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Effect of lidocaine administration at the nucleus locus coeruleus level on lateral hypothalamus-induced antinociception in the rat

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ABSTRACT

Several lines of evidence have shown that stimulation or inactivation of lateral hypothalamus (LH) produces antinociception. In this study, we assessed the role of nucleus locus coeruleus (LC) in antinociceptive response induced by LH stimulation or inactivation in the rat. The cholinergic agonist carbachol (125 nmol/0.5 μl saline) or lidocaine (2%; 0.5 μl) was unilaterally microinjected into the LH with the LC inactivation concurrently. Antinociceptive responses were obtained by tail-flick test and represented as maximal possible effect (MPE) at 5, 10, 15, 20, 30 and 60 min after drug administration. The results showed that microinjection of carbachol into the LH significantly induced antinociception at 5 and 10 min ($p<0.001$). This effect was significantly blocked by microinjection of lidocaine into the LC. On the other hand, microinjection of lidocaine into LH-induced antinociception at 5 ($p<0.01$) and 10 ($p<0.05$) min after administration. However, inactivation of the LC following the LH inactivation increased MPE at 5 min after injection. These findings support the conclusion that antinociception produced by LH stimulation or inactivation involves two separate mechanisms. It seems that analgesic response induced by LH stimulation is mediated in part by the subsequent activation of spinally projecting noradrenergic neurons in the LC cell group.

1. Introduction

It appears that the lateral hypothalamus (LH) is implicated as a part of descending system involved in the modulation of nociceptive transmission at the level of spinal cord dorsal horn. Electrical stimulation of LH is associated with inhibition of the spinal nociceptive tail-flick reflex (Aimone and Gebhart, 1987) and transmission (Carstens et al., 1983). It supports a role for the LH in system(s) of descending spinal inhibition. It has also been shown that microinjection of the cholinergic agonist carbachol into the LH (LH stimulation) increases the response latencies on both the tail-flick and foot-withdrawal tests (Holden and Naleway, 2001). Moreover, reversible inactivation of the LH by lidocaine induces analgesic effect in formalin test (Tasker et al., 1987). These findings support the hypothesis that the LH is involved in nociceptive modulation; however it is less clear how the LH accomplishes this modulation. It seems that LH-induced antinociception appears to be mediated in part through interaction with brainstem nuclei. For example, electrical stimulation of the LH produces antinociception that is mediated in part by neurons in the periaqueductal gray (PAG) (Behbehani et al., 1988) and the nucleus raphe magnus (NRM) (Aimone et al., 1988). Stimulation of the LH elicits antinociception via (possibly glutamatergic system) relays to the PAG and NRM, which ultimately trigger the activation of descending noradrenergic pathways (Aimone et al., 1988; Behbehani et al., 1988; Cechetto and Saper, 1988; Dafny et al., 1996; Franco and Prado, 1996; Holden and Naleway, 2001). It has been shown that the electrical stimulation of the LH increases nociceptive response latencies (Aimone and Gebhart, 1987) that are reversed by intrathecal α_2-adrenergic antagonists (Holden and Naleway, 2001). The same group of investigators has recently showed that antinociception produced by activating neurons in the LH is mediated in part by the subsequent activation of spinally projecting neurons in the rostral ventromedial medulla (Holden and Pizzi, 2008).

Although previous studies demonstrated that the LH has direct projections to the spinal cord dorsal horn, none of these spinally projecting neurons have been shown to contain noradrenaline (Cechetto and Saper, 1988; Jones, 1992; Yeomans and Proudfit, 1992), nor do noradrenaline-containing neurons occur in the spinal cord dorsal horn (Carlsson et al., 1964). Although Tasker et al. (1987) have indicated that LH inactivation-induced antinociception might be produced by a blockade of ascending pathways between the hindbrain and forebrain structures such as the medial forebrain bundle (MFB), several studies showed that noradrenergic neurons within the brainstem are involved in LH stimulation-induced antinociception (Holden and Naleway, 2001; Holden et al., 2002; Jones, 1991; Proudfit, 1988). The spinal cord is innervated both by adrenergic cell clusters in medullary nuclei, and noradrenergic nuclei localized in pontine

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regions such as the nucleus locus coeruleus (LC) or the A6 (Millan, 2002). The LC or the A6 cell group in the pons is the major site of noradrenergic cell bodies in the brain (Ungerstedt, 1971).

The LC has a significant role in noradrenergic pain modulation. In this regard, there are some reports in which its chemical or electrical activation produces antinociception that can be inhibited by spinal administration of alpha-2-adrenoceptor antagonists (Jones, 1991; Proudfit, 1988). Painful stimulation induces impulse discharge (Hirata and Aston-Jones, 1994) and release of neurotransmitters (Sajedianfard et al., 2005; Singewald and Philippu, 1998) in the LC, and its bilateral lesion has a pain facilitatory effect in inflamed but not in healthy animals (Tsuruoka and Willis, 1996). Considering these controversies in the mechanisms involved in LH-induced antinociception by stimulation and/or inactivation through other brainstem structures, we aimed to examine the effect of reversible inactivation of the LC on LH stimulation- or inactivation-induced antinociception in the rat.

2. Materials and methods

2.1. Animals and surgical preparation

Sixty four male Wistar rats (230–280 g) were housed three per cage and allowed free access to rats chow and 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 groups were unilaterally prepared with a guide cannula implantation (23 gauge needle) at least 5–7 d 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 cannulae were stereotaxically (Stoelting, stereotaxic apparatus, USA) implanted in the LC and/or LH. The coordinates for these regions were determined by the rat brain atlas of Paxinos and Watson, (2007) as AP = −9.8 mm caudal to bregma, Lat = +1.3 mm lateral to midline, DV = −7.2 mm ventral from the skull surface for LC (guide cannula was 1 mm above the appropriate injection place) and for the LH was AP = −2.8 caudal to bregma, Lat = +1.2 and DV = −8.6 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 recovery period (5–7 d), a stainless steel obdurator was inserted into 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.

2.2. Drug administration

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 LC and/or LH. The injector cannula was connected to a 1-μl Hamilton syringe by polyethylene tubing (PE-20) and 0.5 μl of drug solution or vehicle infused over 50 s and was left for the 60 s extra time and followed by replacement of the obdurator. Carbachol (Sigma-Aldrich, USA) and Lidocaine (Sigma-Aldrich, USA) were dissolved in normal saline at the dose of 125 nmol and 2% concentration ratio, respectively. Both drug solutions were freshly prepared on the test day and infused in a 0.5 μl volume at the rate of 0.1 μl/10 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.

2.3. Nociceptive testing

The nociceptive threshold was measured by the tail-flick apparatus (Harvard Apparatus, 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 in each time point. 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 that yields baseline TFL values in the range of 3–4 s (about 35% of maximal light intensity). 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

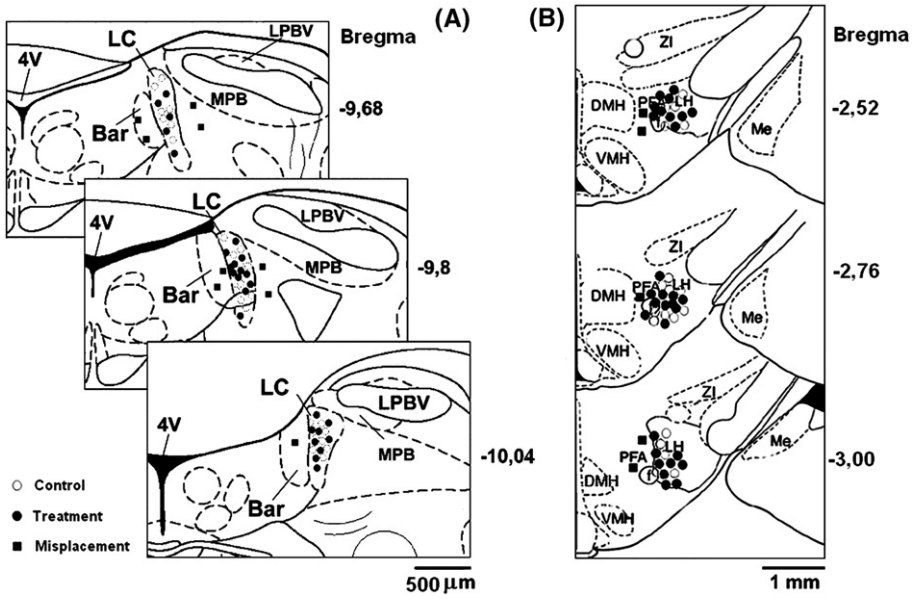

Fig. 1. Schematic diagrams adapted from atlas (Paxinos and Watson, 2007) showing the locations of the microinjection sites into the (A) nucleus locus coeruleus (LC) and (B) lateral hypothalamus (LH). Numbers indicated the distance in mm from Bregma. Abbreviations: 4 V, 4th ventricle, Arc, arcuate nucleus; f, fornix; Bar, Barrington's nucleus; DMH, dorsomedial hypothalamus; LH, lateral hypothalamus; LPBV, lateral parabrachial nucleus; MBP, medial parabrachial nucleus; Me, medial amygdala nucleus; PFA, perifornical area; VMH, ventomedial hypothalamus; ZI, zona incerta.

or percentage of maximal possible effect (%MPE) which was calculated from the following formula:

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

2.4. Experimental protocol and data analysis

In this study, there were 8 groups ($n = 8$ for each group) as follows: (1–3) control groups contain intact, sham and saline groups for determining the baseline TFLs, surgical manipulation and microinjection volume effects, respectively; (4) LH stimulation group that received 125 nmol carbachol (Holden and Naleway, 2001; Holden and Pizzi, 2008; Holden et al., 2002) unilaterally in the LH; (5–6) LH or LC inactivation groups that unilaterally received lidocaine 2% in the LH or LC; (7) LH stimulation and LC inactivation group that lidocaine was microinjected into the LC following the carbachol microinjection into the LH and (8) LH and LC inactivation group that lidocaine was microinjected into the LC following lidocaine microinjection into the LH in order to find out the role of the LC inactivation in descending pain modulatory pathway from the LH. In all above control and experimental groups, TFLs were recorded at 5, 10, 15, 20, 30 and 60 min after saline, carbachol and/or lidocaine administration.

The results obtained are expressed as mean ± SEM (standard error of the mean). The mean TFL (s) or MPE (%) values in all groups were subjected to one-way and/or two-way ANOVA followed by protected post-hoc tests for multiple comparisons, as needed. *p*-values less than 0.05 were considered to be statistically significant.

2.5. Histological verification

After completion of the experiments, animals were deeply anesthetized with ketamine and xylazine, they were transcardially perfused with 0.9% saline and 10% formaldehyde solution; animals died as a result of hemorrhage and toxic effect of formaldehyde. Then after sectioning, neuroanatomical location of cannulae tips was confirmed using the rat brain atlas (Paxinos and Watson, 2007). The data reported here are only from animals in which the placements of cannulae were histologically verified, fourteen rats were excluded due to cannula misplacement (Fig. 1).

3. Results

3.1. Nociceptive effects in control rats

The average baseline TFL in intact group was 3.51 ± 0.24 s at the first trial. Two-way ANOVA followed by Tukey's test revealed that there were no significant differences in mean TFLs among the intact, sham and saline control (saline microinjected into the LH and LC in a volume of 0.5 µl) groups in all time set intervals [Factor treatment: $F(2144) = 0.1692$, $p = 0.8445$; Factor time: $F(6144) = 1.643$, $p = 0.1395$ and Interaction: $F(12,144) = 0.1444$, $p = 0.9997$; Fig. 2]. Therefore, all experimental animals were compared with saline group as a control and its TFL results considered as baseline in all set intervals.

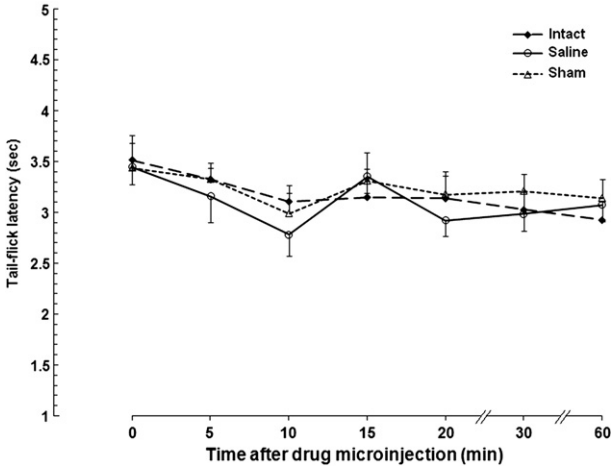

Fig. 2. The mean tail-flick latencies in the intact, sham and saline control groups during 60-min period. Each point is the mean ± SEM for 8–10 rats.

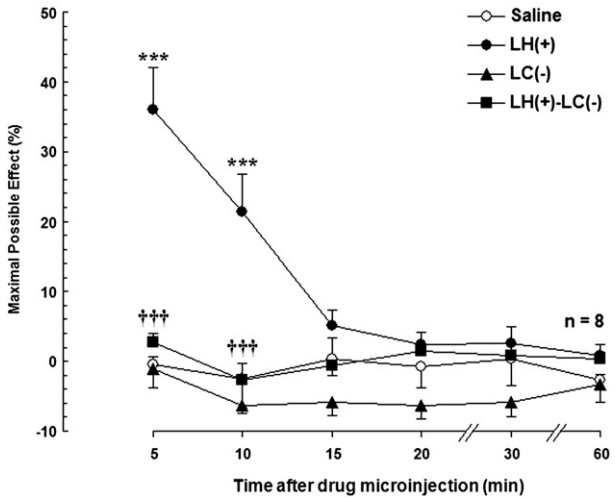

Fig. 3. Antinociceptive responses induced by the lateral hypothalamus (LH) stimulation and the nucleus locus coeruleus (LC) inactivation during 60-min period after drug administration. Saline-treated animals unilaterally received saline into the LC and LH. Each point is the mean ± SEM for 8–10 rats. LH(+) = LH stimulation, LH(+)LC(−) = LH stimulation and LC inactivation, LC(−) = LC inactivation. *** $p < 0.001$ compared to saline group. ††† $p < 0.001$ compared to LH stimulation group.

3.2. Effect of reversible inactivation of the LC on antinociceptive response of carbachol-induced stimulation in the LH

Two-way ANOVA followed by Bonferroni's test indicated significant differences in antinociceptive responses to carbachol microinjected

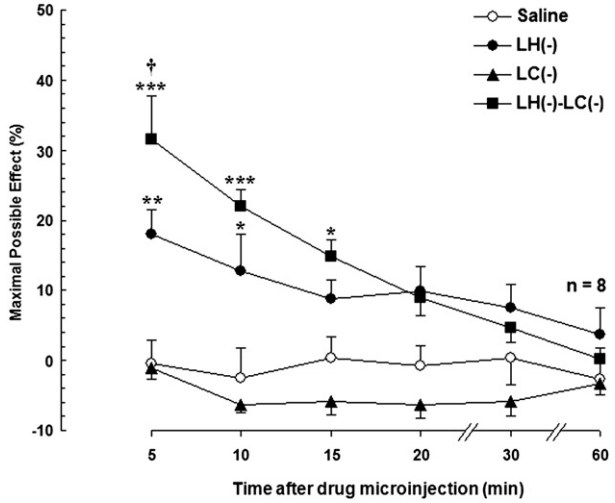

Fig. 4. Antinociceptive responses induced by the lateral hypothalamus (LH) and the nucleus locus coeruleus (LC) inactivation during 60-min period after drug administration. Saline-treated animals unilaterally received saline into the LC and LH. Same data for LC(−) group appeared in Fig. 3 were used in this figure as well. Each point is the mean ± SEM for 8–10 rats. LH(−) = LH inactivation, LH(−)LC(−) = LH and LC inactivation, LC(−) = LC inactivation. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared to saline group. † $p < 0.05$ compared to LH inactivation group.

into the LH alone or when lidocaine concurrently administrated into the LC at post-injection times as compared to those of respective saline-treated group [Factor treatment: $F(3134)=35.83$, $p<0.0001$; Factor post-injection time: $F(5134)=7.268$, $p<0.0001$ and Interaction: $F(15,134)=6.06$, $p<0.0001$]. Fig. 3 shows that sole microinjection of carbachol (125 nmol/0.5 μl saline unilaterally) into the LH provoked an increase in MPEs (LH stimulation-induced antinociception) at 5 and 10 min after carbachol administration ($p<0.001$). Noteworthy, at 5 and 10 post-injection times, LH stimulation-induced antinociception was blocked by concurrent microinjection of lidocaine (reversible inactivation) into the LC ($p<0.001$). Moreover, there were no significant differences in MPEs between LH stimulation and LC inactivation group and saline treated animals in all set intervals.

3.3. Effect of reversible inactivation of the LC on the antinociceptive response of LH inactivation by lidocaine

Fig. 4 shows that microinjection of lidocaine 2% (0.5 μl unilaterally) into the LH results in an increase in MPEs only at 5 and 10 min ($p<0.05$) post-drug administration. Two-way ANOVA followed by Bonferroni's test indicated significant differences in antinociceptive responses to the lidocaine-induced reversible inactivation of the LH alone and when lidocaine concurrently microinjected to the LC at post-injection times as compared to those of respective saline-treated group [Factor treatment: $F(3160)=43.15$, $p<0.0001$; Factor post-injection time: $F(5160)=6.824$, $p<0.0001$ and Interaction: $F(15,160)=2.820$, $p=0.0006$]. However, concurrent microinjection of lidocaine into the LC could not remove analgesic effect of the LH inactivation in LH(−)LC(−) group. In this group, the %MPE significantly increased at 5-min point after drug administration as compared to that of the solely LH inactivation [LH(−)] group ($p<0.05$; Fig. 4). However, reversible inactivation of LC alone [LC(−)] could not affect the TFLs in any time intervals after administration of lidocaine into the LC.

On the other hand, data obtained in Fig. 5 indicates that MPEs in the LH stimulation group were higher than those in the LH inactivation group at 5 (35.91 ± 6.1% vs. 18.05 ± 3.4%) and 10 (21.38 ± 5.3% vs. 12.83 ± 5.1%) time points after drug administration. However, individual group comparisons revealed that there was a significant difference in carbachol-induced antinociception as compared to lidocaine-induced antinociception at 5-min point ($t_{(13)}=2.416$, $p<0.05$; Fig. 5).

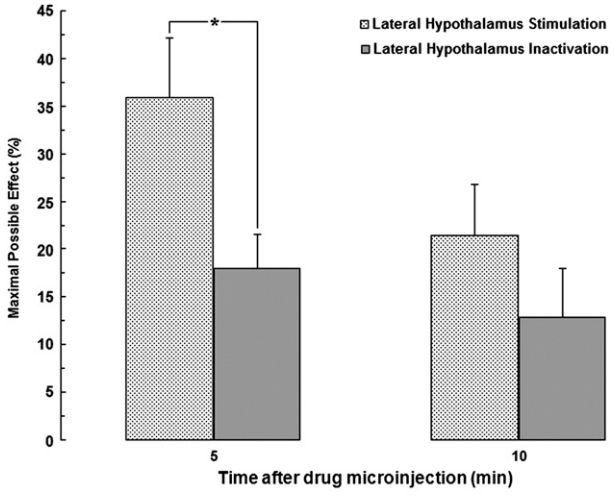

Fig. 5. The maximal possible effect of carbachol microinjected into the lateral hypothalamus (LH), was significantly more than lidocaine into the LH at 5 min after the microinjection. Each point is the mean ± SEM for 6–8 rats. LH(+) = LH stimulation, LH(−) = LH inactivation. * $p<0.05$.

4. Discussion

The purpose of this study was to evaluate the involvement of the LC in the antinociceptive responses induced by LH stimulation or inactivation in the rat. The major findings are: (1) chemical stimulation or inactivation of LH by carbachol- or lidocaine-induced antinociceptive effect in tail-flick test, respectively (2) administration of lidocaine into the LC blocked the carbachol- but not the lidocaine-induced antinociception (3) microinjection of lidocaine into the LC in the absence of LH-induced antinociception elicited a non-significant decrease in response latencies when compared to latencies of rats receiving saline.

The results of the first set of experiments in the present study are matched and are in agreement with those from other studies which have provided evidence that the LH mediates antinociception through noradrenergic brainstem neurons. In essence, the present study emphasizes the role of LC in this phenomenon. Based on previous findings the LH electrical stimulation or microinjection of carbachol in this area produces antinociception that is mediated by spinally projecting noradrenergic neurons (Aimone and Gebhart, 1987; Holden and Naleway, 2001; Holden et al., 2002). Inhibition of the spinal nociceptive tail-flick reflex (Aimone and Gebhart, 1987) and spinal nociceptive transmission (Carstens et al., 1983) by electrical stimulation in the LH supports a role for the LH in descending spinal inhibitory systems. However, the stimulation of LH activates two populations of spinally descending noradrenergic neurons that have opposing effects on nociception. One of these populations inhibits nociception by activating α_2-adrenoceptors in the spinal cord dorsal horn, while the other facilitates nociception by activating α_1-adrenoceptors (Holden and Naleway, 2001).

On the other hand, the microinjection of lidocaine into the midbrain, pons, or medulla resulted in a significant increase in the stimulation threshold in the LH for inhibition of the tail-flick reflex. This indicates that the neuronal pathways which mediate descending inhibition from the LH might pass through these areas (Aimone et al., 1988). Previous studies showed that intrathecal administration of alpha-2-adrenoceptor antagonists reduces the analgesia induced by the chemical or electrical activation of LC (Jones, 1991; Proudfit, 1988). Painful stimulation induces impulse discharge (Hirata and Aston-Jones, 1994) and release of neurotransmitters (Sajedianfard et al., 2005; Singewald and Philippu, 1998) in the LC, and its bilateral lesion has a pain facilitatory effect in inflamed but not in healthy animals (Tsuruoka and Willis, 1996).

There are some controversial findings on the role of LC in LH-induced antinociception. For instance, it has been reported that microinjections in the dorsolateral pons into and adjacent to the locus coeruleus/subcoeruleus (LC/SC) area did not affect the stimulation threshold in the LH (Aimone et al., 1988). Findings of this study implicate pontine fibers of passage, rather than spinally projecting noradrenergic neurons, as mediators of antinociception from focal electrical stimulation of the LH, and leave an unanswered question of how the LH produces antinociception (Aimone et al., 1988). West et al. (1993) demonstrated sub-strain differences in innervations of the dorsal horn by LC or the A7 cell group between Sprague–Dawley rats from Harlan and Sprague–Dawley rats from Sasco. They showed that in the Harlan rats, the LC projected to the dorsal horn and the A7 cell group projected to the ventral horn. In Sasco rats, it was reversed. Holden and Naleway (2001) used female Sasco-derived type Sprague–Dawley rats, so it is assumed that the A7 cell group was involved in LH-induced antinociception mediated by alpha-adrenergic receptors. Aimone et al. (1988) used male Sprague–Dawley rats but the derivation is not specified. It seems like this sub-strain difference, and also the sex difference, may be important facts in this matter. Behavioral evidence in our study suggests that there might be a connection from LH to LC to the dorsal horn in the male Wistar rats which is supported by the anatomical evidence (Bourgin et al., 2000).

However, these controversies may be mediated by some other factors such as the kind of LH stimulation (chemical or electrical stimulation), and/or lidocaine injection sites (LC/SC region not in LC) and experimental models; Aimone et al. (1988) and Holden and Naleway (2001) both used the light anesthesia model while our study was done in awake rats.

Several lines of evidence indicate that antinociceptive response is inducible by reversible inactivation of the LH by lidocaine in addition to LH stimulation by carbachol. Therefore, our findings in this set of experiment are consistent with the previous works (Holden and Naleway, 2001; Holden et al., 2002; Tasker et al., 1987). The use of lidocaine to produce a time-limited, reversible, functional block in the brainstem of the rat has been described by Sandkuhler and Gebhart (1984). Antinociception induced by lidocaine microinjection into the LH was reported previously (Tasker et al., 1987). In the present study, we showed that there was an antinociceptive effect of lidocaine microinjected into the LH in the tail-flick test as well. This analgesic effect could not be blocked by LC inactivation. Pain as a multi-dimensional experience involves sensory processing as well as higher brain functions such as emotion, motivation and cognition (Tasker et al., 1987). The earlier studies have produced antinociception by electrical stimulation of brain areas and have been concerned only with descending pathways. There are also ascending pathways between hindbrain and forebrain structures, such as the MFB, that connect various regions of the limbic system with each other and with a variety of forebrain, midbrain and hindbrain areas (Carpenter and Sutin, 1983). Fibers projecting rostrally to the hypothalamus from the NRM and other regions of the medulla have been traced electro-physiologically (Lumb and Wolstencroft, 1985) and anatomically (Sakumoto et al., 1978; Takagi et al., 1980). Therefore, with regard to the aforementioned findings, it seems that lidocaine affects both cell bodies and ascending fibers of passage, nonspecifically blocking neuronal conduction/transmission (Aimone et al., 1988) while carbachol, as a cholinergic agonist, affects only the cell body of neurons. However, it is possible that the effect of lidocaine in LH inactivation is due to suppression of the descending fibers that may tonically affect the LC.

In another set of experiment, inactivation of the LH and the LC together provided more antinociceptive effect than inactivation of the LH alone, while lidocaine in the LC alone actually produced a mild pronociceptive response. Holden and Naleway (2001) reported that the LH stimulation produces either antinociception or pronociception depends on which alpha-adrenoceptor subtype is activated in the dorsal horn. LC neurons are spinally descending, and release norepinephrine into the dorsal horn. This opposing response might come into play in our experiment.

On the other hand data indicates that MPEs in the LH stimulation group were higher than those in the LH inactivation group. Such a difference may be related to a function of the LH, difference in site of effect of two drugs that was mentioned above but also to the onset of action for lidocaine versus carbachol. Reversible inactivation of LC alone could not impress the TFL times in our study. It has been shown that the degree of spontaneous activity of descending noradrenergic pathways appears to be modest. However, as a function of the internal and external environment of the organism, changes in their activity fulfill a major contribution to fluctuations in the intensity of descending inhibition (Millan, 2002). The lack of behavioral significance following inactivation of the LC, without the LH activation, is probably supposed that LC input to the dorsal horn is not tonically active in Wistar rats. It may be due to sub-strain differences mentioned above involved in this phenomenon.

In this study, we did not determine the kind of neurotransmitters released in the LC during LH stimulation and the neurotransmitters involve in this antinociceptive effect are unraveled. We suggest that further studies need to elucidate the mechanism of this phenomenon. Due to densely orexinergic fibers from the LH to LC (Bourgin et al., 2000) and analgesic effects of orexin reported in previous studies (Bingham et al., 2001; Mobarakeh et al., 2005; Suyama et al., 2004; Watanabe et al., 2005; Yamamoto et al., 2002), we speculate that LH stimulation-induced antinociception may be related to the release of this neurotransmitter in the LC but our study did not provide evidence that orexinergic fibers synapse on spinally projecting adrenergic neurons in the LC. In conclusion, the data from the present study support the hypothesis that LH-induced antinociception is mediated in part by neurons in the LH which innervate or activate spinally projecting noradrenergic neurons located in the LC catecholamine cell group in the pontine tegmentum area. However further investigations are necessary to prove this hypothesis.

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