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A selective CCK_B receptor antagonist potentiates μ-, but not δ-opioid receptor-mediated antinociception in the formalin test

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Abstract

The endogenous peptides enkephalins and cholecystokinin appear to play an opposite role in the control of pain. In this work, the effect of the selective CCK_B receptor antagonist PD-134,308 on antinociceptive effects induced by morphine or by a complete inhibitor of enkephalin-metabolizing enzymes, RB 101, was studied using the formalin test. In mice, s.c. injection of formalin into the dorsal surface of the hindpaw had a biphasic effect: an early nociceptive response followed by a late response. Morphine (2 mg/kg i.p.) caused naloxone (0.5 mg/kg s.c.) but not naltrindole (0.5 mg/kg s.c.) reversible antinociceptive responses in the early and late phases of the assay, suggesting a preferential involvement of μ-opioid receptors in these responses. In contrast, RB 101 (50 mg/kg i.p.) produced antinociceptive effects in the early and late phases which were both antagonized by the δ-selective opioid receptor antagonist naltrindole (0.5 mg/kg s.c.). The antinociceptive response elicited by morphine on the late but not the early phase of the formalin test was potentiated by the CCK_B antagonist PD-134,308 (1 mg/kg i.p.). This compound was unable to facilitate the analgesic effects produced by RB 101 on both phases, in contrast to what was observed in the hot plate test with mice and the tail flick test with rats. Therefore, in the formalin test with mice, the facilitating effects of opiate-induced analgesia by CCK_B receptor antagonists seem to be restricted to μ-opioid receptor-mediated responses.

Keywords: RB 101 (mixed enkephalin-degrading enzyme inhibitor); Morphine; CCK_B receptor antagonist; Formalin test; Naloxone; Naltrindole; (Mouse)

1. Introduction

Anatomical studies have shown that enkephalins and cholecystokinin (CCK) have a strikingly similar distribution within many regions of the central nervous system (CNS). This overlapping distribution of the peptides and their respective receptors in pain-processing regions of the brain and the spinal cord (Gall et al., 1987; Pohl et al., 1990) has focused attention on the role of CCK in nociception (Faris et al., 1983). In several antinociceptive tests, using acute thermal nociceptive stimuli (hot plate and tail flick tests), CCK_B receptor antagonists have been shown to strongly potentiate opioid analgesia induced by RB 101 (Maldonado et al., 1993; Valverde et al., 1994), a systemically active mixed enkephalin-degrading enzyme inhibitor (Fournié-Zaluski et al., 1992). In these tests, the facilitation effect of CCK_B receptor antagonists was 2–8 times higher with RB 101 than with morphine (Valverde et al., 1994). Selective CCK_B receptor antagonists were also found to potentiate morphine-induced analgesia for various nociceptive stimuli (chemical, electrical, thermal and mechanical) (review in Baber et al., 1989). However, in rats with inflammation induced by carrageenin, CCK receptor antagonists did not enhance the antinociceptive effects of morphine (Stanfa and Dickenson, 1993). In contrast, in a neuropathic pain model CCK_B receptor antagonists were shown to increase the effectiveness of morphine (Xu et al., 1993). These findings indicate that various animal models relevant to clinical situations must be used to evaluate the promising association of opiates and CCK receptor antagonists in the management of pain.

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The formalin test is often considered an appropriate model of clinical pain because the nociceptive stimulus, tissue injury by injection of the irritative chemical reagent, induces continuous pain. Moreover, the formalin test is less drastic and thus more acceptable than chronic nociception generated in animals by adjuvant-induced polyarthritis or dorsal rhizotomy-induced autotomy. However, the formalin test is a very sensitive assay, giving positive responses not only to opiates but also to various compounds including aspirin or paracetamol (Abbott et al., 1982; Calcagnetti et al., 1988; Murray and Cowan, 1991; Garret et al., 1991; Giordano, 1991; Sakurada et al., 1993; Corrêa and Calixto, 1993). Injection of diluted formalin into an animal's hindpaw produces a biphasic response with an early (first phase) and a late (second phase) response. It has been postulated that the rapid response is caused by a direct effect of formalin on nociceptors while the late response is due to inflammation (Hunskaar et al., 1986; Hunskaar and Hole, 1987; Rosland et al., 1990).

The aim of the present study was therefore to evaluate the modulation of opioid-induced analgesia by endogenous CCK in the formalin test in mice. For this purpose, the antinociceptive responses induced by morphine or by endogenous enkephalins, protected by RB 101, were measured in presence of the selective CCK_B receptor antagonist PD-134,308 (Hughes et al., 1990).

2. Materials and methods

2.1. Animals

Male CD_1 albino mice (22–24 g) (Charles River, France) were used. The animals were supplied with food and water ad libitum and housed in groups (n = 20) in a temperature (22 ± 1°C) and humidity (45–55%) controlled environment at least 2 days before the experiments were started. Each animal was used only once. All antinociceptive variables were recorded between 10:00 a.m. and 8:00 p.m.

2.2. Chemicals

RB 101 (H_2N-CH(CH_2-CH_2-*S*-CH_3)-CH_2-S-S-CH_2-CH($CH_2\Phi$)-CONH-CH($CH_2\Phi$)-$COOCH_2\Phi$) was synthesized in the laboratory as previously described (Fournié-Zaluski et al., 1992) and dissolved in the following vehicle: ethanol (10%)–cremophor EL (10%)–distilled water (80%). Cremophor EL, a derivative of castor oil and ethylene oxide, was purchased from Sigma Chemical (France). Naltrindole hydrochloride (17-cyclopropylmethyl-6,7-dehydro-4,5α-epoxy-3,14-dihydroxy-6,7,2′,3′-indolomorphinan) (Portoghese et al., 1988) and PD-134,308 (Hughes et al., 1990) were synthesized in the laboratory following previously reported methods. Naloxone hydrochloride and morphine hydrochloride were purchased from Mallet (France) and from Coopération Pharmaceutique Française (Paris, France), respectively. Naltrindole, naloxone, and morphine were dissolved in saline, and PD-134,308 was suspended in carboxymethyl cellulose 0.5%.

2.3. Formalin test

The test, adapted from Hunskaar et al. (1985), was carried out in a glass cylinder chamber (16 cm high, 16 cm diameter). The mice were placed in the test chambers for 30 min. After this period of adaptation, 20 μl of 5% formalin was injected subcutaneously (s.c.) into the dorsal surface of the right hindpaw of the mouse,

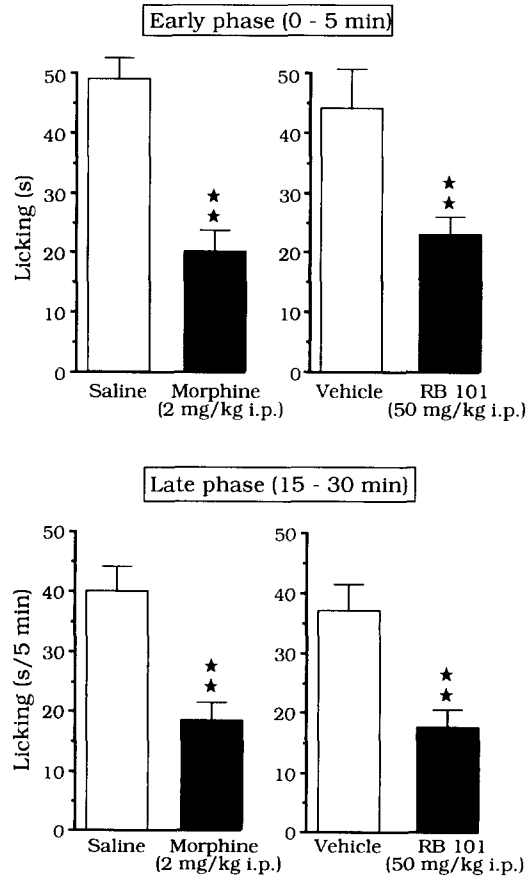


Fig. 1. Antinociceptive effects in the formalin test of morphine (2 mg/kg) and RB 101 (50 mg/kg), and of respective vehicles, injected i.p. 15 min before the test. Nociceptive behavior in both early (0–5 min) and late phases (15–30 min) after 5% formalin s.c. injection into the dorsal surface of the hindpaw was measured as the amount of time spent licking or biting the injected paw. The results are expressed as means ± S.E.M. (n = 7–10 for each group). ** $P < 0.01$ as compared to respective control group (Dunnett's t-test).

using a 26-gauge needle connected to a microsyringe. Each mouse was immediately returned to the observation chamber after injection and its nociceptive response was recorded. Recording of the early response (early phase) started immediately and lasted 5 min (0–5 min) and recording of the late response (late phase) started 15 min after formalin injection and lasted for 15 min (15–30 min). In both phases only licking or biting of the injected hindpaw was defined as a nociceptive response and the total duration of response was registered by a means of a stopwatch during the test period.

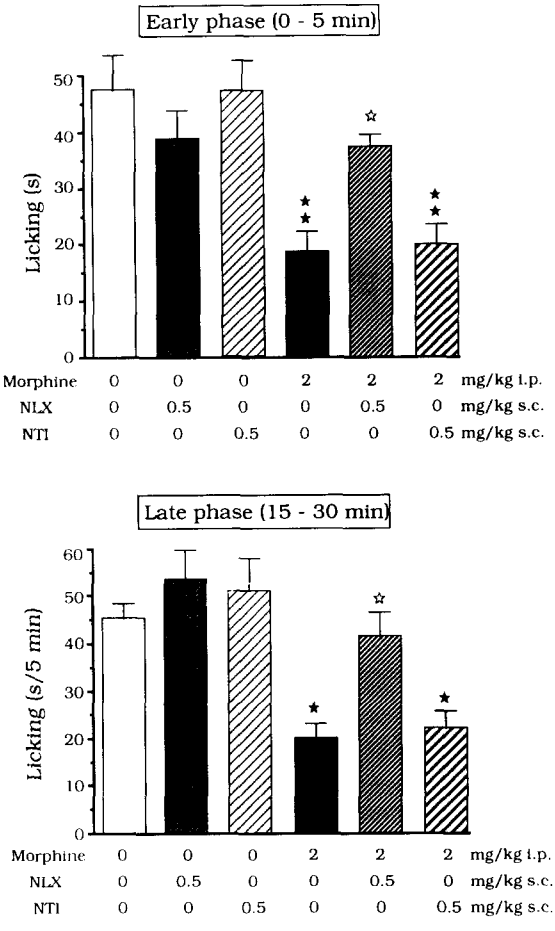


Fig. 2. Effects of naloxone (NLX) (0.5 mg/kg s.c.) and naltrindole (NTI) (0.5 mg/kg s.c.) on the antinociceptive responses observed after i.p. administration of morphine (2 mg/kg) in the formalin test in mice. Opioid antagonists were administered 5 min before i.p. injection of saline or morphine and 20 min before the test. Nociceptive behavior in both early (0–5 min) and late phases (15–30 min) after 5% formalin s.c. injection into the dorsal surface of the hindpaw was measured as the amount of time spent licking or biting the injected paw. The results are expressed as means ± S.E.M. ($n = 8$–10 for each group). $^{*}P < 0.05$, $^{**}P < 0.01$ as compared to control, $^{\star}P < 0.05$ as compared to the same dose of morphine without antagonist (Newman-Keuls test).

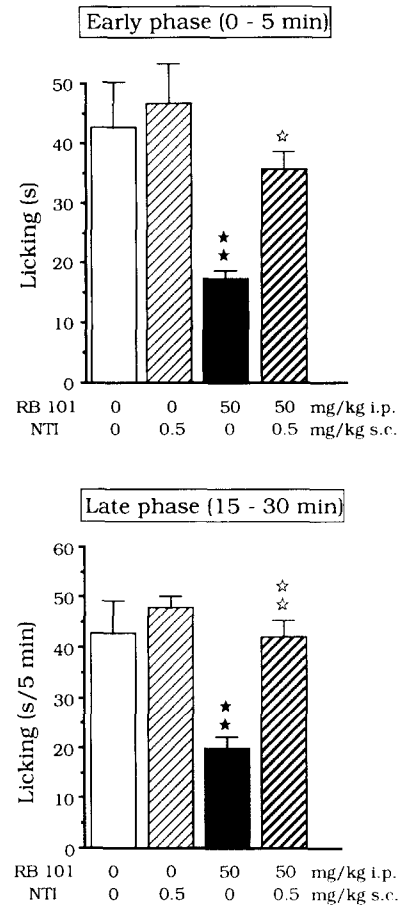


Fig. 3. Effects of naltrindole (NTI) (0.5 mg/kg s.c.) on the antinociceptive responses observed after i.p. administration of RB 101 (50 mg/kg) in the formalin test in mice. Opioid antagonist was administered 5 min before i.p. injection of vehicle or RB 101 and 20 min before the test. Nociceptive behavior in both early (0–5 min) and late phases (15–30 min) after 5% formalin s.c. injection into the dorsal surface of the hindpaw was measured as the amount of time spent licking or biting the injected paw. The results are expressed as means ± S.E.M. ($n = 8$–10 for each group). $^{**}P < 0.01$ as compared to control, $^{\star}P < 0.05$, $^{\star\star}P < 0.01$ as compared to the same dose of RB 101 without antagonist (Newman-Keuls test).

2.4. Systemic administration

RB 101, morphine and control vehicle were administered i.p. in a volume of 0.1 ml/10 g in mice, 15 min before the injection of formalin. The antagonists naltrindole and naloxone were injected s.c., and PD-134,308 was administered i.p., in a volume of 0.1 ml/10 g, 20 min and 30 min before the injection of formalin, respectively.

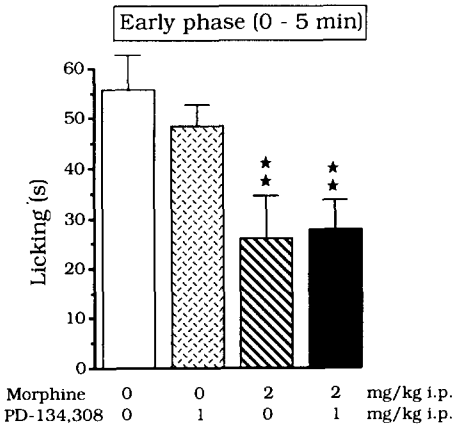
2.5. Analysis of data

Statistical analysis was carried out by a one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test or Newman-Keuls test for multiple comparisons. The level of significance was set at $P < 0.05$.

3. Results

3.1. Effects of morphine and RB 101 on the nociceptive responses induced by 5% formalin

In mice pretreated with saline or vehicle (10% EtOH–10% cremophor–80% distilled water), the response to s.c. injection of 5% formalin followed a biphasic pattern, an early phase (0–5 min after formalin administration) and a late phase (15–30 min after formalin injection). Morphine at 2 mg/kg, injected i.p. 15 min prior to the s.c. injection of 5% formalin into the dorsal surface of the hindpaw, induced a significant antinociceptive effect in the early phase (0–5 min after formalin injection ($F(1,19) = 9.640$, $P < 0.01$)) and the late phase (15–30 min after formalin injection ($F(1,19) = 5.678$, $P < 0.01$)) (Fig. 1).



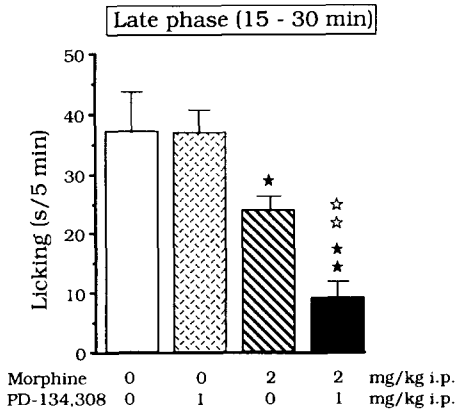
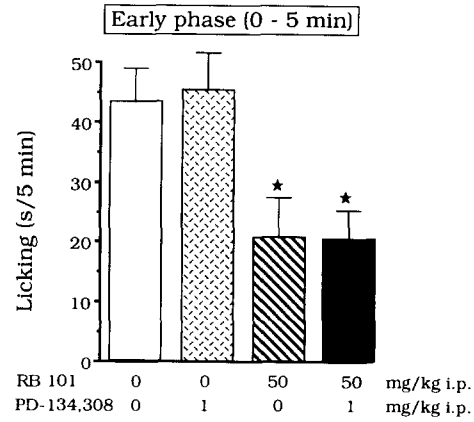


Fig. 4. Effects in the formalin test of morphine (2 mg/kg i.p., 15 min before the test), PD-134,308 (1 mg/kg i.p., 30 min before testing), and a combination of the two drugs. Nociceptive behavior in both early (0–5 min) and late phases (15–30 min) after 5% formalin s.c. injection into the dorsal surface of the hindpaw was measured as the amount of time spent licking or biting the injected paw. The results are expressed as means ± S.E.M. ($n = 9$–11 for each group). $^{*}P < 0.05$, $^{**}P < 0.01$ as compared to control group, $^{\star\star}P < 0.01$ as compared to morphine-treated group (Newman-Keuls test).



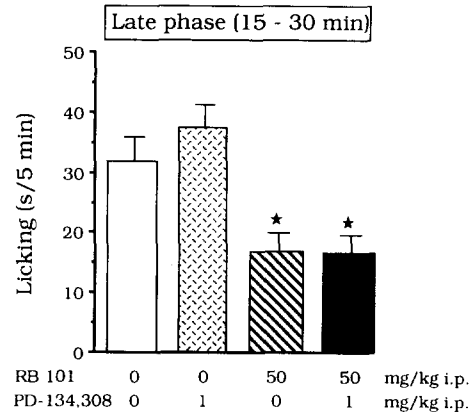


Fig. 5. Effects in the formalin test of RB 101 (50 mg/kg i.p., 15 min before the test), PD-134,308 (1 mg/kg i.p., 30 min before testing), and a combination of the two drugs. Nociceptive behavior in both early (0–5 min) and late phases (15–30 min) after 5% formalin s.c. injection into the dorsal surface of the hindpaw was measured as the amount of time spent licking or biting the injected paw. The results are expressed as means ± S.E.M. ($n = 9$–11 for each group). $^{*}P < 0.05$ as compared to control group (Newman-Keuls test).

As shown in Fig. 1, antinociceptive effects were also observed after i.p. administration of RB 101 at 50 mg/kg in the early phase (0–5 min after formalin injection ($F(1,12) = 10.75$, $P < 0.01$)) and the late phase (15–30 min after formalin injection ($F(1,12) = 5.401$, $P < 0.01$)).

3.2. Effects of naloxone and naltrindole on the antinociceptive responses observed after i.p. administration of morphine or RB 101 in the 5% formalin test

As shown in Fig. 2, the antinociceptive effects of morphine (2 mg/kg i.p., 15 min before formalin injection) observed in both the early (0–5 min after formalin administration) and late (15–30 min after formalin injection) phases were completely antagonized by naloxone (0.5 mg/kg s.c.) administered 20 min before formalin. In contrast, the highly selective δ-opioid receptor antagonist naltrindole (0.5 mg/kg s.c., 20 min

before formalin injection) did not modify the antinociceptive responses induced by morphine (Fig. 2).

As shown in Fig. 3, naltrindole completely antagonized the antinociceptive responses observed after i.p. administration of RB 101 (50 mg/kg) in both early and late phases.

3.3. Effects of pretreatment with PD-134,308 (i.p.) on the antinociceptive responses observed after administration of morphine or RB 101 in the 5% formalin test

As shown in Fig. 4, PD-134,308 (1 mg/kg i.p., a dose at which it did not induce significant effects on either the early or late phases) administered 25 min before formalin, enhanced the antinociceptive effect of morphine only in the late phase. The antinociceptive responses observed after administration of morphine in the early phase were not potentiated by the selective CCK_B receptor antagonist.

In contrast, no significant effects of PD-134,308 on the antinociceptive responses induced by administration of RB 101 were observed in either of these phases (Fig. 5).

4. Discussion

This study shows that intraperitoneal administration of RB 101 or morphine results in antinociceptive responses in both early and late phases of the formalin test. This result extends the efficiency of RB 101 in alleviating acute thermal and electrical (Noble et al., 1992; review in Roques et al., 1993) nociceptive stimuli to those induced by chemical reagents. In addition this study confirms that complete protection of endogenous enkephalins from degradation in vitro and in vivo (Waksman et al., 1985; Bourgoin et al., 1986; Ruiz-Gayo et al., 1992) produces analgesic responses in almost all morphine-sensitive noxious tests, including those resulting from inflammation (Maldonado et al., 1994). An about 25 times higher concentration of RB 101 than of morphine is required to give the same antinociceptive response in both phases of the test.

The mechanisms involved in the action of morphine and RB 101 appear to be different. Thus, morphine-induced analgesia is completely blocked by prior administration of the rather μ-specific opioid receptor antagonist naloxone, but not by the selective δ-opioid receptor antagonist naltrindole (0.5 mg/kg s.c.) in both phases of the test. This shows that the antinociceptive responses elicited by the alkaloid are mainly, if not exclusively, due to μ-opioid receptor stimulation. In contrast, the effects of RB 101 are blocked by prior administration of naltrindole, suggesting a critical role of δ-opioid receptors in enkephalin-induced antinociceptive effects in both early and late phases of the formalin test. This latter result is in good agreement with Sullivan et al. (1989), who showed that endogenous enkephalins protected by the mixed enzyme inhibitor kelatorphan (Fournié-Zaluski et al., 1984) strongly inhibit responses of convergent neurons to nociceptive afferent inputs induced by s.c. formalin through δ-opioid receptor activation.

Administration of the selective CCK_B receptor antagonist PD-134,308 (Hughes et al., 1990) resulted in potentiation of morphine antinociception in the late phase but not in the early phase of the formalin test. In contrast, the antinociceptive responses observed in both phases of the assay following administration of RB 101 were not modified by prior administration of PD-134,308. These results suggest that the facilitation of opioid analgesia observed in the late phase of the formalin test, resulting from blockade of the CCK_B receptors tonically recruited by endogenous CCK, is restricted to μ-opioid receptor-mediated antinociceptive responses. Accordingly, in electrophysiological studies, Magnuson et al. (1990) have shown that CCK-8 selectively prevents the inhibition of C-fibre- evoked activity induced by intrathecal administration of the μ-opioid receptor agonist DAMGO (Tyr-D-Ala-Gly-(NMe)Phe-Gly-ol), but not that resulting from injection of the δ-opioid receptor agonist DSTBULET (Tyr-D-Ser(*O*-tertiobutyl)-Gly-Phe-Leu-Thr). Moreover, Wang and Han (1990) have found that administration of CCK-8 reduces binding to μ- but not to δ-opioid receptors. This is in agreement with the results of behavioral experiments showing that analgesia produced by injection of μ-opioid receptor agonists, but not by δ-opioid receptor agonists, is markedly antagonized by injection of CCK-8 (Wang et al., 1990).

As the early phase of the formalin test could correspond to a direct stimulation by the irritating chemical of the peripheral nociceptive endings (review in Dickenson, 1991), the lack of facilitation by PD-134,308 of the μ-opioid antinociceptive responses induced by morphine at this period of the assay could result from (i) an absence or a too low concentration of CCK_B receptors at the nociceptor level, (ii) an insufficient tonic release of endogenous CCK, assuming that CCK binding sites could be present at this peripheral level. In contrast, the potentiation by PD-134,308 of the morphine effect in the late phase suggests that CCK-8, probably tonically released at the CNS level during the formalin-induced inflammatory processes, counteracts the analgesic effect of exogenously administered opiate.

In contrast to results observed in the hot plate test with mice and the tail flick test with rats (Maldonado et al., 1993), the CCK_B receptor antagonist is unable to potentiate the antinociceptive effect produced by RB 101 in the formalin assay. This is despite the fact that enkephalin-degrading enzymes, μ- and δ-opioid

receptors, as well as CCK_B receptors are present throughout the CNS of rodents (Waksman et al., 1985; Hill and Woodruff, 1990) where strong tonic release and phasic release of endogenous enkephalins have been observed (Bourgoin et al., 1986; Ruiz-Gayo et al., 1992). This suggests, in agreement with pharmacological and biochemical experiments (Magnuson et al., 1990; Wang et al., 1990), that in the conditions of the test, the binding of enkephalins to δ-opioid receptors and subsequent transduction processes in CNS areas involved in the observed antinociceptive responses are not influenced by activation of CCK_B receptors. This seems not to be the case in other tests. Thus, naltrindole-reversible antidepressant-like effects induced by RB 101 in the conditioned immobility suppression test in mice are significantly potentiated by CCK_B receptor antagonists which have been also shown to be active alone (Derrien et al., 1994).

In conclusion, the results of this study show that the facilitating effects of opioid-induced analgesia by CCK_B receptor antagonists are restricted to antinociceptive responses induced by exogenous opiates acting on μ sites whose localization (spinal and/or supraspinal) is the subject of further studies in the laboratory. Indeed, it is well established that, in humans and monkeys, CCK receptors present in the spinal cord are mainly of A-type, while in rodents they are of the B-type (Hill and Woodruff, 1990; Ghilardi et al., 1992). Thus, if the spinal cord receptors are involved in these facilitating effects it would be interesting to verify if CCK_A receptor antagonists are able to facilitate morphine analgesia in primates, in the same manner as CCK_B receptor antagonists in rodents.

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