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Original Article

Role of the AMPA receptors of paragigantocellularis lateralis nucleus in the inflammatory pain modulation in male rat

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Abstract

Introduction: The 17β-estradiol acts as a neurosteroid in the brain and modulates nociception by binding to the estrogen receptors and also by allosteric interaction with other membrane-bound receptors like glutamate receptors. Paragigantocellularis lateralis nucleus (LPGi) is one of the important brain regions implicated in the pain modulation. So, this study was designed to evaluate the possible involvement of the membrane-bound AMPA receptors of LPGi nucleus in the pain modulatory effect of intra-LPGi 17β-estradiol in the male rats.

Methods: In order to study the pain modulatory effect of intra-LPGi microinjection of 17β-estradiol, cannulation into the LPGi nucleus was performed. Then, 500 nl of saline, 17β-estradiol and CNQX- the AMPA receptor antagonist- were unilaterally administered into the right LPGi by injection cannula and Hamilton syringe. In addition, for assessing the role of the AMPA receptors in the pain modulation by 17β-estradiol, 17β-estradiol was injected 15 min after the intra-LPGi administration of CNQX. Then, 50 µl of 4% formalin was subcutaneously injected into the plantar surface of contralateral hind paw and the number of paw jerking behavior was observed for 60 min.

Results: The results showed that intra-LPGi injection of 0.8 µmol of 17β-estradiol attenuated the chronic phase (P<0.001) of paw jerking behaviour. CNQX significantly prevented the antinociceptive effect of intra-LPGi 17β-estradiol both in the acute (P<0.05) and in the chronic phase (P<0.001) of formalin test.

Conclusion: Considering the results of this study, it can be concluded that the analgesic effect of intra-LPGi injection of 17β-estradiol might be mediated via AMPA receptors.

Keywords:
17β-Estradiol;
Paragigantocellularis lateralis nucleus;
AMPA receptors;
Formalin test

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Introduction

Pain is modulated by several central circuits, one of these paths is the descending noradrenergic pain modulatory system (Millan, 1997). The Paragigantocellularis lateralis nucleus (LPGi), located in the rostral ventrolateral medulla, is an important brain region implicated in the pain modulation by descending noradrenergic system (Azhdari-Zarmehri

et al., 2013b). The LPGi nucleus is lengthened in the medulla and receives its afferents from vestibular nucleus, tractus solitarus, lemniscus nucleus and lateral hypothalamus (Azhdari-Zarmehri et al., 2013b). LPGi neurons send their projection fibers to important nuclei such as ventral tegmental tract, arcuate nucleus, caudal raphe nucleus and locus coeruleus (LC) nucleus (Andrezik et al., 1981). The LPGi nucleus provides main glutamatergic excitatory inputs of LC nucleus (Bernard et al., 2011). The involvement of LPGi nucleus in the cardiovascular control (Van Bockstaele et al., 1993), adjustment of sleep-wake cycle, respiratory system regulation (Arita et al., 1988), sexual behavior (Fathi-Moghaddam et al., 2006), consciousness (Van Bockstaele et al., 1993) and pain modulation (Arita et al., 1988; Azhdari-Zarmehri et al., 2013a; Azhdari-Zarmehri et al., 2013b; Erami et al., 2012; Fathi-Moghaddam et al., 2006; Van Bockstaele et al., 1993) are reported. Painful stimuli excite the primary sensory neuron which are projected into the LPGi nucleus. Then, the processed pain and sensory information relayed from LPGi nucleus into the LC nucleus (Singewald and Philippu, 1998). Hence, LPGi nucleus and its glutamatergic projection – including both NMDA and AMPA receptors - to LC nucleus is an important stage in the descending pain modulatory system (Aston-Jones et al., 1991).

The 17β-estradiol is a neuroactive steroid which synthesized in the central nervous system from the cholesterol through an aromatase-dependent conversion of testosterone (Grassi et al., 2010). Pain modulation by 17β-estradiol is well documented (Craft et al., 2004). 17β-estradiol enhances synaptic excitability by increasing the extent of AMPA receptor-mediated currents (Wong and Moss, 1992). The fast beginning of augmented excitability and its inhibition by CNQX, (an AMPA receptor antagonist) supported a postsynaptic effect and an expression by non-NMDA receptor-channels (Foy et al., 1999).

Reinforcement of nociceptive transmission, under pathological conditions, results in hyperalgesia, allodynia or chronic pain (Engelman et al., 1999). Glutamate is a key excitatory neurotransmitter in the central nervous system (Wang et al., 2010). In the spinal cord dorsal horn, glutamate mediates much of the pain signaling and may be responsible for induction of central sensitization associated with altered nociception (Engelman et al., 1999). AMPA receptors are one of the ionotropic glutamate receptor subtypes and intermediate the most of fast excitatory synaptic transmission in the central nervous system (Hayashi, 2014). AMPA receptors are involved in the pain modulation in the primary nociceptive afferents, spinal dorsal and ventral horns and in various brain areas (Gangadharan et al., 2011). Glutamate is released from both peripheral nociceptive neurons and excitatory interneurons onto postsynaptic NMDA and non-NMDA (such as AMPA and kainite) glutamate receptor subtypes (Engelman et al., 1999). GluR-A and GluR-B-containing AMPA receptors are important receptor subtypes for modulating the nociceptive information by descending pathways (Hartmann et al., 2004). There are documents that augmentation of amplitude of AMPA receptor-mediated currents by 17β-estradiol treatment increases the synaptic excitability of CA1 area of hippocampus (Wong and Moss, 1992).

Because of the LPGi nucleus involvement in the pain modulation (Aston-Jones et al., 1991) and considering the interaction between 17β-estradiol and AMPA receptors in the modulation of pain (Khakpay et al., 2010), the present study was designed to assess the extent of the involvement of the AMPA receptors in the pain modulation by 17β-estradiol in the LPGi nucleus of male rats.

Materials and methods

Animals

This study was performed using adult male Wistar rats in the range of 200–270 g obtained from Razi Institute (Hesarak Karj, Iran). Animals were held under a 12/12 h light/dark cycle and at a temperature of 22–24°C. Water and food were available ad libitum. The experiments were done 8 am till 16 pm. 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.

The animals were randomly divided into 6 groups, including the control (intact animals), sham (just cannulation of the LPGi nucleus without intra-LPGi injections), saline (intra-LPGi injection of saline as solvent), E2 (intra-LPGi injection of 0.8 μmol 17β-estradiol), CNQX (intra-LPGi injection of 30 nmol CNQX), E2/CNQX (intra-LPGi injection of CNQX 15 min prior the administration of 17β-estradiol) groups.

Surgery

The animals were softly handled 15 min/day for a week prior the experiments for adaptation. On the day of the surgery, ketamine (60 mg/kg) and xylazine (7.5 mg/kg) were intraperitoneally injected for anesthetizing the animals. A guide cannula (23 gauge) equipped with a 30 gauge stylet was stereotaxically implanted in the right LPGi [coordinates from bregma: AP: -11.9 mm, L: ±1.6 mm, DV: 10.4 mm (Paxinos and Watson, 2005)]. A stainless steel bolt and acrylic cement (Dentimax, the Netherlands) were used for attaching the guide cannula to the skull. All animals were left to improve for a week before behavioral tests.

Drugs

The 4% formalin (Purchased from the Dr. Mojallaly's company) solution was subcutaneously injected into the plantar surface of 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 AMPA receptor antagonist, CNQX methiodide [30 nmol; (Khakpay et al., 2010)] were purchased from Sigma (Sigma Chemicals, St. Louis, MO, USA). The 17β-estradiol and CNQX were dissolved in the normal saline.

Injections

Intra- LPGi administration of treatment were performed as previously described by Khakpay et al. (2014). Since nociceptive information ascend contralaterally and formalin was injected into the left hind paw, all drugs were unilaterally injected into the right LPGi nucleus. Injections were performed through the guide cannula by means of an injection needle (30 gauge) linked to a polyethylene tubing connected to a 0.5 µl Hamilton microsyringe (Hamilton, Switzerland). The injection needle was exchanged by the stylet and its tip was 0.2 mm outside the guide cannula. In line with our previous studies (Khakpay et al., 2014), all substances were administered in a volume of 500 nl. The needle was detached and the stylet replaced one minute after infusing the substance.

Formalin test

The formalin test was performed as defined by Malmberg and Yaksh (1992). Animals were accommodated to the testing room and chamber for 20 min/day, for 2 days prior to the experiment. In order to study the involvement of the AMPA receptors in the antinociceptive effect of 17β-estradiol, CNQX was injected 15 min prior 17β-estradiol administration and then formalin test was carried out 15 min after 17β-estradiol application. The 50 µl of 4% formalin was intraplantarly injected into the left hind paw (Khakpay et al., 2014). Subsequently, the animal was put in the test chamber (a square transparent plexiglas cage, 30 cm × 30 cm × 30 cm) and the number of paw jerking behavior was observed and counted by experimenter for 60 min (Aloisi et al., 1998; Khakpay et al., 2014; Wheeler-Aceto and Cowan, 1991). The formalin-induced paw jerking was recorded over 5 min intervals (Aloisi et al., 1998) and separated into two phases: first phase (acute phase) develops immediately after injection, continues for about 7 min and second phase (chronic phase) begins about 15 min after injection and lasts for approximately 45 min. By the end of the experiments, the rats were sacrificed by CO_2 chamber, the brains were removed and checked for the correct cannula placement in the LPGi nucleus. Only data acquired from animals with accurate location of cannula were included in the analysis.

Statistical analysis

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

Results

There was no significant differences between the sham operated (LPGi cannulation without intra-LPGi injections), the saline (intra-LPGi injections of saline) as well as the control groups (intact animals) and thus were not included in the results. The acute phase reflects the mean response of 0-7 minutes after formalin injection and the chronic phase reflects the mean response between 15 and 60 minutes post-formalin injection.

Effects of 17β-estradiol on formalin-induced responses

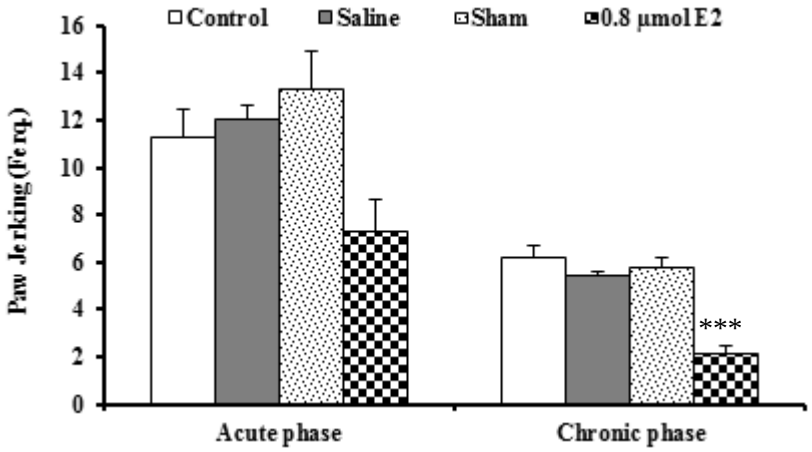

Fig.1. Effect of intra-LPGi injection of 0.8 µmol 17β-estradiol on paw jerking behaviour following 50 µl of 4% formalin injected into the plantar surface of the left hind paw. The graph shows data for the acute and the chronic phase of formalin-induced responses in comparison with control, sham and saline-injected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

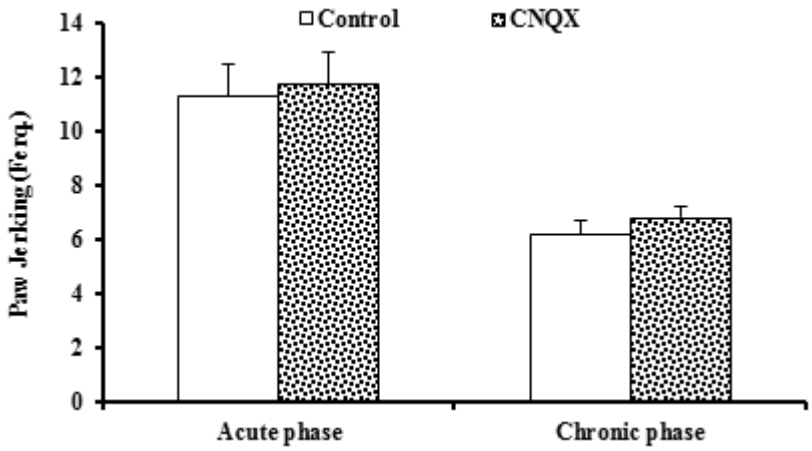

Fig.2. Nociceptive response (paw jerking) during the acute and the chronic phase of the formalin test in rats treated with CNQX (30 nmol) 15 min before formalin injection (4%, 50 µl). There was no significant difference between the control and the CNQX groups. The data are represented as mean ± SEM for six rats. 30 nmol CNQX = CNQX.

The chronic phase of paw jerking frequency was significantly reduced by intra-LPGi injections of 0.8 µmol 17β-estradiol (P<0.001, Fig. 1). These results indicate that this dose of 17β-estradiol has antinociceptive effect on the paw jerking response.

Effects of CNQX on formalin-induced responses

For clarifying the mechanism of the antinociceptive effect of 17β-estradiol and the involvement of AMPA receptors, we tried to discover a dose of AMPA antagonists (CNQX) without any significant influence on pain perception. Hence, intra-LPGi injections of 30 nmol of CNQX had no significant differences with the control group (Fig. 2). In other words, CNQX had no pronociceptive effect and could not interfere with antinociceptive effect of 17β-estradiol.

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

In order to study the possible involvement of membrane-bound AMPA receptors in the antinociceptive effect of 17β-estradiol, CNQX were administered 15 min before the injection of 17β-estradiol and pain-related behavior was investigated following formalin injection.

Pre-treatment of LPGi nucleus with 30 nmol CNQX not only significantly counteracted the antinociceptive effect of 0.8 µmol 17β-estradiol on paw jerking frequency both in the acute (P<0.05) and in the chronic phases (P<0.001) of formalin-induced pain

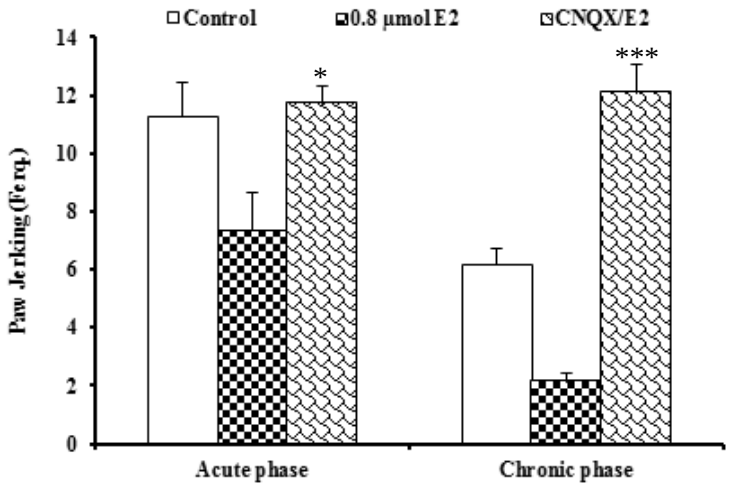

Fig.3. Effect of CNQX (AMPA receptor antagonists) pre-treatment on the antinociceptive effect of intra-LPGi injection of 17β-estradiol. CNQX (30 nmol) was administered 15 min before intra-LPGi injection of 0.8 µmol 17β-estradiol and formalin test was done 15 min after 17β-estradiol injection (E2/CNQX group). Data are presented as mean ± SEM for six rats and significant differences between the 17β-estradiol and CNQX/E2 groups are shown by the * and the *** which represents (P<0.05) and (P<0.001), respectively. 17β-estradiol = 0.8 µmol E2 and CNQX/17β-estradiol = CNQX /E2.

(Fig. 3), but also it induced hyperalgesia.

Discussion

In line with our previous findings, the results of this study indicated that 17β-estradiol treatment of LPGi nucleus decreased the formalin-induced paw jerking response only in the second phase of formalin test. The AMPA receptor antagonist, CNQX, completely reversed the 17β-estradiol-induced attenuation of paw jerking response. Since the antinociceptive effect of 17β-estradiol was entirely abolished by CNQX pre-treatment, the membrane-bound AMPA receptors are possibly involved in the antinociceptive effect of intra-LPGi injection of 17β-estradiol.

In the present experiments, intra-LPGi administration of 0.8 µmol of 17β-estradiol had a significant antinociceptive effect on the second phase of formalin-induced paw jerking response. It can be hypothesized that intra-LPGi injection of 17β-estradiol affects either estrogen receptors and/or the membrane-bound AMPA receptors of LPGi nucleus which are able to modulate pain-induced neural activity in the spinal and supraspinal circuits. Also, our previous study revealed that the administration of 17β-estradiol into the LPGi nucleus has analgesic effect in the formalin-induced inflammatory pain (Khakpay et al., 2014). Therefore, the reduction of the second phase of the inflammatory response can confirm that estradiol application in the LPGi nucleus affects the processing of the nociceptive stimulus in the LPGi nucleus. The activation of AMPA receptors of LPGi nucleus activates the glutamatergic projection from LPGi nucleus into the LC nucleus (Singewald and Philippu, 1998). The stimulation of LC nucleus excites the noradrenergic descending pain modulatory fibers and induces analgesia at the level spinal cord (Aston-Jones et al., 1991).

Besides its hormonal secretion, 17β-estradiol is produced in the central nervous system from cholesterol by an aromatase-dependent transformation of testosterone (Grassi et al., 2010). Therefore, 17β-estradiol acts as a neuroactive steroid and quickly modulates the synaptic transmission and plasticity in the adult central nervous system, even outside areas associated with reproductive behavior (Grassia et al., 2012; Hajszan et al., 2008; Isgor and Sengelaub, 2003; Sakuma, 2009). The 17β-estradiol augments the extent of long-term potentiation in the hippocampus by allosteric interaction with glutamate and GABA neurotransmitter receptors in various brain regions (Grassi et al., 2010). Wong and Moss (1992) suggested that intra-CA1 administration of 17β-estradiol increased synaptic excitability by enhancing the amplitude of AMPA receptor-mediated responses. So, this study was planned to examine whether the AMPA receptors of LPGi nucleus are involved in the pain modulation by 17β-estradiol. Therefore we tried to find a dose of CNQX, a specific antagonist of the AMPA receptors (Foy et al., 1999; Khakpay et al.,

2010), without any significant nociceptive effect. In the present study, 30 nmol intra-LPGi CNQX did not show any significant nociceptive response and it was chosen for the next experiments.

Malinow and Malenka reported that the AMPA-type glutamate receptors are crucial for development of plasticity at central synapses (Malinow and Malenka, 2002). Plasticity of synaptic transmission in the neural circuits following inflammation in periphery or nerve damage is a substantial component of the cellular basis of pathological chronic pain (Woolf and Salter, 2000). This phenomena are not restricted to the synapse between primary nociceptive afferents and spinal projection neurons but are possibly operative in various brain regions that process the sensory and emotional components of pain including the thalamus, somatosensory cortex, anterior cingulate cortex, hippocampus and the amygdala (Gebhart, 2004; Hartmann et al., 2004).

In this study, pre-treatment of LPGi nucleus with CNQX reversed the 17β-estradiol-induced decrement of the second phase of the paw jerking response. Consequently, our results indicated that a portion of this effect is possibly mediated through allosteric interactions and/or directly binding to the membrane-bound AMPA receptors. Our previous study showed that the other part of the antinociceptive effect of 17β-estradiol on the inflammatory pain might be mediated by the intracellular estrogen receptors (Khakpay et al., 2010; Khakpay et al., 2014). Consistent with the results of this study, Wong and Moss (1992) reported that 17β-estradiol treatment of CA1 part of hippocampus increased synaptic excitability by enhancing the magnitude of AMPA receptor-mediated receptors. Similar to these results, Wang et al. (2010) indicated that phosphorylation regulation of AMPA receptor subunits, play a key role in the nociceptive processing of the spinal cord. Gordon and Soliman (1996) reported that intrathecal injection of CNQX inhibits the full development of thermal hyperalgesia for up to 10 days when administered immediately prior to, and for up to 3 days after peripheral nerve damage. Also local, cutaneous application of CNQX attenuates late phase of nociceptive behaviors in the rat formalin test. Consistent with our findings, Tong and MacDermott (2006) suggested that blockade of Ca^{2+}-permeable AMPA receptors in the rat spinal cord reduces the development of hyperalgesia and allodynia related to the peripheral injury. In contrast to our results, khakpay et al. (2010) indicated that CNQX did not show any effect on pain-modulatory effect of 17β-estradiol in the LC nucleus. Also, khakpay et al. reported that the antinociceptive effect of 17β-estradiol in the LPGi nucleus might be mediated via GABAA - but not AMPA- receptors (Khakpay et al., 2017). Hartmann et al. (2004) concluded that AMPA receptors are seriously involved in activity-dependent changes in synaptic processing of painful inputs. In contrast to the results of this study, Gordon and Soliman (1996) reported that AMPA receptor antagonists have the capability to decrease nociceptive transmission at several levels throughout the nociceptive axis.

Conclusion

In conclusion, our data suggest that 17β-estradiol-induced analgesia in the LPGi nucleus is possibly mediated by non-estrogen receptors. With regard to the membrane-bound receptors, AMPA receptors seems to be involved in the 17β-estradiol-mediated antinociception in the LPGi nucleus, but it needs more investigation by molecular and electrophysiological approaches. Our previous study revealed that a part of this analgesic effect in the formalin-induced inflammatory pain is mediated through intracellular estrogen receptors (Khakpay et al., 2014).

Acknowledgments

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Conflict of interest

The authors have declared no conflict of interest.

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