Neuroscience Letters 451 (2009) 165–169

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Effects of electrolytic lesion of dorsolateral periaqueductal gray on analgesic response of morphine microinjected into the nucleus cuneiformis in rat

Abbas Haghparast*, Leila Ahmad-Molaei

Neuroscience Research Center, Shahid Beheshti University, M.C., P.O. Box 19615-1178, Tehran, Iran

ARTICLE INFO

Article history:
Received 1 December 2008
Received in revised form
25 December 2008
Accepted 29 December 2008

Keywords:
Nucleus cuneiformis
Dorsolateral periaqueductal gray
Morphine antinociception
Electrolytic lesion
Tail-flick test
Formalin test

ABSTRACT

The periaqueductal gray (PAG) and nucleus cuneiformis (CnF), like the rostral ventromedial medulla, have functional roles in descending pain-inhibitory pathway related to morphine antinociception. There is not any evidence concerning the role of different regions of the PAG on antinociceptive effect of morphine administered into the CnF in pain modulatory system. In the present study, we investigate whether electrolytic lesion of dorsolateral periaqueductal gray (dl-PAG) influence the analgesic effect of morphine microinjected into the CnF. 71 adult male Wistar rats weighting 230–280 g cannulated bilaterally into the CnF, concurrently lesion of dl-PAG was done. The tail-flick and formalin tests were performed to measure pain and antinociceptive effect of morphine microinjected into the CnF (2.5 μg/0.3 μl saline per side). The tail-flick latency was measured at 15, 30, 45, 60 and 75 min following morphine microinjection. In formalin test, pain behavior was recorded for 60 min in early (0–5 min) and late (15–60 min) phases after formalin injection. Each rat was given a subcutaneous 50-μl injection of formalin 2.5% into plantar surface of hind paw following morphine administration. The results showed that dl-PAG lesion attenuated the effect of morphine microinjected into the CnF both in tail-flick and formalin tests while dl-PAG lesion solely did not alter basal pain behavior as compared to control group. In conclusion, our results suggest the existence of a direct or indirect projection from CnF to the dl-PAG at least at the level of the morphine antinociception in pain modulation.

The periaqueductal gray (PAG) region of the midbrain of vertebrates including rodents and humans is recognized as one of several sites involved in the modulation of nociceptive sensory input through descending controls to the level of spinal cord [2,25]. Beitz [5] showed that PAG receives some mesencephalic inputs from the nucleus cuneiformis (CnF). Projections to caudal CnF were also observed from all subdivisions of the PAG, the deep layers of superior colliculus as well as the caudal levels of CnF [29]. Retrograde tracer studies in animals have shown these two nuclei to be connected with each other [6,30]. Some anatomical and physiological studies have demonstrated that a major source of afferents to nucleus raphe magnus (NRM) has critical role in morphine antinociception in pain modulatory system, arise from a continuous band of cells located within the PAG [7]. Human imaging studies have shown that the PAG [7] and CnF [30] are activated during visceral and somatic pain [11]. The cuneiformis nucleus, a reticular nucleus of the midbrain, located just ventrolateral to the periaqueductal gray, plays a key role in the development of experimental secondary hyperalgesia via possible facilitatory pro-nociceptive mechanisms and these two nuclei involved in a network mediating anti- and pro-nociception, as largely shown from animal data [12,24,28].

Our previous studies have shown that morphine when microinjected into the CnF produced powerful analgesia in tail-flick [13–15] as a model of acute pain. In a pilot study which was carried out in this laboratory, it was revealed that spontaneous activity of CnF neurons significantly increased following formalin test as a model of chronic pain. In addition, our previous study, it was indicated that bilateral electrolytic lesion of NRM [14] but not ventrolateral periaqueductal gray diminished analgesic response of morphine microinjected into the CnF (unpublished data). Regarding to the above mentioned studies that imply there are functional link between PAG and CnF, it is the aim of this study to investigate the role of dorsolateral periaqueductal gray as another part of PAG in antinociceptive response of morphine microinjected into the nucleus cuneiformis in rat.

The experiments were performed on 71 Wistar rats (Pasteur Institute, Iran) weighing 230–280 g. Animals were kept under standard laboratory conditions, with tap water and regular rat chow *ad libitum*. They were individually housed in a temperature and humidity-controlled vivarium on 12-h light/dark cycle. All experiments executed with the Guide for the Care and Use of Laboratory

* Corresponding author. Tel.: +98 21 2243 1624; fax: +98 21 2243 1624.
E-mail address: haghparast@yahoo.com (A. Haghparast).

0304-3940/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2008.12.058

Animals (National Institute 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-gauge 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 dorsolateral periaqueductal gray (dl-PAG) (0.1 mA, 45 s, DC current) was made by an anodal microelectrode. The coordinates for the different regions of brain were determined from Paxinos and Watson [23] 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 the CnF (guide cannulae were 1 mm above the appropriate injection place) and for the dl-PAG was AP = −7.1 caudal to bregma, Lat = ± 0.6 and DV = −5 mm 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.

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). Morphine sulfate (Temad Co, Iran) was dissolved in saline at the dose of 2.5 μg/0.3 μl saline [14]. 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 and was left for the 60 s extra time and followed by replacement of the obturator. Testing was conducted at the same day times. Formalin 2.5% was made from 1 part formaldehyde (∼36.6%; Formaldehyde, Merck) and 13.64 parts saline for subcutaneous injection into the plantar surface of one hind paw. Since all of microinjections were performed bilaterally in this study.

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 light intensity source was manually set at about 35% of maximal intensity that yields baseline TFL values in the range of 3–4 s. The equipment was calibrated in order to obtain two consecutive baseline tail-flick latencies between 3 and 4 s. If at any time the animal failed to flick its tail within 12 s (cut-off point), the tail was removed from the coil to prevent damage to the skin [14]. TFLs (s) are expressed either as raw data or percentage of maximal possible effect (%MPE) which was calculated from the following formula:

$$\%\text{MPE} = \frac{\text{Post-drug latency (s)} - \text{baseline latency (s)}}{\text{Cut-off value (s)} - \text{baseline latency (s)}} \times 100$$

where post-drug latency for morphine was recorded at 15-min intervals (15, 30, 45, 60 and 75 min) after drug microinfusion into the CnF. To evaluate the sensitivity of animals to nociceptive stimulus, we consider the individual TFL before drug treatment as a pain threshold.

Animals were placed individually in an open Plexiglas chamber (35 cm × 35 cm × 35 cm) with a mirror angled at 45° positioned behind to allow an unobstructed view of the paws by the observer. The animals were habituated to the observation chamber for 30 min prior to the experimental sessions. Each rat was given a subcutaneous 50-μl injection of 2.5% formalin into the plantar surface of one hind paw using a 27-gauge needle, randomly. Observations to determine nociceptive responses began upon placing the rat into the box and continued for the next 60 min. The nociceptive behavior was used to quantify nociceptive effects of drugs by assigning weight to the pain-related behaviors [8,10,16,20]. A nociceptive score was determined for each 5-min block during that period by measuring the amount of time spent in each of the four behavioral categories: 0, the position and posture of the injected hind paw is indistinguishable from the another hind paw; 1, the injected paw has little or no weight placed on it; 2, the injected paw is elevated and is not in contact with any surface; 3, the injected paw is licked, bitten or shaken. Then, a weighted nociceptive score, ranging from 0 to 3 was calculated by multiplying the time spent in each category by the category weight, summing these products and dividing by the total time (300 s) for each 5-min block of time

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

By utilizing this method, an ordinal scale [8] of nociceptive scores was generated with a range of 0–3. The first 5 min considered as an early phase (0–5 min) and the late phase was defined from 15 to 60 min after formalin injection in hind paw.

In this study tail-flick and formalin tests carried on separate groups, four groups for each test. (1) dl-PAG sham-lesion + saline as a control group that bilaterally received saline into the CnF; (2) dl-PAG sham-lesion + morphine as a morphine control group that bilaterally received morphine into the CnF; (3) dl-PAG lesion-group + saline that received bilaterally saline into the CnF and (4) lesion-group + morphine that received morphine into the CnF. Morphine or saline were microinjected into the CnF, 5–7 days following the electrolytic lesion of dl-PAG.

The results obtained are expressed as mean ± SEM (standard error of mean). The mean TFLs in all groups were subjected to one-way and/or two-way ANOVA followed by protected Tukey's or Bonferroni's test for multiple comparisons respectively, as needed. The mean maximal possible effect of morphine was subjected to un-paired student *t*-test for comparison of two independent groups at each 15-min intervals. In formalin test, an average of the scores obtained in the first 5 min (0–5 min) was considered as phase 1, and the area under the curve (AUC) of pain scores obtained using trapezoidal rule during 15–60 min after formalin injection was considered as phase 2. In order to evaluate the nociceptive responses, AUC for each phase of formalin test was calculated as raw pain scores × time by linear trapezoidal method [18,26] and a single value for each phase used in statistical analyses. The calculated and normalized AUC (as compared to control group) values in all groups were subjected to one-way and/or two-way ANOVA followed by protected Tukey's or Bonferroni's test for multiple comparisons respectively, as needed. *P*-values less than 0.05 were considered to be statistically significant.

After completion of the experiments, rats were deeply anesthetized with ketamine and xylazine and were transcardially perfused with 0.9% saline and 10% formaldehyde solution prior to sectioning. Then, rats sacrificed and their brains were removed. Animals were the neuroanatomical location of cannulae tips and lesion places were confirmed using the rat brain atlas [23]. The data reported here are only from animals in which the placement of cannulae and lesion sites were histologically verified (Fig. 1).

The average baseline TFL in these experiments was 3.81 ± 0.15 s. In this set of experiments, bilateral electrolytic lesion of dl-PAG on antinociceptive response of morphine microinjected into the CnF was examined. Two-way ANOVA for repeated measures over time followed by Bonferroni's test for the data shown in Fig. 2A revealed significant differences between morphine and lesions in 15-min post-injection times [treatment main effect: $F(3,114) = 35.38$, $P < 0.0001$, time main effect $F(4,114) = 2.834$, $P < 0.05$, treatment × time interaction $F(12,114) = 1.211$, $P = 0.2842$]. Data obtained in tail-flick test showed that morphine microinjected into the CnF, significantly increased TFLs during 15–60 min after injection and bilateral electrolytic lesions in the dl-PAG could notably decreased the morphine-induced antinociception (Fig. 2A). On the other hand, there were no significant differences in TFLs