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Original Article

The antinociceptive effect of 17β-estradiol in the nucleus paragigantocellularis lateralis of male rats may be mediated by the NMDA receptors

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Abstract

Introduction: The nucleus paragigantocellularis lateralis (LPGi) is involved in the descending pain modulation. The neurostreoid, 17β-estradiol found in the PGi nucleus and modulates nociception by binding to estrogen receptors and also by allosteric interaction with NMDA receptors. In this study, the role of NMDA receptors in the 17β-estradiol-induced pain modulation was investigated by assessing the inflammatory pain responses changes after blockade of the LPGi nucleus' NMDA receptors.

Methods: In order to study the antinociceptive effect of intra-LPGi microinjection of 17β-estradiol, a guide cannula was implanted into the right LPGi nucleus. 500 nl of drugs were administered 15 minutes prior to formalin (50 µl of 4%) injection. Then, formalin-induced paw jerking behaviour was recorded for 60 min. For assessing the role of the NMDA receptors in the pain modulation by 17β-estradiol, it was injected 15 min after the intra-LPGi administration of 0.5 nmol of AP5 (the NMDA receptor antagonist); and paw jerking frequency was recorded for 1 h.

Results: The results of the present study showed that intra-LPGi injection of 0.8 µmol of 17β-estradiol attenuated the chronic phase ($P<0.001$) of paw jerking behaviour. AP5 significantly reduced the antinociceptive effect of intra-LPGi 17β-estradiol both in the acute ($P<0.001$) and in the chronic phase ($P<0.001$) of formalin test.

Conclusion: According to the results of this study, it can be concluded that the analgesic effect of intra-LPGi injection of 17β-estradiol on the formalin-induced inflammatory pain might be mediated via NMDA receptors.

Keywords:
17β-estradiol;
Paragigantocellularis lateralis nucleus;
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Introduction

Hyperalgesia following peripheral tissue or nerve damage is related to the increment of the sensitivity of nociceptors at the site of injury. It depends on the N-methyl-d aspartate (NMDA) receptor-mediated central changes in synaptic excitability (Parsons,

2001). The NMDA receptors play a key role in central pain transduction mechanisms (Soleimannejad et al., 2007). These receptors are selectively blocked by the drug APV (2-amino-5-phosphonovaleric acid) (Kandel et al., 2012; Ghasemi et al., 2014). The NMDA receptors are composed of NR1, NR2 (A, B, C, and D), and NR3 (A and B) subunits, which determine the functional properties of native NMDA receptors (Petrenko et al., 2003). Excitatory amino acids (EEAs) bind the NMDA receptors (Ji and Traub, 2002). There are evidences that EAAs mediate nociceptive inputs to the spinal cord (Yashpal et al., 2001). EAAs like glutamate are found in the nerve terminals of spinal nociceptive neurons and released in the spinal cord by peripheral noxious stimuli, hereby act on the NMDA receptors (Yashpal et al., 2001).

The LPGi is a reticular nucleus in the ventral portion of the rostral medulla oblongata, where it has a role in descending pain modulation through the spinal cord (Erami et al., 2012; Soleimani et al., 2013; Azhdari-Zarmehri et al., 2014; Shamsizadeh et al., 2014; Azhdari-Zarmehri et al., 2015). The LPGi nucleus is stretched in the medulla oblongata and receives its afferents from vestibular nucleus, tractus solitarus, lemniscus nucleus, and lateral hypothalamus (Azhdari-Zarmehri et al., 2013). The LPGi neurons send their efferents to important nuclei such as ventral tegmental tract, arcuate nucleus, caudal raphe nucleus, and locus coeruleus (LC) (Andrezik et al., 1981). The LPGi nucleus is involved in the cardiovascular regulation (Van Bockstaele et al., 1993), control of sleep-wake cycle, respiratory system (Arita et al., 1988), sexual behavior (Fathi-Moghaddam et al., 2006), consciousness (Van Bockstaele et al., 1993), dependence and addiction (Azizi et al., 2005), as well as pain modulation (Arita et al., 1988; Van Bockstaele et al., 1993; Fathi-Moghaddam et al., 2006; Erami et al., 2012; Azhdari-Zarmehri et al., 2013). The LPGi neurons respond to painful stimuli and relay the processed pain and sensory information into the LC nucleus. Therefore, the LPGi nucleus plays a key role in the processing of pain information associated with descending pain modulation (Aston-Jones et al., 1991).

Besides of their well-known hormonal mode of action, estrogens such as 17β-estradiol influence brain function by direct effects on the neuronal membranes (Balthazart and Ball, 2006). Estrogenic steroids, especially 17β-estradiol, is synthesized in the nervous system from cholesterol through an aromatase-dependent conversion of testosterone (Grassi et al., 2010). The pain modulatory role of 17β-estradiol is shown well (Craft et al., 2004). 17β-estradiol interacts with glutamate and GABA neurotransmitter receptors in various brain regions. 17β-estradiol modulates nociception by binding to its receptors and also by allosteric interaction with other membrane-bound receptors like glutamate receptors (Potes et al., 2006).

Considering the key role of LPGi nucleus in the modulation of pain (Aston-Jones et al., 1991), and the interaction between 17β-estradiol and NMDA receptors in the modulation of pain (Potes et al., 2006; Khakpay et al., 2010b), the present study was designed to assess the possible involvement of the membrane-bound NMDA receptors in the pain modulatory effect of intra-LPGi injection of 17β-estradiol in the male rats.

Materials and methods

Animals

Experiments were performed on adult male Wistar rats weighing 200–270 g purchased from Razi Institute (Hesarak Karj, Iran). Animals were housed at a room temperature of 22–24◦C, with free access to water and food under a 12/12 h light/dark cycles. The experiments were carried out during the light phase. All research and animal care procedures were performed according to the guidelines on the use of laboratory animals and approved by Tabriz University ethical committee for animal research.

Surgery

The animals were gently handled 5 min/day for a week before the experiment for acclimatization. On the day of the surgery, the rats were anesthetized with intraperitoneal injection of ketamine (60 mg/kg) and xylazine (7.5 mg/kg). A guide cannula (23 gauge) equipped with a 30 gauge stylet was stereotaxically implanted into the right LPGi [coordinates from Bregma: AP: -11.9 mm, L: ±1.6 mm, DV: 10.4 mm (Paxinos and Watson, 2005)]. The guiding cannula was attached to the skull with a stainless steel screw and acrylic cement (Dentimax, the Netherlands). All animals were left to recover for 5–7 days prior to behavioral testing.

Drugs

The 4% formalin (Purchased from the Dr. Mojallaly's company) solution was injected subcutaneously into the left hind paw [50 µl (Khakpay et al., 2014)]. Water soluble cyclodextrin-encapsulated 17β-estradiol [0.8 µmol; (Aloisi and Ceccarelli, 2000; Khakpay et al., 2014)], and AP5 - the NMDA receptor antagonist - [0.5 µmol; (Khakpay et al., 2010a)] were purchased from Sigma (Sigma Chemicals, St. Louis, MO, USA). 17β-Estradiol and AP5 were dissolved in normal saline.

Injections

Intra- LPGi injections were done as previously described by Aloisi and Ceccarelli (Aloisi and Ceccarelli, 2000). Considering both contralateral ascending of nociceptive information and left hind paw as the site of formalin injection, all injections were unilaterally done in the right side through the guide cannula using an injection needle (30 gauge) connected by polyethylene tubing to a 0.5 µl Hamilton microsyringe (Hamilton, Switzerland). The injection needle was replaced by the stylet and its tip was 0.2 mm beyond the guide cannula. According to our previous investigations (Khakpay et al., 2014), all substances were injected in a volume of 500 nl. The needle was removed and the stylet replaced sixty seconds after infusing the substance.

Formalin test

The animals were randomly divided into 6 groups, including the control group (intact animals), the second group or sham (only cannulation into the LPGi nucleus), the third group (saline intra-LPGi injection of saline as solvent), the fourth group (intra-LPGi injection of 0.8 µmol17β-estradiol), the fifth group [intra-LPGi injection of 0.5 µmol AP5 (2-amino-5-phosphonovalerate)], and the sixth group (intra-LPGi injection of 0.5 µmol AP5, 15 min before the intra-LPGi administration of 0.8 µmol 17β-estradiol).

Diluted formalin was intraplantarly injected to induce nociceptive responses. Animals were adapted to the experimental room and test chamber for 20 min/day, for 2 days before the experiment. In order to study the involvement of the NMDA receptors in the antinociceptive effect of 17β-estradiol, AP5 was injected 15 min prior to 17β-estradiol administration, and then formalin test (Dubuisson and Dennis, 1977) was done 15 min after 17-estradiol injection. Therefore, 50 µl of 4% formalin solution was subcutaneously injected into the plantar surface of the left hind paw using a 30 gauge needle (Khakpay et al., 2014). Following the formalin injection, the animals were then immediately returned to their observation box (a square transparent plexiglas cage, 30 cm × 30 cm × 30 cm) and the total time spent the licking and flexing behaviors were recorded over 5 min intervals (Wheeler-Aceto and Cowan, 1991; Aloisi et al., 1998; Khakpay et al., 2010b; Khakpay et al., 2014). The responses observed were divided into two phases: first phase (0–7 min) and second phase (15–60 min) (Mahmoudi and Zarrindast, 2002; Khakpay et al., 2014). By the end of the experiment, the rats were sacrificed by diethyl ether and the brains were removed and checked for the correct cannula placement in the LPGi. Only data obtained from animals with correct placement of cannula were included in the analysis.

Statistical analysis

All results were analyzed by SPSS software and presented as mean ± S.E.M. One-way analysis of variance (ANOVA) followed by Post Hoc Tukey's test was used to compare differences between treatments. $P < 0.05$ was considered statistically significant.

Results

Animals belonging to the sham operated (The LPGi cannulation without intra-LPGi injections) and saline groups (intra-LPGi injections of saline) did not show any significant differences compared with the control group (intact animals); therefore, theywere not included in the results. The mean response of the first 7 min post-formalin injection was considered as the acute phase and the mean response over 45 min between 15 and 60 min post-formalin injection was considered as the chronic phase.

Effects of 17β-estradiol on formalin-induced responses

Intra-LPGi injections of 0.8 µmol 17β-estradiol significantly reduced paw jerking frequency in the chronic phase ($P<0.001$, Fig.1). The results indicate that 17β-estradiol has antinociceptive effect in this dose on the paw jerking response; thus, this concentration was used for the subsequent

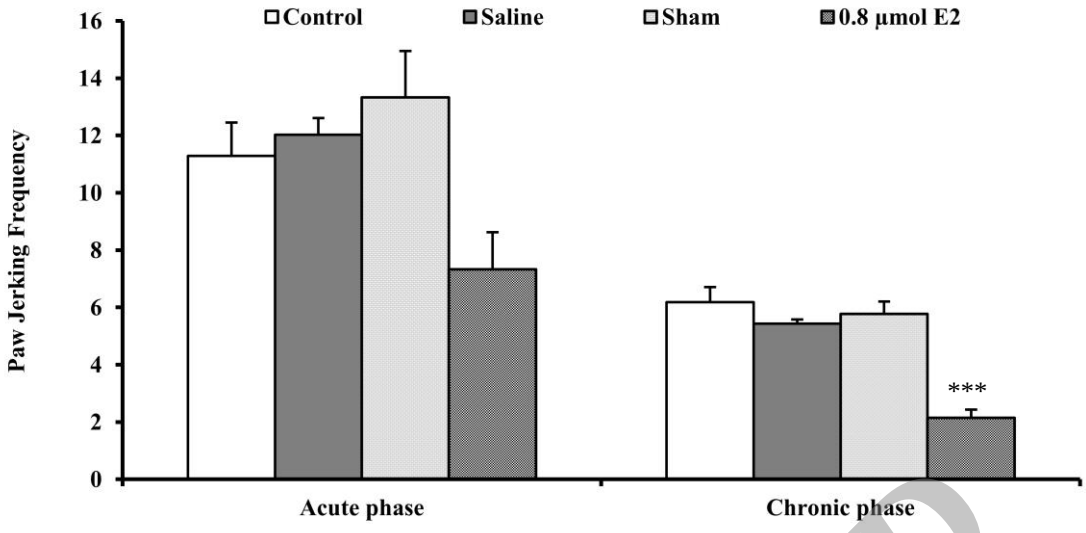

Fig.1. Effect of intra-LPGi injection of 0.8 µmol 17β-estradiol on paw jerking behavior following 50 µl of 4% formalin injected into the plantar surface of the left hind paw. The graph shows data for the acute and the chronic phase of formalin-induced responses in comparison with control, sham and saline-injected animals. The nociceptive response is presented by mean ± SEM of paw jerking frequency of six rats per group.
*** indicates significant difference from control group (P<0.001). 0.8 micromol 17β-estradiol = 0.8 µmol E2.

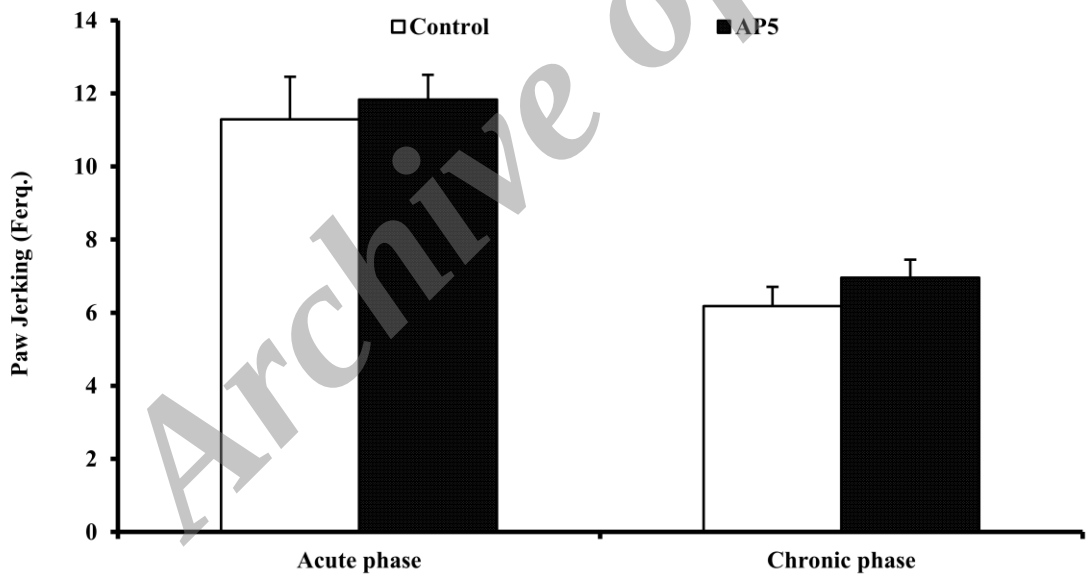

Fig.2. Nociceptive response (paw jerking) during the acute and the chronic phase of the formalin test (4%, 50 µl) in rats treated with AP5 (0.5 nmol) 15 min before formalin injection. The data are represented as mean ± SEM for six rats. 0.5 nanomol AP5 = 0.5 nmol AP5

experiments.

Effects of AP5 on formalin-induced responses

To clarify the mechanism of the antinociceptive effect of 17β-estradiol and the receptors involved, we tried to find a dose of NMDA antagonists without any significant effect on nociception. Consequently, intra-LPGi injections of 0.5 nmol of AP5 did not show any significant differences compared with the control group (Fig. 2). In other words, AP5 had no pronociceptive effect and could not interfere with analgesic effect of 17β-estradiol.

Effects of NMDA receptor antagonists on the antinociceptive effect of 17β-estradiol

For studying the possible involvement of NMDA

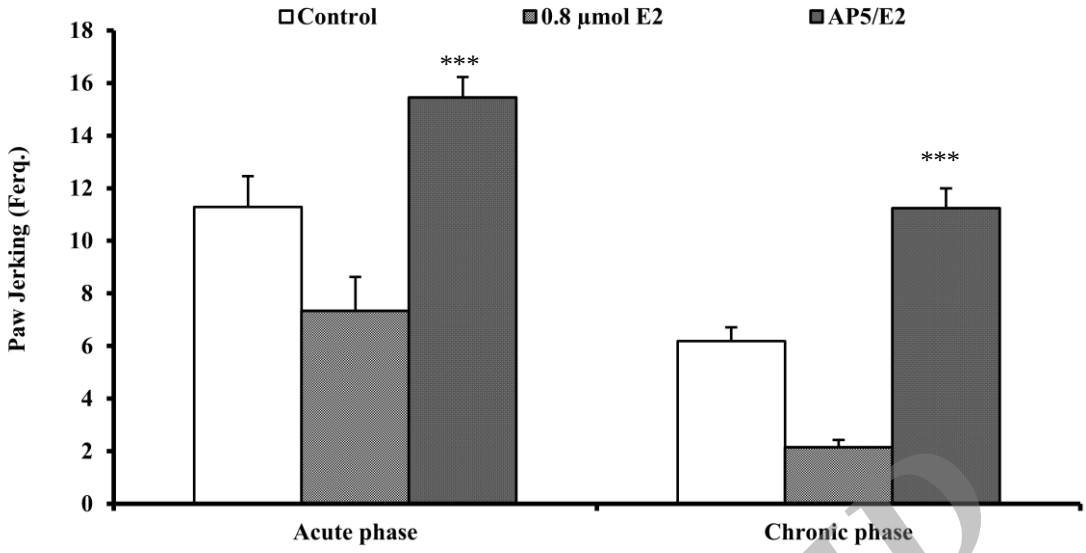

Fig.3. Effects of NMDA receptor antagonist on the paw jerking responses of 17β-estradiol. AP5 (0.5 nmol) were administered 15 min before intra-LPGi injection of 0.8 µmol 17β-estradiol. Data are presented as mean ± SEM for six rats. *** indicates significant difference of the E2/AP5 group from the 17β-estradiol group ($P < 0.001$). 0.8 micromol 17β-estradiol = 0.8 µmol E2 and 17β-estradiol/AP5 = E2/AP5

receptors in the antinociceptive effect of 17β-estradiol, AP5 were applied 15 min before the injection of 17β-estradiol and pain-related behavior was examined following formalin injection.

Pre-treatment with 0.5 nmol AP5 significantly reverse the effect of 0.8 µmol intra-LPGi 17β-estradiol on paw jerking frequency in the both phases of formalin-induced pain ($P<0.001$, Fig.3).

Discussion

In the present study, intra-LPGi injection of 17β-estradiol was used to assess the effect of this neuroactive steroid on centrally mediated behavioral responses to nociceptive stimulus. Our results indicated that 17β-estradiol treatment attenuated the chronic phase of paw jerking behaviour. According to the previous findings of our laboratory, 17β-estradiol microinjection into the LPGi nucleus induces strong analgesia. A part of this analgesic effect is mediated by estrogen receptors (Khakpay et al., 2014). 17β-Estradiol acts as a neuroactive steroid which plays a key role in the pain modulation (Khakpay et al., 2010a; Khakpay et al., 2010b). It controls nociception through binding to its classic receptors and by allosteric interaction with other membrane-bound receptors such as glutamate and $GABA_A$ receptors (Potes et al., 2006; Khakpay et al., 2010b). Estradiol increases the spinal processing of visceral nociception by increasing the NMDA receptor NR1 subunit expression and increasing site-specific receptor phosphorylation on the NR1 subunit contributing to an increase in the NMDA receptor activity (Tang et al., 2008).

In the present study, we hypothesized that the analgesic effect of intra-LPGi injection of 17β-estradiol on the formalin-induced inflammatory pain may be probably mediated via the NMDA receptors. Consistent with our findings, Khakpay et al. (2010) revealed that the analgesic effect of intra-LC injection of 17β-estradiol on the formalin-induced responses is mediated via the interaction with membrane-bound NMDA receptors (Khakpay et al., 2010b). However some studies failed to identify any changes in the NMDA receptor protein levels following 17β-estradiol administration (Snyder et al., 2011; Nebieridze et al., 2012). Therefore, it is unclear how 17β-estradiol modulates the NMDA receptor-mediated effects on the pain modulation (Nebieridze et al., 2012).

Moreover, our results indicated that NMDA receptor antagonist could not significantly affect both the acute and the chronic phases of formalin test. The NMDA receptors play a critical role in nociceptive processing (Yashpal et al., 2001). Peripheral NMDA receptors are also involved in inflammatory somatic and visceral pain (Parsons, 2001). NMDA receptor

antagonists attenuate pain behaviors in models of neuropathic and tonic pain, when applied to the CNS (Yashpal et al., 2001). Intrathecal (i.t.) administration of selective NMDA receptor antagonists produces antinociceptive effects in both phasic and tonic nociceptive tests in rats, as well as decrement of hyperalgesia associated with inflammatory or neuropathic injury in rats (Yashpal et al., 2001). Glutamate receptors - including NMDA, AMPA, and Kainate receptors- of the medullary dorsal reticular nucleus play a key role during the development and maintenance of formalin-induced secondary allodynia (Ambriz-Tututi et al., 2013). Activation of NMDA receptors via glycine sites at the supraspinal level induces an antinociceptive effect on both acute and chronic pain (Ito et al., 2014). Pre-emptive administration of ketamine -a NMDA receptor antagonist- obviously prevents the pain behavior response during the second phase of formalin test (Long et al., 2013). St-Ht31 (stearated Ht31 peptide which inhibits AKAPs/PKA interaction) inhibits the NMDAR-mediated nociceptive transmission and effectively ameliorated CFA-induced inflammatory pain (Wang et al., 2015). The NMDA receptors of amygdala may be involved in the modulation of the minocycline-induced potentiation of morphine analgesia in the tail-flick test (Ghazvini et al., 2015). The peptide NMDA receptor antagonist SHG improves opioid antinociception, but this improvement is dependent on the animal model, behavioral endpoint, and opioid (Hama and Sagen, 2014).

To decipher the mechanism of actions of neuroactive steroids, the NMDA receptor action has attracted the most attention, in recent decades. Therefore, the aim of this study was to find out whether pretreatment of LPGi nucleus with a NMDA receptor antagonist reduces the 17β-estradiol-induced antinociceptive behaviors. For this purpose, we tried to find a dose of antagonist without any significant nociceptive effect. Microinjection of 0.5 nmol of AP5 into the LPGi nucleus did not have any significant nociceptive effect in the formalin test. Therefore, 0.5 nmol AP5 was chosen as the ideal dose.

In the present study, pretreatment of LPGi nucleus with AP5 reversed the 17β-estradiol-induced decrement in the paw jerking behavior. Our results showed that a part of the analgesic effect of intra-LPGi 17β-estradiol on the formalin-induced inflammatory pain is probably mediated by NMDA receptors. Pretreatment of the LPGi nucleus with AP5 significantly reversed both the acute phase and the chronic phase of the paw jerking behaviour. Consistent with the results of this study, soleimannejad et al (2007) reported that microinjection of the NMDA receptor antagonist AP5 into the dentate gyrus region of the hippocampus attenuated pain behaviors both in the acute and in the tonic phases of the formalin test (Soleimannejad et al., 2007). Coderre and Van Empel (1994) showed that during the late phase of the formalin test, the spinal cord neurons release excitatory amino acids and the NMDA receptor subtypes are activated (Coderre and Van Empel, 1994). Similar to our results, they concluded that intrathecal injection of selective NMDA antagonists prevents the nociceptive behavior of the late phase of formalin test (Coderre and Van Empel, 1994). Mangiferin, a glucosylxanthone from Mangifera indica, shows ability to decrease tonic pain in the formalin test. Acute administration of MG reduced licking/biting exclusivity in the tonic phase of formalin test. This effect was enhanced by non-competitive NMDA antagonist ketamine (Garrido-Suárez et al., 2014). Ito et al (2014) reported that using the tail-flick test, intracerebroventricular administration of D-serine, an endogenous co-agonist at the glycine sites of NMDA receptors, elicited an antinociceptive effect on thermal nociception. In agreement with the results of the current study, they suggested that activation of NMDA receptors via glycine sites at the supraspinal level induces an antinociceptive effect on both acute and tonic pain (Ito et al., 2014). A few reports have described that the intrathecal administration of NMDA in rodents can also induce various types of analgesic responses (Lee et al., 2004). Also, intrathecal NMDA has produced a delayed antinociceptive response in the tail flick test in rats (Lee et al., 2004).

Conclusion

In conclusion, our data revealed that intra-LPGi injection of 17β-estradiol is sufficient to produce strong analgesia. The antinociceptive effect of 17β-estradiol was prevented by AP5. These data suggest that 17β-estradiol-induced analgesia in the LPGi nucleus is probably mediated by non-estrogen receptors. With regards to the membrane-bound receptors, the NMDA receptors seem to be involved

in the 17β-estradiol-mediated antinociception in the LPGi nucleus, but further investigations by molecular and electrophysiological approaches are still required.

Acknowledgments

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Conflict of interest

The authors have declared no conflict of interest.

References

Aloisi AM, Ceccarelli I. Role of gonadal hormones in formalin-induced pain responses of male rats: Modulation by estradiol and naloxone administration. Neuroscience 2000; 95: 559-566.

Aloisi AM, Ceccarelli I, Lupo C. Behavioural and hormonal effects of restraint stress and formalin test in male and female rats. Brain Res Bull 1998; 47: 57-62.

Ambriz-Tututi M, Palomero-Rivero M, Ramirez-López F, Millán-Aldaco D, Drucker-Colín AR. Role of glutamate receptors in the dorsal reticular nucleus in formalin-induced secondary allodynia. Eur J Neurosci 2013; 38: 3008-3017.

Andrezik JA, Chan-Palay V, Palay SL. The nucleus paragigantocellularis lateralis in the rat. Anat Embryol 1981; 161: 373-390.

Arita H, Kogo N, Ichikawa K. Locations of medullary neurons with non-phasic discharges excited by stimulation of central and/or peripheral chemoreceptors and by activation of nociceptors in cat. Brain Res 1988; 442: 1-10.

Aston-Jones G, Chiang C, Alexinsky T. Discharge of noradrenergic locus coeruleus neurons in behaving rats and monkeys suggests a role in vigilance. Prog Brain Res 1991; 88: 501-520.

Azhdari-Zarmehri H, Reisi Z, Vaziri A, Haghparast A, Shaigani P, Haghparast A. Involvement of orexin-2 receptors in the ventral tegmental area and nucleus accumbens in the antinociception induced by the lateral hypothalamus stimulation in rats. Peptides 2013; 47: 94-98.

Azhdari-Zarmehri H, Semnanian S, Fathollahi Y. Orexin-a modulates firing of rat rostral ventromedial medulla neurons: An in vitro study. Cell J (Yakhteh) 2015; 17: 163-70.

Azhdari-Zarmehri H, Semnanian S, Fathollahi Y, Pakdell FG. Tail flick modification of orexin-a induced changes of electrophysiological parameters in the rostral ventromedial medulla. Cell J 2014; 16: 131-40.

Azizi H, Semnanian S, PAKDEL FG. Effect of rolipram, a type 4-specific phosphodiesterase inhibitor, on unit activity of paragigantocellularis neurons and withdrawal signs in morphine dependent rats. Cell J (Yakhteh) 2005; 7: 35-42.

Balthazart J, Ball GF. Is brain estradiol a hormone or a neurotransmitter? Trends Neurosci 2006; 29: 241-249.

Coderre TJ, Van Empel I. The utility of excitatory amino acid (eaa) antagonists as analgesic agents. I. Comparison of the antinociceptive activity of various classes of eaa antagonists in mechanical, thermal and chemical nociceptive tests. Pain 1994; 59: 345-352.

Craft RM, Mogil JS, Aloisi AM. Sex differences in pain and analgesia: The role of gonadal hormones. Eur J Pain 2004; 8: 397-411.

Dubuisson D, Dennis SG. The formalin test: A quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain 1977; 4: 161-174.

Erami E, Azhdari-Zarmehri H, Ghasemi-Dashkhasan E, Esmaeili MH, Semnanian S. Intra-paragigantocellularis lateralis injection of orexin-a has an antinociceptive effect on hot plate and formalin tests in rat. Brain Res 2012; 1478: 16-23.

Fathi-Moghaddam H, Kesmati M, Pour Kargar HM. The effect of paragigantocellularis lateralis lesion on conditioned place preference (cpp) in presence or absence of a 2 adrenergic agonist (clonidine) in male rats. Acta Physiol Hung 2006; 93: 33-40.

Garrido-Suárez BB, Garrido G, Castro-Labrada M, Merino N, Valdés O, Rodeiro I, et al. Anti-hypernociceptive effect of mangiferin in persistent and neuropathic pain models in rats. Pharmacol Biochem Behav 2014; 124: 311-319.

Ghasemi M, Phillips C, Trillo L, De Miguel Z, Das D, Salehi A. The role of nmda receptors in the pathophysiology and treatment of mood disorders. Neurosci Biobehav Rev 2014; 47: 336-358.

Ghazvini H, Rezayof A, Ghasemzadeh Z, Zarrindast MR. M-opioid and n-methyl-d-aspartate receptors in the amygdala contribute to minocycline-induced potentiation of morphine analgesia in rats. Behav Pharmacol 2015; 26: 383-392.

Grassi S, Frondaroli A, Scarduzio M, Dutia MB, Dieni C, Pettorossi VE. Effects of 17β-estradiol on glutamate synaptic transmission and neuronal excitability in the rat medial vestibular nuclei. Neuroscience 2010; 165: 1100-1114.

Hama A, Sagen J. Selective antinociceptive effects of a combination of the n-methyl-d-aspartate receptor peptide antagonist [ser1] histogranin and morphine in rat models of pain. Pharmacol Res Perspect 2014; 2: e00032.

Ito M, Yoshikawa M, Ito K, Matsuda M, Jin XL, Takahashi S, et al. Antinociceptive effect of intracerebroventricular administration of d-serine on formalin-induced pain. J Anesth 2014; 28: 228-234.

Ji Y, Traub RJ. Differential effects of spinal cnqx on two populations of dorsal horn neurons responding to colorectal distension in the rat. Pain 2002; 99: 217-222.

Kandel ER, Schwartz JH, Jessell TM, SA S, AJ H. Principles of neural science. Vol 15th ed: NewYork:

Academic Press, 2012.

Khakpay R ,Barani S, Hatami Nemati H. The antinociceptive effect of 17β-estradiol in the paragigantocellularis lateralis of male rats is mediated by estrogenic receptors. Physiol Pharmacol 2014; 18: 215-223.

Khakpay R, Semnanian S, Javan M, Janahmadi M .The effect of intra-locus coeruleus injection of 17β-estradiol on inflammatory pain modulation in male rat. Behav Brain Res 2010a; 214: 409-416.

Khakpay R, Semnanian S, Javan M, Janahmadi M. Is the pain modulatory action of 17β-estradiol in locus coeruleus of male rats is mediated by gabaa receptors? Physiol Pharmacol 2010b; 14: 252-261.

Lee HJ, Choi HS, Jung CY, Ju JS, Kim SK, Bae YC, et al. Intracisternal nmda produces analgesia in the orofacial formalin test of freely moving rats. Prog Neuro-Psychopharmacol Biol Psychiatry 2004; 28: 497-503.

Long I, Suppian R, Ismail Z. The effects of pre-emptive administration of ketamine and norbni on pain behavior, c-fos, and prodynorphin protein expression in the rat spinal cord after formalin-induced pain is modulated by the dream protein. Korean J Pain 2013; 26: 255-264.

Mahmoudi M, Zarrindast MR. Effect of intracerebroventricular injection of gaba receptor agents on morphine-induced antinociception in the formalin test. J Psychopharmacol 2002; 16: 85-91.

Nebieridze N, Zhang XL, Chachua T, Velíšek L, Stanton PK, Velíšková J. B-estradiol unmasks metabotropic receptor-mediated metaplasticity of nmda receptor transmission in the female rat dentate gyrus. Psychoneuroendocrinology 2012; 37: 1845-1854.

Parsons CG. Nmda receptors as targets for drug action in neuropathic pain. Eur J Pharmacol 2001; 429: 71-78.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates. NewYork: Academic press, 2005.

Petrenko AB, Yamakura T, Baba H, Shimoji K. The role of n-methyl-d-aspartate (nmda) receptors in pain: A review. Anesth Analg 2003; 97: 1108-1116.

Potes CS, Neto FL, Castro-Lopes JM. Administration of baclofen, a γ-aminobutyric acid type b agonist in the thalamic ventrobasal complex, attenuates allodynia in monoarthritic rats subjected to the ankle-bend test. J Neurosci Res 2006; 83: 515-523.

Shamsizadeh A, Soliemani N, Mohammad-Zadeh M, Azhdari-Zarmehri H. Permanent lesion in rostral ventromedial medulla potentiates swim stress-induced analgesia in formalin test. Iran J Basic Med Sci 2014; 17: 209-215.

Snyder MA, Cooke BM, Woolley CS. Estradiol potentiation of nr2b-dependent epscs is not due to changes in nr2b protein expression or phosphorylation. Hippocampus 2011; 21: 398-408.

Soleimani N, Erami E, Abbasnejad M, Shamsizadeh A, Azhdari-Zarmehri H. Effect of transient inactivation of rostral ventromedial medulla on swim stressinduced analgesia in formalin test in rats neda. Physiol Pharmacol 2013; 17: 116-124.

Soleimannejad E, Naghdi N, Semnanian S, Fathollahi Y, Kazemnejad A. Antinociceptive effect of intra-hippocampal ca1 and dentate gyrus injection of mk801 and ap5 in the formalin test in adult male rats. Eur J Pharmacol 2007; 562: 39-46.

Tang B, Ji Y, Traub RJ. Estrogen alters spinal nmda receptor activity via a pka signaling pathway in a visceral pain model in the rat. Pain 2008; 137: 540-549.

Van Bockstaele EJ, Akaoka H, Aston-Jones G. Brainstem afferents to the rostral (juxtafacial) nucleus paragigantocellularis: Integration of exteroceptive and interoceptive sensory inputs in the ventral tegmentum. Brain Res 1993; 603: 1-18.

Wang WT, Pan GQ, Zhang ZY, Suo ZW, Yang X, Hu XD. Ht31 peptide inhibited inflammatory pain by blocking nmda receptor-mediated nociceptive transmission in spinal dorsal horn of mice. Neuropharmacology 2015; 89: 290-297.

Wheeler-Aceto H, Cowan A. Neurogenic and tissue-mediated components of formalin-induced edema: Evidence for supraspinal regulation. Agents Actions 1991; 34: 264-269.

Yashpal K, Fisher K, Chabot JG, Coderre TJ. Differential effects of nmda and group i mglur antagonists on both nociception and spinal cord protein kinase c translocation in the formalin test and a model of neuropathic pain in rats. Pain 2001; 94: 17-29.