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Similar involvement of several brain areas in the antinociception of endogenous and exogenous opioids

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Abstract

The complete inhibitor of the enkephalin degrading enzymes, RB 101, *N*-{(*R*,*S*)-2-benzyl-3[(*S*)-(2-amino-4-methylthio)butyldithio]-1-oxopropyl}-L-phenylalanine benzyl ester, which crosses the blood-brain barrier, induced antinociceptive effects similar to those of exogenous opiates. The almost complete absence of tolerance and dependence after chronic administration of RB 101 is therefore due to limited stimulation of opioid receptors by 'protected' endogenous enkephalins. In order to clarify the mechanisms involved in these response, we have investigated the participation of several brain structures in the antinociceptive effects induced by systemic administration of morphine or RB 101. Rats were implanted with bilateral cannulae into the ventro-basal thalamus, central amygdala and periaqueductal gray matter, or with a cannula into the raphe magnus nucleus. The antinociceptive responses induced by systemic morphine or RB 101 were measured by using the tail-electrical stimulation test, where three different thresholds were determined: motor response, vocalization and vocalization post-discharge. The ability of the opioid receptor antagonist methylnaloxonium to block these antinociceptive responses was evaluated after local injection into the different brain structures. The blockade of morphine- and RB 101-induced antinociception was similar, and was stronger when methylnaloxonium was injected into the periaqueductal gray matter and raphe magnus nucleus than when it was injected into the ventro-basal thalamus and amygdala. These results suggest that brain structures related to the control of pain seem to be the same for the antinociception induced by exogenous opiates and endogenous opioids.

Keywords: Enkephalin; Methylnaloxonium; RB 101; Antinociception; Amygdala; Thalamus, ventro-basal; Central gray matter; Raphe magnum nucleus

1. Introduction

The different neural pathways and structures implicated in the control of pain and in the mediation of opiate analgesia are well known (Basbaum and Fields, 1984; Besson and Chaouch, 1987; Lipp, 1991; Fabian and Ableitner, 1995). These include a group of fibers that are involved in the ascendent transmission from the dorsal horn of the spinal cord to the thalamus. Nociceptive fibers project from the thalamus to several telencephalic areas, including the limbic system (Basbaum and Fields, 1984; Lipp, 1991). The limbic system, and particularly the amygdaloid complex, has been reported to be involved in the emotional-affective interpretation of and autonomic reactions to noxious events (LeDoux, 1987; Bernard et al., 1992; Manning and Mayer, 1995). Another group of structures integrates the descending inhibitory neuronal pathway which selectively inhibits dorsal horn nociceptive neurons (Willis, 1984; Delander and Wahl, 1989). This descending system includes the midbrain periaqueductal gray and several nuclei of the rostral ventral medulla, such as the midline nucleus raphe magnus. Both the periaqueductal gray matter and raphe magnum nucleus are densely and reciprocally interconnected with the central nucleus of the amygdala (Beitz, 1982; Reichling and Basbaum, 1991). The periaqueductal gray matter contains high levels of endogenous opioids and receptors (Waksman et al., 1986; Mansour et al., 1987) and plays a critical role in the control of pain. The raphe magnum nucleus represents the major source of axons projecting via the dorso-lateral funiculus to the spinal cord (Basbaum and Fields, 1984).

RB 101, *N*-{(*R*,*S*)-2-benzyl-3[(*S*)-(2-amino-4-methylthio)butyldithio]-1-oxopropyl}-L-phenylalanine benzyl ester, a mixed inhibitor of the enkephalin-degrading enzymes which is able to cross the blood-brain barrier, has been synthesized (Fournié-Zaluski et al., 1992). This compound

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inhibits both neutral endopeptidase and aminopeptidase N, the two enzymes involved in the inactivation of the endogenous enkephalins, and produces naloxone-reversible antinociception after systemic administration in the same nociceptive tests where morphine has been found to be active (Noble et al., 1992a). However, chronic treatment with RB 101, as well as other peptidase inhibitors, induces different effects to those of morphine on the development of tolerance and dependence. Thus, no tolerance to the antinociceptive response or cross-tolerance with morphine was induced in the case of chronic administration of RB 101 (Noble et al., 1992b). In addition, chronic central perfusion with RB 38 A, another mixed inhibitor of enkephalin catabolism, only produced moderate physical dependence, much less severe than that induced by exogenous opioids (Maldonado et al., 1990), and no major signs of withdrawal were observed in animals chronically treated with RB 101 (Noble et al., 1994).

Taking into account the different responses obtained after acute and chronic administration of peptidase inhibitors and exogenous opiates, we investigated the brain mechanisms involved in the antinociception induced in the tail-electrical stimulation test in rats by activation of the endogenous opioid system with the RB 101. For this purpose, the ability of the hydrophilic opioid receptor antagonist methylnaloxonium to antagonize the antinoci-

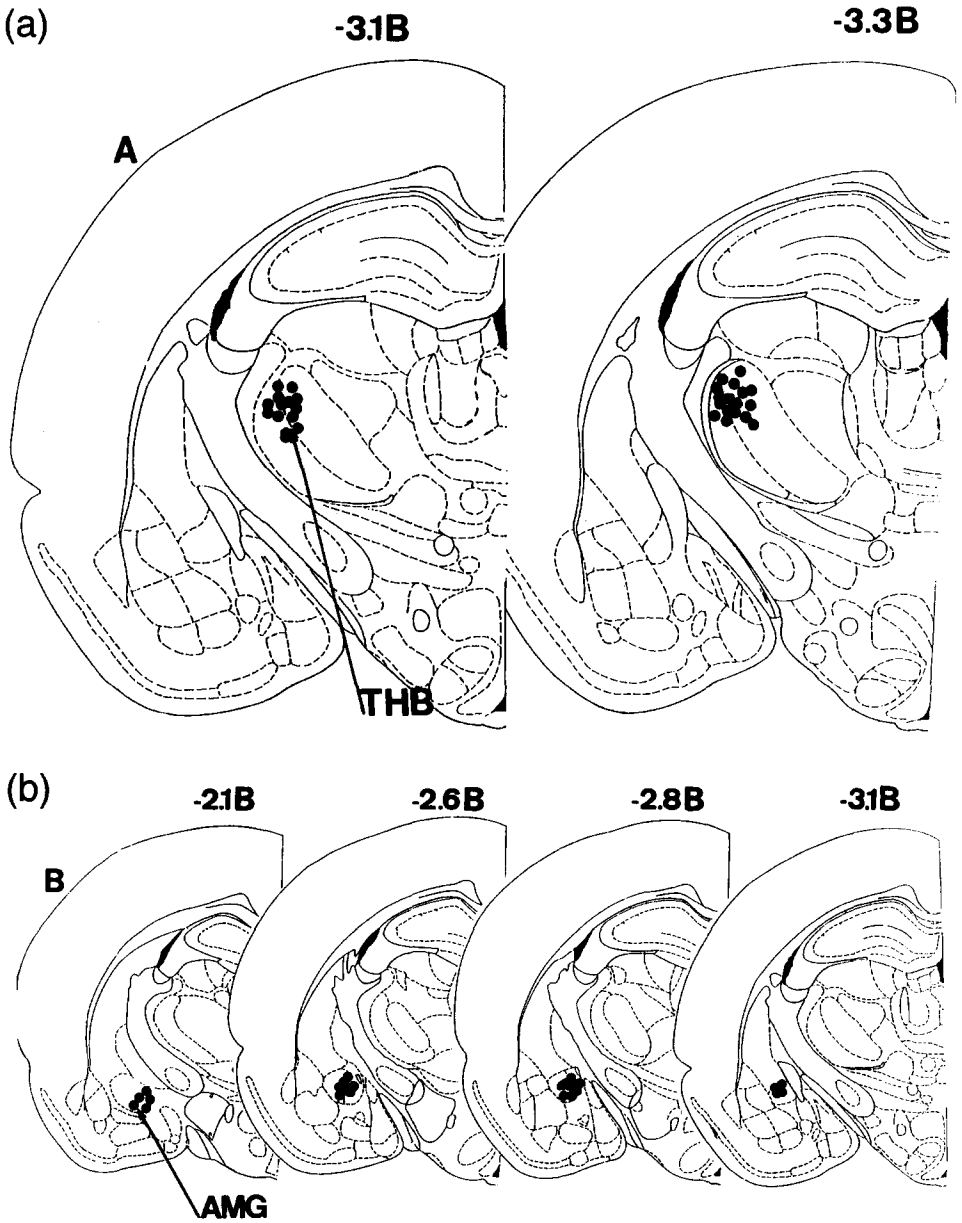

Fig. 1. Representative coronal sections based on the atlas of Paxinos and Watson (1986). Black circles correspond to the final site of injection into: (A) complex ventro-basal of thalamus (THB), Bregma: −3.1; −3.3; (B) central amygdaloid nucleus (AMG), Bregma: −2.1; −2.6; −2.8; −3.1; (C) periaqueductal gray matter (PAG), Bregma: −7.3; −7.8; −8.3; −8.8; (D) raphe magnum nucleus (RMG), Interaural: −0.7; −0.8; −1.1.

ceptive effects induced by systemic administration of morphine or RB 101 was evaluated after its local administration into the most relevant structures linked to the control of nociceptive responses. This includes the ventro-basal complex of thalamus, and the amygdala, which is involved in the ascendent pathways and emotional integration, and the periaqueductal gray matter and the raphe magnus nucleus, which belong to the ascending pathways.

2. Materials and methods

2.1. Animals and surgery

Male Sprague-Dawley rats (Depré) weighing 200–220 g were housed in groups of 5 with food and water available ad libitum. The animals were anesthetized with chloral hydrate (400 mg/kg, i.p.), mounted in a stereotaxic apparatus (Unimécanique, France) and unilateral (raphe magnum nucleus) or bilateral (complex ventro-basal of thalamus, central amygdaloid nucleus and periaqueductal gray matter) stainless-steel cannula guides were implanted 3 mm above the final site of injection. The cannulae were secured to the skull with stainless-steel screws and dental cement. The cannula guides were kept clear with wire stylets. The coordinates, according to Paxinos and Watson (1986), were: complex ventro-basal of thalamus = A −3.2 from bregma, L ± 3.2, V −6.1 from skull; central amygdaloid nucleus = A −2.5 from bregma, L ± 4.2, V −8.1 from skull; periaqueductal gray matter = A −8.1 from bregma, L ± 0.5, V −5.7 from skull; raphe magnum nucleus = A −0.7 from interaural line, L 0, V −10.2

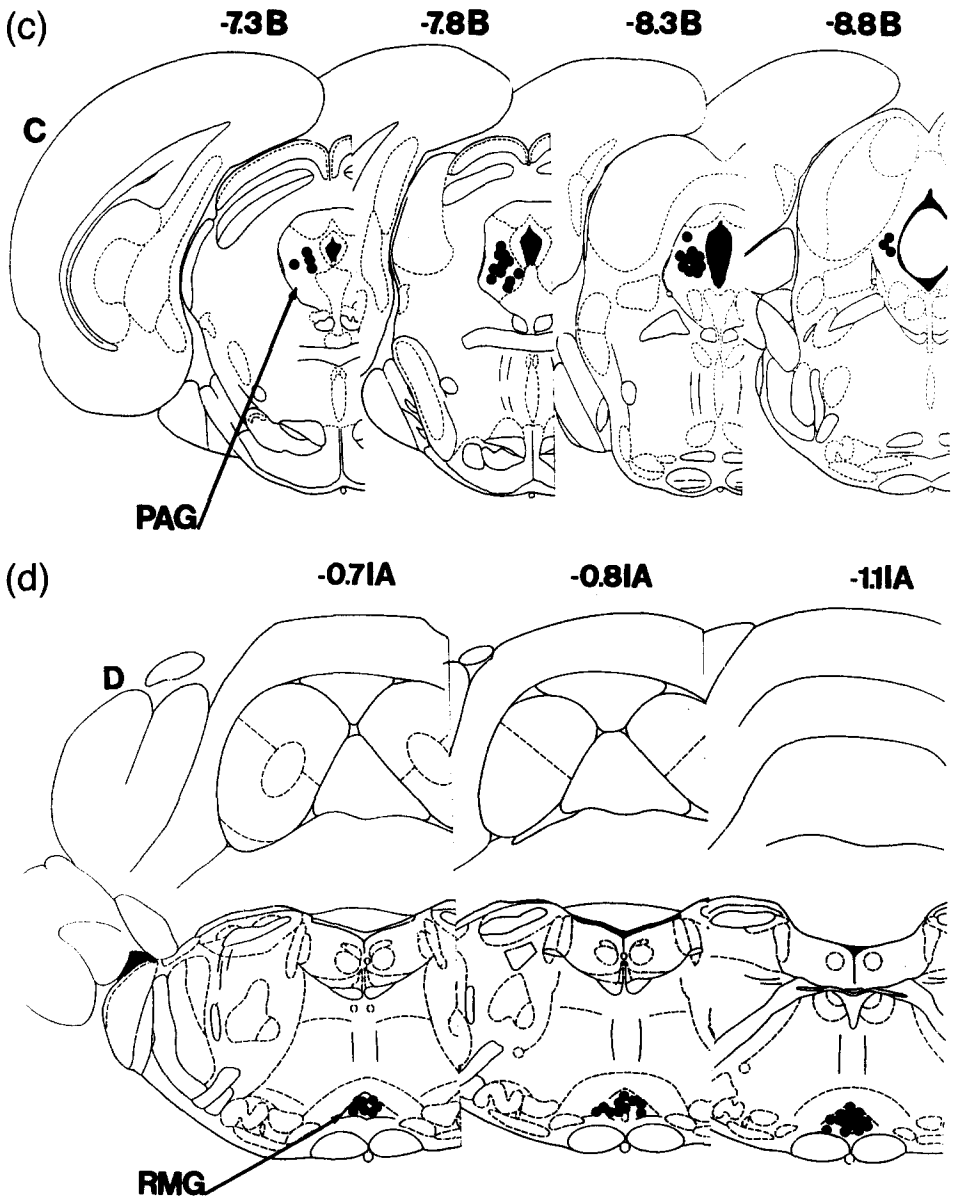

Fig. 1 (continued).

from skull. Rats were tested 6–8 days after surgery. Each animal was tested only once, and the observer was 'blind' to the treatment. Surgery, antinociceptive tests and care of the animals were in accordance with ethical guidelines recommended by Zimmermann (1983).

2.2. Microinjection into structures

Unilateral (raphe magnum nucleus) or bilateral (complex ventro-basal of thalamus, central amygdaloid nucleus and periaqueductal gray matter) injections into the different brain structures were given with an injection apparatus consisting of 30.5 gauge stainless-steel needles, attached to a 10 μl microsyringe (Hamilton) by polyethylene tubing. Methylnaloxonium (0.3 or 1 μg) and control solution (saline) were administered by an infusion pump in a constant volume of 1 μl (0.5 μl per side), at rate of 0.0083 μl/s, followed by a 1-min diffusion period. In the case of raphe magnum nucleus, the volume was 0.5 μl and the rate 0.00415 μl/s. Before each injection, the cannula guides were cleared of debris with a dental needle cut to the exact length of the guide.

2.3. Drugs

RB 101, *N*-{(*R*,*S*)-2-benzyl-3[(*S*)(2-amino-4-methylthio)butyl dithio]-1-oxopropyl}-L-phenylalanine benzyl ester (Fournié-Zaluski et al., 1992) and methylnaloxonium were synthesized in our laboratory. Cremophor EL was purchased from Sigma Chemical (France), and morphine hydrochloride from Francopia (France). RB 101 was dissolved in the following vehicle: ethanol (10%), cremophor EL (10%) and distilled water (80%). Methylnaloxonium and morphine were dissolved in saline (0.9% NaCl). Drugs were injected systemically by i.v. route through a tail-vein and were administered in a volume of 0.1 ml per 100 g of body weight.

2.4. Tail-electrical stimulation test

The rats were placed in a horizontal aerated plexiglas cylinder. Two electrodes were implanted s.c. at the base of the tail and connected to a stimulator (IZ Hugo Sach Elektronik, March-Hugstetten, Germany) which delivered the current (10 ms rectangular pulse, 60 cycles/s) for 2 s. The intensity of the stimulation was increased by 0.5 V until a response was observed. The duration of the intertrial period was 30 s. As described by Carroll and Lim (1960), thresholds for three reactions were measured: (1) regional reflexes (tail withdrawal and hind limb movements); (2) vocalization during the electrical stimulation; (3) vocalization occurring briefly after the nociceptive stimulus had ceased (post-discharge vocalization).

2.5. Pharmacological treatment

Dose-response curves of morphine (1, 3 and 9 mg/kg, i.v.) and RB 101 (10, 20 and 40 mg/kg, i.v.) were first established by using the tail-electrical stimulation test. The dose of each drug showing a significant antinociceptive effect on the three thresholds measured was then chosen to associate with doses of methylnaloxonium, which did not induce any pharmacological effects, administered locally into the different brain structures. Thus, morphine (9 mg/kg), RB 101 (20 mg/kg) or vehicle was acutely administered, i.v., to different groups of rats 10 min before the test. Methylnaloxonium (0.3 and 1 μg) or vehicle was centrally administered 15 min before the test.

2.6. Histology

Rats were killed with an overdose of chloral hydrate. The brains were removed, frozen and cut in a cryostat. The slices (50 μm) were stained with cresyl violet and the location of the injection site was determined according to the atlas of Paxinos and Watson (1986) (Fig. 1). The data from rats that were not correctly implanted were eliminated from the data calculations. This selection was performed without knowledge of the individual behavioral response of each animal.

2.7. Analysis of data

Individual group comparisons were made using a one-way analysis of variance (ANOVA). Post-hoc individual treatment effects were analyzed using the Scheffé *F*-test. The level of significance was $P < 0.05$.

3. Results

3.1. Dose-dependent antinociceptive responses induced by systemic administration of morphine and RB 101

Systemic morphine administration (1, 3, 9 mg/kg, i.v.) induced a dose-dependent increase in the three nociceptive thresholds measured. One-way ANOVA revealed a significant dose-effect relation for the motor response ($F(3,22) = 4.394$, $P < 0.05$), the vocalization during stimulation ($F(3,22) = 12.84$, $P < 0.001$), and the vocalization post-discharge ($F(3,22) = 30.407$, $P < 0.001$). Post-hoc comparisons showed significant responses for the three types of reactions only at the dose of 9 mg/kg (motor response, vocalization, and vocalization post-discharge), whereas 3 mg/kg was effective in modifying the vocalization and the vocalization post-discharge, but not the motor response. No significant effect was observed in any threshold when morphine was administered at the dose of 1 mg/kg (Fig. 2).

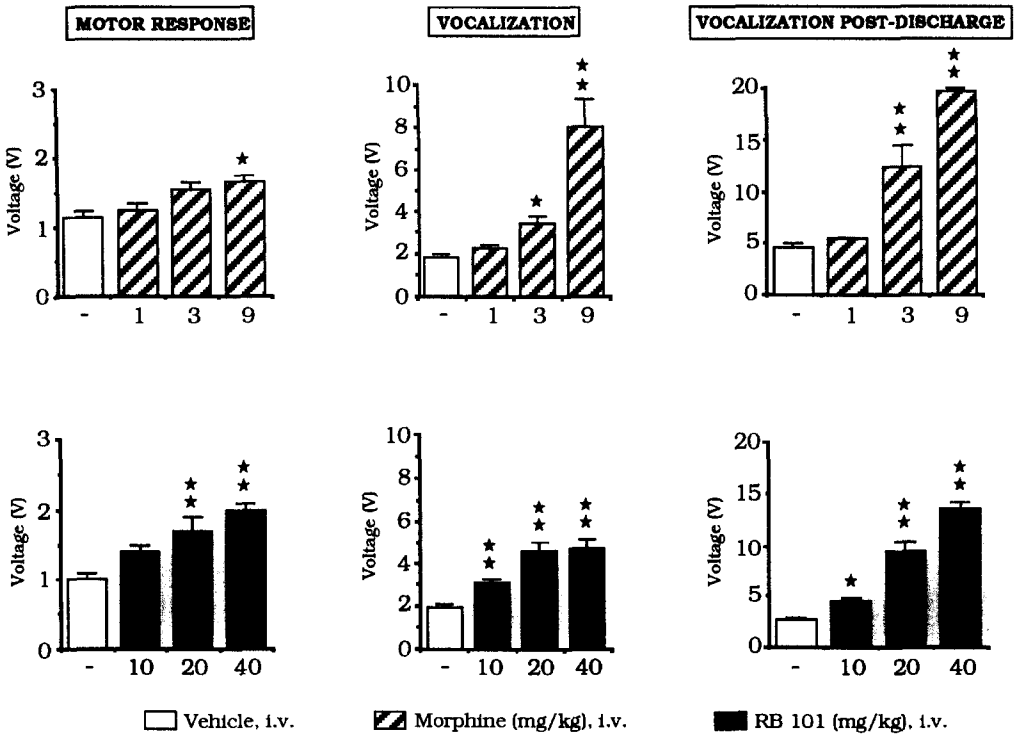

Fig. 2. Effects of morphine and RB 101 on the three responses elicited by tail-electric stimulation test. Morphine (stripped columns) and RB 101 (black colums) were administered (i.v.) at the doses indicated, 10 min before the test. The results are expressed as means ± S.E.M. (volts). * $P < 0.05$, ** $P < 0.01$ vs. saline (Scheffé F-test).

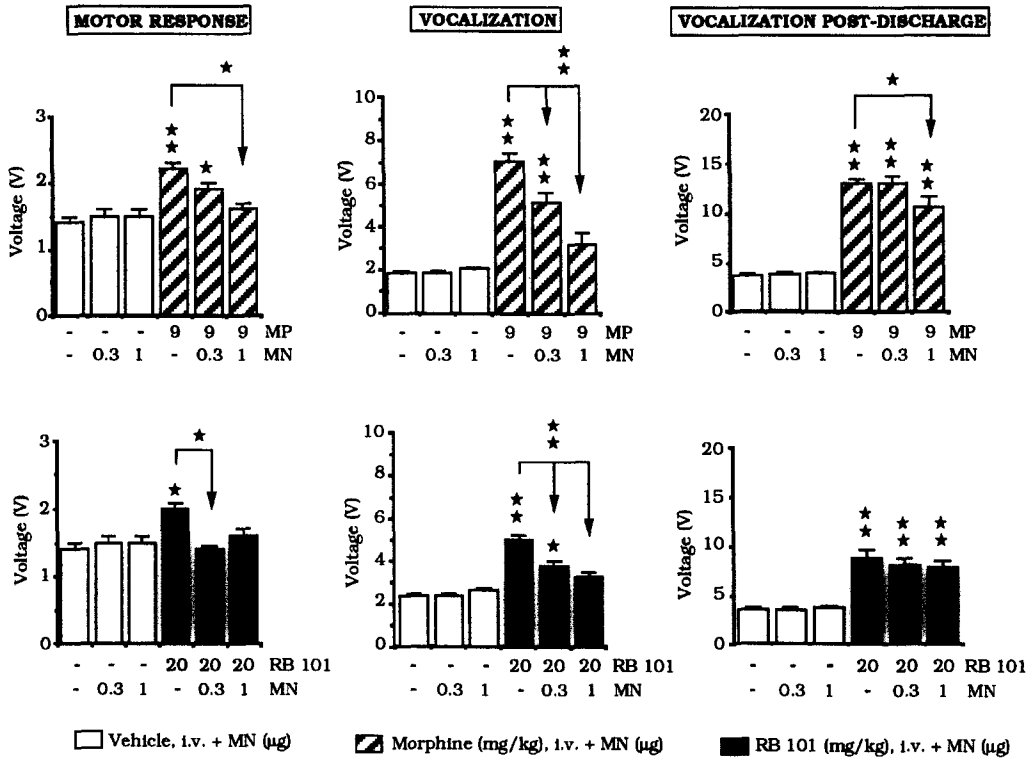

Fig. 3. Effects of methylnaloxonium injection into the ventro-basal thalamus on antinociceptive responses induced in the tail-electric stimulation test by, systemically administered (i.v.), morphine or RB 101. Methylnaloxonium (MN) was injected 15 min before the test. Morphine (MP) (stripped columns) and RB 101 (black columns) were injected 10 min before testing. The results are expressed as means ± S.E.M. (volts). * $P < 0.05$, ** $P < 0.01$ vs. saline when placed on the columns; vs. morphine when placed on the arrows (Scheffé F-test).

Systemic administration of RB 101 (10, 20 and 40 mg/kg, i.v.) resulted in a dose-dependent antinociceptive effects on the three thresholds measured. One-way ANOVA showed a significant treatment effect with RB 101 for the motor response ($F(3,28) = 9.333$, $P < 0.001$), the vocalization during the stimulation ($F(3,28) = 17.578$, $P < 0.001$), and the vocalization post-discharge ($F(3,28) = 48.582$, $P < 0.001$). Post-hoc comparisons revealed that the lowest dose of RB 101 used (10 mg/kg) has a significant effect on vocalization and vocalization post-discharge, whereas the doses of 20 and 40 mg/kg had a significant effect on the three types of reaction observed (Fig. 2).

3.2. Effects of methylnaloxonium injected into the complex ventro-basal of thalamus on antinociception induced by systemic administration of morphine or RB 101

3.2.1. Systemic administration of morphine

One-way ANOVA indicated a significant treatment effect on the three threshold values: motor response ($F(5,42) = 4.641$, $P < 0.01$), vocalization during the stimulation ($F(5,42) = 26.705$, $P < 0.0001$), and vocalization post-discharge ($F(5,42) = 52.099$, $P < 0.0001$). Post-hoc comparisons revealed a significant antinociceptive effect of morphine (9 mg/kg) on the three thresholds. The antinociceptive response induced by morphine on vocalization during discharge was significantly antagonized after the administration of methylnaloxonium at the dose of 0.3 μg, and completely blocked with 1 μg of methylnaloxonium. The morphine effect on vocalization post-discharge was unaffected by methylnaloxonium at the dose of 0.3 μg and only slightly reduced by the dose of 1 μg of methylnaloxonium (Fig. 3).

3.2.2. Systemic administration of RB 101

One-way ANOVA showed a significant treatment effect on the threshold values of motor response ($F(5,42) = 2.792$, $P < 0.05$), vocalization ($F(5,42) = 15.631$, $P < 0.0001$), and vocalization post-discharge ($F(5,42) = 17.226$, $P < 0.0001$). Post-hoc comparisons indicated a significant antinociceptive effect of RB 101 (20 mg/kg) on the three nociceptive responses. The RB 101-increased motor response was reversed by methylnaloxonium at the doses of 0.3 and 1 μg. The effect induced by RB 101 on vocalization during discharge was reversed by methylnaloxonium at the dose of 0.3 μg and abolished by the highest dose of the opiate antagonist (1 μg). The antinociceptive effect of RB 101 on vocalization post-discharge was not modified after the administration of methylnaloxonium (0.3 and 1 μg) (Fig. 3).

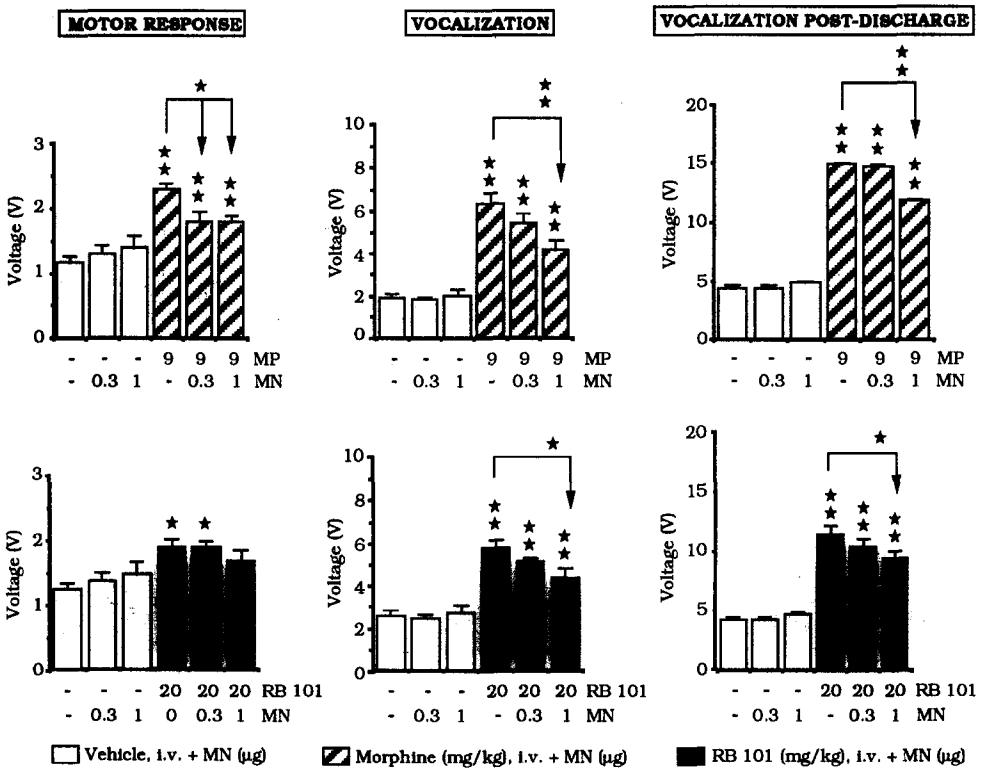

Fig. 4. Effects of methylnaloxonium injection into the central amygdala on antinociceptive responses induced in the tail-electric stimulation test by, systemically administered (i.v.), morphine or RB 101. Methylnaloxonium (MN) was administered 15 min before the test. Morphine (MP) and RB 101 were injected 10 min before testing. The results are expressed as means ± S.E.M. (volts). * $P < 0.05$, ** $P < 0.01$ vs. saline when placed on the columns; vs. morphine when placed on the arrows (Scheffé F-test).

3.3. Effects of methylnaloxonium injected into the central amygdaloid nucleus on antinociception induced by systemic administration of morphine or RB 101

3.3.1. Systemic administration of morphine

One-way ANOVA revealed a significant treatment effect on the threshold values of motor response ($F(5,42) = 10.005$, $P < 0.001$), vocalization ($F(5,42) = 28.969$, $P < 0.001$), and vocalization post-discharge ($F(5,42) = 71.540$, $P < 0.001$). Post-hoc comparisons showed a significant antinociceptive effect of morphine (9 mg/kg) on the three thresholds. The antinociceptive effect of morphine on the motor response was reversed after the local injection of methylnaloxonium (0.3 and 1 μg). Methylnaloxonium administered at the dose of 1 μg significantly reversed the effect induced by morphine on the vocalization during discharge, but this blockade was not significant when methylnaloxonium was injected at the dose of 0.3 μg. The morphine-increased vocalization post-discharge threshold was defreased by methylnaloxonium at the dose of 1 μg but was unaffected with the dose of 0.3 μg of methylnaloxonium (Fig. 4).

3.3.2. Systemic administration of RB 101

One-way ANOVA indicated a significant treatment effect on motor response ($F(5,42) = 3.653$, $P < 0.01$), vocalization ($F(5,42) = 18.654$, $P < 0.0001$), and vocalization post-discharge ($F(5,42) = 33.758$, $P < 0.0001$). Post-hoc comparisons revealed a significant antinociceptive effect of RB 101 (20 mg/kg) on the three thresholds. The RB 101-increased motor response was decreased by the highest dose of methylnaloxonium (1 μg) but was not modified when the opiate antagonist was injected at the dose of 0.3 μg. The effects induced by RB 101 on vocalization and vocalization post-discharge were reduced by the administration of methylnaloxonium at the dose of 1 μg, but significant antinociception was still present (Fig. 4).

3.4. Effects of methylnaloxonium injected into the periaqueductal gray matter on antinociception induced by systemic administration of morphine or RB 101

3.4.1. Systemic administration of morphine

One-way ANOVA revealed a significant treatment effect on motor response ($F(5,42) = 10.369$, $P < 0.0001$), vocalization ($F(5,42) = 19.265$, $P < 0.0001$), and vocalization post-discharge ($F(5,42) = 37.551$, $P < 0.0001$). Post-hoc comparisons showed a significant antinociceptive effect of morphine (9 mg/kg) on the three thresholds measured. The morphine-induced increase in the motor response was completely blocked after methylnaloxonium injection (0.3 and 1 μg). The effects of morphine on vocalization and vocalization post-discharge were antago-

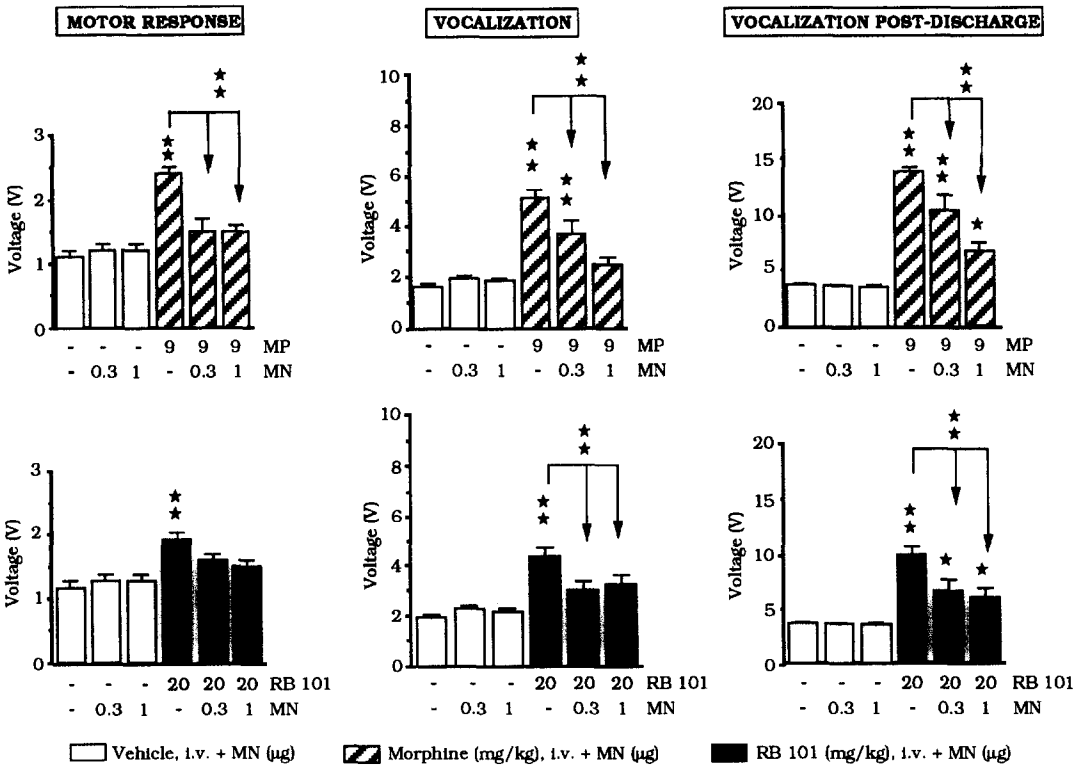

Fig. 5. Effects of methylnaloxonium injection into the periqueductal gray matter on antinociceptive responses induced in the tail-electric stimulation test by, systemically administered (i.v.), morphine or RB 101. Methylnaloxonium (MN) was injected 15 min before the test. Morphine (MP) and RB 101 were administered 10 min before testing. The results are expressed as means ± S.E.M. (volts). * $P < 0.05$, ** $P < 0.01$ vs. saline when placed on the columns; vs. morphine when placed on the arrows (Scheffé F-test).

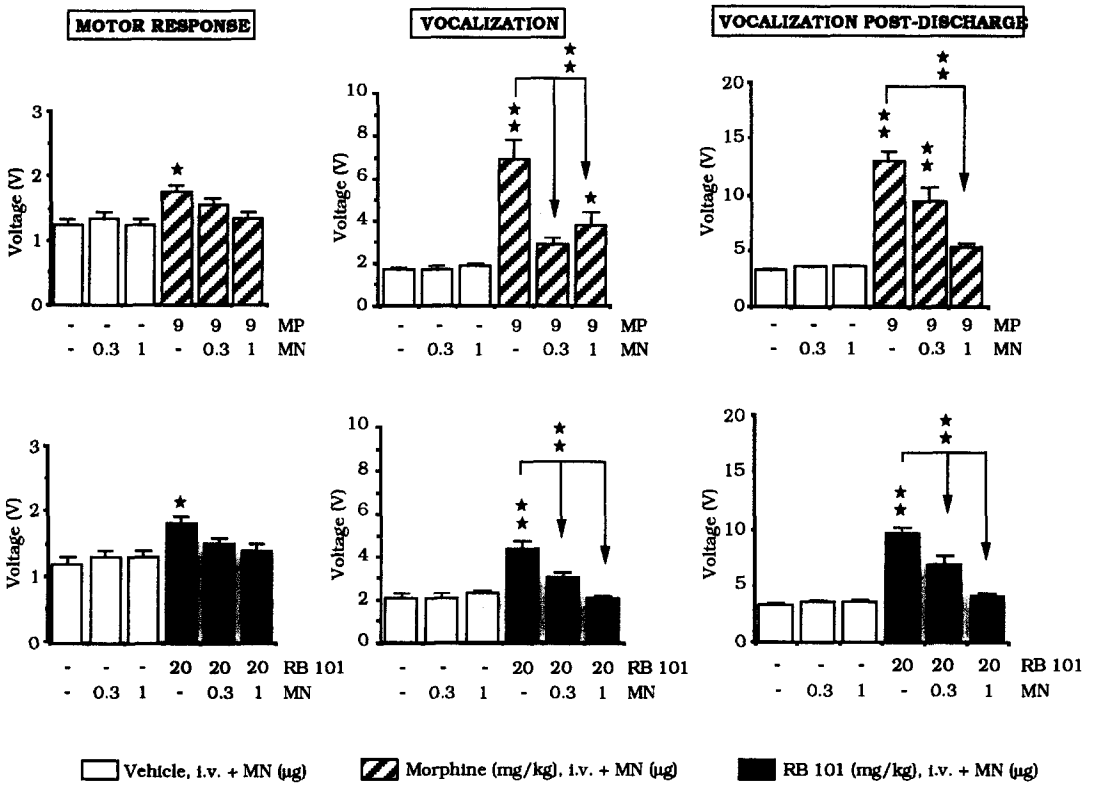

Fig. 6. Effects of methylnaloxonium injection into the raphe magnus nucleus on antinociceptive responses induced in the tail-electric stimulation test by, systemically administered (i.v.), morphine or RB 101. Methylnaloxonium (MN) was injected 15 min before the test. Morphine (MP) and RB 101 were injected 10 min before testing. The results are expressed as means ± S.E.M. (volts). * $P < 0.05$, ** $P < 0.01$ vs. saline when placed on the columns; vs. morphine when placed on the arrows (Scheffé F-test).

nized by the doses of 0.3 and 1 μg of methylnaloxonium (Fig. 5).

3.4.2. Systemic administration of RB 101

One-way ANOVA indicated a significant treatment effect on motor response ($F(5,40) = 4.056$, $P < 0.01$), vocalization ($F(5,40) = 7.780$, $P < 0.0001$), and vocalization post-discharge ($F(5,40) = 15.013$, $P < 0.0001$). Post-hoc comparisons indicated a significant antinociceptive effect of RB 101 (20 mg/kg) on the three nociceptive thresholds. The antinociceptive effects induced by RB 101 on motor response and vocalization were completely antagonized by methylnaloxonium administration (0.3 and 1 μg). A significant antagonism was also induced by methylnaloxonium (0.3 and 1 μg) on RB 101-increased vocalization post-discharge threshold, but residual antinociception was still present (Fig. 5).

3.5. Effects of methylnaloxonium injected into the raphe magnum nucleus on antinociception induced by systemic administration of morphine or RB 101

3.5.1. Systemic administration of morphine

One-way ANOVA revealed a significant treatment effect on the threshold values of motor response ($F(5,41) = 3.077$, $P < 0.05$), vocalization ($F(5,41) = 18.220$, $P < 0.0001$), and vocalization post-discharge ($F(5,41) = 28.382$, $P < 0.0001$). Post-hoc comparisons indicated a significant effect of morphine (9 mg/kg) on the three threshold values. The morphine antinociceptive effects on motor response and vocalization during discharge were blocked by local administration of methylnaloxonium (0.3 and 1 μg). The effect of morphine on vocalization post-discharge was reversed by methylnaloxonium at 1 μg (Fig. 6).

3.5.2. Systemic administration of RB 101

One-way ANOVA indicated a significant treatment effect on the three threshold values: motor response ($F(5,41) = 3.299$, $P < 0.05$), vocalization ($F(5,41) = 10.908$, $P < 0.0001$), and vocalization post-discharge ($F(5,41) = 13.269$, $P < 0.0001$). Post-hoc comparisons showed significant antinociceptive responses of RB 101 (20 mg/kg) on the three nociceptive thresholds. The RB 101 induced increases in motor response, vocalization and vocalization post-discharge were all antagonized after the local injection of methylnaloxonium (0.3 and 1 μg) (Fig. 6).

4. Discussion

The participation of different brain structures in the antinociception produced by exogenous administration of

opioid receptor agonists or by the activation of the endogenous opioid system by injection of an inhibitor of enkephalin catabolism, RB 101, was investigated in the present study. For this purpose, the hydrophilic opiate receptor antagonist methylnaloxonium was microinjected locally into several central structures, and its ability to antagonize the antinociceptive responses of RB 101 and morphine was evaluated. The brain structures that were investigated in this study are involved in the different neuronal pathways responsible for the control of pain: complex ventro-basal of thalamus, ascendent transmission of nociceptive stimuli; central amygdaloid nucleus, emotional-affective reactions to pain; periaqueductal gray matter and raphe magnum nucleus, descending inhibitory pathways (Basbaum and Fields, 1984; Jensen and Yaksh, 1986; Lipp, 1991; Reichling and Basbaum, 1991; Fabian and Ableitner, 1995; Manning and Mayer, 1995).

Previous studies have investigated the neuroanatomical substrate involved in opiate antinociception by microinjecting opiate receptor agonists and antagonists into different brain regions (Yaksh and Rudy, 1978; Yeung and Rudy, 1980; Hill, 1981; Beitz, 1982; Jensen and Yaksh, 1986). However, lipophilic opiate compounds were used in most of these studies, which usually have a wide and rapid diffusion after local injection. In this study, the quaternary opioid receptor antagonist methylnaloxonium was selected because prior investigations showed that the non-lipophilic drugs, microinjected into the brain remain better localized to a discrete site without a rapid local diffusion or systemic redistribution compared with more lipophilic agents, like naloxone. For instance, labeled methylnaloxonium was localized at the site of injection for 60 min, whereas labeled naloxone remained at the site of injection for only 10 min under the same conditions (Schroeder et al., 1991). Consequently, the response observed after the local injection of this hydrophilic antagonist in a given brain structure should reflect more selective functional roles for the opiate receptors located therein. Methylnaloxonium has been previously used to clarify the role of neuroanatomical structures involved in other specific pharmacological responses linked to opiates, such as rigidity (Weinger et al., 1991), or the expression of the somatic symptoms of morphine withdrawal syndrome (Maldonado et al., 1992).

In the present study, the tail-electrical stimulation test was chosen to measure the antinociceptive responses. This test allows evaluation of three nociceptive thresholds, that seem to be related to pain regulating systems existing at distinct levels of the central nervous system. Thus, the post-discharge vocalization response has been proposed to be organized in diencephalic or limbic structures and to reflect the affective aspect of noxious stimulus, whereas the motor and simple vocalization responses have been suggested to be organized at the spinal cord and caudal brainstem levels, respectively, (Hoffmeister, 1968). Both morphine and RB 101 produced a potent antinociception in the tail-electrical stimulation test, in a dose-dependent manner (Noble et al., 1992a). The similar effectiveness of morphine and endogenous enkephalins is consistent with their close affinities for opioid binding sites, and suggests that, at the concentrations used, the related opioid receptor activition is the same (Roques et al., 1993). However, relatively large doses of both compounds were required to obtain a clear elevation of the three thresholds evaluated. The most sensitive response to the antinociceptive effects of morphine and RB 101 was the vocalization post-discharge, whereas the spinal reflex (motor response) was the least modified by these compounds. Consequently, the effectiveness of endogenous and exogenous opioids was directly related to the degree of integration of the nociceptive response, revealing the importance of supraspinal mechanisms in opioid antinociceptive responses. The doses chosen of morphine and RB 101 to be antagonized by methylnaloxonium, 9 and 20 mg/kg, i.v., respectively, were the lowest effective dose of each of compounds that induced a significant inhibition of the three responses measured in the tail-electrical stimulation test.

The antinociceptive effects induced by the systemic administration of morphine were partially or completely blocked by local injection of methylnaloxonium, depending of the brain structure where the opiate antagonist was administered. This antagonism was particularly strong when methylnaloxonium was microinjected into the periaqueductal gray matter, central amygdaloid nucleus and raphe magnum nucleus, and less intense in the case of the complex ventro-basal of thalamus, where only the highest dose of methylnaloxonium was able to block the effect of morphine on motor response and vocalization, and to slightly attenuate the effect on vocalization post-discharge. The relatively large area of projection of nociceptive fibers into the thalamus may contribute to the lack of effect of methylnaloxonium, which being highly hydrophilic probably does not diffuse to an area large enough to completely block morphine-induced antinociception.

In agreement with the present results, central administration of the opiate receptor antagonist naloxone into the lateral ventricle has been previously reported to produce a rightward shift of antinociception induced by systemic morphine injection (Yeung and Rudy, 1980). In addition, other studies have shown a powerful effect on spinally and supraspinally mediated nociception by the local intracerebral microinjection of opiate compounds (Dickenson et al., 1979; Jensen and Yaksh, 1986; Yaksh et al., 1976). Indeed, morphine and other opiates induced antinociception when administered in several brain structures including lateral ventricle, periaqueductal gray matter, raphe magnum nucleus, nucleus reticularis paragigantocellularis, somatosensory cortex, hypothalamus, central amygdaloid nucleus and thalamus (Herz et al., 1970; Helmstetter et al., 1993). The periaqueductal gray matter has been reported to be particularly sensitive to the induction of antinociception by local microinjection of opioids (Yaksh and Rudy, 1978; Jensen and Yaksh, 1986), in agreement with the strong

blockade of morphine antinociception induced by the administration of methylnaloxonium into this structure. The periaqueductal gray matter was also the only site at which morphine microinjection completely blocked the spinally mediated tail-flick response, suggesting that this structure modulates sensory actions at the spinal level (Herz et al., 1970; Yaksh and Rudy, 1978; Jensen and Yaksh, 1986). A marked antinociceptive effect was also previously found after morphine microinjection in the amygdala and the raphe magnum nucleus, but not into the thalamus (Herz et al., 1970; Helmstetter et al., 1993). Indeed, after the microinjection of 40 μg of morphine into the thalamus the nociceptive threshold remains almost unaffected (Herz et al., 1970). Furthermore, autoradiographic mapping with [^{14}C]2-deoxyglucose showed that intracerebroventricular injection of the μ-opioid receptor agonist DAMGO (Tyr-DAla-Gly-(Me)Phe-Gly-ol) decreased glucose utilization in the thalamus, in contrast with the increase observed in other regions involved in the control of pain, such as periaqueductal gray matter, raphe magnum nucleus and amygdala (Fabian and Ableitner, 1995). All these previous findings are in agreement with our finding of a reduced ability of methylnaloxonium to antagonize morphine-analgesia when microinjected into the thalamus.

RB 101, as well as other enkephalin catabolism inhibitors, has been previously reported to induce a naloxone-reversible antinociceptive response in the tail-electrical stimulation test (Noble et al., 1992a). These effects were shown to be due to the inhibition of the peptidases involved in the enkephalin metabolism, leading to a large increase in the concentration of endogenous opioid peptides in brain areas involved in the modulation of nociceptive inputs (Waksman et al., 1985; Bourgoin et al., 1986; Ruiz-Gayo et al., 1992). The decrease induced by methylnaloxonium in RB 101 antinociceptive response was, as in the case of morphine, dependent on the brain structure in which the opioid receptor antagonist was microinjected. The response was more strongly blocked when methylnaloxonium was administered into the periaqueductal gray matter and the raphe magnum nucleus as compared to the complex ventro-basal of thalamus and the central amygdaloid nucleus.

Opioid receptor agonists, such as morphine are expected to directly interact with opioid receptors in all the brain regions where these receptors are present, independently of the nature of the nociceptive stimulus. Thus, morphine's efficacy is related to the level of receptor stimulation required to inhibit a particular nociceptive message (Basbaum and Fields, 1984; Besson and Chaouch, 1987). The efficacy of peptidase inhibitors is also related to the degree of receptor activation, but in this case the stimulation of the opioid receptors will directly depend on the tonically or phasically sustained extracellular concentration of endogenous enkephalins. The permanent release of endogenous opioids is probably different in the various brain regions involved in the control of pain, and the increase in the release of these peptides triggered by exposure to a nociceptive stimulus is very likely dependent on the nature of the stimulus (Roques and Fournié-Zaluski, 1986; Yaksh and Chipkin, 1989; Roques et al., 1993).

In summary, the present results confirm that endogenous enkephalins, when completely protected from their catabolism by mixed inhibitors, such as RB 101, elicit similar pain suppressive effects as exogenous opiates. Similar brain structures seem to be involved in the antinociception induced by exogenous and endogenous opioids. Some structures related to the elaboration of the emotional reactions to pain, such as complex ventro-basal of thalamus and central amygdaloid nucleus, are less involved in these antinociceptive responses, probably as consequence of a lower release of endogenous enkephalins in these areas.

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