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Two distinctive antinociceptive systems in rats with pathological pain

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Abstract

A common obstacle in clinical management of pathological pain is the poor response to opioid analgesics. We now report that Δ^9-tetrahydrocannabinol (Δ^9-THC)-induced antinociception remained effective in rats with pathological pain. The selective central cannabinoid receptor antagonist SR141716A, but not the generic opioid receptor antagonist naloxone, blocked the Δ^9-THC antinociception. Moreover, there is no cross-tolerance between the antinociceptive effects of morphine and Δ^9-THC in pathological pain states. The results indicate that Δ^9-THC antinociception is both effective and independent of opioid receptors in rats with pathological pain. Thus, the cannabinoid analgesic system may be superior to opioids in alleviating intractable pathological pain syndromes.

Keywords: Antinociception; Cannabinoids; Morphine; Pathological pain

It has been recognized that the analgesic effects of opioids, a powerful class of analgesics long utilized in clinical pain management, are diminished in pathological pain states such as neuropathic pain after nerve injury [1,5,7,9]. This reduced effectiveness of opioid analgesics significantly hampers the ability to manage intractable, debilitating pathological pain syndromes. Recent investigations have indicated that pathological pain states and morphine tolerance share common cellular and intracellular mechanisms [8]. Both result in activation of the *N*-methyl-D-aspartate (NMDA) receptor in cells in the superficial spinal cord dorsal horn, which leads to intracellular cascades involving activation of protein kinase C and nitric oxide and the modification of NMDA and μ-opioid receptor-ion channel complexes [8]. These findings suggest that factors triggering pathological pain syndromes may also activate intracellular cascades contributing to the development of opioid tolerance, causing reduced effectiveness of opioid analgesia in pathological pain states.

Cannabinoids are a known class of analgesics [14]. Cannabinoids produce antinociception in animal acute pain models through both spinal and supraspinal mechanisms [6,16–18]. Intrathecal administration of SR 141716A, a selective central cannabinoid receptor antagonist, also produces hyperalgesia in naive mice indicating the existence of a tonic cannabinoid analgesic system in rodents [11,12]. In addition, WIN-55, 212-2, a cannabinoid receptor agonist, reduces hyperalgesia in a rat model of neuropathic pain [4]. Thus, contrary to reduced opioid analgesia in pathological pain states, it is possible that the effectiveness of cannabinoid analgesia remains intact under such conditions. We tested these hypotheses in three sets of experiments utilizing a well-established rat model of neuropathic pain resulting from chronic constriction nerve injury (CCI) [2].

We first examined whether the antinociceptive effect (bringing foot-withdrawal latency above the baseline) of Δ^9-tetrahydrocannabinol (Δ^9-THC) would differ in CCI and sham rats. We selected Δ^9-THC as the agent because it represents the generic spectrum of cannabinoids. Male Sprague–Dawley rats (350–400 g) were anesthetized with intraperitoneal 50 mg/kg sodium pentobarbital. The rat's right sciatic nerve was exposed and four loose ligatures (4-0 chromic gut) were made around the dissected nerve.

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Rats in sham groups underwent the same surgical procedure except for the sciatic nerve ligation. Each rat also was implanted with an intrathecal (i.t.) catheter with the tip at the lumbar spinal area immediately following nerve ligation or sham operation.

Thermal hyperalgesia to radiant heat was assessed by using the paw-withdrawal test [7]. Three test trials were made for each rat's hind paw. Cumulative dose-response curves [7] were generated as follows. Δ^9-THC was injected intrathecally in increasing logarithmic (log) doses such that each subsequent cumulative dose was increased by a 0.3 log unit. Post-treatment paw-withdrawal latencies were determined at 20 min after Δ^9-THC injection. This procedure was repeated until either the rat did not move its hind paw within the cutoff time (18 s) or there was no further increase in paw-withdrawal latencies from one dose to the next. Data derived from the paw-withdrawal test were analyzed using ANOVA followed by Waller–Duncan K ratio *t*-tests. ED_{50} values was defined as a Δ^9-THC dose which results in 50% of the maximal possible antinociceptive effects (%MPAE = (test latency − baseline latency/cut-off time − baseline latency) × 100%). ED_{50} values and 95% confidence intervals were then computed.

Eight days after surgery, CCI but not sham rats developed hyperalgesia. Because of the development of hyperalgesia, the baseline foot-withdrawal latency was reduced in CCI rats. This reduction of baseline latency in CCI rats makes it difficult to compare the antinociceptive effects of Δ^9-THC between CCI and sham rats, since the baseline latency in these two groups are not comparable. In order to make meaningful comparisons of Δ^9-THC antinociception between CCI and sham rats, the pre-treatment foot-withdrawal latency in CCI rats must be brought to a level comparable to that of sham rats. In a previous study [7], we used the non-competitive NMDA receptor antagonist MK-801 to reverse thermal hyperalgesia in CCI rats thereby normalizing the baseline latency between CCI and sham rats. A similar procedure was used in the present experiment to normalize the baseline latency in CCI rats by i.t. treatment with 10 nmol MK-801 30 min before Δ^9-THC in both CCI and sham rats. Importantly, neither the baseline latency of sham rats nor the antinociceptive effect of Δ^9-THC (data not shown) was affected by MK-801 indicating that MK-801 does not change nociception in this model. MK-801 also did not change the baseline latency of the contralateral hind paw of CCI rats indicating a selective reduction of hyperalgesia by MK-801.

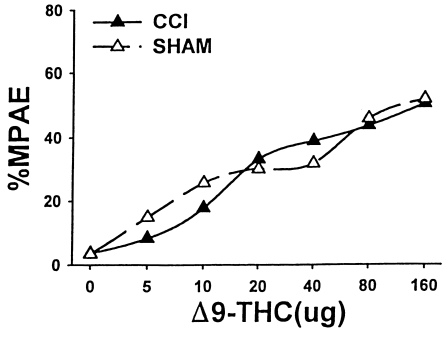

Fig. 1. The antinociceptive effect of Δ^9-THC did not diminish in CCI rats. The decrease in baseline latency (hyperalgesia) in CCI rats was normalized to the level comparable to that of sham rats by i.t. 10 nmol MK-801 given 30 min before Δ^9-THC to both CCI and sham rats (data not shown). Under these circumstances, i.t. Δ^9-THC produced effective antinociception (raising the baseline latency above the nociceptive threshold) in both CCI and sham rats. % Maximal possible antinociceptive effects = %MPAE ((test latency − baseline latency/cut-off time − baseline latency) × 100%).

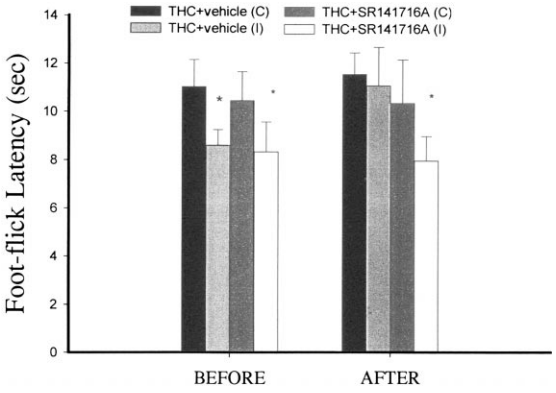

Fig. 2. SR 141716A blocked the effect of Δ^9-THC on thermal hyperalgesia. Intrathecal injection of SR 141716A (5 mg/kg, i.p.) but not vehicle, given 10 min before Δ^9-THC (80 μg), effectively blocked the effect of Δ^9-THC on thermal hyperalgesia. That is, thermal hyperalgesia remained in CCI rats treated with both Δ^9-THC and SR 141716A as compared between the sides ipsilateral (I) and contralateral (C) to nerve ligation. $^*P < 0.05$, as compared with the contralateral foot-withdrawal latencies.

Under these circumstances, i.t. Δ^9-THC produced effective antinociception in both CCI and sham rats. In both CCI and sham rats, there was a significant increase in nociceptive threshold above the baseline in response to the cumulative doses of Δ^9-THC used in the experiment (Fig. 1, $n = 6$–8/group), and the increase in nociceptive threshold did not differ between these two groups ($P > 0.05$). The result indicates that the efficacy of Δ^9-THC antinociception remains comparable to that of sham rats in CCI rats. No significant side effects of Δ^9-THC (such as a local motion disorder) were observed within the cumulative Δ^9-THC dose range used in the experiment.

In order to determine the type of receptor(s) responsible for the effect of Δ^9-THC, we examined whether the effect of Δ^9-THC could be reserved by antagonists of the central cannabinoid receptor (SR 141716A), generic opioid receptors (naloxone), or the κ-opioid receptor (Nor-109). SR 141716A (5 mg/kg, i.p.), given 10 min before i.t. Δ^9-THC (80 μg, $n = 6$/group), blocked the antinociceptive effect of Δ^9-THC as compared with the vehicle group ($P < 0.05$, Fig. 2). Since SR 141716A is an antagonist against the central cannabinoid receptors and Δ^9-THC was given intrathecally, the result indicates that the Δ^9-THC effect is likely to be

mediated through the spinal cannabinoid receptors. However, neither naloxone (10 mg/kg, i.p.) nor Nor-109 (160 nmol, i.t.), also given 10 min before i.t. 80 μg Δ^9-THC ($n = 6$–8/group), blocked the effect of Δ^9-THC (data not shown). Thus, the effect of Δ^9-THC is unlikely to be mediated through opioid receptors.

We then examined whether there is cross-tolerance between morphine and Δ^9-THC antinociception in the CCI model. Fourteen days after once daily i.t. Δ^9-THC (80 μg) but not vehicle treatment ($n = 6$–8/group), tolerance to Δ^9-THC antinociception developed as compared to that on day 1 (Fig. 3, upper panel). The antinociceptive effect of a single i.t. dose of morphine (10 μg), however, did not differ between the Δ^9-THC and vehicle groups on day 15 ($P > 0.05$, Fig. 3, lower panel). Similarly, 10 days after i.t. morphine (10 μg, once daily) but not saline treatment, tolerance to morphine antinociception developed as compared to that on day 1 (Fig. 4, upper panel). The antinociceptive effect of a single i.t. injection of Δ^9-THC (160 μg) also did not differ between the morphine and vehicle groups on day 11 ($P > 0.05$, Fig. 4, lower panel). The combination of these results indicates that there is no cross-tolerance of antinociception between Δ^9-THC and morphine in the present study.

Data from the previous [7] and present studies indicate that the antinociceptive effects of Δ^9-THC and morphine

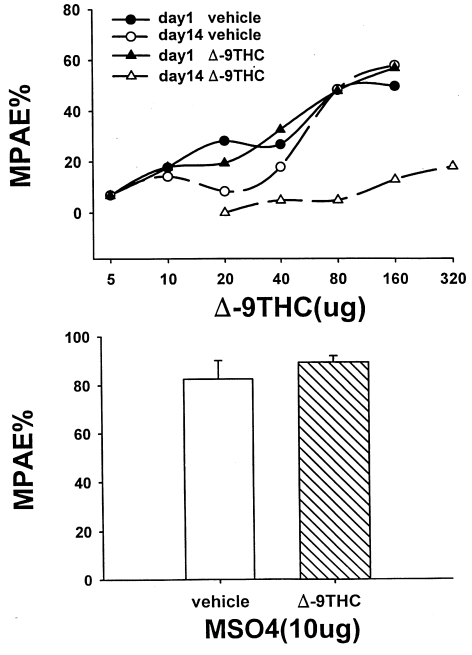

Fig. 3. No cross-tolerance of antinociception was observed between Δ^9-THC and morphine. Upper panel, 14 days after once daily i.t. treatment with Δ^9-THC (80 μg) but not vehicle, tolerance to Δ^9-THC antinociception (the rightward shift of the Δ^9-THC dose-response curve) developed as compared with the vehicle group. Lower panel, the antinociceptive effect after a single i.t. dose of morphine (10 μg), however, did not differ between the Δ^9-THC and vehicle groups on day 15. %MPAE = % maximal possible antinociceptive effects.

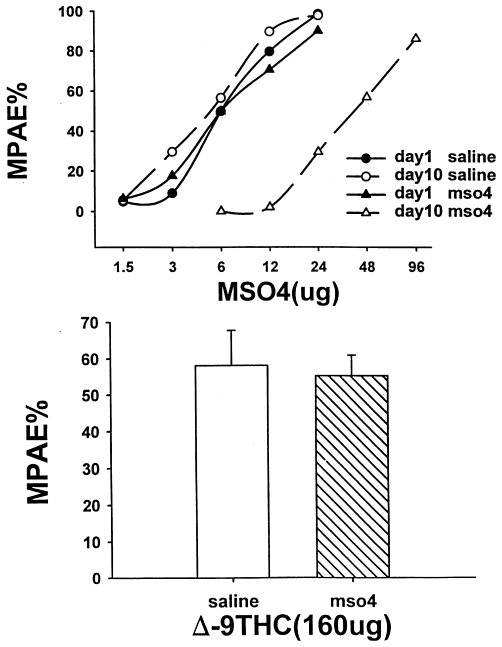

Fig. 4. No cross-tolerance of antinociception was observed between morphine and Δ^9-THC. Upper panel, 10 days after once daily i.t. treatment with morphine (10 μg) but not saline, tolerance to morphine antinociception (the rightward shift of morphine dose-response curve) developed as compared with the vehicle group. Lower panel, the antinociceptive effect after a single i.t. dose of Δ^9-THC (160 μg) did not differ between the morphine and vehicle groups on day 11. %MPAE = % maximal possible antinociceptive effects.

differ in pathological pain states. These differences include changes in antinociceptive efficacy and, interactions between the opioid and cannabinoid systems in physiological versus pathological pain states. With regard to changes in antinociceptive efficacy, both morphine and Δ^9-THC are effective antinociceptive agents in animals without pathological pain, albeit Δ^9-THC is less potent than morphine [6,7,16,18]. In pathological pain states, however, the antinociceptive effect of morphine was reliably reduced with a significant rightward shift of the morphine dose-response curve in CCI rats [7]. In contrast, the antinociceptive effect of Δ^9-THC in CCI rats remains comparable to that of sham rats, i.e., the efficacy of Δ^9-THC antinociception is not reduced in rats with pathological pain. It should be noted that the same experimental paradigm was employed to compare antinociception induced by morphine [7] and Δ^9-THC between CCI and sham rats. While the antinociceptive potency of morphine and Δ^9-THC could not be compared directly from these data, it appears clear that the efficacy of Δ^9-THC antinociception remains unaffected in CCI rats with pathological pain.

Interactions between the cannabinoid and opioid systems also differ in physiological and pathological pain states. In the absence of pathological pain, pre-treatment with morphine increases the antinociceptive effect of Δ^9-THC [10,13], whereas pre-treatment with Δ^9-THC enhances

morphine antinociception [15]. Such interactions may be related to the release of endogenous opioids [10] and/or changes in central cannabinoid receptors [13]. In pathological pain states seen in this CCI model, Δ^9-THC antinociception is not enhanced by repeated morphine treatment, nor is morphine antinociception affected by repeated Δ^9-THC treatment. In addition, there is no cross-tolerance between morphine and Δ^9-THC and no antagonism of Δ^9-THC antinociception by opioid antagonists in CCI rats, suggesting that cannabinoids and opioids function independently in CCI rats. It is thus likely that two distinctive analgesic systems exist in rats with pathological pain.

The mechanisms underlying differences between morphine and Δ^9-THC antinociception in pathological pain states remain to be determined. Some information from previous studies [1,5,7–9] may provide insights into this issue. Pathological pain has been shown to activate the NMDA receptor within the spinal cord, which leads to intracellular cascades involving activation of protein kinase C and nitric oxide [8]. These pain-related changes in the spinal cord have been shown to decrease the efficacy of morphine antinociception [8]. On the contrary, cannabinoids reduce NMDA receptor-mediated activity since anandamide (an endogenous cannabinoid) diminishes NMDA-induced Ca^{2+} influx [3]. Indeed, Δ^9-THC antinociception is unaffected by MK-801 in the present study. As such, activation of NMDA receptors in pathological pain states, which affects the efficacy of morphine antinociception, is unlikely to influence the efficacy of cannabinoid antinociception. The differences between opioids and cannabinoids in relation to the NMDA receptor may attribute to the differences in the effectiveness of cannabinoid and opioid antinociception in pathological pain states. The continuing effectiveness of cannabinoid antinociception demonstrated here in an experimental pathological pain state suggests that cannabinoids may be useful for the management of currently intractable, debilitating clinical pathological pain syndromes.

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