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Electrolytic lesion of the nucleus raphe magnus reduced the antinociceptive effects of bilateral morphine microinjected into the nucleus cuneiformis in rats

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ABSTRACT

Several lines of investigation show that the rostral ventromedial medulla is a critical relay for midbrain regions, including the nucleus cuneiformis (CnF), which control nociception at the spinal cord. There is some evidence that local stimulation or morphine administration into the CnF produces the effective analgesia through the nucleus raphe magnus (NRM). The present study tries to determine the effect of morphine-induced analgesia following microinjection into the CnF in the absence of NRM. Seven days after the cannulae implantation, morphine was microinjected bilaterally into the CnF at the doses of 0.25, 1, 2.5, 5, 7.5 and 10 μg/0.3 μl saline per side. The morphine-induced antinociceptive effect measured by tail-flick test at 30, 60, 90 and 120 min after microinjection. The results showed that bilateral microinjection of morphine into the CnF dose-dependently causes increase in tail-flick latency (TFL). The 50% effective dose of morphine was determined and microinjected into the CnF (2.5 μg/0.3 μl saline per side) in rats after NRM electrolytic lesion (1 mA, 30 s). Lesion of the NRM significantly decreased TFLs, 30 ($P < 0.01$) and 60 ($P < 0.05$) but not 90–120 min after morphine microinjection into the CnF, compared with sham-lesion group. We concluded that morphine induces the analgesic effects through the opioid receptors in the CnF. It is also appeared that morphine-induced antinociception decreases following the NRM lesion but it seems that there are some other descending pain modulatory pathways that activate in the absence of NRM.

It has been known for over 30 years that the brain possesses networks that modulate the processing of nociceptive information. Analgesic descending pathways from midbrain to dorsal horn of the spinal cord play a crucial role in the transmission of nociceptive information from the periphery to central nervous system. The rostral ventromedial medulla (RVM), which includes nucleus raphe magnus (NRM), is one of the most important components of the brainstem descending antinociceptive pathway that modulates the transmission of nociceptive impulses in the level of spinal cord [13]. The nuclei in the RVM play well-established roles in the control of nociception. Behavioral studies recently appear that the RVM is required for enhanced inflammatory, neuropathic pain and opiate withdrawal following prolong administration of morphine [5,28,35]. The nucleus cuneiformis (CnF) is a region of considerable functional interest which lies in the reticular nucleus of midbrain extending ventrally to the colliculi in the dorsolateral part of mesencephalic tegmentum [16]. Previous studies have revealed that the CnF is one of the regions that provide a major source of afferents to the NRM [4,38]. The CnF is connected with many areas of the reticulospinal nuclei [11]. Anatomical and behavioral data from the CnF indicate that this nucleus plays an important role in sensory and motor integration relevant to pain transmission [18,19,38]. Ipsilateral CnF fibers caudally coursed the ventral pontine reticular formation to innervate NRM and nucleus magnocellularis [38]. Previous studies have indicated the powerful analgesic effect elicited from morphine microinjected into the CnF [18] and NRM [7,10].

The NRM plays a major role in opiate-induced analgesia and it has been demonstrated that lesions of the NRM prevents the analgesic effects of morphine [30,31]. It is hypothesized that opioid-dependent descending inhibitory system is activated due to disinhibition of brainstem output neurons [25]. In addition, naloxone administered into the NRM attenuates the analgesia produced by systemic administration of morphine or electrical stimulation of this nucleus [33]. However, some controversial investigations indicate that this nucleus causes the facilitation as well as inhibition of nociception. Le bars et al. showed that injection of morphine into the NRM potentiates the responses of dorsal horn neurons [23]. In contrast, another investigation has shown that injection of morphine into the NRM attenuates the responses of dorsal horn neurons to noxious stimuli and electrical stimulation of nerves [10].

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It is also observed that electrical stimulation of some other brain regions such as periaqueductal gray (PAG) is effective in inhibiting the responses of lateral cervical nucleus neurons to noxious stimulus [8]. As the CnF is a rostrocaudal extensive nucleus and the above-mentioned studies support the hypothesis that functional link between NRM in brainstem and CnF concerning the pain modulation. Therefore, in this study, we try to examine the effects of electrolytic lesion of the nucleus raphe magnus on morphine-induced antinociception by bilateral microinjection of morphine into the nucleus cuneiformis in rats.

One hundred and eight male Wistar rats (230–280 g) were housed three per cage and allowed free access to chow and tap water. The vivarium was maintained on a 12:12-h light/dark cycle at a room controlled temperature (23 ± 1 °C). All experiments were executed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 80-23, revised 1996) and were approved by the Research and Ethics Committee of Shahid Beheshti University (M.C.). Experimental animals were prepared with bilaterally guide cannulae implantation (23 guage needle) at least 5–7 days before their use. The rats were anesthetized with intraperitoneal (i.p.) injection of ketamine 10% (100 mg/kg) and xylazine 2% (10 mg/kg) and two cannulae were stereotaxically (Stoelting, stereotaxic apparatus, USA) implanted in the CnF, then an electrolytic lesion of NRM (1 mA, 30 s, dc current) was made by an anodal microelectrode. The coordinates for the different regions of brain were determined from Paxinos and Watson [27] as AP = −8.4 mm caudal to bregma, Lat = ± 1.9 mm lateral to midline, DV = −6.3 mm ventral from the skull surface for CnF (guide cannulae were 1 mm above the appropriate injection place) and for NRM was AP = −11.2 caudal to bregma, Lat = 0.0 and DV = −9.3 ventral from the skull surface. The guide cannulae were secured in place using two stainless steel screws anchored to the skull and dental acrylic cement. At the period of recovery (5–7 days) a stainless steel obdurator was inserted into the each guide cannula to prevent occlusion. Penicillin-G 200,000 IU/ml (0.2–0.3 ml/rat, single dose, intramuscular) and acetaminophen (1/100 in drinking water, 48 h) were administered immediately after surgery.

Microinjections were performed by lowering a stainless steel injector cannula (30 gauge needle) with a length of 1 mm longer than the guide cannulae into the CnF. The injector cannula was connected to a 1-μl Hamilton syringe by polyethylene tubing (PE-20) and 0.3 μl of drug solution or vehicle infused over 45 s and was left for the 60 s extra time and followed by replacement of the obdurator. Morphine sulfate (Temad Co., Iran) was dissolved in saline at the doses of 0.25, 1, 2.5, 5, 7.5 and 10 μg/0.3 μl per side. Morphine solutions were prepared freshly on test day and infused in a 0.3-μl volume at the rate of 0.1 μl/15 s counted on a timer-controlled micrometer. The movement of an air bubble in the PE-20 tubing confirmed drug flow. Testing was conducted at the same day times. It is postulated that the doses of ≥15 μg/0.3 μl saline per side were eliminated in the present protocol due to increase the incidence of lethality.

The nociceptive threshold was measured by the tail-flick apparatus (Harvard, USA). The heat was applied in succession after the 3, 5 and 7 cm from the caudal tip of the tail. The value of each tail-flick latency (TFL) time was calculated on the average of three consecutive TFL tests. The reaction time between the onset of heat stimulus and the movement of tail was determined by an automatic sensor as TFL. The light source was set at an intensity (35%) that yields baseline TFL values in the range of 3–4 s. If animal did not respond to heat stimulus after 12 s (cut-off point), the tail was removed from the heat radiant to prevent the tissue damages. TFLs (s) are expressed either as raw data or as percentage of maximal possible effect (MPE%).

In this study, there were 12 groups as follows: (1–3) control groups contain intact, sham-operated and saline groups for determining the baseline TFLs, surgical manipulation and microinjection volume effects, respectively, (4) sham-lesion group, (5–10) morphine dose-response groups (0.25, 1, 2.5, 5, 7.5 and 10 μg/0.3 μl saline per side) that we determined the 50% effective dose (ED50%) of morphine microinjected into the CnF, (11) NRM-lesion group that electrolytic lesion was applied and received saline into the CnF and (12) In the last experiment, in order to find out the role of NRM in descending pain modulatory pathway from the CnF, morphine was microinjected into the CnF 5–7 days following the electrolytic lesion of NRM. After recovery period, the ED50 of morphine was bilaterally infused in the CnF. In all above control and experimental groups, TFLs were recorded at 30, 60, 90 and 120 min after morphine or saline microinfusion as an index of analgesia.

The results obtained are expressed as mean ± standard error of mean (S.E.M.). The mean TFLs in all groups were subjected to one-way and/or two-way ANOVA followed by protected Tukey's or Dunnett's test for multiple comparisons, as needed. *P*-values less than 0.05 were considered to be statistically significant.

After completion of the experiments, rats were sacrificed and the cannulae and lesion sites were confirmed. Animals were deeply anesthetized with ketamine and xylazine. Then, they were transcardially perfused with 0.9% saline and 10% formaldehyde solution

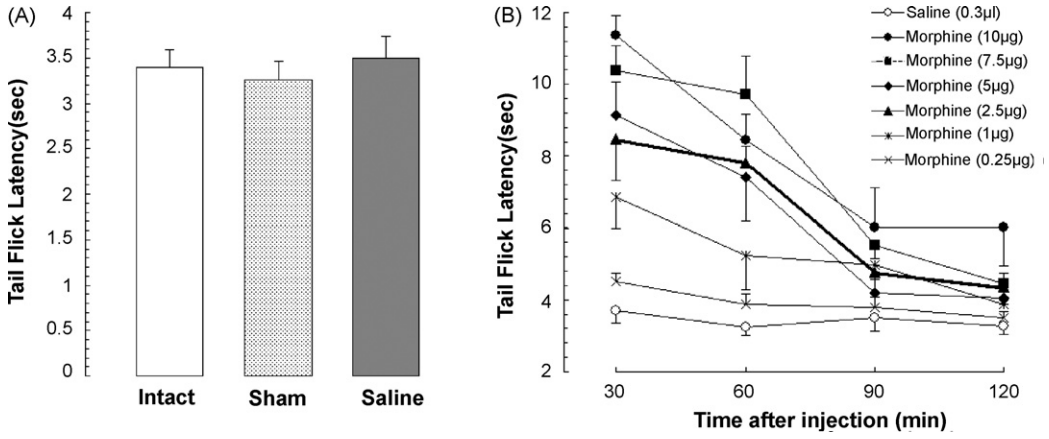

Fig. 1. (A) The mean tail-flick latencies (TFLs) in the intact, sham and saline groups. (B) Effect of different doses of morphine when microinjected into the nucleus cuneiformis on the TFLs at 30–120 min intervals after injection. A dose-dependent decrease occurred in the TFLs with lasting the time after morphine microinjection. Except two lowest doses of morphine, the others significantly increased the TFLs compared to saline-treated animals at 30 and 60 min after microinjection. The 50% effective dose of morphine was close to 2.5 μg/0.3 μl saline per side (bold line). Each point is the mean ± S.E.M. for 7–10 rats.