

# **Effect of Cuneiformis Nucleus Inactivation by Lidocaine Microinjection on the Analgesic Response of Morphine in Rats**

Gholamreza Sepehri<sup>\*1</sup> and Mohammad Naser Shafeiee<sup>2</sup>

<sup>1</sup>*Dept. of Physiology and Pharmacology, and Neuroscience Research Center, Kerman University of Medical Sciences, Kerman;* <sup>2</sup>*Dept. of Physiology, Mashhad University of Medical Sciences, Mashhad, Iran*

Received 15 June 2005; revised 14 August 2005; accepted 22 October 2005

## **ABSTRACT**

**The present study was performed to evaluate the analgesic effect of morphine microinjection into the cuneiformis nucleus (CnF) and the effect of inactivation of this area by lidocaine on pain modulation. Rats were anaesthetized by thiopental (45-60 mg/kg/i.p.) and placed in a stereotaxic instrument, and then a guide cannula was implanted just one mm above the CnF. Following surgery and recovery period, various doses of morphine (10, 20 and 40 µg/0.5 µl saline) and lidocaine 5% (0.5 µl) were microinjected into the CnF. Antinociceptive response was measured by tail flick latency (TFL) and maximal possible effect (% MPE) for 25 min at 5-min intervals, before and after any injection in control and experimental groups. The results of this study showed that morphine microinjection into the CnF increased TFL in a dose-dependent manner. TFL was also increased significantly after lidocaine microinjection. However, co-microinjection of morphine and lidocaine increased TFL which was less than morphine microinjection alone. The intravenous morphine injection with lidocaine microinjection increased TFL significantly, as compared to morphine microinjection. These effects were reversed by naloxone administration. In summary, the results of this study showed that morphine microinjection into the CnF caused a significant analgesic response which indicates that CnF may be involved in pain modulation. *Iran. Biomed. J. 10 (1): 21-26, 2006***

*Keywords:* Cuneiformis nucleus (CnF), Morphine, Lidocaine, Tail flick latency (TFL), Pain modulation

## **INTRODUCTION**

**P**revious studies showed that modulation of pain transmission occurs through activation of several descending pain inhibitory pathways [1-3]. Many studies showed that different nuclei in the brain including periaquiductal gray (PAG) rostral ventromedial medulla (RVM), the amygdala, medullary nucleus raphe magnus (NRM) and cuneiformis nucleus (CnF) are among the target for modulation of pain transmissions [4-6]. CnF, a reticular nucleus of the midbrain, extending ventrally to the colliculi in the dorsolateral part of the mesencephalic tegmentum, is located just ventrolateral to the PAG, projects excitatory neurons to NRM [4, 7]. It is proposed that CnF, which is a glutamatergic pathway, mostly receives afferent connections from the forebrain and the midbrain (including the amygdala, PAG, NRM) and

which in turn densely projects to the B3 area in medulla may be involved in modulation of pain transmission [4, 8, 9]. The CnF modulates the nociceptive effects of morphine through the descending pathways [5, 9-11].

Microinjection of opioid agonists into many of these sites (PAG, RVM etc.), inhibits spinal withdrawal reflexes elicited by noxious stimuli [1, 5, 6]. Although the involvement of CnF in modulation of pain transmission is reported by other investigators [4, 5, 8-10], the contribution of CnF in pain modulation and the antinociceptive response of morphine microinjection into this nucleus have not determined yet. So, this study was performed to evaluate the analgesic response of CnF to the morphine microinjection and the effects of its inactivation by lidocaine on the antinociceptive effects of morphine microinjection.

<sup>\*</sup>Corresponding Author; Tel: (+98-341) 211 3187; Fax: (+98-341) 211 3034; E-mail: gsepehri@yahoo.com

## MATERIALS AND METHODS

**Animals.** Ninety male NMRI rats, weighing 200-250 g, at the time of experiment were purchased from Razi Research Institute (Karaj, Iran). The animals were housed two into a cage in hanging baskets on a rack for one week to accommodate the environment. The animal room followed a standard 12 h light/dark cycle. The animals were tested at approximately the same time during their light cycle each day. The temperature of the animal room was kept constant ( $22 \pm 2^\circ\text{C}$ ) and the rats had free access to food and water.

**Surgery.** Animals were anesthetized with thiopental Na (45-60 mg/kg/i.p.) and placed in a stereotaxic frame. The position of CnF nucleus was estimated according to Paxinos and Watson atlas [12]. A guide cannula (5.3 mm in length) was inserted and stereotactically positioned in the area of CnF (AP = 8.3 mm, L = 1.7 mm and D = 6.3 mm with references to bregma and the cortical surface) and secured to skull with dental cement. The animals were allowed to recover from surgery for at least one week prior to the initiation of experimental protocol, to accommodate for handling stress. Microinjection of normal saline, lidocaine and morphine were made during 5 min period through a 33-gauge injection cannula that extended 3 mm bellow the 25-gauge guide cannula tip. Lidocaine microinjection (0.5  $\mu\text{l}$ ) causes inactivation of an area with 500  $\mu\text{m}$  diameter for 5-30 min [13, 14].

**Analgesic test.** Tail flick apparatus [Sparco, Iran] were used to evaluate the analgesic response to 9 groups of rats [intact, sham-operated, saline (0.5  $\mu\text{l}$  microinjection), morphine (10, 20 and 40  $\mu\text{g}/0.5 \mu\text{l}$  microinjection), Lidocaine 5% (0.5  $\mu\text{l}$  microinjection) and intravenous morphine (2 mg/kg)]. A light beam from tail flick apparatus was focused on a fixed point of the tail, 2-3 cm from the tip, and the latency of tail flick was measured. The beam intensity was adjusted so that the average control withdrawal latency was 3-4 s. A 10-s cut-off time was employed to avoid skin damage. Data were expressed as tail flick latency (TFL), or as percentage of maximum possible effect (%MPE), which was calculated as follows [15]:

$$\%MPE = \frac{\text{Post drug latency (s)} - \text{pre- drug latency (s)}}{\text{Cut- off (10 s)} - \text{pre- drug latency (s)}}$$

The TFL time in intact (group I) and sham-operated rats (group II) was measured for 20 min. at 5-min intervals (0, 5, 10, 15, 20). TFL time in treated groups was also measured before and after saline or drug treatment for 25 min. at 5-min intervals (2, 7, 12, 17, 22, 27). The TFL time in each group is expressed as the mean  $\pm$  SEM of 7-15 rats in each group. Naloxone, 2 mg/kg/i.v., was used after morphine to antagonize the analgesic effects of morphine in the groups that received morphine (microinjection or i.v.) and then TFL was measured in naloxone treated animals.

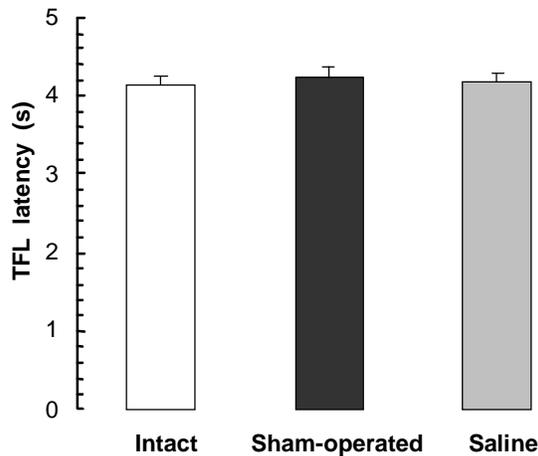
**Histology.** At the completion of the experiment, pontamine sky blue microinjection (0.5-1  $\mu\text{L}$ ) into the CnF was done through the guide cannula. Then, the rats were sacrificed with a lethal dose of thiopental (100 mg/kg/i.p.) and the brains were removed and fixed with 10% formalin for 3 days. Then, the brain slices (50-100  $\mu\text{m}$ ) were prepared and examined for the site of injection into the CnF according to the atlas of Paxinos and Watson [12]. Data were used only if the drugs were injected directly into the CnF.

**Statistics.** Data are the mean  $\pm$  SEM of 7-15 rats in each group. TFL time and %MPE in treated groups were analyzed by analysis of variance (ANOVA) followed by post hoc-Tukey. Repeated measures ANOVA were used to evaluate the group differences to antinociceptive effects, followed by post hoc Tukey if the group differences were significant. Paired student's *t*-test was used to evaluate the differences in mean of TFL or % MPE. The results were considered significant when  $P < 0.05$ .

## RESULTS

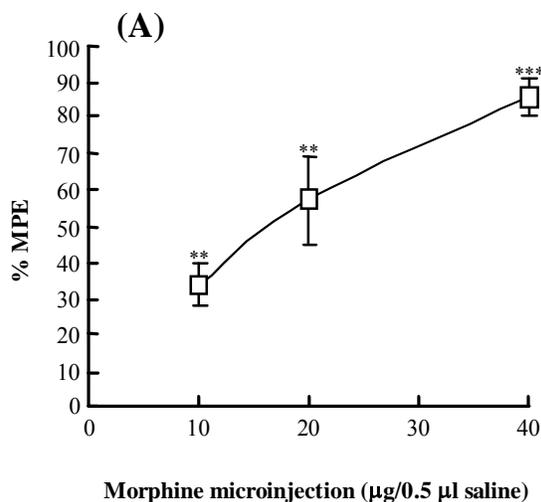
**TFL time in control groups.** Control groups were included intact, sham-operated (cannulated) and saline treated groups. The TFL time was not significantly different in control groups (Fig. 1). So, the saline treated group, which received 0.5  $\mu\text{L}$  normal saline by microinjection into the CnF, was regarded as control group. The TFL time in saline treated group was  $4.11 \pm 0.16$  s.

**Dose-response effects of morphine microinjection into the CnF.** Morphine was microinjected into CnF at doses of 10, 20 and 40  $\mu\text{g}/0.5 \mu\text{L}$  saline and the analgesic response was



**Fig. 1.** Mean of tail flick latency (TFL) time in intact, sham-operated and saline-treated rats. Normal saline (0.5  $\mu$ l) was injected into the cuneiformis nucleus in saline-treated rats. In sham-operated rats, a cannula was inserted into cuneiformis nucleus but no drug was microinjected. Data are expressed as the Mean  $\pm$  SEM of 10 rats in each group. No significant differences were observed in 3 groups of rats.

evaluated by tail flick apparatus. The analgesic response to the microinjection of 10  $\mu$ g morphine was started 2 min after microinjection and the maximum analgesic response was at 27 min after microinjection (% MPE =  $33.80 \pm 5.59$ ) which was significantly different from saline treated groups (Fig. 2), ( $P < 0.0001$ ).

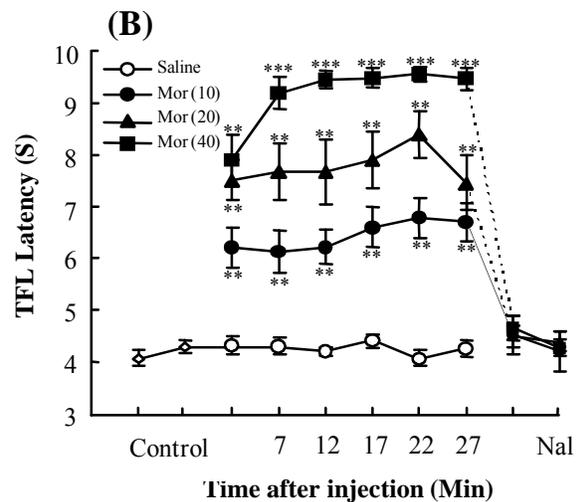


The % MPE increased to  $57.29 \pm 12.8$  and  $85.93 \pm 5.33$  after microinjection of 20 and 40  $\mu$ g of morphine, respectively. Naloxone (2 mg/kg/i.v.) reversed the analgesic response of morphine microinjection (10, 20 and 40  $\mu$ g/0.5  $\mu$ l microinjection) (Fig. 2B).

The results showed that the analgesic response to morphine microinjection into the CnF in rats is dose-dependent (Fig. 2A). The ED 50% of morphine microinjection (20  $\mu$ g/0.5  $\mu$ l saline) was calculated from morphine dose response curve.

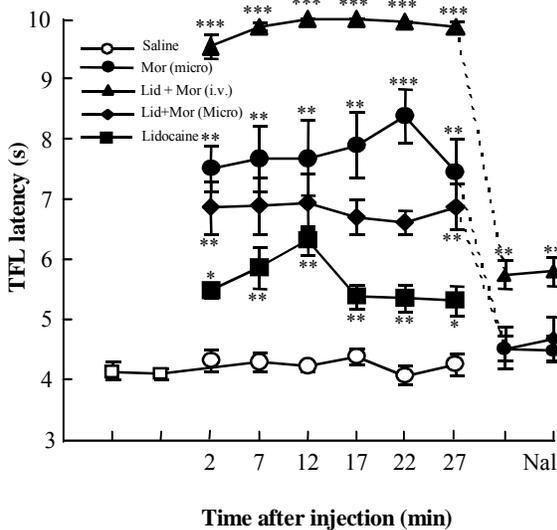
**TFL time after lidocaine microinjection into the CnF.** The lidocaine microinjection caused an increase in TFL time which was significantly different from control group,  $P < 0.0001$ , (Fig. 3). The maximum analgesic response to lidocaine was observed 12 min after microinjection.

**The effect of inactivation of CnF by lidocaine on the analgesic response of morphine microinjection.** CnF was inactivated by lidocaine microinjection into the CnF, and after one min, morphine (20  $\mu$ g/0.5  $\mu$ l saline) was injected into the CnF. The TFL time was significantly increased after lidocaine and morphine microinjection as compared to control ( $6.92 \pm 0.31$  sec in drug-treated v.s.  $4.57 \pm 0.3$  sec in saline treated groups). % MPE increased to  $44.73 \pm 6.76$  in drug treated groups.



**Fig. 2.** Analgesic response to different doses of morphine microinjection into the CnF in rats. (A), % MPE after morphine microinjection; (B), tail flick latency time after morphine microinjection (10  $\mu$ g, 20  $\mu$ g and 40  $\mu$ g) into the cuneiformis nucleus. Naloxone (2 mg /kg/ I.v.) was injected as a pure opioid antagonist to all rats. Data are the mean  $\pm$  SEM of at least 7 rats in each group.  $P$  values as compared to control  $**P < 0.01$ ,  $***P < 0.0001$ .

The ANOVA followed by post hoc Tukey showed that the TFL time in lidocaine and morphine-treated group was significantly increased compared to saline-treated group ( $P < 0.0001$ ). Naloxone (2 mg/kg/i.v.) reversed the analgesic response to morphine and lidocaine microinjection (Fig. 3). The effect of inactivation of CnF by lidocaine on the analgesic response of intravenous morphine injection, the CnF was inactivated by lidocaine 5% (0.5  $\mu$ l) microinjection. Then the TFL time was evaluated after intravenous morphine (2 mg/kg) injection in rats. The TFL time was increased significantly after lidocaine and morphine treatment, and the maximum response was observed after 27 min. (TFL =  $9.86 \pm 0.5$  s, %MPE =  $96.97 \pm 2.43$ ). Repeated measures ANOVA followed by post hoc Tukey showed a significant increase in analgesic response in treated rats as compared to control ( $P < 0.0001$ ). Naloxone (2 mg/kg/i.v.), a  $\mu$  receptor opioid antagonist, reversed the analgesic response of i.v. morphine injection partially, but not completely (Fig. 3).



**Fig. 3.** The effects of cuneiformis nucleus inactivation by lidocaine on the tail flick latency response to thermal stimuli and analgesic response to morphine (microinjection and i.v.). ○, Saline microinjection; □, lidocaine 5% microinjection; ●, Morphine (20  $\mu$ g) microinjection; ◆, lidocaine 5% + morphine (20  $\mu$ g) microinjection; ▲, lidocaine microinjection + morphine (2 mg/kg i.v.). Tail flick latency was measured 5 times every 5 min intervals and then naloxone (2 mg/kg/i.v.) was used as a pure opioid antagonist. Data are expressed as the mean  $\pm$  SEM of at least 7 rats in each group.  $P$  values as compared to saline group. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.0001$ .

## DISCUSSION

The CnF is considered as a part of the pain system which modulates the pain through its anatomical connections with other part of the pain modulating system, especially PAG and RVM which are the major targets for supraspinal analgesic actions of opioids [8, 9]. Although the anatomical connections of CnF with other pain modulating systems have been studied, the contribution of CnF in pain modulation and the antinociceptive response of morphine microinjection into this nucleus have not determined yet. [4]. So, in this study, the effects of reversible inactivation of CnF on the analgesic response of morphine microinjection into the CnF have been evaluated to identify the role of CnF in pain modulation, and as we know, this is a novel work in this area.

In the present study, the microinjection of morphine into the CnF showed a dose-dependent increase in TFL time and analgesic response in rats, which was reversed by naloxone (2 mg/kg/i.v.). These results indicate that CnF has opioid receptors which are involved in pain modulation, and analgesic effect of morphine is mediated through its binding with opioid receptors, especially  $\mu$  receptors [16-18]. Since the reversal of the analgesic response of morphine with naloxone, which antagonizes the opioid receptors, has been proved in other parts of the CNS pain modulating system, including PAG, these results are in agreement with the results of previous studies, which offer that CnF also has opioid receptors involved in pain modulation [1, 19, 20]. Other studies showed that CnF, through its connection with PAG, can modulate pain indirectly via a powerful effect on raphe nucleus and magnocellular nucleus in RVM [5, 9, 21]. About 75% of neurons in raphe nucleus and magnocellular nucleus respond to the electrical stimulation of CnF and caused analgesia through the posterior horn of spinal cord, which indicate that CnF plays a role in pain modulation through RVM [5].

Many studies have been reported the decrease in TFL time and an increase in hyperalgesia following lidocaine microinjection into the PAG and RVM [22, 23]. But, lidocaine microinjection into the CnF caused an increase in TFL time until 12 min after microinjection, contrary to our expectation, as compared with controls. The precise mechanism(s) by which lidocaine microinjection into the CnF increases the TFL time is not known, but it could be related to the inactivation of glutamatergic transmission by lidocaine which causes an increase

in TFL time [1, 5, 24]. The change in pain response following lidocaine administration and the involvement of alpha-adrenoreceptors has been observed in other CNS nuclei, such as anterior pretectal nucleus, so it is possible that lidocaine may affect pain modulation through adrenergic system in the CnF [25, 26]. Previous studies reported that systemic morphine administration will stimulate opioid receptors and caused a marked increase in analgesic response which was reversed completely by naloxone [19, 23]. In this study, systemic morphine (2 mg/kg/i.v.) after lidocaine microinjection caused a marked analgesic response. However, this effect was not reversed completely by naloxone and TFL time in morphine treated rats after lidocaine microinjection was significantly greater than control (sham operated) rats after naloxone treatment, (Fig. 3), because naloxone administration blocked the opioid receptors in CnF and abolishes the analgesic response of morphine microinjection. However, naloxone did not affect the increase in TFL following lidocaine microinjection into the CnF, which could be mediated through the glutamatergic pathway in CnF [1, 5, 10, 11, 24, 27].

In summary, the results of this study showed that morphine microinjection into the CnF caused a significant analgesic response in a dose-dependent manner, which indicates that CnF has opioid receptors that are involved in pain modulation. Further investigation is needed to determine the type of opioid receptors in CnF and their interaction with other brain nuclei which are involved in pain modulation.

#### ACKNOWLEDGMENTS

This project was granted by Kerman Neuroscience Research Council of Kerman University of Medical Sciences (Kerman, Iran).

#### REFERENCES

1. Mc Garaughty, S. and Heinricher, M.M. (2002) Microinjection of morphine into various amygdaloid nuclei differentially affects nociceptive responsiveness and RVM neuronal activity. *Pain* 96: 153-162.
2. Bodnar, R.J. (2000) Spinal circuitry mediating opioid antinociception: Antagonist and synergy studies in multiple sites. *J. Biomed. Sci.* 7: 181-194.
3. Fields, H.L. (2000) Pain modulation: expectation, opioid analgesia and virtual pain. *Prog. Brain Res.* 122: 245-253.
4. Bernard, J.F., Peschanski, M. and Besson, J.M. (1989) Afferents and efferents of the rat cuneiformis nucleus: an anatomical study with reference to pain transmission. *Brain Res.* 490: 181-185.
5. Richter, R.C. and Behbehani, M.M. (1991) Evidence for glutamic acid as a possible neurotransmitter between the mesencephalic nucleus cuneiformis and the medullary nucleus raphe magnus in the lightly anesthetized rat. *Brain Res.* 544: 279-286.
6. Mitchell, J.M., Lowe, D. and Fields, H.L. (1998) The contribution of the rostral ventromedial medulla to the antinociceptive effects of systemic morphine in restrained and unrestrained rats. *Neuroscience* 87(1): 123-133.
7. Gioia, M. and Bianchi, R. (1987) The cytoarchitecture of the nucleus cuneiformis. A Nissl and Golgi study. *J. Anat.* 155: 165-176.
8. Carlson, J.D., Jacono, R.P. and Maeda, G. (2004) Nociceptive excited and inhibited neurons within the pedunculopontine tegmental nucleus and cuneiformis nucleus. *Brain Res.* 1013 (2): 182-187.
9. Zemlan, F.P. and Behbehani, M.M. (1988) Nucleus Cuneiformis and pain modulation: anatomy and behavioral pharmacology. *Brain Res.* 453: 89-102.
10. Morgan, M.M., Heinricher, M.M. and Fields, H.L. (1992) Circuitry linking opioid-sensitive nociceptive modulatory systems in periaqueductal gray and spinal cord with rostral ventromedial medulla. *Neuroscience* 47: 863-871.
11. Pan, Z.Z. and Fields, H.L. (1996) Endogenous opioid-mediated inhibition of putative pain-modulating neurons in rat rostral ventromedial medulla. *Neuroscience* 74 (3): 855-862.
12. Paxinos, G. and Watson, C. (1986) The rat brain in stereotaxic coordinates. 2<sup>nd</sup> ed., San Diego, California, Academic Press Inc.
13. Gelez, H., Archer, E., Chesneau, D., Magallon, T. and Fabre-Nys, C. (2004) Inactivation of the olfactory amygdala prevents the endocrine response to male odour in anoestrus ewes. *Eur. J. Neurosci.* 19 (6): 1581-1590.
14. Sarihi, A., Fathollahi, Y., Motamedi, F., Naghdi, N. and Rashidi-Pour, A. (2003) Effects of lidocaine reversible inactivation of the median raphe nucleus on long-term potentiation and recurrent inhibition in the dentate gyrus of rat hippocampus. *Brain Res.* 962 (1-2): 159-168.
15. Cicero, T.J., Nock, B. and Meyer, E.R. (1996) Gender-related differences in the antinociceptive properties of morphine. *J. Pharmacol. Exp. Ther.* 279: 767-773.
16. Zhu, W. and Pan, Z.Z. (2004) Synaptic properties and post synaptic opioid effects in rat central amygdala neurons. *Neuroscience* 127 (4): 871-879.

17. Bie, B., Fields, H.L., Williams, J.T. and Pan, Z.Z. (2003) Role of alpha1- and alpha2- adrenoceptors in the nucleus raphe Magnus in opioid analgesia and opioid abstinence-induced hyperalgesia. *J. Neurosci.* 23 (21): 7950-7957.
18. Bie, B. and Pan, Z.Z. (2003) Presynaptic mechanism for anti-analgesic and anti-hyperalgesic actions of kappa-opioid receptors. *J. Neurosci.* 23 (19): 7262-7268.
19. Huang, X., Tang, J.S., Yuan, B. and Jia, H. (2001) Morphine applied to the ventrolateral orbital cortex produces a naloxone-reversible antinociception in the rat. *Neurosci. Lett.* 299 (3): 189-192.
20. Harte, S.E., Lagman, A.L. and Borszez, G.S. (2000) Antinociceptive effects of morphine injected into the nucleus parafascicularis thalami of the rat. *Brain Res.* 874 (1): 78-86.
21. Behbehani, M.M. and Zemlan, F.P. (1989) Response of nucleus raphe magnus neurons to electrical stimulation of nucleus cuneiformis: role of acetylcholine. *Brain Res.* 369: 110-118.
22. Pertovaara, A., Wei, H. and Hamalainen, M.M. (1996) Lidocaine in the rostroventromedial medulla and the periaqueductal gray attenuates allodynia in neuropathic rats. *Neurosci. Lett.* 218: 127-130.
23. Urban, M.O. and Smith, D.J. (1994) Nuclei within the rostral ventromedial medulla mediating morphine antinociception from the periaqueductal gray. *Brain Res.* 652: 9-16.
24. Heinricher, M.M., McGaraughty, S. and Farr, D.A. (1999) The role of excitatory amino acid transmission within the rostral ventromedial medulla in the antinociceptive actions of systemically administered morphine. *Pain* 81: 57-65.
25. Dudley, M.W., Butcher, L.L., Kammerer, R.C. and Cho, A.K. (1981) The action of xylamine on central noradrenergic neurons. *J. Pharmacol. Exp. Ther.* 217: 834-840.
26. Villarreal, C.F., Kina, V.A. and Prado, W.A. (2004) Participation of brainstem nuclei in the pronociceptive effect of lesion or neural block of the anterior pretectal nucleus in a rat model of incisional pain. *Neuropharmacology* 47 (1): 117-127.
27. Benderson, J.B., Fields, H.L. and Barbaro, N.M. (1990) Hyperalgesia during naloxone-precipitated withdrawal is associated with increased on-cell activity in the rostral ventromedial medulla. *Somatosens Mot. Res.* 7 (2): 185-203.