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Research Report

Intra-paragigantocellularis lateralis injection of orexin-A has an antinociceptive effect on hot plate and formalin tests in rat

Elaheh Erami[a], *Hassan Azhdari-Zarmehri*[a,b,*], *Elmira Ghasemi-Dashkhasan*[a,b], *Mohammad-Hossein Esmaeili*[a,b], *Saeed Semnanian*[c]

[a]*Cellular and Molecular Research Center, Qazvin University of Medical Sciences, Qazvin, Iran*
[b]*Department of Physiology, School of Medicine, Qazvin University of Medical Science, Qazvin, Iran*
[c]*Department of Physiology, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran*

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ABSTRACT

In the present study, the effect of orexin-A (ORXA) microinjection into the paragigantocellularis lateralis (LPGI) on nociceptive behaviors, using hot-plate and formalin tests as thermal and chemical models of pain in rat, was examined. Also, we determined whether the pretreatment with SB-334867, a selective OX1-receptor antagonist, would prevent the antinociceptive effect of orexin-A. ORXA (0.1–100 nM/0.5 μL) microinjected into the LPGi nucleus, dose-dependently decreased the formalin induced nociceptive behaviors and also produced a dose-dependent antinociceptive effect in the hot-plate test. Pretreatment with a selective orexin receptor 1 (OX1R) antagonist, SB-334867, also inhibited the effect of ORXA on formalin induced nociceptive behaviors while the SB-334867 (100 μM) alone had no effect on formalin test. These data demonstrated that the ORXA-induced antinociception in formalin test is mainly mediated through the OX1R in LPGi which might play a potential role in processing the pain information associated with descending pain modulation.

1. Introduction

Orexin-A (ORXA, a cyclic 33-amino acid peptide also known as hypocretin-1) and orexin-B (ORXB, or hypocretin-2, a linear, 28-amino acid peptide) are produced from the precursor protein prepro-orexin in neurons and restricted to a few regions of the lateral hypothalamus (LH) (Peyron et al., 1998; Sakurai et al., 1998). ORXA and ORXB activate the two G-protein coupled receptors, orexin 1 receptor (OX1R) and orexin 2 receptor (OX2R) (Sakurai et al., 1998). Several lines of evidence imply that the orexin is involved in reward, addiction (Aston-Jones et al., 2009, 2010; Harris et al., 2005), and nociceptive sensory processes (Azhdari Zarmehri et al., 2008, 2011; Bingham et al., 2001; Mobarakeh et al., 2005; Yamamoto et al., 2002). ICV injection of ORXA (hypocretin-1) has been shown to elicit analgesic responses processes (Bingham et al., 2001; Mobarakeh et al., 2005; Yamamoto et al., 2002) and OX1R is involved in responsiveness to both pain and stressful stimuli (Sofi-Abadi et al., 2011; Watanabe et al., 2005). However, the locations of central sites that may mediate

*Corresponding author at: Cellular and Molecular Research Center, Qazvin University of Medical Sciences, Qazvin, Iran. Fax: +98 281 3336005.
E-mail address: hasan.azhdari@gmail.com (H. Azhdari-Zarmehri).

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these effects have not been clearly elucidated. The orexin receptors and orexinergic projections are localized in regions previously shown to play a role in pain modulation such as periaqueductal gray (PAG), locus coeruleus, and paragigantocellularis lateralis (LPGI) (Mondal et al., 1999; Peyron et al., 1998). The LPGi in the medulla is implicated in several functions including cardiovascular control, respiration, pain, and analgesia (Van Bockstaele et al., 1989; Van Bockstaele and ston-Jones, 1995; Zhou et al., 1993, 1995. Furthermore, the LH appears to modify nociception, in part, through the brain stem (Behbehani et al., 1988; Holden and Pizzi, 2008) and there are even several studies in which the interaction of LH with LPGi is documented (Andrezik et al., 1981; Li and Lovick, 1985). Nevertheless, the role of ORXA within LPGI in pain control has not been determined yet. In our previous study, we have shown that the ORXA has a moderate effect on PAG in formalin induced nociceptive behaviors (Azhdari Zarmehri et al., 2011). There is widespread projections of orexinergic pathway and orexin receptors expression in LPGI (Ciriello et al., 2003; Mondal et al., 1999; Peyron et al., 1998). Therefore, in this study, the effect of ORXA microinjection into LPGI on formalin induced nociceptive behaviors and hot-plate test was investigated. To further verify the role of OX1Rs in the LPGi, the effect of the selective OX1R antagonist on antinociceptive effect of ORXA was also examined in formalin test.

2. Results

2.1. *Intra-LPGi application of ORXA decreased formalin-induced nociceptive behaviors*

Formalin injection into the plantar surface of the right hind paw produced typical biphasic nociceptive responses (Abbott et al., 1995; Dubuisson and Dennis, 1977). The first and second phases were separated by a brief inter-phase where the nociceptive behaviors were sporadically observed in the vehicle group. The columns represent the mean nociceptive score at each phase: phase 1 (minutes 1–7), inter-phase (minutes 8–14), and phase 2 (minutes 15–60) for all figures. Only those rats with microinjection site and diffusion located within the nucleus were included in the results (Fig. 1, data from 100 nM orexin-A are shown). To determine the appropriate dose of ORXA for microinjection into the LPGi and showing the antinociceptive effect in formalin test, the orexin microinjection into the LPGi started from 0.1 nM concentration (Fig. 2C). In part B of the figure, the average pain scores of different doses of ORXA 0.1 nM ($n=4$), 0.0.5 nM ($n=5$), 10 nM ($n=4$), 50 nM ($n=6$), and 100 nM in 0.5 μl at different phases of formalin test is shown. The antinociceptive effect of ORXA was observed from 10 nM in phase 1 and 1 nM in phase 2 of the formalin test. Intra-LPGi microinjection of 10, 50, and 100 nM of ORXA caused a decrease in formalin-induced nociceptive behaviors in phase 1 as compared to saline-treated rats (Fig. 2C, $P<0.05$ by ANOVA), and the effect was dose-dependent. Similarly, the intra-LPGi microinjection of ORXA (1, 10, 50, and 100 nM) also caused a decrease in formalin-induced nociceptive behaviors in phase 2 as compared to saline-treated rats (Fig. 2C, $P<0.05$ by ANOVA), and again the effect occurred in a dose-dependent manner (Fig. 2C). To compare the antinociceptive effects of ORXA with morphine, the latter was microinjected (50 nM/0.5 μl, $n=6$) into the LPGi and the outcome was a vigorous decrease in formalin-induced nociceptive behaviors in phase 1, inter-phase, as well as the phase 2 of the formalin test (Fig. 2A and B). The antinociceptive effect of intra-LPGi injection of ORXA (100 nM) was similar to that of morphine in the formalin test. All microinjections were made into the right side of the LPGi and the results presented here only include the animals for which the confinement of injection to the LPGi region was confirmed and the data from all dubious injections were rejected. When the peptide was microinjected into the reticular formation surrounding the LPGi, it failed to produce any obvious effect on formalin induced nociceptive behaviors, indicating that the site for peptide action was within the LPGi rather than the adjacent medullary reticular formation.

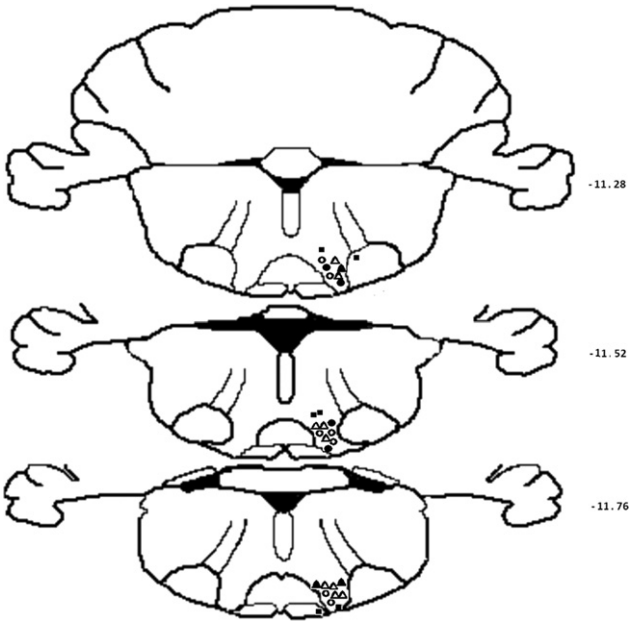

Fig. 1 – Location of injections as percentage of maximum possible effect (%MPE) on the LPGi nucleus: black square for 0–10%, black circle for 10–25%, white circle for 25–50%, black triangle for 50–75%, and white triangle for over 75% effect. The plane is modified to a series of three sections for the brain area from the atlas of Paxinos and Watson (The Rat Brain in Stereotaxic Coordinates, fourth ed. Academic Press, New York; Paxinos and Watson, 2005) the approximate coordinate indicated is in millimeters posterior to the bregma (data shown are only from 100 nM ORXA in formalin test).

2.2. *Effects of ORXA injection into the LPGi nucleus on hot-plate test*

According to the formalin test experiments, after LPGi microinjection of ORXA, different doses of ORXA (1–100 nM), to compare the antinociceptive effect of this drug on hot-plate test, was also administered. Intra-LPGi microinjection of ORXA at 50 and 100 nM concentrations significantly increased the latency in the hot-plate test at 15 ($P<0.01$), 30 ($P<0.01$) and 60