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A role for central cannabinoid and opioid systems in peripheral Δ^9-tetrahydrocannabinol-induced analgesia in mice

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Abstract

Δ^9-tetrahydrocannabinol elicits analgesia in rodents by both spinal and supraspinal mechanisms. Pharmacological data point to a link between cannabinoids and the opioid system. The lack of specific cannabinoid receptor antagonists has hindered the investigation of the physiological relevance of the cannabinoid system in nociception control. In this work we characterized the effect of the new cannabinoid receptor antagonist, SR-141,716 A (*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazolecarboxamide hydrochloride), on Δ^9-tetrahydrocannabinol-induced analgesia. pA_2 values in the tail-flick and in lick and jump responses in the hot-plate tests were 9.59, 8.72 and 10.21, respectively. Slope values of pA_2 plots were not different from -1 indicating competitive antagonism. The involvement of the opioid system in Δ^9-tetrahydrocannabinol-induced analgesia was investigated by using naloxone as well as δ (naltrindole)- and κ (nor-binaltorphimine)-opioid receptor antagonists. Intrathecal norbinaltorphimine antagonized the effect of Δ^9-tetrahydrocannabinol. The effect of Δ^9-tetrahydrocannabinol was also blocked by administration of dynorphin A-(1–8) antiserum in the same test.

Keywords: Analgesia; Dynorphin; Opioid; Δ^9-Tetrahydrocannabinol; SR-141,716 A; Nor-binaltorphimine

1. Introduction

Cannabinoids are a distinct class of psychoactive compounds which produce a wide array of effects including hypothermia, depressed motor activity, hypotension, inhibition of intestinal motility and antinociception (review in Dewey, 1986). Δ^9-tetrahydrocannabinol is the most psychoactive cannabinoid in marijuana. High-affinity binding sites for Δ^9-tetrahydrocannabinol have been characterized both in the rat central nervous system (CNS) (Devane et al., 1988) and in peripheral organs (Munro et al., 1993). These receptors have been cloned and their structures have been found to be consistent with those of the G protein receptor family (Matsuda et al., 1990).

The wide distribution of cannabinoid receptors (Howlett et al. (1990) and references cited therein) and their corresponding mRNA (McLaughlin and Abood, 1993) throughout the CNS gives neuroanatomical support to the large spectrum of pharmacological effects elicited by cannabinoids. The recent 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 cannabinergic pathways endowed with physiological relevance. However the lack of a specific cannabinoid receptor antagonist has hindered the elucidation of the specific pharmacological properties of cannabinoids, and most work in this field has been directed at investigating the relationship between cannabinoids and other neurotransmitter or neuromodulator systems. The development of a new cannabinoid receptor antagonist has opened up new prospects in this field of research (Rinaldi-Carmona et al., 1994).

An interaction between cannabinoid and opioid systems in the modulation of nociception has been the focus of much attention in recent years (Smith et al., 1994; Welch and Stevens, 1992; Welch, 1993; Welch et al., 1995; Vela et al., 1995a). Pharmacological data point to a link between cannabinoids and the opioid system. In this context, Δ^9-tetrahydrocannabinol inhibits, in a non-competitive manner, the binding of μ- and δ-, but not κ-opioid receptor ligands to rat brain membranes (Vaysse et al.,

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1987). In addition, analgesia and the rewarding properties of cannabinoids have been reported to be blocked, at least partially, by the opioid receptor antagonist naloxone (Wilson and May, 1975; Bhargava, 1976a; Bhargava, 1976b; Tulunay et al., 1981; Gardner and Lowinson, 1991). Moreover, rodents chronically treated with crude hashish extracts or with purified Δ^9-tetrahydrocannabinol develop an opioid-like withdrawal syndrome after acute naloxone administration (Kaymakçalan et al., 1977). Interestingly, naloxone has been found to induce opioid withdrawal signs in rats perinatally exposed to Δ^9-tetrahydrocannabinol. Consistent with this effect, both Δ^9-tetrahydrocannabinol and anandamide decrease opioid withdrawal symptoms in rodents (Hine et al., 1975; Vela et al., 1995b). However the ability of the opioid receptor antagonist naloxone to antagonize cannabinoid-induced analgesia seems to depend on the nature of the cannabinoid drug as well as on the route of administration used.

In this work we investigated the effect of the new cannabinoid receptor antagonist SR-141,716 A (*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazolecarboxamide hydrochloride) on the antinociceptive responses elicited by Δ^9-tetrahydrocannabinol both in the tail-flick test and in the hot-plate test in mice. The involvement of the opioid system, and the characterization of the opioid binding sites involved in these responses, were evaluated by using selective opioid receptor antagonists. The action of a selective dynorphin-(1–8) antiserum was also tested.

2. Materials and methods

2.1. Animals

Male Swiss albino mice (Interfauna Ibérica, Spain) weighing 25–27 g were housed in groups under a 12 h light/dark cycle 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

Naloxone, naltrindole and Cremophore EL were purchased from Sigma (USA). Nor-binaltorphimine was obtained from RBI (USA). Morphine was donated by the Ministerio de Sanidad y Consumo (Spain). Δ^9-tetrahydrocannabinol was donated by the National Institute for Drug Abuse (USA). SR-141,716 A was a generous gift of M. Mossé (Sanofi Recherche, France). Dynorphin A-(1–8) antiserum was obtained from Peninsula Laboratories (UK).

2.3. Analgesic assays

Antinociceptive activity was measured in 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 on the tail was measured. Animals were restrained and 1/2 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 s 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. 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, 5 min or 1 h before Δ^9-tetrahydrocannabinol respectively. SR-141,716 A was administered i.p. in a mixture of distilled water/ethanol/Cremophore EL (8 : 1 : 1), 30 min before Δ^9-tetrahydrocannabinol. Dynorphin A-(1–8) antiserum or non-immunized rabbit serum was administered i.t. in 5 μl of distilled water 2 h before Δ^9-tetrahydrocannabinol. I.t. and i.c.v. injections were performed as described by Hylden and Wilcox (1980) and Haley and McCormick (1957), respectively.

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 the maximal analgesic response. For pA_2 calculation dose ratios (X) of ED_{50} values (ED_{50} in the presence of antagonist/ED_{50} in the absence of the antagonist) were calculated and the log ($X - 1$) plotted against the negative logarithms of antagonist expressed in mol/kg. pA_2, CL and slopes were determined by using the method of Tallarida and Murray (1986).

3. Results

As illustrated in Fig. 1, i.v., Δ^9-tetrahydrocannabinol induced a dose-dependent increase in the tail-flick latency,

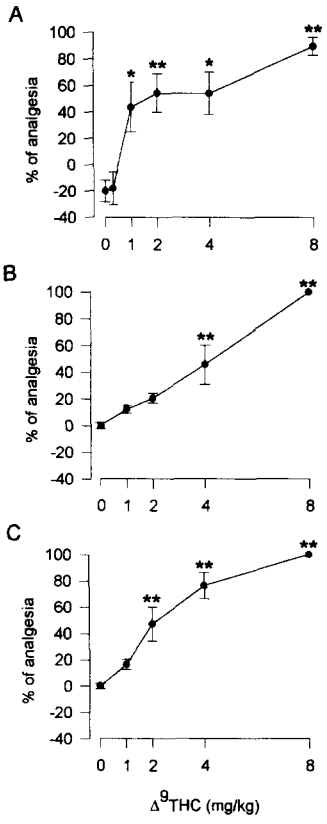

Fig. 1. Response to a thermal stimulus in the tail-flick (A) and hot-plate (B, jumping; C, paw-lick) tests for mice treated with Δ^9-tetrahydrocannabinol (Δ^9-THC). Animals were i.v. injected with different doses of the drug 20 min prior to the test. % of analgesia corresponds to [(test latency − control latency)/(cut-off time − control latency)] × 100. Values in the graph are expressed as means ± S.E.M. and correspond to results for 8–10 mice. * $P < 0.05$; ** $P < 0.01$. Newman-Keuls' test.

as well as in paw-lick and jump latencies in the hot-plate test. Analgesia was measured 20 min after i.v. administration of the drug. This time corresponds to the maximal response (data not shown). ED_{50} values at this time were 2.08 mg/kg (0.87–4.99), 2.47 (1.63–3.74) and 4.71 (2.85–7.76) for tail-flick, paw-lick and jump latencies, respectively.

The time course of the blocking of the Δ^9-tetrahydrocannabinol antinociceptive response by the new cannabinoid receptor antagonist SR-141,716 A (i.p., 2 mg/kg) was examined in the tail-flick test. SR-141,716 A was administered, i.p., at different intervals (15, 30, 60 and 90 min) before Δ^9-tetrahydrocannabinol. Maximal antagonism was found in animals receiving SR-141,716 A 30 min before Δ^9-tetrahydrocannabinol (data not shown). The potency of the cannabinoid antagonist in blocking the Δ^9-tetrahydrocannabinol analgesic effect was tested at this time at the doses of 0.04, 0.2, 0.1 and 1 mg/kg in both the tail-flick and the hot-plate tests (paw-lick and jumping responses). As illustrated in Fig. 2, SR-141,716 A shifted to the right the dose-response curve of Δ^9-tetrahydrocannabinol in both the tail-flick and the hot-plate tests (paw-lick and jumping). ED_{50} values in the presence of the antagonist, pA_2 values and pA_2 slopes are shown in Table 1. Slope values in all the tests were not different from −1, which is indicative of competitive antagonism (Hayashi and Takemori, 1971).

The effect of opioid receptor antagonists on Δ^9-tetrahydrocannabinol-induced analgesia was examined in the tail-flick and the hot-plate tests (jumping). The effect of the opioid receptor antagonist naloxone was tested at the doses of 10, 5, 3, 1 and 0.1 mg/kg (Fig. 3). Naloxone given at 10 mg/kg fully blocked the effect of Δ^9-tetrahydrocannabinol, both in the tail-flick ($F(3,24) = 13.38$; $P < 0.01$) and in the hot-plate tests (jumping, $F(3,24) = 16.94$; $P < 0.01$). 5, 3 or 1 mg/kg of naloxone partially blocked the effect of Δ^9-tetrahydrocannabinol in the tail-flick ($F(3,29) = 37.8$; $P < 0.01$; $F(3,29) = 38.9$; $P < 0.01$ and $F(3,25) = 13.09$; $P < 0.05$ for the doses of 5, 3 and 1

Table 1
ED_{50}, apparent pA_2 and pA_2 slopes for the antagonist effect induced by SR-141,716 A on the antinociceptive activity of Δ^9-tetrahydrocannabinol in the presence of different doses of the cannabinoid receptor antagonist drug

Test	Dose of SR 141716A (mg/kg)	ED_{50} of agonist (95% CL) (mg/kg)	Apparent pA_2 values (95% CL)	Slope of pA_2 (95% CL)	No. of mice
Jump	0.00	2.96 (1.66–5.27)			50
	0.10	6.47 (3.88–12.37)	10.21	−0.36	30
	0.20	11.68 (8.08–16.89)	(7.46–12.96)	(−1.21–0.48)	42
	1.00	14.16 (11.40–17.56)			48
Lick	0.00	2.13 (1.32–3.41)			50
	0.04	4.40 (2.78–6.96)	8.72	−0.47	42
	0.10	5.64 (3.73–8.52)	(7.11–10.33)	(−1.27–0.62)	30
	0.20	9.97 (6.05–16.43)			42
	1.00	13.83 (9.02–21.00)			48
Tail-flick	0.00	3.12 (1.88–5.51)			50
	0.04	4.26 (2.38–7.63)	9.59	−0.56	42
	0.10	8.91 (4.94–16.05)	(5.13–14.04)	(−4.11–2.99)	30
	0.20	13.68 (6.84–27.36)			42
	1.00	14.94 (9.82–22.71)			48

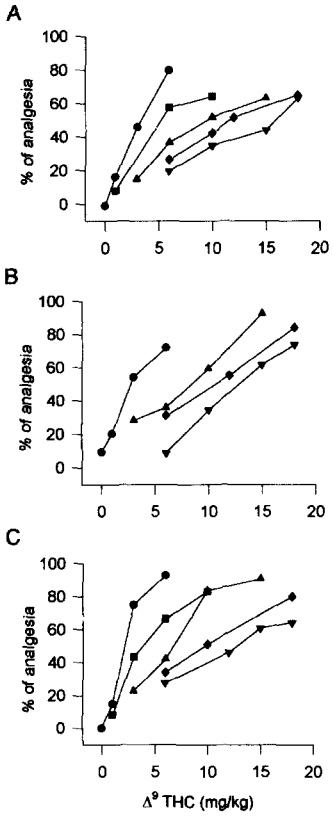

Fig. 2. Dose-response relationship for the antinociceptive activity of Δ^9-tetrahydrocannabinol (Δ^9-THC) in the presence of different doses (●, control curve; ■, 0.04 mg/kg; ▲, 0.1 mg/kg; ◆, 0.2 mg/kg; ▼, 1 mg/kg) of SR-141,716 A. Latencies of the response to thermal stimulus in the tail-flick (A) and hot-plate (B, jumping; C, paw-lick) tests were studied. Drugs were given 50 min (SR-141,716 A) and 20 min (Δ^9-tetrahydrocannabinol) prior to the test. % 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. A group of 8–16 mice was used for each one of the single values in the graph. Error bars have been omitted.

mg/kg respectively) and the hot-plate tests (jumping, $F(3,29) = 18.04$; $P < 0.01$; $F(3,29) = 13.56$; $P < 0.01$; $F(3,27) = 24.49$; $P < 0.05$ for the doses of 5, 3 or 1 mg/kg respectively) (Fig. 3). However when given at the dose of 0.1 mg/kg, naloxone did not modify the antinociceptive effect of Δ^9-tetrahydrocannabinol (tail-flick, $F(3,20) = 3.61$; hot-plate, $F(3,22) = 6.43$).

The δ-opioid receptor antagonist naltrindole, given at the dose of 0.1 mg/kg 30 min before testing, failed to block the effect of Δ^9-tetrahydrocannabinol in both tests (tail-flick, $F(3,27) = 1.3$; hot-plate, $F(3,28) = 12.96$) (data not shown). The dose and time administration of naltrindole can be considered as δ-selective and were chosen on the basis of previous works (Baamonde et al., 1992).

The κ-opioid receptor antagonist nor-binaltorphimine was given i.c.v. at the dose of 70 μg/mouse or i.t. at the doses of 10, 20 or 40 μg/mouse. Neither i.c.v. (tail-flick, $F(3,26) = 20.44$; hot-plate, $F(3,30) = 80.93$) nor i.t. nor-binaltorphimine at the lowest dose (tail-flick, $F(3,19) = 10.29$; hot-plate $F(3,19) = 95.46$) antagonized the effect of Δ^9-tetrahydrocannabinol. In contrast, i.t. nor-binaltorphimine, given at the doses of 20 or 40 μg/mouse, fully blocked the effect of Δ^9-tetrahydrocannabinol in the tail-flick test ($F(3,21) = 12.49$: $P < 0.05$; $F(3,26) = 9.04$; $P < 0.01$ for the doses of 20 and 40 μg/mouse respectively) and partially in the hot-plate test ($F(3,27) = 18.56$; $P < 0.05$; $F(3,26) = 22.32$, $P < 0.01$ for the doses of 20 and 40 μg/mouse respectively) (Fig. 4). Doses used for nor-binaltorphimine were chosen on the basis of their ability to block analgesic responses induced by the κ-opioid receptor agonist U-50,488H (Takemori et al., 1988).

I.t. administration of a dynorphin A-(1–8) rabbit antiserum partially blocked the effect of i.v. Δ^9-tetrahydrocannabinol in the tail-flick test at the dose of 50 ($F(3,26) = 16.79$; $P < 0.01$; Fig. 5) or 100 μg/mouse ($F(5,34) = 42.50$; $P < 0.01$). In contrast, dynorphin antiserum did not modify the analgesic effect of Δ^9-tetrahydrocannabinol in

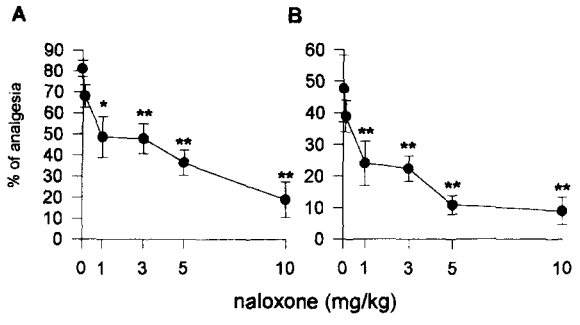

Fig. 3. Effect of the opioid receptor antagonist naloxone (0.1, 1, 3, 5 and 10 mg/kg; s.c.; 40 min before the test) on the analgesic effects of Δ^9-tetrahydrocannabinol. Δ^9-tetrahydrocannabinol was given i.v. 20 min prior to the test at the doses of 3 mg/kg (A, tail-flick response) or 4.5 mg/kg (B, jumping response). % 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.

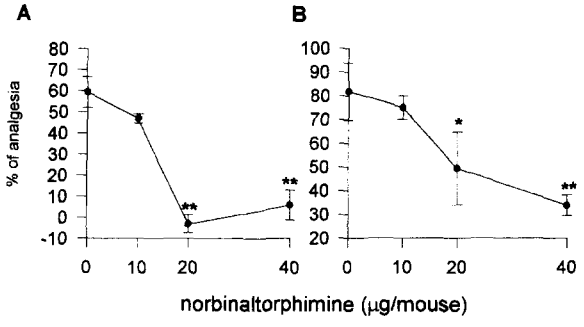

Fig. 4. Effect of the κ-opioid receptor antagonist nor-binaltorphimine (nor-BNI; 20 μg/mouse; i.t.; 25 min before the test) on the analgesic effect of Δ^9-tetrahydrocannabinol. Δ^9-tetrahydrocannabinol was i.v. administered 20 min prior to the test at the dose of 3.5 mg/kg (A: tail-flick latency) or 4.5 mg /kg (B: jumping response). % 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.

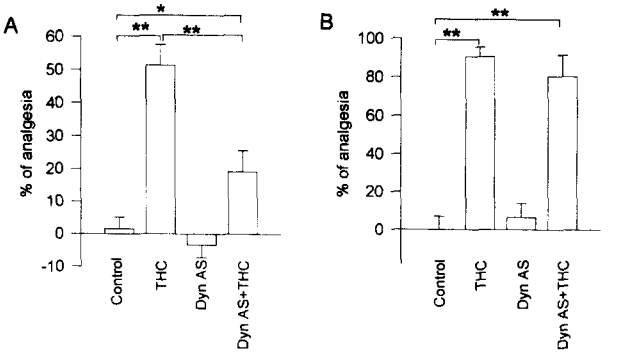

Fig. 5. Effect of dynorphin A-(1–8) rabbit antiserum (Dyn AS; 50 μg/mouse; it) on the hypoalgesic effect of Δ^9-tetrahydrocannabinol (THC). Dynorphin antiserum was given 120 min before Δ^9-tetrahydrocannabinol. Δ^9-tetrahydrocannabinol was i.v. administered, at the dose of 3.5 mg/kg, 20 min prior to the test. A: tail-flick test; B: hot-plate test (jump). % 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 hot-plate test at the dose of 50 μg ($F(3,31) = 29.09$; Fig. 5) or 100 μg/mouse ($F(5,34) = 10.48$). Non-immunized rabbit serum did not modify Δ^9-tetrahydrocannabinol-induced analgesia in the tail-flick test or in the hot-plate test (data not shown).

4. Discussion

In this work we have shown that i.v. administered Δ^9-tetrahydrocannabinol elicited analgesia, both in the tail-flick test and in the hot-plate test in mice, in a dose-dependent manner (Fig. 1). Such an effect was fully competitively antagonized, in both tests, by the cannabinoid receptor antagonist SR-141,716 A, given i.p. The effect of Δ^9-tetrahydrocannabinol was partially blocked by the opioid antagonist naloxone at the dose of 1 mg/kg (s.c.) as well as by the κ-opioid receptor antagonist nor-binaltorphimine (nor-binaltorphimine) given i.t. Naloxone, given at 0.1 mg/kg (s.c.), nor-binaltorphimine given icv and the δ-opioid receptor antagonist naltrindole given s.c. did not modify the effect of Δ^9-tetrahydrocannabinol.

The cannabinoid antagonist SR-141,716 A induced a parallel shift to the right of the dose-response curve of Δ^9-tetrahydrocannabinol, suggesting competitive antagonism. Apparent pA_2 values (Fig. 2; Table 1) for the cannabinoid antagonist SR-141,716 A (Rinaldi-Carmona et al., 1994), determined in the tail-flick and hot-plate tests (paw-lick and jump latencies) were similar for the three responses examined, indicating the involvement of the same receptor. The slopes of the pA_2 plots were not significantly different from the theoretical value of −1, which is consistent with a type of competitive antagonism (Hayashi and Takemori, 1971). These data show the involvement of cannabinoid receptors in the antinociceptive properties elicited by Δ^9-tetrahydrocannabinol in these tests, and support the existence of a distinct cannabinergic nociceptive pathway, which may be an eventual pharmacological target for pain relief.

The partial blockade of Δ^9-tetrahydrocannabinol-induced analgesia (Fig. 3) by the non-selective opioid receptor antagonist naloxone (1 mg/kg, s.c.) suggests the involvement of the opioid system in this response. The involvement of the endogenous opioid system in cannabinoid-induced analgesia is a controversial matter. Initial work in this field showed that naloxone (Chesher et al., 1973; Bloom et al., 1977; Sanders et al., 1979) as well as the irreversible opiate receptor antagonist chlornaltrexamine (Tulunay et al., 1981) blocked the antinociceptive effects elicited by cannabinoid-related compounds. In addition, cross-tolerance between the analgesic effects of morphine and Δ^9-tetrahydrocannabinol has also been described (Bloom and Dewey, 1978; Thorat and Bhargava, 1994). Nevertheless, other authors (Yaksh, 1981; Martin, 1985) have shown that naloxone fails to block the antinociceptive effects of cannabinoid drugs. In line with this, Welch and Stevens (1992) reported that naloxone does not antagonize antinociception induced by i.t. Δ^9-tetrahydrocannabinol. The discrepancy between our results and those reported in the literature could be related to the type of cannabinoid drug used as well as to the administration route. This may also be linked to the existence of more than one cannabinoid binding site subtype in the CNS, as recently proposed by Smith et al. (1994). The hypothetical existence of several cannabinoid receptor subtypes is supported by the results of binding studies showing that cannabinoid compounds bind to central and peripheral cannabinoid receptors with different affinities (Lynn and Herkenham, 1994). It could be speculated that if more than one cannabinoid binding site exists in the CNS, its relative affinity for the different cannabinoid drugs as well as its anatomical distribution throughout the CNS would account for the differences mentioned above. Interestingly, the putative endogenous ligand of the cannabinoid receptor, anandamide, displays only micromolar affinity for cannabinoid receptors (Devane et al., 1992; Smith et al., 1994). This could indicate that anandamide binds, with different affinities, to two cannabinoid receptor populations, with the higher affinity binding site being the less abundant receptor subtype.

The complete antagonism observed with a dose of naloxone as high as 10 mg/kg shows that a dose-response relationship can be established and supports the argument that opioids are involved in Δ^9-tetrahydrocannabinol-induced analgesia. Since naloxone blocks μ-opioid mediated responses at lower doses (0.1–1 mg/kg), these data seem to indicate the involvement of δ- or κ-, rather than μ-opioid receptors in Δ^9-tetrahydrocannabinol-induced analgesia, as previously suggested by Ferri et al. (1986).

In order to assess the relative contribution of each (μ-,

δ- or κ-) opioid receptor to the antinociceptive effects of i.v. Δ^9-tetrahydrocannabinol, we tested the following selective opioid receptor antagonists: naltrindole (δ), low doses of naloxone (μ) and nor-binaltorphimine (κ). In our study only the κ-opioid receptor antagonist nor-binaltorphimine, given i.t. but not i.c.v., fully blocked the effect of Δ^9-tetrahydrocannabinol in the tail-flick test. These results are in agreement with those previously reported by Welch (1993). The effect of Δ^9-tetrahydrocannabinol was only blocked partially in the hot-plate test (jump latency). The different response elicited by i.t. nor-binaltorphimine in the hot-plate and in the tail-flick tests points to the involvement of spinal κ-opioid receptors since spinal mechanisms are mainly involved in the inhibition of the tail-flick reflex elicited by analgesic drugs in the tail-flick test. In contrast, the increase in the jump latency in the hot-plate test seems to be related to supraspinal rather than to spinal mechanisms. Our results are in accordance with those reported by Welch's group (Welch and Stevens, 1992; Welch, 1993) and suggest that, at a spinal but not at supraspinal level, Δ^9-tetrahydrocannabinol-induced analgesia could be related to the activation of the endogenous opioid system. Our data point to κ-opioid spinal receptors as being uniquely involved in these responses. As suggested by Welch (1993), the blocking of cannabinoid-induced antinociception by nor-binaltorphimine might also represent a nonopioid action of nor-binaltorphimine since the doses required are higher than those needed to block the effect of κ opioid receptor agonists. However such a possibility seems to be unlikely since binding experiments have demonstrated the lack of significant affinity of nor-binaltorphimine for cannabinoid receptors (Vaysse et al., 1987). In addition, Smith et al. (1994) have recently reported the existence of cross-tolerance between the analgesic effects of Δ^9-tetrahydrocannabinol and κ-opioid receptor agonists.

Blockage of Δ^9-tetrahydrocannabinol-induced analgesia by i.t. administered dynorphin A-(1–8) rabbit antiserum points to the activation of the endogenous opioid system by Δ^9-tetrahydrocannabinol and suggests that this drug may evoke dynorphin-related peptide release in the spinal cord. This result can account for the involvement of κ-opioid receptors in Δ^9-tetrahydrocannabinol-induced analgesia. Interestingly, dynorphin antiserum only blocked Δ^9-tetrahydrocannabinol-analgesia in the tail-flick test, but not in the hot-plate test. As discussed in the case of the effects of i.t. nor-binaltorphimine, this suggests the involvement of κ-opioid spinal mechanisms in the response elicited by Δ^9-tetrahydrocannabinol.

In summary, our results support the existence of a cannabinergic pathway involved in nociception which may be a pharmacological target to relieve pain. In addition, the analgesic response induced by cannabinoids could be related to the release of dynorphin-related peptides at a spinal cord level.

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