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Opioid Receptors of the Central Amygdala and Morphine-Induced Antinociception

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ABSTRACT

Background: The amygdala is a forebrain region, which is known as a modulator of pain sensation. The amygdala, particularly the central nucleus, has high concentrations of enkephalins relative to dynorphins and has high concentrations of opioid receptors. We here studied the role of central nuclei of amygdala in morphine antinociception. **Methods:** In this stud**y,** we used 130 male Wistar rats (200- 250g). Bilateral two guide cannula were inserted into central nuclei of amygdala. The drugs were administrated via intra central-amygdala and intraperitoneal. The antinociceptive effect was measured by formalin test. **Results:** Bilateral microinjections of morphine (50 and 100 μg/rat) into the central nuclei of amygdala elicited powerful suppression of nociceptive behaviors in both phases of formalin test. The intraperitoneal administration of naloxone (1 and 2 mg/kg) decreased significantly the antinociception induced by the intra-amygdaloid injection of morphine. Our data also showed that microinjection of naloxone (50 and 100 μg/rat) into the central nuclei of amygdala could reduce the analgesic effects of systemic morphine (7 mg/kg). On the other hand, bilateral neurotoxic lesions of the central nuclei of amygdala attenuated the antinociception induced by subcutaneous or intra-amygdaloid injection of morphine. **Conclusion:** These findings suggest that morphine analgesia in the formalin test depends on ascending connections to the forebrain, probably the amygdala. *Iran. Biomed. J. 11 (2): 75-80, 2007*

Keywords: Amygdala, Morphine, Antinociception, Naloxone, Formalin test

INTRODUCTION

The amygdala is a subcortical complex of nuclei, which contributes to antinociception elicited by both psychological factors (fear) and exogenous opioid agonists. The amygdala receives direct projections from the regions of the thalamus [1] and Para brachial nucleus that are innervated by pain pathway. Although the relative importance and functional involvement of different amygdala nuclei in antinociception is not adequately studied but there is body of evidence that the central nucleus, which belongs to dorsomedial part of the amygdala, is one of the most important brain structures contributing to the antinociceptive processes [2]. The amygdala, particularly the central nucleus, has high concentrations of enkephalins relative to dynorphins and has high concentrations of opioid receptors in binding studies [3] and chemical studies [4]. Morphine's primary sites of action have been shown to lay within the midbrain periaqueductal gray matter (PAG) and spinal cord while other brain-stem sites of action have been suggested including the nucleus reticular is paragiganto cellularis. Furthermore, there is some evidence implicating the amygdala as a mediator of morphine anti-nociception, since microinjections of morphine into this structure are reported to produce analgesia in some pain tests [5]. On the other hand, lesions of the amygdala affect the ability of systemically administered opioid agonists like morphine to produce antinociception [6] as well as certain forms of stress-induced analgesia [2]. One test in which analgesia appears to be dependent of forebrain mechanism is the formalin test. Lesions of the brainstem and spinal cord can affect analgesia in the formalin test [7]. For these reasons, we here studied the possible role of central nuclei of

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amygdala in morphine antinociception in the formalin test. In this study, we used glutamic acid to destroy neurons originating from the central nucleus of the amygdala. Then the effects of these lesions on morphine antinociception in both (acute and chronic) phases of the formalin test were examined.

MATERIALS AND METHODS

Subjects. Male Wister rats (n = 130, Pasteur Institute of Iran, Tehran) weighing 200-250 g were used. All animals were housed under a 12-h light/dark schedule, with lights on at 8:00. Food and water were available at all times except during testing. Throughout the work, the guidelines proposed by the Committee on Research and Ethical Issues of International Association for the Study of Pain for investigations in experimental pain in animals were followed. Animals were used only once and euthanized immediately after the experiment. All testings were conducted during the light portion of the cycle.

Surgery. Rats were anesthetized with sodium pentobarbital (50 or 48 mg/kg, i.p.). Using standard stereotaxic equipment, stainless steel injection cannulae (30 gauges) were lowered bilaterally into the central nuclei of amygdala according to the atlas of Paxions and Watson, rat brain atlas [8]. Central nucleus coordinates: antero-posterior, -2.5 mm; posterior to bregma lateral, ± 4.4 mm and ventral, -8.0 mm below the skull surface. The cannulae were connected via polyethylene tubing (PE-10) to an infusion pump (Harvard Apparatus USA). After 5 minutes, either L-Glutamic acid (300 μg/rat) or vehicle alone was slowly infused into the target site over a 5-minute period such that the final volume of injection was 150 nl. The guide cannulae were anchored with jeweler's screws, and the incision was closed with dental acrylic cement. After surgery, the guide cannulae left in place until injections were made. All animals were allowed to recover from the surgical procedure until they had returned to 100% of their preoperative body weight (at least 7 days) before the first nociceptive testing began.

Nociceptive testing. After a 7-day recovery period, pain related behaviors were measured by formalin test. Nociceptive scoring was carried out with the rat free to move around in a Plexiglas observation box. The box dimensions were 32 × 32 × 32 cm, and a mirror below the floor angled at 45° allowed for an unobstructed view of the rat's paws. Animals were allowed to acclimatize for 30 min before formalin injection. Formalin (2.5%; 25 μl) was injected into the dorsal surface of the right hind paw. Immediately after formalin injection, animals were placed individually in the observation box. Scoring of nociceptive behaviors started immediately and continued for the next 50 minutes. We here used the weighted-scores or rating scale method proposed by Dubuisson and Dennis [9] in nociception was quantified using the rating scale method by assigning weights to the following categories of pain-related behaviors: the animal walks or sits normally without favoring the injected paw (weight = 0), the animal walks or sits while placing some, but not full, pressure on the injected paw (weight = 1), the animal walks or sits while maintaining the paw completely elevated off the floor (weight = 2), the animal licks, bites or vigorously shakes the injected paw (weight = 3). A weighted average nociceptive score was obtained for each 5-minute test interval by multiplying the number of seconds the animal spent in each category by its assigned weight, summing these products and dividing by the total time (300 s).

$$\text{Nociceptive Score} = (t_0\times 0) + (t_1\times 1) + (t_2\times 2) + (t_3\times 3)/(t_0+t_1+t_2+t_3)$$

By utilizing this method, an ordinal scale of nociceptive scores is generated with a possible range of 0-3.

Drugs. The following drugs were used: glutamic acid (Sigma, USA), morphine sulphate (Mac Farlan Smith Ltd., UK), naloxone hydrochloride (Tocris, UK). All solutions were prepared immediately before use.

Histology. At the end of the study, animals in the lesion group were perfused intracardially with physiological saline followed by 10% formalin. Brains were sectioned and stained with Pontamine Blue for microscopic verification of lesion placement by using the atlas of Paxinos and Watson's atlas of rat brain [10]. Only data from rats that received histologically verified injections were included for analyses.

Statistical analysis. ANOVAs followed by Newman-Keuls test were used for analysis of the data. Differences between means were considered statically significant if $P<0.05$. Each point is the Mean ± SEM of 6 rats.

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RESULTS

Antinociception induced by intra-amygdaloid injection of morphine in the presence or absence of naloxone. Figure 1 shows that the injection of morphine (100 µg/rats) into the central nuclei of amygdala produced analgesia significantly in animals in the early and late phases of formalin test. There was also an interaction between naloxone with morphine in the early phase [F (2, 30) = 10.8, $P<0.0001$] (Fig. 1A) and the late phase [F (2, 30) = 22.6, $P<0.0001$] (Fig. 1B). Further analysis also demonstrated that naloxone (1 and 2 mg/kg) decreased significantly the analgesic effect of morphine (100 µg/rat) in the early phase [F (2, 30) = 10.15, $P<0.0001$] (Fig.1A) and the late phase [F (2,30) = 23.88, $P<0.0001$] (Fig. 1B). Further analysis also demonstrated that naloxone (1 and 2 mg/kg) decreased significantly the analgesic effect of morphine (100 µg/rat) in the early phase [F (2, 30) = 10.15, $P<0.0001$] (Fig.1A) and the late phase [F (2, 30) = 23.88, $P<0.0001$] (Fig.1B).

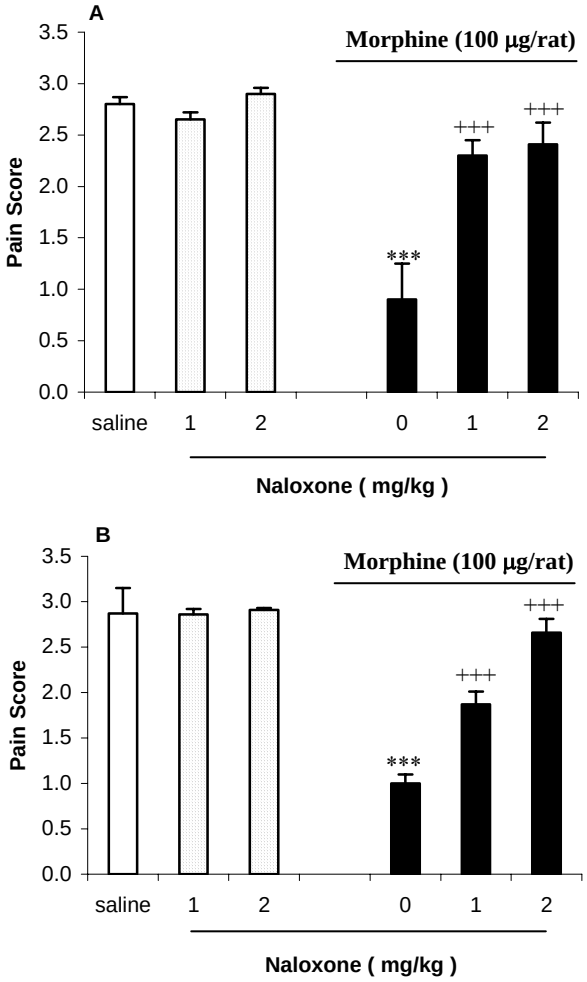

Fig. 1. Antinociceptive effect of bilateral intra-amygdaloid injection of morphine (dark bars) in the presence (1 and 2 mg/kg) or absence (saline or 0 mg/kg) of naloxone in formalin test. Animals received either intra-amygdaloid injection of 100 µg/rat morphine (dark bars) 15 min after the intraperitoneal administration of naloxone (0, 1 and 2 mg/kg). Morphine or saline was injected 5 min before formalin injection. Antinociception was recorded 0-5 min (A: Early Phase) and 15-50 min (B: Late Phase) after formalin injection. Data are expressed as Mean ± S.E.M. of 6 animals. ***$P<0.001$ as compared with saline control group. $^{+++}P<0.001$ as compared with morphine control group.

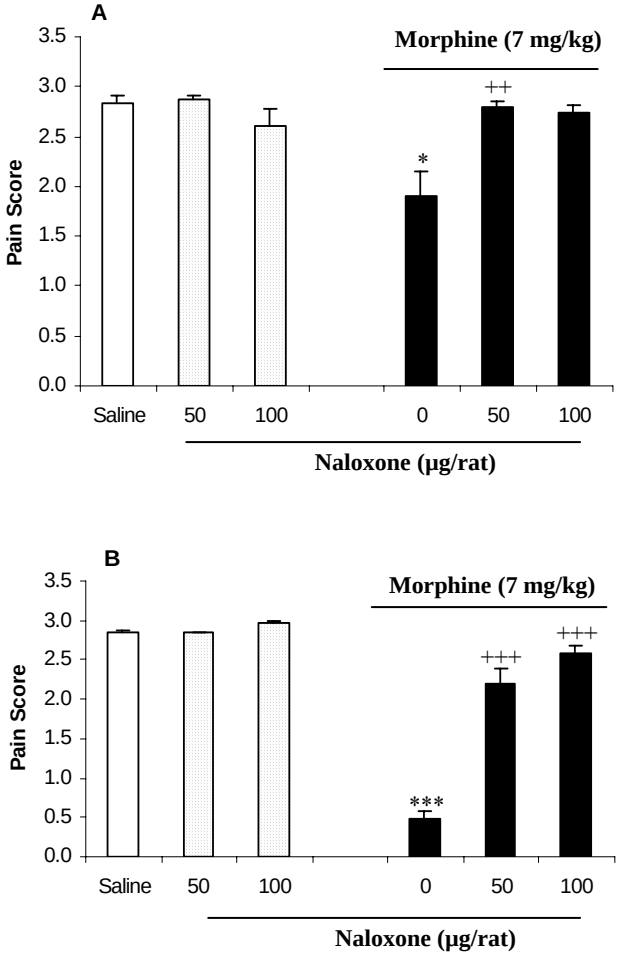

Fig. 2. Antinociceptive effect of systemic injection of morphine (dark bars) in the presence (50 or 100 µg/rat) or absence (saline or 0 µl/rat) of intra-amygdaloid injection of naloxone in formalin test. Animals received either intra-amygdaloid injection of naloxone (0, 50 and 100 µg/rat) 5 min before the subcutaneous administration of morphine (7 mg/kg). Morphine or saline was injected 15 min before formalin injection. Antinociception was recorded 0-5 min (A: Early Phase) and 15-50 min (B: Late Phase) after formalin injection. Data are expressed as Mean ± S.E.M. of 6 animals. *$P<0.05$, ***$P<0.001$ as compared with saline control group. $^{++}P<0.01$, $^{+++}P<0.001$ as compared with morphine control group.

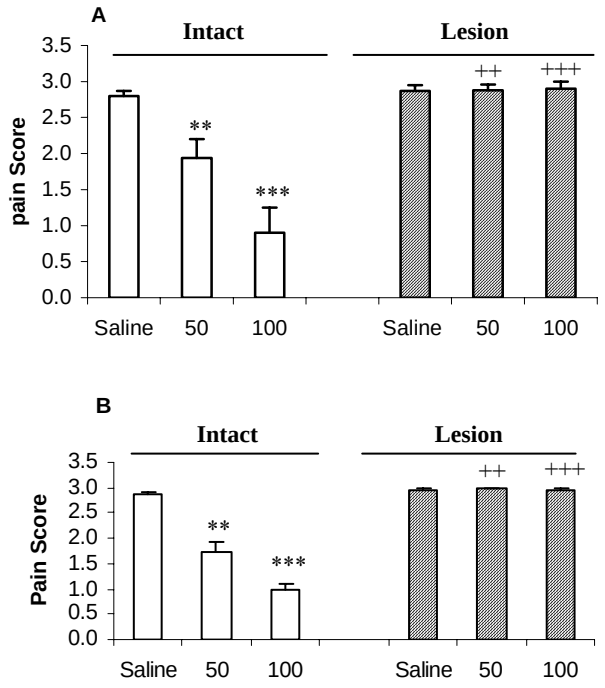

Fig. 3. Antinociceptive effect of bilateral intra-amygdaloid injection of morphine in intact and lesion rats. Intact or lesion animals received intra-amygdaloid injection of morphine (50 and 100 µg/rat) or saline (1 µg/rat) 5 min before formalin injection. Antinociception was recorded 0-5 min (A: Early Phase) and 15-50 min (B: Late Phase) after formalin injection. Data are expressed as Mean ± S.E.M. of 6 animals. ***P*<0.01, ****P*<0.01 as compared with saline intact group. ^{++}P<0.001, ^{+++}P<0.001 as compared with morphine intact group.

Effects of intra-amygdaloid injection of naloxone on morphine antinociception in formalin test. The effects of microinjection of different doses of naloxone on the analgesia induced by the subcutaneous administration of morphine have been shown in Figure 2. One-way ANOVA revealed that pretreatment of animals with the injection of naloxone (50 and 100 µg/rats) into the central nuclei of amygdala decreased the antinociceptive effect of morphine (7mg/kg) in the early phase [F (3, 20) = 12.16, *P*<0.0001] (Fig. 2A) and the late phase [F (3, 20) = 36.8, *P*<0.001] (Fig. 2B).

Effects of central amygdala lesions on morphine analgesia in formalin test. Figure 3 shows the analgesic effects of intra-amygdaloid injection of morphine (50 and 100 µg/rat) in the lesion and intact rats. Two-way ANOVA indicated an interaction between morphine effect in the lesion and intact groups in the early phase [F (2, 30) = 14.94, *P*<0.0001] (Fig. 3A) and the late phase [F (2, 30) = 176.45, *P*<0.0001] (Fig. 3B). It has also shown that microinjection of morphine (50 and 100 µg/rat) could produce analgesia in a dose-dependent manner in both phases of formalin test in intact groups (*P*<0.001), but not in the lesion rats.

Figure 4 shows the effect of bilateral lesions of central nuclei of amygdala on the analgesia induced by systemic administration of morphine. One-way ANOVA indicated that morphine (7 and 10 mg/kg) could produce dose-dependent antinociception in intact animals in the early phase [F (4, 25) = 10.72, *P*<0.0001] (Fig. 4A) and the late phase [F (4, 25) = 70.76, *P*<0.0001] in intact groups (Fig. 4B). Furthermore, subcutaneous injection of morphine (10 mg/kg) induced significant analgesia in lesion rats in the late phase (*P*<0. 001) (Fig. 4B).

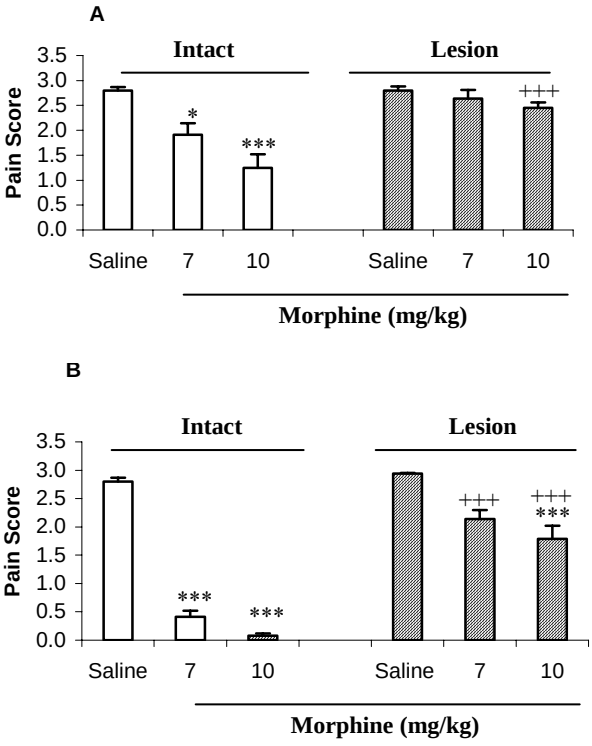

Fig. 4. Antinociceptive effect of systemic morphine in intact and lesion rats. Intact or lesion animals received subcutaneous injection of morphine (7 and 10 mg/kg) or saline (1 ml/kg) 15 min before formalin injection. Antinociception was recorded 0-5 min (A: Early Phase) and 15-50 min (B: Late Phase) after formalin injection. Data are expressed as Mean ± S.E.M. of 6 animals. **P*<0.1, ****P*<0.01 as compared with saline group. ^{+++}P<0.001 as compared with morphine intact group.

DISCUSSION

Our study demonstrates that the central nucleus of amygdala contributes to the antinociceptive effect of

systemic and/or intra-amygdaloid administration of morphine. However, the present findings indicate that bilateral lesions in the central amygdala nuclei block the analgesia induced by morphine in rats.

Our results show that microinjection of morphine into the central nucleus of amygdala can produce analgesia in rats in both phases of formalin test. There are opioid receptors in most of forebrain and midbrain regions connected with corticomedial amygdala [11]. Although neuropharmacological investigations demonstrate that PAG and posterior hypothalamic area as well as the spinal cord are morphine's primary sites of action, other site may also be involved in the production of morphine-induced antinociception during formalin test. The present data are in accordance with previous studies indicating that the central amygdale may contribute to the induction of morphine antinociception in the formalin test [6]. The amygdale is a collection of anatomically and functionally diverse nuclei [12] that are receiving increasing attention as modulator of pain sensation [13]. The central amygdaloid nucleus is thought to be the main output of the amygdala, which involves in arousal, expression of emotions and inducing antinociception processes [14]. Our data showed that the intraperitoneal administration of naloxone significantly attenuated the antinociception induced by the intra-central amygdale injection of morphine. Since systemic naloxone antagonizes the response morphine in the central amygdala, the involvement of opioid receptor mechanisms seems likely. Besides, our data demonstrate that microinjection of naloxone into the central amygdala nuclei inhibits the antinociceptive activity of systemically administered morphine, which supports the involvement of amygdala in morphine antinociception. Considering that the central amygdala contributes to the production of morphine antinociception in the formalin test [6], it seems likely that the inhibition opioid receptors of the central amygdala by naloxone block the response of morphine. Although Manning and Franklin's research [15] indicated that the microinjection of naloxone into the central amygdale failed to attenuate morphine analgesia, but the present data suggest that opioid receptors within the central amygdala are critical for eliciting the analgesic effect of systemic morphine. This view is supported by the fact that amygdala, particularly the central nucleus has high concentration of opiate receptors [16]. This also indicates that opioidergic transmission in the central amygdala plays a critical role in the analgesia effect of systemic morphine in the formalin test.

On the other hand, our findings indicate that systemic administration of naloxone reduces analgesia induced by morphine, microinjected into the central nuclei of amygdala. Besides, previous studies showed that intra-amygdaloid injection of morphine elicited an increase in the release of enkephalins and beta-endorphin in the amygdala**,** [17] PAG and nucleus accumbens [18] which was antagonized by naloxone. On the other hand PAG may be a relay station for the effects of stimulating the central amygdala [19].

Furthermore, our results demonstrate that bilateral lesions of central amygdala nuclei reduce morphine antinociception in formalin test. However, our findings are consistent with previous researches, which have shown that amygdala plays a significant role in pain and analgesia [6, 10]. Additionally, some studies have indicated that lesions of the central nucleus of amygdala abolish classically conditioned antinociception as assessed on both the tail-flick and formalin tests.

There are several possible mechanisms by which the amygdala might affect nociception [20-22]. The amygdala may also be a part of the forebrain pain modulating system, and that the lesions disrupted ascending pain projection to the other telencephalic or cortical pain processing regions. On the other hand, Bernard and Besson [23] have provided convincing data that the central amygdala responds to stimulation of peripheral pain fibers via a pathway through parabrachial nucleus. The mechanism by which amygdala inactivation reduces morphine antinociception is unclear. Nevertheless, it is likely that pain modulating circuits in the PAG, rostral ventro-medial medulla and spinal cord are affected by amygdala inactivation in both the tail-flick and formalin tests under normal conditions.

Interestingly, our study demonstrates that morphine (10 mg/kg I.P.) induces analgesia in the late phase of formalin test in lesioned rats. This result may suggest that antinociception can be re-instated in lesioned groups by raising the dose of systemic morphine. However, it could be argued that the re-instatement of antinociception may be due to an increased contribution of spinal cord or peripheral sites of action while the dose of systemic morphine is raised. Several lines of evidence, however, suggest that neurons originating from the central nucleus of amygdala contribute to the induction of morphine antinociception during the formalin test but the precise role of the amygdala in pain perception is still unknown, and the extent to which

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the amygdala is involved in other types of pain remains to be determined.

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