THE NEUROBIOLOGY OF CANNABINOID ANALGESIA

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Summary

The discovery of cannabinoid receptors and their putative endogenous ligands raises questions as to the nature of the effects produced by cannabinoids on neural circuits that mediate pain and whether endogenous cannabinoids produced by the brain or in the periphery serve naturally to modulate pain. A sizable body of previous work showed that cannabinoid agonists suppress pain behavior in a variety of models of acute and chronic pain. However, at appropriate doses, cannabinoids also profoundly suppress motor behavior (see Sañudo-Peña et al., this volume), which complicates the interpretation of behavioral analgesia since a motor response is the endpoint of virtually all such studies. Studies conducted in this laboratory used biochemical and neurophysiological measures to determine whether cannabinoids suppress nociceptive neurotransmission. The results showed that cannabinoids suppress nociceptive neurotransmission at the level of the spinal cord and the thalamus. These effects are reversible, receptor mediated, selective for painful as opposed to nonpainful somatic stimuli, and track the behavioral analgesia both in time course and potency.

Key Words: pain, analgesia, cannabinoids

Suppression of nocuous stimulus-evoked expression of c-fos in the spinal cord by cannabinoids. Hunt et al. (1) demonstrated that noxious stimuli elicit the expression of the immediate early gene c-fos in the spinal dorsal horn. Spinal c-fos expression in response to painful levels of stimulation is a good measure of spinal pain processing because analgesics such as morphine suppress this response. Our first study of the effects of cannabinoids on the neural processing of pain examined whether spinal c-fos expression induced by injections of dilute formalin in the hindpaw (2) was affected by cannabinoids (3).

The cannabinoid agonist WIN 55,212–2 suppressed pain-induced spinal expression of c-fos (Fig 1). The specificity of this effect and its mediation by cannabinoid receptors were suggested by the dose–dependency, the inactivity of the receptor–inactive enantiomer, and the lack of effect in cannabinoid–tolerant animals.

Suppression of nocuous stimulus-evoked firing of spinal wide dynamic range and nociceptive specific neurons. The suppression of pain–induced expression of immediate early genes by a cannabinoid suggested that cannabinoids do in fact inhibit spinal processing of pain, but many questions remained

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which are better addressed using neurophysiological methods. One of the major questions we addressed using electrophysiology was whether cannabinoids suppress noxious stimulus-evoked firing in the two main types of nociceptive neurons: wide dynamic range (WDR) and nociceptive specific (NS) neurons (4–7, 26). These studies were carried out in urethane-anesthetized rats using either WIN 55,212-2 or CP55,940. Three types of noxious stimuli have been used: noxious pressure (applied by a computer-controlled air cylinder), noxious heat (applied by a Peltier thermode) or painful C-fiber strength electrical stimulation (4–7, 26).

![Graph showing dose-response functions](image)

**Fig. 1.**
Dose–response functions showing the number of neurons in various spinal cord laminae that exhibited pain-evoked Fos-like immunoreactivity in animals pretreated with vehicle or a cannabinoid agonist. The number of immunoreactive cells was first determined for each animal by calculating the mean for three sections qualitatively exhibiting the greatest number of labeled cells. Data shown represent the mean number of labeled cells per section ± SEM across subjects. Each animal received an i.p. injection of either a vehicle control solution or WIN 55,212–2 (5 or 10 mg/kg) 10 minutes prior to formalin injection. Two hours later, the spinal cords were processed for immunocytochemistry. The number of immunoreactive neurons was significantly decreased by the drug in all laminae except III and IV.

Low doses (62.5 μg/kg to 500 μg/kg, i.v.) of the cannabinoid agonists produced a profound inhibition of noxious stimulus-evoked firing of wide dynamic range and nociceptive specific spinal dorsal horn neurons (Figs 2–3). Further work indicated that these effects of cannabinoids are mediated by cannabinoid receptors (Figs 4, 8, 9). In these experiments, nociceptive neurons were isolated and the animals were then pretreated with the competitive CB1 cannabinoid receptor antagonist SR141716A. These animals failed to show the decrease in nociceptive responding produced by the agonist (Fig 4). In other experiments (Figs 8, 9) the inactive enantiomer (WIN 55,212–3) failed to alter the nociceptive responses.

**Cannabinoid analgesia or anesthesia?** The single unit recordings of WDR and NS neurons do not provide a complete characterization of the effects of cannabinoids on somatosensory processing because they cannot distinguish between analgesia (suppression of pain responses) and anesthesia (suppression of all somatosensory input). In order to distinguish between these two possibilities, we examined whether cannabinoid agonists alter the responses of non-nociceptive neurons to mild stimuli (5, 7). Non-nociceptive neurons were identified by their responses to mild taps with a wooden probe or mild pressure stimuli, and by their failure to exhibit increased responses to noxious levels of stimulation, i.e. their maximum firing rates were achieved at non-noxious levels of stimulation. In contrast to their suppression of evoked firing in nociceptive neurons, cannabinoid agonists did not alter the
evoked activity of non-nociceptive neurons in the spinal cord or the ventroposterolateral nucleus of the thalamus, a major termination zone of the spinothalamic tract (Fig. 5). This finding suggests that cannabinoids selectively suppress the reactions of nociceptive neurons. From these studies we conclude that cannabinoids produce true antinociception, not anesthesia.

Cannabinoid suppression of nociceptive responses in the ventroposterolateral thalamus. The classical ascending pain transmission pathway is the lateral spinothalamic tract, which terminates in the ventroposterolateral nucleus of the thalamus, an area that contains many nociceptive neurons. Additional evidence of the suppression by cannabinoids of nociceptive neurotransmission can thus be derived by examining their effects in this important brain region. We found that the effects of cannabinoids in this area of the brain are very similar to those observed in the spinal cord suggesting that some or most of the nociceptive neurons recorded were thalamic projection neurons (4–7). In these experiments we used a graded pressure stimulus, which allowed us to construct stimulus–response functions. As shown in figure 6, we found that cannabinoids and morphine produce similar effects in shifting the stimulus–response function downward. Furthermore, at higher doses, the cannabinoid flat-
tended the stimulus-response function. This indicates that these neurons lost their normal ability to encode the strength of noxious stimuli in their firing rate.

![Example of suppression of noxious pressure-evoked firing by WIN 55,212-2 in a spinal nociceptive specific neuron.](image)

**Fig. 3.**

Example of suppression of noxious pressure-evoked firing by WIN 55,212-2 in a spinal nociceptive specific neuron. Left Panel: Action potential digitized and printed from a Tektronix TDS210 oscilloscope. Middle Panel: Response to pinch; stroking with an artist brush and non-noxious pressure were without effect. Right Panels: Histograms showing responses (mean of 3 applications) of a noxious computer-controlled pressure stimulus similar to that used in our previous work 4-6). Note the marked decrease in firing 30 min post administration of WIN 55,212-2 (rightmost panel). The firing returned to normal within 90 min.

![Graph showing suppression of noxious activity by cannabinoid](image)

**Fig. 4.**

Pretreatment with SR141716A, the competitive antagonist for the central cannabinoid receptor (CB1), blocks the suppression of noxious heat-evoked activity by the classical cannabinoid CP 55,940 (125 µg/kg i.v.). *Significant difference from antagonist pre-treatment group, p<0.05.

![Graph showing effect of CP 55,940 on non-noxious pressure](image)

**Fig. 5.**

CP 55,940 (125µg/kg i.v.) fails to suppress activity evoked by non-noxious pressure in a non-nociceptive mechanosensitive cell recorded in the lumbar dorsal horn. A mild pressure stimulus was applied for 3 sec as indicated. LEFT: Pre-injection levels of evoked activity. RIGHT: Evoked activity following administration of CP 55,940. The raster plot shows the time of occurrence of each action potential relative to the stimulus onset. Successive stimulation trials are represented by the horizontal rows of dots in the raster plot. The peristimulus time histogram, shown in black, summarizes the response to the stimulus pre and post drug. A similar experiment in the VPL yielded very similar results (see ref. 7).

**Relationship between effects of cannabinoids on nociceptive neurons and behavior:** If the observed neurophysiological changes account for the antinociceptive effects of cannabinoids, one would expect to find the same potency and time course for the changes in physiology and behavior. These stud-
ies could not be performed simultaneously because all of the electrophysiology experiments discussed above were conducted in anesthetized rats. In order to simulate these experiments and still obtain behavioral responses, we tested lightly anesthetized rats with the same apparatus used for delivering controlled noxious pressure in the physiology experiments. The time course of the suppression of the behavioral responses (limb withdrawal) was virtually identical to the time course of the suppression of neurophysiological responses (see Fig 7, ref. 7). Likewise, the potency of the cannabinoid agonist in suppressing thalamic nociceptive neuronal responses was virtually identical to its potency in suppressing thermal nocifensor (tail-flick) reflexes (Fig 7). These findings support the conclusion that cannabinoids effectively and selectively suppress neuronal responses to pain.

![Graph](attachment:image)  
**Fig. 6.** Mean stimulus—response functions of a nociceptive VPL neuron following administration of (●) vehicle, WIN55,212-2 ([▲] 0.0625, [■] 0.125, or [●] 0.25 mg/kg) or (+) morphine. The lowest dose of the drug (0.0625 mg/kg) reduced the overall firing but did not alter the slope of the stimulus—response function. Morphine (0.5 mg/kg) showed a similar effect. Significant decreases in slope occurred at higher doses of WIN 55,212-2 (0.125 and 0.25 mg/kg). Post-injection slope values and confidence limits for estimation of β are shown to the right of each regression line. Note that the confidence intervals of the slope for the dose of 0.250 mg/kg include 1 indicating that the firing rate of the neuron no longer encoded the level of applied pressure.

**Cannabinoid antinociception mediated by descending modulation, direct spinal, and peripheral actions.** All of the experiments discussed above utilized intravenous administration of the drug which provides no information on its site of action. Therefore, we conducted experiments to determine whether the effects were the result of local actions in the spinal cord, the result of descending modulatory influences, or both. In some experiments the drug was administered by the intracerebroventricular (i.c.v.) route. Microinjections of 20 μg WIN 55,212-2 suppressed noxious stimulus—evoked responses of WDR neurons in the spinal cord (Fig 8, ref. 5). This finding indicated that cannabinoids suppress spinal nociceptive processing, in part by supraspinal descending influences. These results are consistent with previous findings from this laboratory and others. We previously demonstrated that intraventricular administration of a cannabinoid suppresses a spinal nocifensor reflex (27). It is difficult to imagine how such an effect could be obtained other than by a descending modulatory influence. Furthermore, Lichtman et al. (8) had shown that spinal administration of the α2 receptor antagonist yohimbine markedly reduced the analgesia induced by systemically administered cannabinoids. In light of the crucial role of brainstem noradrenergic pathways in the modulation of spinal pain responses by opioids (9), this finding was highly suggestive of a descending modulatory action of cannabinoids.
These observations led us to carry out behavioral studies in which we mapped the sites of cannabinoid analgesia in the brain. Cannabinoids were microinjected in numerous brain sites that are involved in pain processing (10–12), and the effects upon the tail flick reflex assessed. We identified 7 sites through which cannabinoids produce effects (Table 1). It is notable that cannabinoids produce analgesia following microinjections in the periaqueductal gray, the amygdala, and the rostral ventrolateral medulla. These interconnected structures are part of a well-established circuit that mediates, in part, the descending pain-suppressive influences of opiates (13). A recent study provided confirmatory neurophysiological evidence that indeed this circuit is involved in cannabinoid analgesia (14). These findings support the notion of common opiate/cannabinoid neural substrates for analgesia and may explain the cross-tolerance sometimes observed between cannabinoid and opiate analgesia (15) in spite of the fact that naloxone has little effect on cannabinoid analgesia (16).

Besides their actions in the brain, cannabinoids act directly in the spinal cord to produce analgesia. This was first observed by Yaksh (17) and studied extensively by Welch's group who demonstrated synergistic interactions with opiates (18). We observed that topical spinal administration of cannabinoids suppresses the stimulus–evoked activity of spinal nociceptive neurons (Fig 9). Cannabinoid receptor mRNA is located in the dorsal root ganglion suggesting that some primary afferent neurons have presynaptic cannabinoid receptors (19), and cannabinoid mRNA is abundant in the spinal dorsal horn suggesting the presence of another cellular mechanism directly on the neuronal somata and dendrites. A presynaptic mechanism of cannabinoid analgesia is suggested by the expression of CB1 mRNA in dorsal root ganglion cells that express neuropeptide markers (23) found in nociceptive primary afferents (24). Moreover, unilateral dorsal rhizotomy (25) and destruction of sensory C-fibers with neonatal capsaicin treatment (23) depletes cannabinoid receptor sites in the dorsal horn. Thus,
spinal cannabinoid receptors are localized, in part, presynaptically on central terminals of pain-sensitive primary afferents. It must be noted, however, that transection of the spinal cord well above the level of recording virtually abolishes the effect of intravenously administered cannabinoids (Fig 10). This would appear to indicate that the descending modulatory influences are dominant. Alternatively, it may be that the main effect of spinally administered cannabinoids occurs by presynaptic actions on the descending tracts which are inactivated by spinal transection.

![Fig. 8.](image)

**Effects of intraventricular administration of WIN55,212-2, WIN55,212-3 or vehicle on noxious heat-evoked firing in lumbar dorsal horn neurons.** Intraventricular administration of WIN55,212-2 produced a significant decrease in noxious heat-evoked firing of WDR neurons compared to vehicle treatment. Treatment with the inactive enantiomer did not differ significantly from treatment with vehicle. Treatment with WIN55,212-3, the enantiomer of the active compound, differed from treatment with WIN55,212-2 by t-test. Values are mean ± SEM for the first 5 post-injection trials. Data are plotted as % of pre-injection levels of evoked activity. Mean pre-injection firing rates were 20.5, 16.8 and 18.3 Hz for vehicle, WIN55,212-2 and WIN55,212-3, respectively. **p<0.01

![Fig. 9.](image)

**Effects of topical administration of WIN55,212-2, WIN55,212-3 or vehicle on noxious heat-evoked firing in lumbar dorsal horn neurons.** WIN55,212-2 suppressed the firing rate relative to treatment with either vehicle or enantiomer. Treatment with the inactive enantiomer did not differ from treatment with vehicle. Values are mean ± SEM for the third block of 5 post-injection trials. Mean pre-injection firing rates were 16.7 ± 2.1, 19 ± 4.8 and 23.5 ± 4.7 Hz for vehicle, WIN55,212-2 and WIN55,212-3, respectively. *Significantly different from vehicle p<0.05.

Hargreaves' group (20) demonstrated that cannabinoids also produce analgesia by peripheral actions. This finding is in line with the presence of cannabinoid receptor mRNA in the dorsal root ganglion since the receptors formed from the mRNA would be expected to travel to the distal as well as the proximal terminals of these pseudo-unipolar neurons. Furthermore, very low doses of anandamide
injected intradermally inhibit the release of a pro-inflammatory factor (calcitonin gene-related peptide) from distal nerve endings, and this is accompanied by inhibition of hyperalgesia consequent to intradermal injections of carrageenan. When the same dose is applied to the contralateral paw to control for systemic uptake of the drug, no such effect occurred. Notably, this effect results from CB1 receptor activation (as expected from the neuronal localization of the receptor), since the effect was blocked with the selective CB1 receptor antagonist SR141716A. Some recent evidence also suggests a role of CB2 receptors in peripheral cannabinoid analgesia (21).

![Fig. 10.](image)

**Fig. 10.** Effects of WIN55,212-2 on noxious heat-evoked activity in lumbar dorsal horn neurons recorded in intact and spinal rats. Post-injection levels of evoked activity were significantly greater in the spinal compared to the intact group, suggesting that the ability of WIN55,212-2 to suppress spinal nociceptive transmission is attenuated following transection. *Significant difference from spinal group p<0.05.

<table>
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N.S.: no significant difference from control. Active sites in bold face. * P < 0.05, Dunnett's post hoc test.
In summary, as of 1990 virtually nothing was known about the mechanisms of cannabinoid analgesia, and even the existence of cannabinoid analgesia was questionable in view of the potentially confounding motor effects of cannabinoids. The discovery of cannabinoid receptors and putative endogenous ligands suggested that endogenous cannabinoids play an important role in the nervous system, with one potential function being the modulation of pain sensitivity. This led to renewed interest in cannabinoid analgesia and the mechanisms by which it occurs. While there is much to be learned, it is now clear that 1) cannabinoids selectively suppress nociceptive neurotransmission; 2) synthetic cannabinoids are equal to morphine in potency and efficacy; and 3) the effects are mediated by actions on descending modulatory tracts, the spinal cord, and the periphery. Perhaps the most pressing future direction is to examine the effects of cannabinoids in chronic pain. This direction is important because prolonged nociceptive stimulation leads to a series of biochemical changes that typically serve in total to enhance pain sensitivity. The scant evidence available suggests that cannabinoids have high efficacy in models of chronic pain, in some cases being superior to morphine (22).

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References