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Potentiation of Δ^9-tetrahydrocannabinol-induced analgesia by morphine in mice: involvement of μ- and κ-opioid receptors

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Abstract

The antinociceptive effect of peripheral Δ^9-tetrahydrocannabinol was examined in mice previously treated with an inactive dose of morphine. The ED_{50} of Δ^9-tetrahydrocannabinol was significantly reduced by morphine, both in the tail-flick test (0.85 vs. 2.10 mg/kg) and in the hot-plate test (1.51 vs. 4.71 mg/kg and 0.73 vs. 2.47 mg/kg in jumping and paw-lick responses, respectively). The synergistic effect between morphine and Δ^9-tetrahydrocannabinol was partially blocked by the cannabinoid receptor antagonist, SR-141,716 A [(*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichorophenyl)-4-methyl-3-pyrazolecarboxamide, hydrochloride)], at a dose of 2 mg/kg (i.p.) as well as by the opioid receptor antagonist naloxone, at the dose of 1 mg/kg (s.c.). Such an effect was also blocked by i.t. nor-binaltorphimine (a κ-selective opioid receptor antagonist) given at 20 μg/mouse as well as by β-funaltrexamine (a μ-selective opioid receptor antagonist) at a dose of 2 nmol/mouse (i.c.v., 24 h before the test). Accordingly, the μ-opioid receptor agonist DAMGO ([D-Ala^2, *N*-Me-Phe^4,Gly-ol^5]enkephalin) potentiated the effect of Δ^9-tetrahydrocannabinol. These data show that the synergism between morphine and Δ^9-tetrahydrocannabinol appears to involve cannabinoid as well as μ-supraspinal and κ-spinal opioid receptors.

Keywords: Analgesia; Morphine; Opioid; Δ^9-Tetrahydrocannabinol; SR-141,716 A; Dynorphin; Nor-binaltorphimine; β-Funaltrexamine; DAMGO ([D-Ala^2, *N*-Me-Phe^4,Gly-ol^5]enkephalin)

1. Introduction

Cannabinoids are a distinct class of psychoactive compounds which produce a large spectrum of pharmacological effects, including antinociception (review in Dewey, 1986). The cannabinoid CB_1 receptor, as well as its corresponding mRNA (Mailleux and Vanderhaeghen, 1992), is widely distributed through the rat central nervous system (CNS). The identification of arachidonylethanolamide (anandamide) as a putative endogenous ligand of the cannabinoid receptor (Devane et al., 1992) and the potential role of this molecule as neurotransmitter or neuromodulator (Di Marzo et al., 1994) suggest the existence of cannabinoid pathways endowed with physiological relevance. The recently developed cannabinoid receptor antagonist SR-141,716 (Rinaldi-Carmona et al., 1994) [(*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichorophenyl)-4-methyl-3-pyrazolecarboxamide, hydrochloride)] has led to the confirmation that the pharmacological effects elicited by cannabinoid-related drugs are specific and due to the activation of cannabinoid receptors (Rinaldi-Carmona et al., 1994; Reche et al., 1996).

The interaction between cannabinoid and opioid systems in nociceptive modulation has been the focus of much attention in recent years (Smith et al., 1994; Welch and Stevens, 1992; Welch, 1993; Welch et al., 1995; Reche et al., 1996). Cannabinoid-induced analgesia has been reported to be blocked by opioid receptor antagonists such as chlornaltrexamine (Tulunay et al., 1981) or naloxone (Bhargava, 1976a,b; Bloom et al., 1977). Recent work by Welch's group has demonstrated that analgesia induced by i.t. Δ^9-tetrahydrocannabinol is sensitive to κ-opioid receptor antagonists (Welch, 1993). In this line we have recently reported that i.v. Δ^9-tetrahydrocannabinol leads to analgesic responses in both the tail-flick test and the hot-plate test, responses which are fully blocked by i.t. nor-binaltorphimine (a selective κ-opioid receptor antagonist) as well as by i.t. dynorphin A-(1–8) antiserum (Reche et al., 1996). The interaction between cannabinoid and opioid nociceptive pathways is also supported by the synergism observed between i.t. Δ^9-tetrahydrocannabinol and i.t. morphine-induced analgesia (Welch and Stevens, 1992). Such an interaction seems to involve κ-spinal opioid recep-

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tors since cross-tolerance between i.t. selective κ-opioid receptor agonists, but not μ- or δ-opioid receptor agonists, and Δ^9-tetrahydrocannabinol occurs in the tail-flick test in mice (Smith et al., 1994). The interaction between the cannabinoid and the opioid systems seems to be restricted to nociception but not to other cannabinoid actions such as hypothermia, hypoactivity and catalepsia (Smith et al., 1994).

In this work we further studied the effect of morphine on the analgesia elicited by i.v. Δ^9-tetrahydrocannabinol. Two analgesiometric tests were used: (i) the tail-flick test, which mainly involves spinal nociceptive mechanisms, and (ii) the hot-plate test, which is more related to supraspinal nociceptive pathways. The characterization of the opioid binding sites involved and their localization were investigated by using selective μ- (β-funaltrexamine), δ- (naltrindole) or κ- (nor-binaltorphimine) opioid receptor antagonists.

2. Materials and methods

2.1. Animals

Male Swiss albino mice (Interfauna Ibérica, Spain) weighing 25–27 g were housed in groups with 12 h light/dark cycles for a week before the experiment. Food and water were available ad libitum. All experiments were carried out between 12 h and 16 h. Each animal was used only once.

2.2. Chemicals

DAMGO ([D-Ala^2, *N*-Me-Phe^4,Gly-ol^5]enkephalin), naloxone, naltrindole and Cremophore EL were purchased from Sigma (USA). Nor-binaltorphimine and β-funaltrexamine were obtained from RBI (USA). Morphine was donated by the Ministerio de Sanidad y Consumo (Spain). Δ^9-Tetrahydrocannabinol was provided by the National Institute for Drug Abuse (USA). SR-141,716 A [(*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichorophenyl)-4-methyl-3-pyrazolecarboxamide, hydrochloride)] was a generous gift of Dr. M. Mossé (Sanofi Recherche, France).

2.3. Analgesic assays

Antinociceptive activity was measured by using the tail-flick (D'Amour and Smith, 1941) and the hot-plate tests (Eddy and Liembach, 1953). In the tail-flick test the time required to respond to a thermal stimulus in the tail was measured. Animals were restrained and one half of the tail was immersed in a water bath maintained at 52°C. Cut-off time was set at 10 s. Control latencies were measured before drug injection for each mouse. Animals with a control latency outside the range of 2–4 s were discarded.

The hot-plate test was performed as described by Eddy and Liembach (1953). A glass cylinder (16 cm high, 16 cm diameter) was used to keep the mouse on the heated surface of the plate, which was kept at a temperature of 55 ± 0.5°C. Two responses were examined: paw-lick and jump latencies. Cut-off times were set at 40 and 240 respectively.

Percentages of analgesia in both tests were calculated as follows: percentage of analgesia = [(test latency − control latency)/(cut-off time − control latency)] × 100.

2.4. Injection procedure

Δ^9-Tetrahydrocannabinol was administered i.v. in a mixture of Cremophore EL/ethanol/water (1:1:18) 20 min before the test. SR-141,716 A was administered in Cremophore EL/ethanol/water (1:1:18) i.p. 50 min before the test. Morphine sulphate was administered i.p., in saline, 30 min before the test. Naltrindole and naloxone were administered s.c., in saline, 30 and 40 min before the test respectively. Nor-binaltorphimine was administered i.t. or i.c.v., in saline, 25 or 80 min before the test respectively. β-Funaltrexamine was administered i.c.v. 24 before the test. DAMGO was administered i.c.v., in saline, 15 min before the test. I.t. and i.c.v. injections were performed as described by Hylden and Wilcox (1980) and Haley and McCormick (1957) respectively. Time of administration and doses of cannabinoid and opioid receptor antagonists were chosen on the basis of previously published studies (Ward et al., 1982; Baamonde et al., 1992; Rinaldi-Carmona et al., 1994; Reche et al., 1996).

2.5. Analysis of data

Comparisons between groups were made by using a one-way analysis of variance (ANOVA). Post-hoc comparisons were made by using the Newman-Keuls test.

ED_{50} values and their 95% confidence limits were calculated by log-probit analysis according to the method of Litchfield and Wilcoxon (1949). The ED_{50} was defined as the dose of the compound required to induce 50% of analgesia.

3. Results

The effect of morphine on the antinociceptive effect of Δ^9-tetrahydrocannabinol was examined in the tail-flick test as well as on paw-lick and jumping responses in the hot-plate test in mice. As illustrated in Fig. 1, i.p. morphine (2 mg/kg, 30 min before the test) shifted the dose-response curve of i.v. Δ^9-tetrahydrocannabinol (i.v., 20 min before the test) to the left for the three responses studied (2.5- and 3-fold shift for tail-flick and hot-plate responses respectively). ED_{50} calculated values for Δ^9-tetrahydrocannabinol alone and in combination with morphine are shown in Table 1.

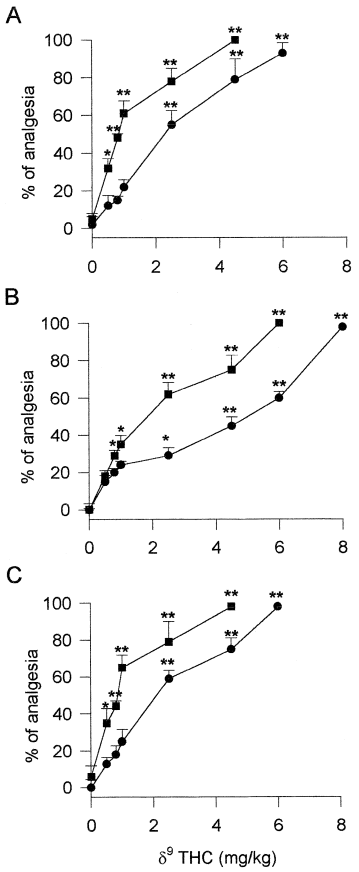

Fig. 1. Dose-response curves for the antinociceptive activity of Δ^9-tetrahydrocannabinol (δ^9-THC) alone (●) and in animals that received an ineffective dose of morphine (■). Analgesic responses were evaluated in the tail-flick (A) and hot-plate (B: jumping; C: paw-lick) tests. Δ^9-Tetrahydrocannabinol was given i.v. 20 min prior to the test. Morphine was given i.p. 30 min before the test. Percentage of analgesia corresponds to [(test latency − control latency)/(cut-off time − control latency)]×100. Values on the graph are expressed as means ± S.E.M. and correspond to values for groups of 8–10 mice. * $P < 0.05$; * * $P < 0.01$. Newman-Keuls' test.

The cannabinoid receptor antagonist SR-141,716 A (2 mg/kg, i.p., 30 min before the test) antagonized the antinociception induced by a combination of Δ^9-tetrahydrocannabinol (1.5 mg/kg, i.v., 20 min before the test) and morphine (2 mg/kg, i.p., 30 min before the test) both in the tail-flick test ($F_{ANOVA}(3,20) = 5.95$; $P < 0.01$) and in the hot-plate test (jump latency) ($F_{ANOVA}(3,20) = 14.67$; $P < 0.01$) (Fig. 2). Under these conditions, neither Δ^9-tetrahydrocannabinol nor morphine, given alone, caused significant analgesia.

Table 1
ED_{50} values in mg/kg of Δ^9-tetrahydrocannabinol (Δ^9-THC) alone and in the presence of 2 mg/kg of morphine

Test	Δ^9-THC ED_{50} (confidence limits)	Δ^9-THC + morphine (confidence limits)
Tail-flick	2.1 (0.87–4.99)	0.85 (0.31–1.27)
Jumping	4.71 (2.85–7.77)	1.51 (1.02–2.71)
Paw-lick	2.47 (1.63–3.73)	0.73 (0.41–1.29)

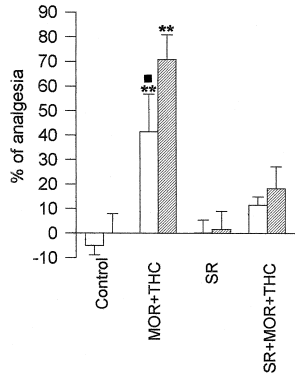

Fig. 2. Effect of the cannabinoid receptor antagonist SR-141,716 A (SR; 2 mg/kg, i.p., 50 min before the test) on the potentiation between Δ^9-tetrahydrocannabinol (THC) and morphine (MOR) in the tail-flick (white columns) and in the hot-plate (jumping) (hatched columns) tests. Percentage of analgesia corresponds to [(test latency − control latency)/(cut-off time − control latency)]×100. Values in the graph are expressed as means ± S.E.M. of 7–9 individual values. * $P < 0.05$; * * $P < 0.01$, compared to the control group. ■ $P < 0.05$, compared to the SR + MOR + THC group. Newman-Keuls' test.

The effect of the opioid receptor antagonist naloxone (1 mg/kg, s.c., 40 min before the test) was also tested on the analgesia induced by the combination of single doses of Δ^9-tetrahydrocannabinol (0.6 mg/kg or 1 mg/kg in the tail-flick or in the hot plate test, respectively, i.v., 20 min before the test) and morphine (2 mg/kg, i.p., 30 min before the test) in the same tests. Naloxone fully blocked the effect of such a combination on the tail-flick ($F_{ANOVA}(3,27) = 4.97$; $P < 0.01$) and jump ($F_{ANOVA}(3,28) = 14.22$; $P < 0.01$) latencies (Fig. 3).

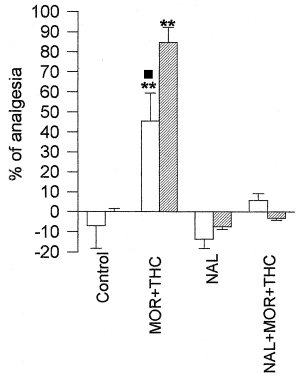

Fig. 3. Effect of the opioid receptor antagonist naloxone (NAL; 1 mg/kg, s.c., 40 min before the test) on the potentiation between Δ^9-tetrahydrocannabinol (THC) and morphine (MOR) in the tail-flick (white columns) and in the hot-plate (jumping) (hatched columns) tests. Percentage of analgesia corresponds to [(test latency − control latency)/(cut-off time − control latency)]×100. Values in the graph are expressed as means ± S.E.M. of 7–9 individual values. * $P < 0.05$; * * $P < 0.01$, compared to the control group. ■ $P < 0.05$, compared to the NAL + MOR + THC group. Newman-Keuls' test.

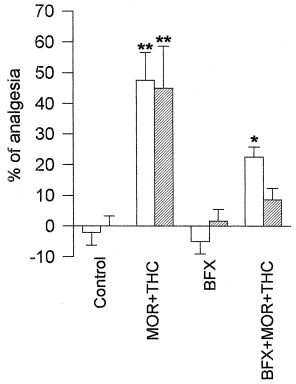

Fig. 4. Effect of the μ-opioid receptor antagonist β-funaltrexamine (BFX; 2 nmol/mouse, i.c.v., 24 h before the test) on the potentiation between Δ^9-tetrahydrocannabinol (THC) and morphine (MOR) in the tail-flick (white columns) and in the hot-plate (jumping) (hatched columns) tests. Percentage of analgesia corresponds to [(test latency − control latency)/(cut-off time − control latency)]×100. Values in the graph are expressed as means ± S.E.M. of 7–9 individual values. * $P < 0.05$; * * $P < 0.01$, compared to the control group. Newman-Keuls' test.

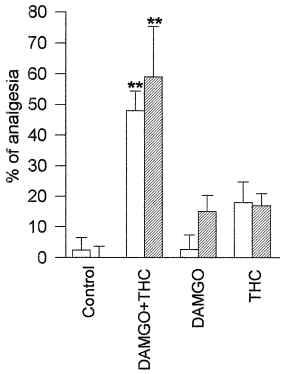

Fig. 6. Effect of the μ-opioid receptor agonist DAMGO on the analgesic effect of Δ^9-tetrahydrocannabinol (THC). DAMGO was administered i.c.v. 15 min before the test. Tail-flick (white columns) and hot-plate (jumping) (hatched columns) tests. Percentage of analgesia corresponds to [(test latency − control latency)/(cut-off time − control latency)]×100. Values in the graph are expressed as means ± S.E.M. of 7–9 individual values. * $P < 0.05$; * * $P < 0.01$. Newman-Keuls' test.

The μ-opioid receptor antagonist β-funaltrexamine (2 nmol/mouse, i.c.v., 24 h before the test) partially blocked the analgesia induced by the combination of morphine (2 mg/kg, i.p., 30 min before the test) and Δ^9-tetrahydrocannabinol (0.6 mg/kg, i.v., 20 min before the test) in the tail-flick test ($F_{ANOVA}(3,29) = 10.24$; $P < 0.01$). Analgesia was fully blocked in the hot-plate test (jump latency) ($F_{ANOVA}(3,29) = 8.05$; $P < 0.01$). (Fig. 4).

The κ-selective opioid receptor antagonist nor-binaltorphimine was given i.c.v. (70 μg/mouse) or i.t. (20 μg/mouse) to animals receiving a combination of Δ^9-tetrahydrocannabinol and morphine. Administered i.c.v., nor-binaltorophimine (1 h 20 min before the test) did not modify the analgesia induced by co-administration of Δ^9-tetrahydrocannabinol and morphine either in the tail-flick test or in the hot-plate test (data not shown). When i.t. administered, nor-binaltorphimine (25 min before the test) fully blocked the potentiation between morphine (2 mg/kg, i.p., 30 min before the test) and Δ^9-tetrahydrocannabinol (0.7 mg/kg, i.v., 20 min before the test) in the tail-flick test ($F_{ANOVA}(3,25) = 13.22$; $P < 0.01$). The antagonism was only partial in the hot-plate test (Δ^9-tetrahydrocannabinol, 1 mg/kg, i.v., 20 min before the test; morphine, 2 mg/kg, i.p., 30 min before the test) ($F_{ANOVA}(3,30) = 78.67$; $P < 0.01$) (Fig. 5).

The δ-selective opioid receptor antagonist naltrindole (0.1 mg/kg, s.c., 30 min before the test) failed to block the potentiation between morphine and Δ^9-tetrahydrocannabinol (data not shown).

Animals treated with ineffective doses of both DAMGO (2 nmol/mouse, i.c.v., 15 min before the test) and Δ^9-tetrahydrocannabinol (1.5 and 0.8 mg/kg for the hot-plate and tail-flick tests respectively, i.v., 20 min before the test) showed a significant level of analgesia both in the tail-flick test (F_{ANOVA} $(3,27) = 21.89$; $P < 0.01$) and in the hot-plate test (jumping, F_{ANOVA} $(3,26) = 7.23$; $P < 0.01$) (Fig. 6).

In experiments carried out with SR-141,716 A or with opioid receptor antagonists, uneffective doses of Δ^9-tetrahydrocannabinol and morphine were estimated for each individual experiment. Data are not given in order to facilitate the interpretation of the graphics.

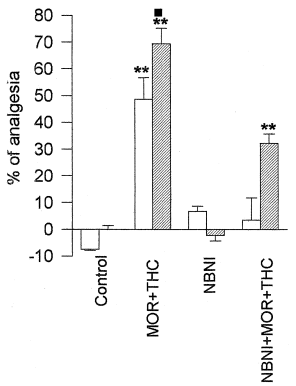

Fig. 5. Effect of the κ-opioid receptor antagonist nor-binaltorphimine (NBNI; 20 μg/mouse, i.t., 25 min before the test) on the potentiation between Δ^9-tetrahydrocannabinol (THC) and morphine (MOR) in the tail-flick (white columns) and in the hot-plate (jumping) (hatched columns) tests. Percentage of analgesia corresponds to [(test latency − control latency)/(cut-off time − control latency)]×100. Values in the graph are expressed as means ± S.E.M. of 7–9 individual values. * $P < 0.05$; * * $P < 0.01$, compared to the control group. ■ $P < 0.05$, compared to the NBNI + MOR + THC group. Newman-Keuls' test.

4. Discussion

The potentiation between Δ^9-tetrahydrocannabinol and morphine has been described by Welch and Stevens (1992) after i.t. administration of both drugs. However, the nature of the interaction between these drugs remains unknown. Several hypotheses have been proposed, including an al-

losteric interaction of cannabinoids with opioid receptors (Vaysse et al., 1987) as well as the co-localization, in the same neurons, of both cannabinoid and opioid receptors (Welch and Stevens, 1992). In our experience, i.v. administration of Δ^9-tetrahydrocannabinol and morphine also elicited potent analgesic responses, which suggests a greater than additive interaction between cannabinoids and opioids, since inactive doses of these drugs elicited analgesia when given in combination to the same animal. The antagonism induced by the opioid receptor antagonist naloxone as well as by the cannabinoid CB_1 receptor antagonist SR-141,217 A indicates the involvement of two different type of receptors in the analgesic response and demonstrates that Δ^9-tetrahydrocannabinol acts specifically on CB_1 receptors (Rinaldi-Carmona et al., 1994; Reche et al., 1996). Our data suggest that these drugs may act on different receptors in a sequence or cascade. In accordance with this, blockade of only one of the receptors involved (i.e. opioid or cannabinoid receptors) by specific antagonists is enough to block analgesia.

The potentiation between morphine and Δ^9-tetrahydrocannabinol was antagonized by the κ-opioid receptor antagonist nor-binaltorphimine given i.t., but not i.c.v. The κ-opioid component of the potentiation between morphine and Δ^9-tetrahydrocannabinol is probably due to Δ^9-tetrahydrocannabinol rather than to morphine. The involvement of κ-opioid binding sites in the antinociceptive effect of morphine is not well established. In fact, it has been demonstrated that nor-binaltorphimine, at high doses, does not antagonize the effect of morphine in the writhing test (Portoghese et al., 1987; Takemori et al., 1988). In addition, pretreatment with s.c. morphine does not induce significant cross-tolerance to the analgesic effect of the κ-opioid receptor agonist U-50,488 H in the tail-flick test (Fujimoto and Holmes, 1990). In contrast, cannabinoid-induced analgesia seems to be related to the spinal κ-opioid system. It has been shown that nor-binaltorphimine blocks Δ^9-tetrahydrocannabinol analgesia after peripheral or intrathecal administration (Welch and Stevens, 1992; Reche et al., 1996), but not after i.c.v. administration to mice (Reche et al., 1996). Additionally i.t. administered antiserum against dynorphin A-(1–8) prevents Δ^9-tetrahydrocannabinol-induced analgesia, supporting the hypothesis of the activation of endogenous dynorphinergic systems by cannabinoids (Reche et al., 1996). This suggests the existence of complex neuronal circuits in which cannabinoid receptors activate endogenous opioid pathways, leading to κ-opioid receptor occupation. The involvement of κ-opioid receptor in the interaction between the opioid system and cannabinoids has already been proposed by Smith et al. (1994) on the basis of the cross-tolerance observed between κ-opioid-selective agonists and Δ^9-tetrahydrocannabinol. Furthermore, these results confirm previous observations (Reche et al., 1996) that point to the involvement of spinal, but not supraspinal, κ-opioid receptors in Δ^9-tetrahydrocannabinol analgesia.

The potentiation between morphine and Δ^9-tetrahydrocannabinol was also reversed by the μ-opioid receptor antagonist β-funaltrexamine, given i.c.v. In contrast to the case of κ-opioid receptors, the activation of μ-opioid receptors should be an action of morphine, since low doses of naloxone do not modify Δ^9-tetrahydrocannabinol analgesia (Reche et al., 1996). The involvement of μ-opioid supraspinal receptors has been confirmed by using subeffective doses of the μ-selective opioid receptor agonist DAMGO administered by the i.c.v. route. This selective agonist, like peripheral morphine, also potentiated the effect of Δ^9-tetrahydrocannabinol.

Although from these data we cannot propose a mechanism for the interaction between morphine and Δ^9-tetrahydrocannabinol, it can be speculated that both cannabinoid and μ- supraspinal opioid receptors activate similar descending inhibitory pathways regulating, at a spinal level, the release of neurotransmitters involved in nociceptive transmission. The activation of spinal dynorphin systems may result in a decrease of substance P release by a presynaptic mechanism (Millan, 1990). Thus, a combination of both Δ^9-tetrahydrocannabinol and morphine may result in the sequential activation of spinal and supraspinal mechanisms, leading to antinociception. In this respect, it has been reported that simultaneous activation of μ-opioid receptors both at a spinal and at a supraspinal level induces large analgesic effects in rats. In a minor extension such an effect is also observed after activation of μ- supraspinal and κ- spinal opioid receptors (Roerig and Fujimoto, 1989), as also seemed to occur in our study.

Taken together, the preceding data demonstrate that the greater than additive effect of morphine on Δ^9-tetrahydrocannabinol analgesia involves cannabinoid as well as μ- and κ-opioid receptors. These results suggest that a combination of both opioid and cannabinoid drugs could be a useful tool for pain relief in clinical treatment. In this respect, an especially attractive alternative could be the design of a new family of analgesic drugs endowed with high affinity for both μ-opioid and cannabinoid CB_1 receptors.

Acknowledgements

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