Basic and Clinical NEUROSCIENCE
Summer 2010, Volume 1, Number 4

Effects of Electrolytic Lesions of the Ventrolateral Periaqueductal Gray and Nucleus Raphe Magnus on Morphine - Induced Antinociception in the Nucleus Cuneiformis

Abbas Haghparast [1,*], Mehdi Ordikhani-Seyedlar [1], Maryam Ziaei [1,2], Pegah Azizi [1], Mohammad Ebrahimzadeh-Sarvestani [1]

1. Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, P.O.Box 19615-1178, Tehran, Iran
2. Department of Psychology, Faculty of Education and Psychology, Shahid Beheshti University, Tehran, Iran

Article info:
Received:16 May 2010
First Revision: 15 June 2010
Accepted: 28 June 2010

Key Words:
Nucleus Cuneiformis;
Ventrolateral Periaqueductal Gray;
Nucleus Raphe Magnus;
Electrolytic Lesion;
Morphine Analgesia

ABSTRACT

Introduction: The nucleus cuneiformis (NCF) and ventrolateral periaqueductal gray (vlPAG), two adjacent areas, mediate the central pain modulation and project to the nucleus raphe magnus (NRM).

Methods: This study examined whether the antinociceptive effect of morphine microinjected into the NCF is influenced by inactivation of vlPAG and NRM in rats. Animals were bilaterally microinjected with morphine (2.5 µg/0.3 µl saline) into the NCF. Electrolytic lesions were made in vlPAG (0.1 mA, 45 sec) and/or NRM (1 mA, 30 sec). Tail-flick latency (TFL) was measured at 30, 60, 90 and 120 min after microinjection.

Results: The results showed that TFLs are significantly decreased in vlPAG+NRM lesions group at 30 ($P<0.001$) and 60 ($P<0.01$) min after intra-NCF administration of morphine whereas TFLs did not affect in solely vlPAG lesion animals. Our findings show that concurrent lesions of NRM and vlPAG completely reversed the analgesic effect of morphine in NCF. However, vlPAG do not play a critical role directly in pain modulatory system elicited from NCF, at least at the level of morphine-induced analgesia.

Discussion: It can be concluded that its interactive effect in descending pain modulation from NCF to NRM should not be neglected.

1. Introduction

The importance of rostral ventromedial medulla (RVM) in nociceptive modulation is well documented, and several lines of evidence point to a role for periaqueductal gray (PAG) in regulating the activity of pain modulating neurons in this region (Flores et al., 2004; Yang et al., 2007). There are two major relay stations in pain modulatory system, the nucleus raphe magnus (NRM) and PAG (Basbaum and Fields, 1984; Besson et al., 1991). The PAG may involve a population of neurons having descending projections to NRM and adjacent reticular nuclei (Williams and Beitz, 1989). Additionally, the ventrolateral periaqueductal gray (vlPAG), as described by Beitz (1995), extends from the region ventral to the horizontal line transecting the center of aqueduct. Both electrical and chemical stimulation of sites throughout the rostrocaudal extent of vlPAG produce potent antinociception. This analgesic effect is mediated in part by the activation of spinally projecting neurons in the RVM (Jones, 1992).

Some anatomical and physiological studies have demonstrated that a major source of afferents to NRM arise

** Corresponding Author:*
Abbas Haghparast, PhD
Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, P.O.Box 19615-1178, Tehran, Iran.
Tel./fax: +98 21 22431624
E-mail: Haghparast@yahoo.com

from a continuous band of cells located within the PAG (Beitz, 1990; Bodnar, 2000) and NCF (Behbehani and Zemlan, 1986; Zemlan and Behbehani, 1988). The NCF begins in the caudal part of midbrain at the level of inferior colliculus extending as far as the rostral part of pons (Gioia and Bianchi, 1987a). The NCF plays an important role in sensory/motor integration relevant to pain transmission (Haghparast et al., 2007a, 2007b, 2008; Zemlan and Behbehani, 1988). Two adjacent nuclei, the PAG and NCF, and their major caudal projection target, RVM, are important components of a descending pain modulatory circuit (Zemlan and Behbehani, 1988). Projections to caudal NCF were observed from all subdivisions of the PAG, the deep layers of superior colliculus as well as the caudal levels of NCF (Zemlan and Behbehani, 1984). Beitz in 1982 showed that PAG receives some mesencephalic inputs from NCF and similarly, they are modulated via opioid receptors (Jensen and Yaksh, 1989). Opioid sensitive cells located in the PAG project to RVM neurons which in turn are capable of inhibiting noxious input at the spinal cord (Basbaum and Fields, 1984). It has been shown that animals with lesions of the PAG or RVM fail to show normal inhibition of nociceptive reflexes (Helmstetter and Tershner, 1994). Additionally, previous studies have shown the role of NRM (Dickenson et al., 1979), PAG (Bernal et al., 2007; de Luca et al., 2003) and NCF (Haghparast et al., 2007a, 2007b, 2008) in analgesic mechanisms for descending pain modulation and their critical involvement in opioid-induced antinociception. The above mentioned studies support the hypothesis that there is a functional link among NCF, PAG and NRM in regard to the descending pain modulation. Furthermore, our recent studies indicated that solely electrolytic lesion of the NRM (Haghparast et al., 2008) and the dorsolateral periaqueductal gray (dlPAG) region (Haghparast and Ahmad-Molaei, 2009) significantly reduced the antinociceptive effect of intra-NCF morphine in rats. Notably, it is well established that the different parts of PAG influence the pain modulation (Loyd and Murphy, 2006, 2009) and vlPAG and NCF are anatomically neighbors (Loyd and Murphy, 2009). Therefore, based on the aforementioned studies and regarding contribution of vlPAG to morphine-induced antinociception, we tried to examine the effects of electrolytic lesions of solely ventrolateral PAG and/or concurrent with nucleus raphe magnus on antinociceptive effect of morphine microinjected into the nucleus cuneiformis in rats.

2. Methods

2.1. Animal Preparation and Stereotaxic Surgery

Eighty five adult male Wistar rats (230-280 g) were used in this study. 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 were executed with the Guide for the Care and Use of Laboratory Animals (National Institute of Health Publication No. 80-23, revised 1996) and were approved by the Research and Ethics Committee of Shahid Beheshti University of Medical Sciences. In this study, the animals were anaesthetized by intraperitoneal (i.p.) ketamine (100 mg/kg) and xylazine (10 mg/kg) and were mounted in a stereotaxic frame (Stoelting, USA) with the incisor bar positioned at -3.3 mm below the horizontal plane. Stainless steel guide cannulae of 0.6 mm outer-diameter was directed bilaterally in accordance with stereotaxic coordinates (Paxinos and Watson, 2007) at the NCF as AP=7.7-8.4 mm caudal to Bregma, L=±1.8-1.9 lateral to midline, DV=5.9-6.3 from the skull surface (guide cannulae were aimed 1 mm above the appropriate injection place). They were sealed with occluding stylette in recovery period (5-7 days). Then, electrolytic lesions of the NRM (1 mA, 30 sec DC current) (Haghparast et al., 2008) and/or vlPAG (0.1 mA, 45 sec DC current) were made by anodal microelectrode. It was lowered into the NRM (AP=10.3-11.3 caudal to bregma, Lat=0.0 and DV=9.1-9.5 ventral from the skull surface) and vlPAG (AP=7.9-8.7 caudal to bregma, Lat=±0.7 lateral to midline, DV=5.9-6.1 ventral from the skull surface).

2.2. Drug Administration

On the day of the experiment a stainless steel needle was directly inserted into the guide cannula, with 1 mm beyond the tip of the latter. 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 sec. Morphine sulfate (Temad Co, Iran) was dissolved in saline freshly on test day and infused in a 0.3 µl volume at the rate of 0.1 µl/15 sec counted on a timer-controlled micrometer. The injector was left in situ for 60 sec after drug administration and was followed by replacement of the occluding stylette.

2.3. Nociceptive Testing

For the purpose of this investigation, morphine analgesia was assessed by the tail-flick test after recovery period. Tail-flick latency (TFL) was recorded at 30, 60, 90 and 120 min after morphine or saline microinjection as an index of analgesia. The heat was applied in succession after the 3, 5 and 7 cm from the caudal tip of the tail. The light source was set at intensity to obtain three consecutive TFLs between 3 and 4 sec. If the animal did not remove its tail from the heater within 12 sec (cut-off point),

the tail was removed from the heat radiant to prevent tissue damage. TFLs (sec) are expressed either as raw data or as percentage of maximal possible effect (MPE%).

2.4. Experimental Procedures and Groups

Eleven experimental groups were used 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; (4-7) vlPAG and vlPAG+NRM sham-lesion groups which bilaterally received saline or morphine in the NCF after recovery period; (8-11) vlPAG and vlPAG+NRM lesion groups that electrolytic lesions were made and bilaterally received saline or morphine in the NCF after recovery period. In all control and experimental groups, TFL was recorded at 30-min intervals after morphine or saline microinjection as an index of analgesia.

2.5. Statistical Analysis

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 Dunnett's test for multiple comparisons, 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 30-min intervals. P-values less than 0.05 were considered to be statistically significant.

2.6. Histological Verification

Upon the completion of behavioral testing, experimental animals were perfused transcardially with heparinized saline followed by buffered 10% Formalin. The brain was removed and stored in buffered Formalin 10% prior to sectioning by using a vibratome (Campden Instruments Ltd, UK). The sections were examined under a stereomicroscope. The most ventral point of the microinjector tips were mapped onto schematics of the appropriate plates using a rat brain atlas. The locations of injection and lesion sites were determined according to the atlas (Paxinos and Watson, 2007). The data reported here are only from animals in which the placement of cannulae and lesion sites were histologically verified (Fig.1 A,B).

3. Results

The average baseline TFL in these experiments was 3.81±0.15 sec. One-way ANOVA revealed that there are no significant differences in TFLs among the intact (n=8), sham-operated (n=8) and a saline control (saline delivered into the NCF in a volume of 0.3 µl/side; n=8) group [$F(2,23)=0.1197$, $P=0.8878$]. So, all experimental animals were compared with saline group as a control and its TFL results considered as baseline in all 30-min intervals.

3.1. Effect of vlPAG electrolytic lesions on antinociceptive effect of morphine microinjected into the NCF

In this set of experiments, electrolytic lesion of vlPAG on antinociceptive response of morphine microinjected into the NCF 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 30-min post-injection times [treatment main effect: $F(3,116)=37.06$, $P<0.0001$, time main effect $F(3,116)=22.04$, $P<0.0001$, treatment×time interaction $F(9,116)=7.136$, $P<0.0001$]. The results showed that morphine microinjected into the NCF, significantly increases TFLs at 30 and 60 min ($P<0.001$) after injection whereas bilateral electrolytic lesions in the vlPAG did not alter the effect of morphine microinjected into the NCF (Fig. 2A). Nevertheless, Fig. 2B showed that the maximal possible effect of morphine, as an analgesic index, is not significantly different in compared with the electrolytic lesions in the vlPAG in both 30 min ($t15=0.1358$, $P=0.8938$) and 60 min ($t15=0.7824$, $P=0.4462$) after the morphine microinjection. Furthermore, there were no significant differences in TFLs between electrolytic lesion and sham-lesion groups in animals that received morphine or saline in the NCF.

3.2. Effect of vlPAG and NRM electrolytic lesions on antinociceptive effect of morphine microinjected into the NCF

In this set of experiments, we examined the effect of concurrent electrolytic lesions of vlPAG and NRM on antinociceptive response of morphine microinjected into the NCF. Two-way ANOVA for repeated measures over time followed by Bonferroni's test resulted a significant treatment (electrolytic lesions of vlPAG and NRM) main effect [$F(3,93)=13.5$, $P<0.0001$], time (morphine post-injection times) main effect [$F(3,93)=5.123$, $P=0.0025$], and treatment×time interaction [$F(9,93)=3.236$, $P=0.0019$] as shown in Fig. 3A. The results showed that morphine microinjected into the NCF, significantly increases TFLs at 30 ($P<0.001$) and 60 min ($P<0.01$) after injection in sham-lesion animals whereas concurrent electrolytic lesions in the vlPAG and NRM could be shifted the TFLs toward the

baseline at 30-min intervals (Fig. 3A). On the other hand, Fig. 3B shows that the concurrent lesions in the vlPAG and NRM significantly decreases the maximal possible effect of morphine microinjected into the NCF at 30 min (t13=3.78, P=0.0023) and 60 min (t13=4.615, P=0.0005) compared with sham-lesion group. Furthermore, the results also showed that lesions of the vlPAG and NRM could not reduce the baseline pain in animals that bilaterally received saline in the NCF.

4. Discussion

Our results showed that lesions solely of bilateral ventrolateral regions of periaqueductal gray are ineffective in the alteration of nociception induced by tail-flick latency test. The ineffectiveness of vlPAG lesions on morphine-induced analgesia in the NCF, as compared to the sham-lesion group, is another evidence for this unexpected finding. On the other hand, concurrent lesions of bilateral vlPAG and NRM significantly decreased TFL following morphine microinjection into the NCF as compared to the same microinjection in sham-lesion group.

Our data are in agreement with the results from previous studies that showed that opioid receptors in the NCF are involved in descending pain modulation (Behbehani and Zemlan, 1986; Beitz, 1990; Haghparast et al., 2007a, 2007b, 2008; Zemlan and Behbehani, 1988). Consistent with other studies (Dostrovsky and Deakin, 1977) the baseline latency did not alter following the vlPAG electrolytic lesions. Electrical stimulation or direct morphine microinjection into the PAG may lead to a disinhibition of descending pain inhibitory pathways (Stiller et al., 1996). It seems that these investigations are in contrast with our data showing there is no significant difference in TFL after bilateral vlPAG lesions. These inconsistencies may be in part due to some technical differences such as kind of lesions (chemical or electrical) and characterizations of the behavioral test or lesion. Several lines of evidence indicated the involvement of μ-opioid receptors in the PAG (Tershner et al., 1995) and RVM (Poore and

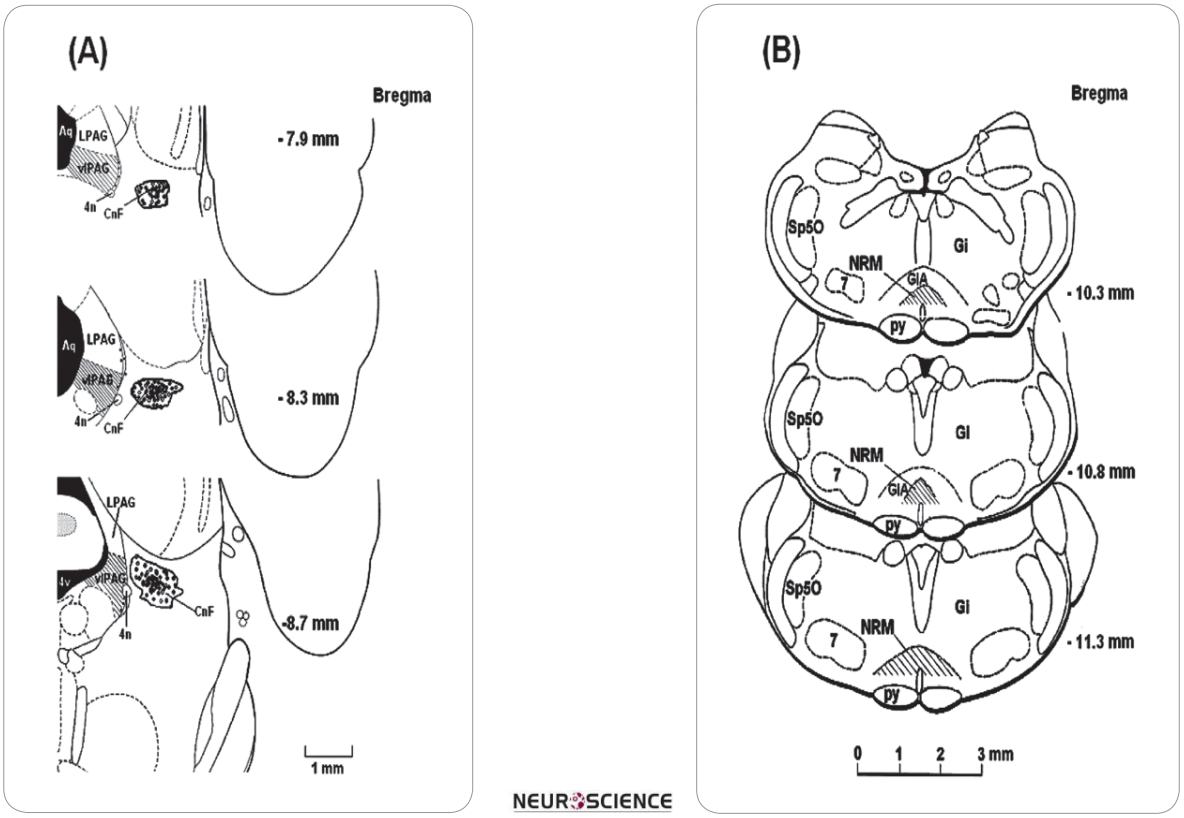
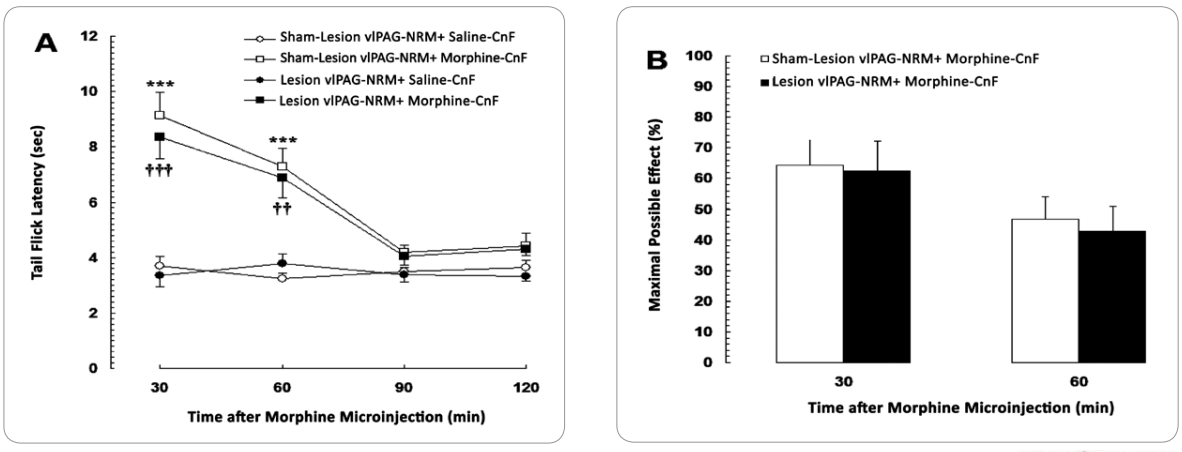


Figure. 1. Schematic microinjection locations and electrolytic lesion sites summarized on three representative coronal sections for (A) the NCF and vlPAG and (B) the NRM. Morphine and saline microinjection sites in the NCF have been shown by filled and open circles, respectively. Hatched regions in vlPAG and NRM indicate the electrolytic lesion areas. 7, Facial nucleus; 4n, Trochlear nerve; 4V, 4th Ventricle; Aq, Aqueduct; NCF, Cuneiform nucleus; Gi, Gigantocellular reticular nucleus; GiA, Gigantocellular reticular nucleus (alpha part); LPAG, Lateral periaqueductal gray; NRM, Nucleus raphe magnus; py, Pyramidal tract; Sp5O, Spinal trigeminal nucleus oralis; vlPAG, Ventrolateral periaqueductal gray.

Helmstetter, 1996) which may reflect general organizational principles related to antinociception at the neural systems. One recent electrophysiological study showed that a large number of PAG cells respond to both chemical and electrical stimulation of the amygdala and that a portion of these responses may be due to release of endogenous opioid peptides within the PAG (da Costa Gomez and Behbehani, 1995). On the contrary, antinociception produced by morphine administration was markedly reduced in the PAG-lesioned rats (Dostrovsky and Deakin, 1977). However, morphine-induced analgesia in the NCF did not alter following vlPAG lesions in the present study. Regarding some similarities between the PAG and NCF in ultrastructural (Gioia and Bianchi, 1987b) and functional (Beitz, 1982) levels, neighboring the vlPAG and NCF (Paxinos and Watson, 2007) and their anatomical projections to the NRM (Jiang and Behbehani, 2001; Zambreanu et al., 2005), we supposed that vlPAG may affect the antinociceptive response of morphine microinjected into the NCF, the same as role of NRM in mediating the morphine-induced analgesia in the NCF (Haghparast et al., 2008). It seems that the relative failure of vlPAG lesions to reduce morphine-induced analgesia may be, in part, due to lack of direct involvement of the population of neurons found in this specific portion of PAG in mediating the antinociceptive actions of morphine in the NCF. A study showed that the PAG, like the RVM, has facilitatory as well as inhibitory influences on nociception (Heinricher et al., 2004). However, Zemlan et al. (1984) concluded that the caudal NCF, PAG and the deep layers of the superior colliculus function in unison to control ventral medullary pain pathways. The present results suggest that there is no considerable projection from NCF to vlPAG at least in the level of morphine-induced antinociception.

On the other hand, our previous data indicated that NRM absence per se was not able to fully revert the analgesic effect of morphine injected in the NCF (Haghparast et al., 2008) but, in the present study, the lesion of both vlPAG and NRM seems to completely revert the analgesic effect of morphine albeit it was non-significant. Moreover, several studies have shown a tonic role for vlPAG in descending pain modulation (Starowicz et al., 2007). Alternatively, it has been shown that the dlPAG plays a critical role in the production of shock-induced hyperalgesia (McLemore et al., 1999). Earlier studies showed that stimulation at ventral PAG areas supported the analgesic effect much shorter than that of dorsal PAG sites (Morgan and Liebeskind, 1987). This indicates that ventral PAG sites and its related regions like vlPAG may have a flexible role in antinociception. Furthermore, it is also likely that the antinociception from PAG stimulation is not equally distributed throughout the body, and that the intensity of the noxious stimulus influences the threshold for stimulation-produced analgesia (Levine et al., 1991). It is possible that neurons, with similar properties to RVM on-cells, in PAG (Heinricher et al., 1987)



*** P<0.001 compared to sham-lesion vlPAG in saline treated rats
†† P<0.01; ††† P<0.001 compared to lesion vlPAG in saline treated rats

Figure. 2. (A) Effect of electrolytic lesion of ventrolateral periaqueductal gray (vlPAG) on antinociceptive response of morphine microinjected into the nucleus cuneiformis (NCF). Non-significant differences were observed in the average of tail-flick latencies at 30-min intervals between the saline vs morphine microinjected into the NCF in both sham-lesion and lesion groups. Morphine microinjection into the NCF significantly increased the TFLs in both vlPAG lesioned and sham-lesioned groups in 30 and 60 min after injection. (B) Maximal possible effect of morphine in vlPAG lesioned and sham-lesioned groups was not significant in 30 and 60 min after microinjection. Each point is the mean ± SEM for 8-9 rats.

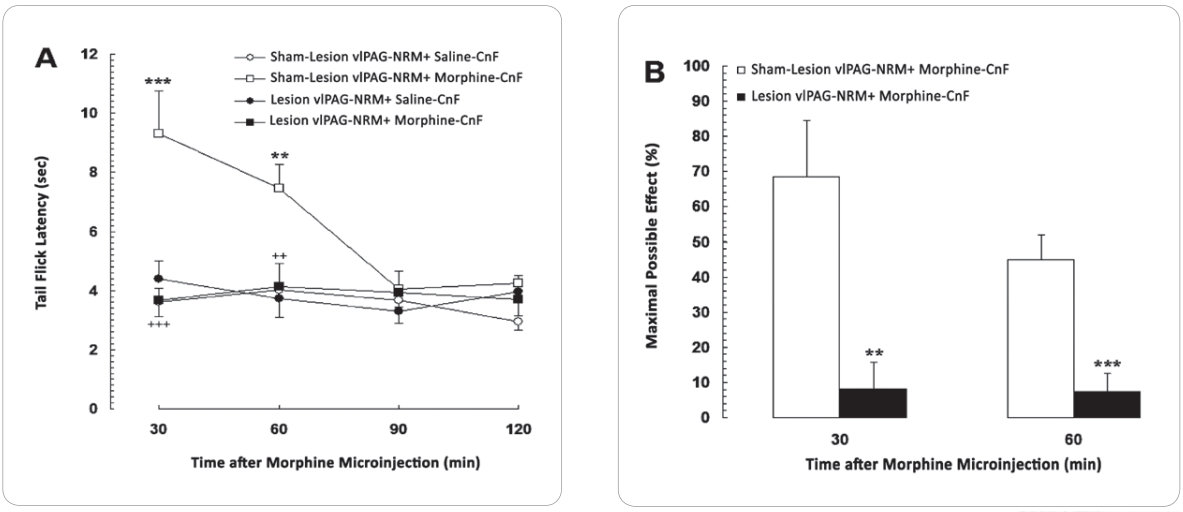
are differently distributed in its subdivisions with less concentration in the vlPAG. As the vlPAG receives opioid projections from other structures such as amygdaloid and hypothalamic complex (da Costa Gomez and Behbehani, 1995; Oliveira and Prado, 2001; Parry et al., 2008) and also the somatosensory cortex and anterior cingulate cortex (Calejesan et al., 2000; Millan, 2002; Tang et al., 2005), it is possible that the crucial role of the vlPAG in the activation of NRM is through the other regions but not via the NCF.

This study showed that concurrent lesions of vlPAG and NRM significantly decrease the morphine-induced analgesia at 30 and 60 min after microinjection of morphine into the NCF. Previous studies reported that there is a functional connection between the PAG and NRM in order to mediate morphine-induced antinociception (Jiang and Behbehani, 2001). On the other hand, the antinociceptive effects of opioids in the PAG or its electrical stimulation are mediated by the RVM (Urban and Smith, 1994; Young et al., 1984), and microinjection of μ-opioid agonist in the PAG suppresses the firing rate of RVM on-cells and causes off-cells to become continuously active (Cheng et al., 1986). It has been also suggested that endogenous opioid peptides are released in the RVM following activation of neurons in the PAG and selectively inhibited on-cells, which presumably have a facilitating action on spinal nociceptive transmission (Pan and Fields, 1996). In the same way, present results show that the vlPAG and NRM concurrent lesions may block the similar mechanisms in both nuclei and the morphine-induced antinociceptive signals, sourced from NCF, is being neglected.

In conclusion, results of this study confirm our previous work that the NCF and NRM containing a neural network in descending pain modulatory pathway in morphine-induced antinociception. At the moment, we found that solely vlPAG did not participate in morphine-induced analgesia in the NCF. However, we think that vlPAG might play its antinociceptive action through the other pathways apart from NCF and it seems that more investigation is needed to further clarify the role of other parts of the PAG (e.g. dorsolateral regions) in morphine-induced antinociception at the level of NCF-NRM.

5. Acknowledgements

This work was supported by the Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.



** P<0.01; *** P<0.001 compared to Sham-Lesion vlPAG and NRM in saline treated rats
++ P<0.01; +++ P<0.001 compared to Lesion vlPAG and NRM in Morphine treated rats

Fig. 3. (A) The comparison of mean tail-flick latencies after microinjection of morphine and saline into the nucleus cuneiformis (NCF) following electrolytic lesion or sham-lesion in the ventrolateral periaqueductal gray (vlPAG) and nucleus raphe magnus (NRM) at 30-min intervals. Concurrent lesions of vlPAG and NRM significantly decreased the TFLs in compared to the sham-lesion group. Morphine microinjected into the NCF in sham-lesion of vlPAG and NRM significantly increased TFLs in compared to the saline-treated rats in 30 and 60 min after injection. (B) Maximal possible effect of morphine microinjected into the NCF was significantly decreased in vlPAG and NRM lesioned animals compared to sham-lesionat 30 and 60 min after microinjection. Each point is the mean ± SEM for 6-9 rats.

References

Basbaum, A.I., Fields, H.L., (1984). Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. Annu Rev Neurosci 7, 309-338.

Behbehani, M.M., Zemlan, F.P., (1986). Response of nucleus raphe magnus neurons to electrical stimulation of nucleus cuneiformis: role of acetylcholine. Brain Res 369, 110-118.

Beitz, A.J., (1982). The organization of afferent projections to the midbrain periaqueductal gray of the rat. Neuroscience 7, 133-159.

Beitz, A.J., (1990). Relationship of glutamate and aspartate to the periaqueductal gray-raphe magnus projection: analysis using immunocytochemistry and microdialysis. J Histochem Cytochem 38, 1755-1765.

Beitz, A.J., (1995). Periaqueductal gray. In: Paxinos, G. (Ed.), In The Rat Nervous System. Academic, New York. pp. 173-182.

Bernal, S.A., Morgan, M.M., Craft, R.M., (2007). PAG mu opioid receptor activation underlies sex differences in morphine antinociception. Behavioural Brain Research 177, 126-133.

Besson, J.M., Fardin, V., Oliveras, J.L., (1991). Antinociception following opioid stimulation of the basolateral amygdala is expressed through the periaqueductal gray and rostral ventromedial medulla. In: Depaulis, A., Bandler, R. (Eds.), The midbrain periaqueductal grey matter. Plenum Press, New York. pp. 121-138.

Bodnar, R.J., (2000). Supraspinal circuitry mediating opioid antinociception: antagonist and synergy studies in multiple sites. J Biomed Sci 7, 181-194.

Calejesan, A.A., Kim, S.J., Zhuo, M., (2000). Descending facilitatory modulation of a behavioral nociceptive response by stimulation in the adult rat anterior cingulate cortex. Eur J Pain 4, 83-96.

Cheng, Z.F., Fields, H.L., Heinricher, M.M., (1986). Morphine microinjected into the periaqueductal gray has differential effects on 3 classes of medullary neurons. Brain Res 375, 57-65.

da Costa Gomez, T.M., Behbehani, M.M., (1995). An electrophysiological characterization of the projection from the central nucleus of the amygdala to the periaqueductal gray of the rat: the role of opioid receptors. Brain Res 689, 21-31.

de Luca, M.C., Brandao, M.L., Motta, V.A., Landeira-Fernandez, J., (2003). Antinociception induced by stimulation of ventrolateral periaqueductal gray at the freezing threshold is regulated by opioid and 5-HT2A receptors as assessed by the tail-flick and formalin tests. Pharmacol Biochem Behav 75, 459-466.

Dickenson, A.H., Oliveras, J.L., Besson, J.M., (1979). Role of the nucleus raphe magnus in opiate analgesia as studied by the microinjection technique in the rat. Brain Res 170, 95-111.

Dostrovsky, J.O., Deakin, J.F.W., (1977). Periaqueductal grey lesions reduce morphine analgesia in the rat. Neuroscience Letters 4, 99-103.

Flores, J.A., El Banoua, F., Galan-Rodriguez, B., Fernandez-Espejo, E., (2004). Opiate anti-nociception is attenuated following lesion of large dopamine neurons of the periaqueductal grey: critical role for D1 (not D2) dopamine receptors. Pain 110, 205-214.

Gioia, M., Bianchi, R., (1987a). The cytoarchitecture of the nucleus cuneiformis. A Nissl and Golgi study. J Anat 155, 165-176.

Gioia, M., Bianchi, R., (1987b). Ultrastructural study of the nucleus Cuneiformis in the cat. J Hirnforsch 28, 375-383.

Haghparast, A., Ahmad-Molaei, L., (2009). Effects of electrolytic lesion of dorsolateral periaqueductal gray on analgesic response of morphine microinjected into the nucleus cuneiformis in rat. Neurosci Lett 451, 165-169.

Haghparast, A., Gheitasi, I.P., Lashgari, R., (2007a). Involvement of glutamatergic receptors in the nucleus cuneiformis in modulating morphine-induced antinociception in rats. Eur J Pain 11, 855-862.

Haghparast, A., Ordikhani-Seyedlar, M., Ziaei, M., (2008). Electrolytic lesion of the nucleus raphe magnus reduced the antinociceptive effects of bilateral morphine microinjected into the nucleus cuneiformis in rats. Neurosci Lett 438, 351-355.

Haghparast, A., Soltani-Hekmat, A., Khani, A., Komaki, A., (2007b). Role of glutamatergic receptors located in the nucleus raphe magnus on antinociceptive effect of morphine microinjected into the nucleus cuneiformis of rat. Neurosci Lett 427, 44-49.

Heinricher, M.M., Cheng, Z.F., Fields, H.L., (1987). Evidence for two classes of nociceptive modulating neurons in the periaqueductal gray. J Neurosci 7, 271-278.

Heinricher, M.M., Martenson, M.E., Neubert, M.J., (2004). Prostaglandin E2 in the midbrain periaqueductal gray produces hyperalgesia and activates pain-modulating circuitry in the rostral ventromedial medulla. Pain 110, 419-426.

Helmstetter, F.J., Tershner, S.A., (1994). Lesions of the periaqueductal gray and rostral ventromedial medulla disrupt antinociceptive but not cardiovascular aversive conditional responses. J Neurosci 14, 7099-7108.

Jensen, T.S., Yaksh, T.L., (1989). Comparison of the antinociceptive effect of morphine and glutamate at coincidental sites in the periaqueductal gray and medial medulla in rats. Brain Res 476, 1-9.

Jiang, M., Behbehani, M.M., (2001). Physiological characteristics of the projection pathway from the medial preoptic to the nucleus raphe magnus of the rat and its modulation by the periaqueductal gray. Pain 94, 139-147.

Jones, S.L., (1992). Descending control of nociception. In: Light, A.R. (Ed.), The Initial Processing of Pain and its Descending Control: Spinal and Trigeminal Systems. Karger, New York. pp. 203-295.

Levine, R., Morgan, M.M., Cannon, J.T., Liebeskind, J.C., (1991). Stimulation of the periaqueductal gray matter of the rat produces a preferential ipsilateral antinociception. Brain Res 567, 140-144.

Loyd, D.R., Murphy, A.Z., (2006). Sex differences in the anatomical and functional organization of the periaqueductal gray-rostral ventromedial medullary pathway in the rat: a potential circuit mediating the sexually dimorphic actions of morphine. J Comp Neurol 496, 723-738.

Loyd, D.R., Murphy, A.Z., (2009). The role of the periaqueductal gray in the modulation of pain in males and females: are the anatomy and physiology really that different? Neural Plast 2009, 462879.

McLemore, S., Crown, E.D., Meagher, M.W., Grau, J.W., (1999). Shock-induced hyperalgesia: II. Role of the dorsolateral periaqueductal gray. Behav Neurosci 113, 539-549.

Millan, M.J., (2002). Descending control of pain. Prog Neurobiol 66, 355-474.

Morgan, M.M., Liebeskind, J.C., (1987). Site specificity in the development of tolerance to stimulation-produced analgesia from the periaqueductal gray matter of the rat. Brain Res 425, 356-359.

Oliveira, M.A., Prado, W.A., (2001). Role of PAG in the antinociception evoked from the medial or central amygdala in rats. Brain Res Bull 54, 55-63.

Pan, Z.Z., Fields, H.L., (1996). Endogenous opioid-mediated inhibition of putative pain-modulating neurons in rat rostral ventromedial medulla. Neuroscience 74, 855-862.

Parry, D.M., Macmillan, F.M., Koutsikou, S., McMullan, S., Lumb, B.M., (2008). Separation of A- versus C-nociceptive inputs into spinal-brainstem circuits. Neuroscience 152, 1076-1085.

Paxinos, G., Watson, C.R., (2007). The Rat Brain in Stereotaxic Coordinates. Elsevier Academic Press, San Diego. pp. 141-149, 162-170.

Poore, L.H., Helmstetter, F.J., (1996). Mu opioids in the RVM contribute to antinociception following DAMGO stimulation of the amygdala. Soc Neurosci Abstr 22, 116.

Starowicz, K., Maione, S., Cristino, L., Palazzo, E., Marabese, I., Rossi, F., de Novellis, V., Di Marzo, V., (2007). Tonic endovanilloid facilitation of glutamate release in brainstem descending antinociceptive pathways. J Neurosci 27, 13739-13749.

Stiller, C.O., Bergquist, J., Beck, O., Ekman, R., Brodin, E., (1996). Local administration of morphine decreases the extracellular level of GABA in the periaqueductal gray matter of freely moving rats. Neurosci Lett 209, 165-168.

Tang, J., Ko, S., Ding, H.K., Qiu, C.S., Calejesan, A.A., Zhuo, M., (2005). Pavlovian fear memory induced by activation in the anterior cingulate cortex. Mol Pain 1, 6.

Tershner, S.A., Helmstetter, F.J., Besson, J.M., (1995). Spinal antinociception following stimulation of the amygdala depends on opioid receptors in the ventral periaqueductal gray. Soc Neurosci Abstr 21, 1169.

Urban, M.O., Smith, D.J., (1994). Nuclei within the rostral ventromedial medulla mediating morphine antinociception from the periaqueductal gray. Brain Res 652, 9-16.

Williams, F.G., Beitz, A.J., (1989). A quantitative ultrastructural analysis of neurotensin-like immunoreactive terminals in the midbrain periaqueductal gray: analysis of their possible relationship to periaqueductal gray-raphe magnus projection neurons. Neuroscience 29, 121-134.

Yang, J., Yang, Y., Xu, H.T., Chen, J.M., Liu, W.Y., Lin, B.C., (2007). Arginine vasopressin induces periaqueductal gray release of enkephalin and endorphin relating to pain modulation in the rat. Regul Pept, 142, 29-36.

Young, E.G., Watkins, L.R., Mayer, D.J., (1984). Comparison of the effects of ventral medullary lesions on systemic and microinjection morphine analgesia. Brain Res 290, 119-129.

Zambreanu, L., Wise, R.G., Brooks, J.C., Iannetti, G.D., Tracey, I., (2005). A role for the brainstem in central sensitisation in humans. Evidence from functional magnetic resonance imaging. Pain 114, 397-407.

Zemlan, F.P., Behbehani, M.M., (1984). Afferent projections to the nucleus cuneiformis in the rat. Neurosci Lett 52, 103-109.