechanism exists for inactivation of endocannabinoid actions in the basolateral amygdala. Both conditioned (Helmstetter 1992; Helmstetter and Bellgowan 1993) and unconditioned (Bellgowan and Helmstetter 1996) stress-induced analgesia depend on intact functioning of the amygdala. These observations, together with the demonstration of cannabinoid-mediated antinociceptive effects following site-specific administration to the basolateral nucleus of the amygdala (Martin et al. 1999a), suggest that endocannabinoids may serve naturally to suppress environmentally induced pain by actions in the amygdala.

4
Antinociception Mediated by CB2Rs

In clinical trials of THC and other cannabinoid agonists for pain pharmacotherapy, unwanted, negative psychotropic effects limit dosing to levels that are probably below those producing maximal analgesic efficacy. These effects are caused by actions of the compounds at CB1Rs in the brain. However, CB2Rs are either absent or expressed in low levels by neural tissues (Munro et al. 1993; Zimmer et al. 1999). This distribution has led to evaluation and validation of CB2Rs as targets for novel pharmacotherapies for pain.

4.1
Localization of CB2Rs that Contribute to Cannabinoid Antinociception

CB2Rs are expressed by cells that are involved in inflammation and thereby pain. Among them are monocytes, polymorphonuclear neutrophils, mast cells, B cells, T cells, and natural killer cells (see Cabral and Staab, this volume). CB2Rs are also found on microglia (Walter et al. 2003), which play an important role in pathological pain states (Zhang et al. 2003). Recent pharmacological evidence also supports the presence of CB2Rs in human and guinea pig vagus nerve (Patel et al. 2003). CB2R immunoreactivity has been detected in dorsal root ganglion cells (Ross et al. 2001a) in cultures derived from neonatal rats. More work is necessary

to identify the phenotypes of cells expressing CB2Rs, especially since levels of CB2R mRNA in neurons of dorsal root (Hohmann and Herkenham 1999a) and trigeminal (Price et al. 2003) ganglia are near background under conditions in which CB_1 mRNA is clearly demonstrated.

4.2 CB2R-Mediated Antinociceptive Effects

CB2R agonists are antinociceptive in models of acute (Malan et al. 2001) and persistent pain (Clayton et al. 2002; Hanus et al. 1999; Hohmann et al. 2004; Ibrahim et al. 2003; Nackley et al. 2003a). Direct evidence of CB2R-mediated antinociceptive effects was reported by Hanus et al. (1999) using HU-308, a highly selective CB2R agonist ($K_i = 22.7$ CB2R vs >10 μM CB1R). They found that HU-308 (50 mg/kg) produced marked decreases in pain behavior in rats receiving hindpaw injections of dilute formalin. This effect occurred without any change in motor function, a centrally mediated effect of CB1R agonists that may predict psychoactivity in humans. HU-308 also reduced the swelling produced by arachidonic acid. The CB2R-selective cannabinoid antagonist SR144528 blocked these effects. Another CB2R agonist, AM1241, has also been shown to induce a CB2R-mediated antinociceptive effect in otherwise untreated rats while failing to elicit centrally mediated side effects such as hypothermia, catalepsy, and hypoactivity (Malan et al. 2001). AM1241 also induces CB2R-mediated suppression of carrageenan and capsaicin-evoked thermal and mechanical hyperalgesia and allodynia (Hohmann et al. 2004; Nackley et al. 2003b; Quartilho et al. 2003) and suppresses carrageenan-evoked Fos protein expression (Nackley et al. 2003a). These effects were blocked by the CB2R-selective antagonist but not by a CB1R-selective antagonist.

Electrophysiological studies also support a role for CB2Rs in suppressing nociception. AM1241 induced CB2R-mediated suppression of C fiber-evoked responses and windup in spinal WDR neurons; this suppression was observed in both the absence and presence of carrageenan inflammation and following local and systemic drug administration (Nackley et al. 2004). The suppressive effects of AM1241 were more pronounced in the presence compared to the absence of inflammation. By contrast, low threshold, purely non-nociceptive spinal neurons did not show sensitization during the development of inflammation and were not altered by AM1241 actions in the periphery (Nackley et al. 2004). Intraplantar administration of anandamide also suppresses mechanically evoked responses in spinal dorsal horn neurons in the carrageenan model of inflammation; these effects were blocked by a CB2R-selective antagonist (Sokal et al. 2003). These data demonstrate that activation of peripheral cannabinoid CB2Rs is sufficient to suppress neuronal activity at central levels of processing in the spinal dorsal horn. Sensory hypersensitivity in animals with nerve injury was also reduced by a CB2R agonist (Ibrahim et al. 2003). In light of the induction of CB2Rs in the spinal dorsal horn by neuropathic pain states, coincident with the appearance of activated microglia, it appears likely that these latter effects are mediated, at least in part, by nonneuronal cells.

The main effect of inflammatory cells in nociception is to sensitize neurons. This occurs in the periphery when the immune response stimulates peripheral cells to secrete mediators that sensitize primary afferent neurons. Substances released by immune cells that sensitize nociceptors include histamine, serotonin, eicosanoids, interleukin 1, tumor necrosis factor-α, and nerve growth factor (Dray 1995; McMahon 1996; Tracey and Walker 1995). Sensitization also occurs in the CNS, and centrally located microglia, which express CB2Rs, may be involved in the sensitization of central nociceptive neurons during inflammation (reviewed by DeLeo et al. 2004).

CB2R agonists reduce the secretion of inflammatory mediators from immune cells. For example, cannabinoids inhibit lipopolysaccharide (LPS)-inducible cytokine mRNA expression in rat microglial cells (Puffenbarger et al. 2000) and cytotoxicity and release of inflammatory mediators from monocytic cells (Klegeris et al. 2003). Activation of CB2Rs localized to mast cells or other immune cells also attenuates the release of inflammatory mediators, including nerve growth factor (Rice et al. 2002) and cytokines (Klegeris et al. 2003) that in turn sensitize nociceptors (Mazzari et al. 1996). In the presence of inflammation, CB2R agonists could thus act locally on immune cells in the periphery and suppress C fiber sensitization. These observations suggest that the effects of CB2R ligands occur via the decreased release of inflammatory mediators from peripheral immune cells in the periphery and microglia in the CNS. However, CB2R modulation of immune responses does not readily account for the effects of AM1241 on windup and C fiber responses in the absence of inflammation and local antinociceptive effects of this compound that are observed in otherwise untreated rats (Malan et al. 2001). Direct effects on CB2Rs localized to primary afferents (Griffin et al. 1997; Patel et al. 2003; Ross et al. 2001a; see also Hohmann and Herkenham 1999a; Price et al. 2003) could provide a parsimonious explanation for the antinociceptive and electrophysiological actions of CB2R agonists observed in the absence of inflammation. Malan's group has recently identified a potential mechanism of action for AM1241; AM1241 is likely to suppress primary afferent activation indirectly by stimulating local release of β-endorphin in peripheral tissue through a CB2R-specific mechanism (Malan et al. 2004).

Besides suggesting a novel pharmacotherapy for pain, these findings suggest that CB2R activation by endocannabinoids would promote anti-inflammatory and antinociceptive effects, some of which may be mediated by non-neuronal cells in the CNS.

5
Pain Modulation by Endocannabinoids

Seven putative endocannabinoids have been identified: (1) anandamide, (2) dihomo-γ-linolenoylethanolamide (HEA), (3) docosatetraenoylethanolamide (DEA), (4) 2-AG, (5) noladin ether, (6) virodhamine, and (7) *N*-arachidonoyldopamine (NADA). The roles of these novel putative endogenous compounds in pain and inflammation have been a recent focus of investigations. The sections above, which described the

relationship between pain circuits, exogenous drugs, and CBRs provide a foundation for understanding how these putative endocannabinoids may operate physiologically to modify pain perception. Proving that a particular endocannabinoid plays such a role requires first the demonstration that it can produce antinociception within the proposed site of action, then the demonstration that it is formed and released in the proposed site under conditions where pain sensitivity is altered. In the following, we review the data for each endocannabinoid with these criteria in mind.

5.1 Anandamide

Anandamide was the first putative endocannabinoid to be identified (Devane et al. 1992) and has therefore been the focus of the majority of investigations of endocannabinoid mechanisms of pain suppression.

5.1.1 Effects of Exogenous Anandamide on Pain Sensitivity

In studies of physiological pain (i.e., pain induced by noxious stimuli in animals free of inflammation, nerve injury, or other pathology), anandamide typically produced antinociceptive effects, but these effects were not blocked by cannabinoid antagonists (Adams et al. 1998; Vivian et al. 1998). This effect was likely due to the rapid metabolism of anandamide by FAAH, since FAAH knockout mice exhibit marked CB1R-mediated analgesic responses to anandamide (Cravatt et al. 2001). However, in animals with nerve injury, at doses of 10 and 100 μg i.v., anandamide reversed neuropathic mechanical hyperalgesia, and this effect was antagonized by the CB1R and CB2R antagonists SR141716A and SR144528.

These findings above are in good agreement with electrophysiological and neurochemical studies of the effects of anandamide on sensory neurons. In 64% of neurons examined, anandamide (10 μM) depressed Aδ fiber-evoked excitatory postsynaptic currents (EPSCs) (Luo et al. 2002). By contrast, an inhibitory action of anandamide on C fiber-evoked EPSCs was observed in only 31% of neurons tested. Anandamide also inhibited the release of neuropeptides evoked by a TRPV1 agonist (Helyes et al. 2003). These findings are consistent with studies of the localization of CBRs (see Sect. 3.2.1) and suggest that anandamide acts primarily on larger caliber peripheral afferent fibers and cells (see Sect. 3.2.1).

5.1.2 Effects of Inhibition of the Putative Anandamide Transporter

Another approach to examining the role of endogenous anandamide in pain has been to employ transport inhibitors such as AM404. Blocking transport would be expected to block the reuptake of anandamide and cause increased levels to

occur in the vicinity of CBRs, with both processes leading to increased occupation of CBRs. Beltramo et al. (1997) showed that administration of AM404 caused the accumulation of anandamide in cultures of cortical neurons and enhanced the hot-plate analgesia produced by systemically administered anandamide. AM404 alone did not alter pain sensitivity, suggesting that anandamide does not act tonically to maintain pain thresholds for thermal stimuli. The paper did not address whether environmentally produced analgesia was affected by AM404 (but see Hohmann et al. 2001).

5.1.3
Modulation of Pain by Endogenous Anandamide

Anandamide appears to participate in endogenous pain modulation by actions in the PAG. Blocking the CB1R with the antagonist SR141716A produced hyperalgesia in the formalin test (Calignano et al. 1998; Strangman et al. 1998) and prevented the analgesia produced by electrical stimulation of the dorsolateral PAG (Walker et al. 1999). These pro-nociceptive actions of the antagonist are reasonable evidence for an antinociceptive action of one or more endocannabinoids, but conclusions along this line are limited by the possible confound with the proposed inverse-agonist activity of current CBR antagonists (Landsman et al. 1997). In order to address directly the questions regarding the role of endocannabinoids that were made inferentially from the actions of an antagonist, the release of anandamide in the PAG was studied using microdialysis (Walker et al. 1999). This method permits collection of neurotransmitters/modulators from the extracellular space, and is therefore an indicator of the release of these modulators. Microdialysis coupled with liquid chromatography/mass spectrometry established that the analgesia producing electrical stimulation or injections of the chemical irritant formalin into the hindpaws of anesthetized rats induced the release of anandamide in the PAG. Thus, it appears that either pain itself, or electrical stimulation leads to the release of anandamide, which acts on CB1Rs in the PAG to inhibit nociception.

5.2
Dihomo-γ-Linolenoylethanolamide and Docosatetraenylethanolamide

HEA and DEA were reported together by Hanus et al. (1993) as cannabinoids similar in structure to anandamide but with different fatty acyl chains: 20:3 (n-6) and 22:4 (n-6) for HEA and DEA, respectively. As they have been studied together often and produce similar results, they are considered together here. Koga et al. (1997) verified the occurrence of these compounds as endogenous to a variety of mammalian tissues by using liquid chromatography/mass spectrometry. A recent study indicates that, along with anandamide, these two compounds are formed in astrocytes, suggestive of a potential role in inflammatory pain (Walter et al. 2002). These compounds possess binding affinities for CB1Rs that are similar to that of anandamide (Felder et al. 1993; Hanus et al. 1993; Vogel et al. 1994). They also inhibit

forskolin-stimulated cyclic AMP (cAMP) and electrically evoked contractions of the mouse vas deferens with potencies similar to that of anandamide (Felder et al. 1993; Pertwee et al. 1994; Vogel et al. 1994). Piomelli et al. (1999) reported that HEA effectively competes against anandamide for the putative anandamide transporter. As with anandamide, DEA exhibits weak activity at the TRPV1 (Ross et al. 2001b). Taken together, the findings indicate that DEA and HEA are naturally occurring compounds in mammals and exhibit a pharmacology that is very similar to that of anandamide. Systemic administration of DEA and HEA causes analgesia to acute thermal stimulation in mice (Fride and Mechoulam 1993), and tolerance develops to this effect (Fride 1995). Whether this effect is CBR-mediated is currently unknown. More work with these poorly studied compounds is warranted.

5.3
2-Arachidonoylglycerol

2-AG was the second endocannabinoid to be identified (Mechoulam et al. 1995; Sugiura et al. 1995). Compared to anandamide, less is known as to what role it may play in pain modulation and whether its effects on nociceptive processing are indeed CB1R-mediated. Intravenous administration of 2-AG caused a suppression of pain behavior in the tail-flick test (Mechoulam et al. 1995). However, the investigators did not test whether the effects could be blocked by CBR antagonists. This leaves open the possibility that active non-CB metabolites may have produced the effect, as was apparently the case with anandamide discussed above. Ben-Shabat et al. (1998) showed that at doses of 2-AG that fail to produce analgesic effects in the hot plate test, the addition of two cannabinoid-inactive endogenous congeners of 2-AG, 2-lineoylglycerol and 2-palmitoylglycerol, caused significant analgesia. These effects were referred to as "entourage effects," a reference to the notion that endogenous mediators of similar structure are often released together and act in concert.

5.4
Noladin Ether

The novel endocannabinoid noladin ether was recently identified by Hanus et al. (2001). Subsequently, its existence in brain was reported by Fezza et al. (2002), but Oka et al. (2003) were unable to detect the compound in the brains of any of several mammalian species by gas chromatography/mass spectrometry. Noladin ether was reported to occur in relatively high amounts in dissected thalamus, but its localization to somatosensory areas of thalamus has not been established. It was reported to occur in much lower amounts in spinal cord (Fezza et al. 2002). Hanus et al. (2001) showed that the compound produces analgesic effects in the hot plate test following systemic administration in mice (20 mg/kg, i.p.). However, as with 2-AG, experiments have not been carried out to determine whether its effects were due to an action at CBRs. More work is needed to verify the formation of this compound in vivo and its potential role in pain modulation.

5.5 Virodhamine

O-Arachidonoylethanolamine was identified in rat brain and named virodhamine (Porter et al. 2002). This compound is similar to anandamide in being formed from arachidonic acid and ethanolamine, but virodhamine contains an ester linkage rather than anandamide's amide linkage. Like anandamide, it appears to act as a partial agonist. However, a microdialysis study suggested that while its tissue concentrations are similar to anandamide, it is released in much higher amounts. The existence of this compound has not been independently verified, and this author has been unable to detect it in rat brain extracts using ultrasensitive LC/MS/MS (liquid chromatography/tandem mass spectrometry) methods developed using the synthetic compound (J.M. Walker, unpublished observations). Additional confirmatory studies of the existence of virodhamine are needed upon which further study of its potential role in pain modulation would be warranted.

5.6 *N*-Arachidonoyldopamine

Another molecule with the arachidonic acid backbone, NADA was recently identified in rat and bovine brain (Huang et al. 2002). It activates CB1Rs and elicits cannabimimetic effects (which include analgesia following systemic administration but not tested with a cannabinoid antagonist) (Bisogno et al. 2000; De Petrocellis et al. 2000; Huang et al. 2002). NADA significantly inhibited innocuous (8, 10 g) mechanically evoked responses of dorsal horn neurons, and these effects were blocked by intraplantar injection of SR141716A (Sagar et al. 2004). In addition, NADA activates TRPV1 receptors and causes hyperalgesia when administered peripherally (Huang et al. 2002). This effect is in contrast to anandamide, which also activates TRPV1 (Smart et al. 2000; Zygmunt et al. 1999), though administration of anandamide typically causes analgesia. The distribution of endogenous NADA in various brain areas differs from that of anandamide, with the highest levels found in the striatum and hippocampus (Huang et al. 2002). It also occurs in the DRG in low levels. Given that NADA is capable of eliciting analgesia upon systemic administration and hyperalgesia upon intradermal injection, it is possible that endogenous NADA activates either TRPV1 or CB1Rs, depending upon location and circumstance.

5.7 Regulation of Endocannabinoids by Fatty Acid Amide Hydrolase

Three putative endogenous cannabinoids, anandamide, 2-AG, and NADA, appear to be susceptible to degradation by FAAH (Cravatt et al. 1996; Deutsch and Chin 1993; Di Marzo et al. 1998; Huang et al. 2002). Immunohistochemical studies show that FAAH is present in the ventral posterior lateral nucleus of the thalamus (Egertová et al. 1998, 2003; Tsou et al. 1998b), the termination zone of the

spinothalamic tract. FAAH is also found in Lissauer's tract, which comprises primary afferent fibers entering the spinal cord, and in small neurons in the superficial dorsal horn, which is the termination zone of nociceptive primary afferents. These observations demonstrate that a mechanism capable of inactivating anandamide, 2-AG, and NADA is present in regions of the CNS related to nociceptive processing and thus suggest a role for these ligands in pain modulation. Of course, the presence of FAAH does not necessarily identify that cell as a site of synthesis of endocannabinoids, as FAAH is a catabolic enzyme and also metabolizes fatty acid amides that act through CBR-independent mechanisms.

5.7.1
Pain Sensitivity and Inflammatory Responses in FAAH Knockout Mice

Cravatt and colleagues (2001; Lichtman et al. 2004) developed transgenic mice lacking FAAH and observed in these mutants enhanced analgesic effects of exogenously administered anandamide (Fig. 4). These effects were reversed by the selective CB1R antagonist/inverse agonist SR141716A. Moreover, these animals exhibit tonic CB1R-mediated analgesia, apparently due to the decreased metabolism of FAAH-susceptible endocannabinoids. These findings support the hypothesis that endocannabinoids susceptible to hydrolysis by FAAH serve to naturally suppress pain sensitivity. The development of FAAH and CB1R knockouts and pharmacological approaches employing subtype selective antagonists or antisense knockdown have been used to evaluate a role of endocannabinoids in pain modulation.

In a subsequent study, mice were generated that expressed FAAH in the nervous system but not in peripheral tissues. These mice exhibited normal pain sensi-

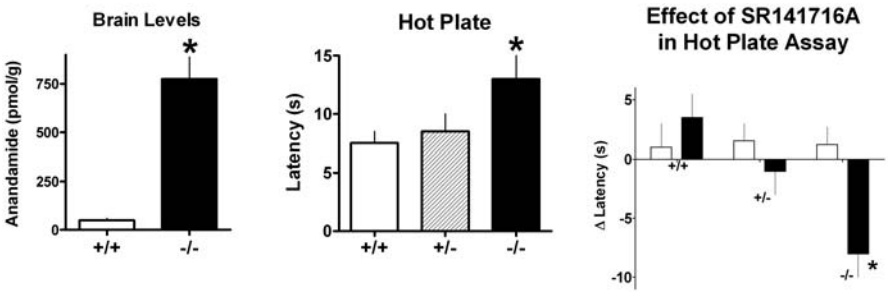

Fig. 4 Marked changes in anandamide levels, hot plate sensitivity, and basal effects of the CB1R antagonist SR141716A in animals lacking the enzyme fatty acid amide hydrolase (FAAH). Wild-type mice (+/+, *left panel*) exhibit relatively low levels of anandamide (50 pmol/g) in brain compared to FAAH knockout mice (–/–) which exhibit 775 pmol/g, indicating that FAAH is the principal mechanism for the metabolism of anandamide. FAAH knockout mice (–/–, *middle panel*) exhibit significantly reduced pain sensitivity under basal conditions compared to wild-type (+/+) and heterozygous (+/-) mice, raising the possibility that the increased levels of anandamide in the knockouts produce a constant state of hypoalgesia. The tonic hypoalgesia observed in the FAAH knockout mice (–/–, *right panel*) is eliminated by the CB1R antagonist SR141716A (*black bars*) compared to vehicle (*white bars*), whereas no significant effect of the antagonist is observed in wild-type (+/+) or heterozygous (–/–) mice. Redrawn from Cravatt et al. (2001)

tivity but a reduced inflammatory response (edema) to carrageenan via a non-cannabinoid mechanism (Cravatt et al. 2004). These findings indicate that the elevated levels of anandamide and other fatty acid conjugates susceptible to FAAH in the nervous system mediate the analgesia observed in FAAH knockouts, while the reduced susceptibility to inflammation is mediated by peripherally elevated lipids acting via a non-CBR mechanism. These data suggest that the central and peripheral FAAH signaling systems regulate discrete phenotypes that may be separately targeted for distinct therapeutic needs.

5.8 Role of Endocannabinoids in the Antinociceptive Actions of Cyclooxygenase Inhibitors

Anandamide is metabolized by cyclooxygenase 2 (COX-2) to form prostaglandin (PG) E2 ethanolamide, PGD_2 ethanolamide, and $PGF_{2\alpha}$ ethanolamide (Kozak et al. 2002; Yu et al. 1997). Ross et al. (2002) demonstrated that PGE_2 ethanolamide binds with nanomolar affinity to prostaglandin EP1, EP2, EP3, and EP4 receptors (K_i (nM) = 5.61 ± 0.1, 6.33 ± 0.01, 6.70 ± 0.13, and 6.29 ± 0.06, respectively; receptor subtypes reviewed by Breyer et al. 2001). Anandamide is not the only derivative of arachidonic acid that is oxygenated by COX-2. The predicted glycerol adduct of PGE_2 is formed upon exposure of 2-AG to recombinant COX-2 (Kozak et al. 2000; Prusakiewicz et al. 2002). The glycerol ester of PGE_2 was recently shown to produce proinflammatory-like effects in macrophage cell line (Nirodi et al. 2004). The above findings indicate that when COX-2 is induced by inflammation, endocannabinoids may be converted from antinociceptive/anti-inflammatory compounds to pro-nociceptive/proinflammatory compounds. This possibility was addressed by Gühring et al. (2002) with the demonstration that the reduction of pain behavior following formalin injection in the hindpaw produced by the COX-2 inhibitor indomethacin was reversed by the CB1R antagonist AM251 but not by PGE_2. This effect was absent in CB1R knockout mice. AM251 also reversed the antihyperalgesic effect of indomethacin subsequent to zymosan-induced inflammation. These findings suggest that COX inhibitors suppress pain, at least in part, by preventing the metabolism of antinociceptive endocannabinoids to pro-nociceptive prostanoids.

5.9 Evidence for Tonic Modulation of Pain via CB1Rs

5.9.1 Pain Sensitivity in CB1R Knockout Mice

Knockouts of the CB1R provided mixed results. Ledent et al. (1999) found that CB1R knockout mice failed to exhibit any of the usual changes produced by exposure to cannabinoids including analgesia. In the absence of any treatment, the basal responses to noxious stimuli in the –/– mice were similar to those of the wild-

type mice, in contrast to another study published the same year on a different CB1R knockout (Zimmer et al. 1999), in which a higher pain threshold in the –/– mice compared to wild-type was observed. The surprising finding of analgesia-like effects of the knockouts in this study are at variance with the other study and are difficult to explain, except to hypothesize different patterns of developmental organization of the pain system in the absence of CB1Rs in the groups of mice used by the two laboratories. It is possible that regulatory changes in other receptor systems occur during development subsequent to the knockout of the CB1R gene and contribute to the behavioral phenotype observed in the transgenic mice.

5.9.2 Effects of Endocannabinoids Assessed with CBR antagonists

Studies of the effects of SR141716A, a specific cannabinoid CB1R antagonist (reviewed by Walker et al. 2000) suggest that endocannabinoids participate in endogenous pain modulation and that this action involves the PAG. Blocking the cannabinoid CB1R with SR141716A produced hyperalgesia in the formalin test (Calignano et al. 1998; Strangman et al. 1998) and blocked the analgesia produced by electrical stimulation of the dorsolateral PAG (Walker et al. 1999). These findings are in line with previous studies (Richardson et al. 1997; Richardson et al. 1998b) that demonstrated hyperalgesia following intrathecal administration of this cannabinoid antagonist or CB1R knockdown with an antisense oligonucleotide. Chapman (1999) found that spinal nociceptive neurons exhibit markedly greater C fiber-mediated responses following low doses of SR141716A (0.1–1 ng in 50 ml applied to spinal cord). The authors of these studies posited that the pain-enhancement by the antagonist results from the blockade of endocannabinoids. However, the conclusions from these and other experiments that use SR141716A in this manner are limited by three factors. First, several reports have suggested that SR141716A acts as an inverse agonist, an effect that would mimic that of blocking endocannabinoids (reviewed by Walker et al. 2000). Second, these studies do not identify any particular endocannabinoid that might be involved in the proposed suppression of pain. Third, not all investigators have observed the pain-enhancing effect of SR141716A (Beaulieu et al. 2000), perhaps due to differences in experimental procedures or baseline differences in activation of the endocannabinoid system. For example, ceiling effects in pain behavior could contribute to failures to observe hyperalgesia in the cited work, which used twice the concentration of formalin that was used by Strangman et al. (1998).

6 Effects of Cannabinoids on Pain in Humans

The human trials of cannabis and Δ^9-THC are few in number and typically small in size. These studies differ in important ways. There are marked differences between studies in dose and dose regimens, and the drug preparations differ, with

some using smoked marijuana and some using Δ^9-THC by the oral or intravenous routes. Some studies used healthy volunteers whereas others used patients with clinical pain of various origins. Therefore, it is important to note that (1) some negative results may have arisen from ineffective doses; (2) the oral route of administration adds variability due to the unpredictable absorption of Δ^9-THC; (3) smoked marijuana contains additional constituents that likely contribute to any observed effects; (4) clinical pain is very different from experimental pain due to plasticity in the neuronal circuits that mediate pain. In light of the fact that the extant materials do not permit one to reach solid conclusions about the utility of direct-acting full cannabinoid agonists as therapeutic agents in pain, it seems best to examine this literature with an eye toward uncovering whatever therapeutic potential exists.

6.1 Experimental Pain

One approach in studying the effects of cannabinoids in pain perception in humans is through paradigms that involve administering controlled painful stimuli to healthy volunteers. An interesting approach used in two papers (Clark et al. 1981; Zeidenberg et al. 1973) aimed at distinguishing between response bias (often referred to as B, β, or Lx) and sensitivity [often referred to as P(A) or d′] to painful stimuli, using the methods of sensory decision theory. In this approach, response bias refers to the tendency of a particular subject to rate events in a more positive or negative direction. This variable is related to cognitive processes reflecting factors such as a person's temperament. Sensitivity refers to the detectability of a stimulus and the subject's ability to distinguish stimuli that are of similar but slightly different intensities. Sensory decision analysis requires a variety of statistical assumptions, which make interpretation of the results more difficult.

Zeidenberg et al. (1973) administered 5 mg (p.o.) of Δ^9-THC to healthy male volunteers between the ages of 25 and 29, and tested them for thermal pain perception to a radiant heat source before and after administration of the drug. They found that d′ or the ability to distinguish between stimuli of different intensities dropped, and this drop occurred both during the period of subjective effects of the drug and, in 3 of 4 subjects, for the subsequent testing period. Response bias exhibited more intersubject variability. The authors noted that the analgesic effects of the drug remained at a time when effects on memory and psycholinguistic parameters were returning to normal levels, suggesting a longer time course for the drug's effect on pain sensitivity.

A second study that used sensory decision theory reached opposite conclusions (Clark et al. 1981). However, in this study tolerance to cannabinoids is confounded with the pain tests. Healthy volunteers were permitted to smoke increasing quantities of marijuana cigarettes (2%, 20 mg Δ^9-THC content per cigarette, supplied by the U.S. National Institute on Drug Abuse). The total number of cigarettes consumed was very high for both the moderate and high consumption groups (average 19.4 cigarettes per day for high consumption, 13.1 for moderate users),

which undoubtedly induced tolerance in the subjects. This confound is so deeply embedded in the experimental design that it is virtually impossible to interpret the data from this experiment.

Raft et al. (1977) used two doses of Δ^9-THC administered intravenously (0.022 and 0.044 mg/kg) in 10 males (ages 18–28) and measured pain induced by two types of noxious stimuli, pressure and electrical. These investigators took the approach of examining the pain threshold (the lowest intensity of stimulation that gives rise to pain) and pain tolerance (the intensity at which pain becomes unbearable). At both doses and for both stimuli the threshold for pain was increased, whereas pain tolerance was not affected. In this and other studies conducted around the same time, the use of threshold and tolerance measures is unfortunate. Clinical pain is normally somewhere in between the two, and it is difficult to assess from the present data what happens in this middle range. Modern approaches would likely use a range of noxious stimuli coupled with ratings of pain intensity, allowing the construction of stimulus–response functions. What is clear from the results of the study by Raft et al. (1977) is that the sensation of pain was entirely absent at some levels of noxious stimulation, but whether this would extend to the clinically relevant levels cannot be assessed from these data. An interesting result from this paper stems from patient reports on pain severity overall. Although the largest decrease in pain threshold occurred with the pressure stimulus at the 0.44 mg/kg dose, most patients rated this condition as the least desirable. It appears that dysphoric effects of Δ^9-THC heightened the overall negativity of the pain. Thus, there is a dissociation between the sensory phenomena and the overall pain experience such that the negative psychotropic effects of Δ^9-THC at the higher dose range overrides the positive effects of the drug on sensory threshold.

Hill et al. (1974) also measured pain thresholds and tolerance. In this single-dose study, healthy male volunteers (ages 21–30, $n = 26$) inhaled marijuana smoke using an apparatus that caused nearly complete combustion of the plant while the subject practiced inhalation in a timed manner. Subjects experienced ascending intensities of electrical stimulation and were asked to report when the stimulation became painful and when it became intolerable. The strength of stimulation was then reversed and the subjects were asked to report when the pain disappeared. The authors found that marijuana smoking lowered the pain threshold as well as pain tolerance. A drawback of this study is the inability to state the dose with any accuracy, a possible basis for the fact that it is at variance with the results of Raft et al. (1977).

A recent study employing topical administration of the cannabinoid agonist HU210 has demonstrated its effectiveness in reducing the magnitude estimation of pain induced in human volunteers following intradermal administration of capsaicin (Rukwied et al. 2003). HU210 also increased the mean heat threshold for pain and reduced tactile allodynia elicited by stimulation with a cotton pad following capsaicin administration. Although pharmacological specificity was not assessed in this work, it is consistent with preclinical studies where mediation by CBRs was confirmed with competitive antagonists (see Sects. 3.2.3 and 4). These data collectively suggest that local administration of a cannabinoid may be employed in humans to suppress pain without psychomimetic side effects.

6.2 Clinical Pain

The studies discussed in this section are the most compelling because the subject population was drawn from patients suffering from significant chronic clinical pain. Chronic pain takes on features that distinguish it from acute pain due to neural plasticity. The changes in sensory processes that take place during periods of prolonged pain serve mainly to amplify the pain. Ongoing painful stimulation leads to peripheral and central sensitization, a process in which the responses to stimulation are enhanced. This leads to allodynia (a painful sensation pursuant to mild tactile stimulation), hyperalgesia (a greater than normal pain sensation to a noxious stimulus), and spontaneous pain. The peripheral mechanisms for different classes of pain (e.g., inflammatory pain versus neuropathic or nerve injury pain) differ. Consequently, different analgesics exhibit different degrees of efficacy in chronic pain of different etiologies. For example, morphine is an excellent analgesic for inflammatory pain, whereas it frequently lacks efficacy in neuropathic pain (Arner and Meyerson 1988). Therefore, studies of clinical pain of different types are necessary precursors to drawing sound conclusions about the possible role of cannabinoids in the pharmacotherapy for pain.

Positive results of cannabinoids have been found in the studies of cancer pain conducted by Noyes and colleagues (Noyes et al. 1975a,b). The larger of the two studies used 36 subjects (26 women and 10 men, mean age 51). These patients reported continuous pain of moderate intensity. In a double-blind random pattern, patients received on successive days placebo, 10 and 20 mg Δ^9-THC, and 60 and 120 mg of codeine. Pain ratings by the patients were used to estimate pain relief and pain reduction scores. The results indicated that 20 mg Δ^9-THC was roughly equivalent to 120 mg codeine. Five of the 36 patients experienced adverse reactions to Δ^9-THC, one following 10 mg Δ^9-THC, four following 20 mg. These side effects undoubtedly limit the amount of analgesia that can be produced by Δ^9-THC. Another report by Noyes (1975) reached similar conclusions with a smaller sample.

Neuropathic pain is a potential target for cannabinoid pharmacotherapies that have been validated at preclinical as well as clinical levels. The cannabinoid Δ^9-THC (dronabinol) has recently been evaluated in multiple sclerosis patients with central neuropathic pain in a double-blind placebo-controlled crossover design (Svendsen et al. 2004). Orally administered dronabinol (10 mg daily for three weeks) lowered median spontaneous pain intensity scores and increased the median pain relief scores relative to placebo treatment. The modest but clear therapeutic effect was associated with improvements on the SF-36 quality-of-life scale with no change in the functional ability of the multiple sclerosis patients. During the first week of treatment, adverse side effects of dronabinol treatment (dizziness, lightheadedness) were more frequent with dronabinol than placebo, but the adverse effects decreased over the therapeutic course, possibly due to tolerance (Svendsen et al. 2004). Nonetheless, the clinical relevance of dronabinol for pain management may be limited by unwanted psychoactive side effects (Svendsen et al. 2004). Results of a randomized, placebo-controlled 21-day intervention trial suggest that smoked and oral cannabinoids do not appear to be unsafe [with respect to hu-

man immunodeficiency virus (HIV) RNA levels, $CD4^+$ and $CD8^+$ cell counts, or protease inhibitor levels] in individuals with HIV infection (Abrams et al. 2003). Cannabinoids also represent a promising therapeutic target in acquired immunodeficiency syndrome (AIDS) and cancer patients where the antiemetic effects of cannabinoids represent a useful therapeutic adjunct in patient populations for whom the emetic effects of opioids are poorly tolerated.

Recent work has aimed at developing cannabinoids that lack psychotropic side effects, which limit dosing. One example of this may be found in the THC and cannabidiol acid derivatives ajulemic acid (CT-3) and HU-320. These compounds were reported to produce anti-inflammatory effects with a reduced side effect profile (Burstein et al. 1998; Burstein et al. 2004; Sumariwalla et al. 2004), perhaps because they possess either poor (ajulemic acid) or virtually no (HU-320) affinity for either CB1R or CB2R. Consequently, the mechanism by which they produce analgesic effects is not clear. In a recent clinical trial of patients suffering from neuropathic pain, ajulemic acid possessed some efficacy (Karst et al. 2003). While many questions about these and similar compounds are awaiting further research, this appears to be an important line of inquiry.

7 Conclusions

Although cannabinoids have been used for pain relief for centuries, the basis for their analgesic effects were poorly understood until recently. During the last decade a prodigious output of research papers from many laboratories has elucidated many of the major features of cannabinoid analgesia. These studies have not only provided a detailed understanding of the network of neural and inflammatory cells that serve as the targets of cannabinoids, the literature has also begun to address the more difficult question of the physiological role of endocannabinoids in pain regulatory circuits. The low levels of CBRs in brainstem regions that control vital heart rate and respiratory function provided an anatomical basis for the low toxicity of cannabinoids (Herkenham et al. 1991). However, the psychoactivity of direct-acting CB1R agonists proved to be a major barrier to their use as therapeutic tools in the pharmacotherapy of chronic pain. More encouraging results have arisen from a number of studies showing positive effects of CB2R agonists, locally administered cannabinoids, inhibitors of the anandamide-degrading enzyme or the putative anandamide transporter, or the use of atypical cannabinoids such as HU-320. Such novel targets for pain pharmacotherapy represent important future directions for research in this field.

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