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Original Article

Hippocampal $GABA_A$ Receptor and Pain Sensitivity during Estrous Cycle in the Rat

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Abstract

Background: Estradiol and progesterone as well as hippocampal $GABA_A$ receptors are believed to play a role in the modulation of pain. The aim of present study was to investigate the effect of intrahippocampal injections of $GABA_A$ receptor agonist (muscimol) and $GABA_A$ receptor antagonist (picrotoxin) on pain sensitivity during estrous cycle.

Methods: Pain sensitivity was evaluated in rats by formalin test during all stages of estrous cycle. Animals were divided into five groups including; 1- control (intact animal); 2- sham 1 receiving 0.75 µl artificial cerebrospinal fluids (ACSF); 3- sham 2 receiving 0.75 µl alcoholic ACSF; 4- experimental 1 receiving 250 or 500 µg/rat of muscimol in 0.75 µl vehicle, and 5- experimental 2 receiving 20 or 30 µg/rat picrotoxin in 0.75 µl vehicle. Data were analyzed by Kruskal-Wallis followed by Tucky's test for pairwise comparisons using a P value of ≤ 0.50 for statistical significance.

Results: Muscimol significantly ($P<0.05$) decreased pain sensitivity in all stages of estrous cycle, and the analgesic effect was higher during proestrus and estrus stages of estrous cycle than that during metestrus and diestrus stages. Picrotoxin significantly ($P<0.05$) increased pain sensitivity in all stages of estrous cycle, and such a hyperalgesic effect was lower during proestrus and estrus stages of estrous cycle than that during metestrus and diestrus stages.

Conclusion: The findings of the present study indicate that the role of hippocampal $GABA_A$ receptor in the control of the pain sensitivity can be modulated by variation in gonadal steroids during different stages of the estrous cycle.
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Keywords ● Pain ● estrous cycle ● muscimol ● picrotoxin ● hippocampus

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Introduction

There is now strong evidence for sex differences in sensitivity to pain and analgesia. These differences imply that gonadal steroid hormones such as estradiol and testosterone modulate the sensitivity to pain and analgesia.[1] Terner et al suggested that the phase of the menstrual cycle might alter the effectiveness of certain opioids administered to relieve pain in women.[2] Shekunova and Bespalov suggested that pain management strategies could be optimized through the use of sex- and estrous cycle-specific techniques.[3]

Inhibitory mechanisms are essential in suppressing the development of allodynia and hyperalgesia in a normal animal, and there is evidence that loss of inhibition can lead to the development of neuropathic pain. A great deal of effort has been expended in attempting to define the role of GABA in mediating the transmission and perception of pain.[4] Lovick and colleagues reported that the plasticity of $GABA_A$ receptor subunit expression occurs during the estrous cycle of the rat.[5] In addition, GABA neurons and receptors are found in supraspinal sites known to coordinate the perception and response to painful stimuli, and this neurotransmitter system has been shown to regulate the control of sensory information processing in the spinal cord.[6] Behavioral studies have indicated that GABAergic modulation is involved in the opioid-induced antinociception in the ventrolateral orbital cortex.[7] Lee and co-workers suggested that although the impairment in spinal GABAergic inhibition may play a role in the mediation of neuropathic pain, it is not accomplished by the quantitative change in spinal elements for GABAergic inhibition, and therefore these elements are not related to the generation of neuropathic pain following peripheral nerve injury.[8]

There are now several reports that a rapid decline in progesterone is associated with changes in $GABA_A$ receptor subunit expression in diverse regions of the female rat brain.[9] There is a sex difference in response to $GABA_A$ receptor-mediated injury in the developing hippocampus, also endogenous estradiol concentrations are the same in neonatal male and female hippocampus.[10] Hippocampal volume was increased by either pain or stress, which may be due to edema.[11] The opposite modulatory effects in acute and chronic pain states suggest that there exists a functional switch for the primary sensory cortex at different stages of pain disease, which is of great significance for the biological adaptation.[12] The aim of the present study was to investigate the effects of intrahippocampal injection of muscimol ($GABA_A$ receptor agonist) and picrotoxin ($GABA_A$ receptor antagonist) on pain sensitivity during estrous cycle.

Materials and Methods

Animals

Thirty five female Sprague Dawly rats weighing 200-220g were used. Food and water were made available ad libitum, under a 12 h light/dark cycle (light on at 6 a.m.) and controlled temperature (20±4°C). The protocol of the study was approved by the institutional Committee for the Care and Use of Animal.

Before experiment, different stages of estrous cycle were detected by microscopic examination of vaginal smear based on the relative frequency of leukocyte, cornified and nucleated epithelial cells.[13] Pain sensitivity was examined by formalin test.[14]

Animals were divided into five groups. 1- control group comprised of intact animal (n=5), 2- sham 1 (n=5) assigned to receive 0.75 µl artificial cerebrospinal fluids (ACSF), 3- sham 2 assigned to receive 0.75 µl alcoholic ACSF (n=5), 4- experimental1 allocated to receive 0.75 µl of muscimol 250 or 500 µg/rat (n=10), and 5- experimental 2 assigned to receive 0.75 µl of picrotoxin 20 or 30 µg/rat (n=10). Picrotoxin was solved in alcohol, so the sham 2 group was used to make the comparison with picrotoxin more rational. The doses of the drugs used were according to one of our previous studies.[15] In all animals, formalin test was performed in all stages of estrous cycle.

Formalin Test

Five minutes after the injection of ACSF or drugs 50 µl of 2.5% formalin solution was injected subcutaneously into the planar surface of hind paw using a gauge 30 needle. Formaline-induced pain was scored in blocks of five minutes every 15 seconds during 60 minutes using the following scoring system. The injected paw is not favored; 0, the injected paw has little or no weight on it; 1, the injected paw is elevated and is not in contact with any surface;2, and the injected paw is licked, bitten or shaken; 3.[16] The records of the first 10 minutes were considered as phase 1 of formalin test, and the records after the first 10 minutes was considered as phase 2 of the test.

Surgery

The rats were anesthetized with IP injections of Ketamine 35 mg/kg and Xylazine 5 mg/kg. Afterwards, they were mounted in a sterotaxic instrument (stoelting, USA) and a cannula (gauge 23) was implanted unilaterally at hippocampus (AP: 3.5 mm behind the Bregma, lateral: 3.1 mm and vertical: 4.5 mm from cerebral cortex). Two screws were placed in the skull, and each cannula was anchored into place with dental cement poured around the outer cannula and screws. A stainless steel bar extending just beyond the tip of the cannula was inserted and left in place until injection. The animals were allowed to recover for at least seven days after the surgery.[17] After all tests, rats were given a lethal administration of ether. The microinjection site was marked by

injection of Cresyl Violet (0.2 μl) into the hippocampus. The brain was removed, placed in formalin (10%). Coronal section was prepared to determine the accuracy of the surgery. The data of animals, in which that cannulation was not correct, were removed.

Data Analysis

Number of rats was determined according to a pilot study using the following formula:

$$n=[(Z\alpha+Z\beta)SD/\text{mean difference}]\times 2$$

using Zα=1.96, Zβ=0.84, SD=0.06, and mean difference of 0.07 yielded a sample size of 4.62 for each group, therefore a sample of five rats were included in each group. Data, presented as mean±SD, were analyzed by Statistical Package for Social Sciences (SPSS, version 18). They were analyzed separately for each group with Kruskal-Wallis nonparametric test. In case of significant results with Kruskal-Wallis test, pairwise comparisons were made using Tukey test. A P value of ≤0.05 was considered statistically significant.

Results

The Effects of Muscimol

Intrahippocampal injection of muscimol (250 μg/rat) significantly decreased the level of pain in phase 1 of formalin test during proestrus (P=0.008) and estrus (P=0.000) stages of estrous cycle (figure 1). Intrahippocampal injection of muscimol (250 and 500 μg/rat) significantly decreased the level of pain in phase 1 of formalin test during metestrus (P=0.000) and diestrus (P=0.000) stages of estrous cycle (figure 1). Intrahippocampal injection of muscimol (250 and 500 μg/rat) significantly decreased the level of pain in phase 2 of formalin test during proestrus (P=0.000), estrus (P=0.004) and metestrus (P=0.000) stages of estrous cycle, whereas no significant change in the level of pain was observed during diestrus stage of estrous cycle (figure 1). The analgesic effect of muscimol was significantly (P=0.004) higher during proestrus and estrus than during metestrus and diestrus stages of estrous cycle (figure 1).

The Effects of Picrotoxin

Intrahippocampal injection of picrotoxin (20 μg/rat) significantly increased the level of pain in phase 1 of formalin test during proestrus (P=0.000), estrus (P=0.035) and diestrus (P=0.003) stages of estrous cycle (figure 2). Intrahippocampal injection of picrotoxin (30 μg/rat) significantly increased the level of pain in phase 1 of formalin test during metestrus

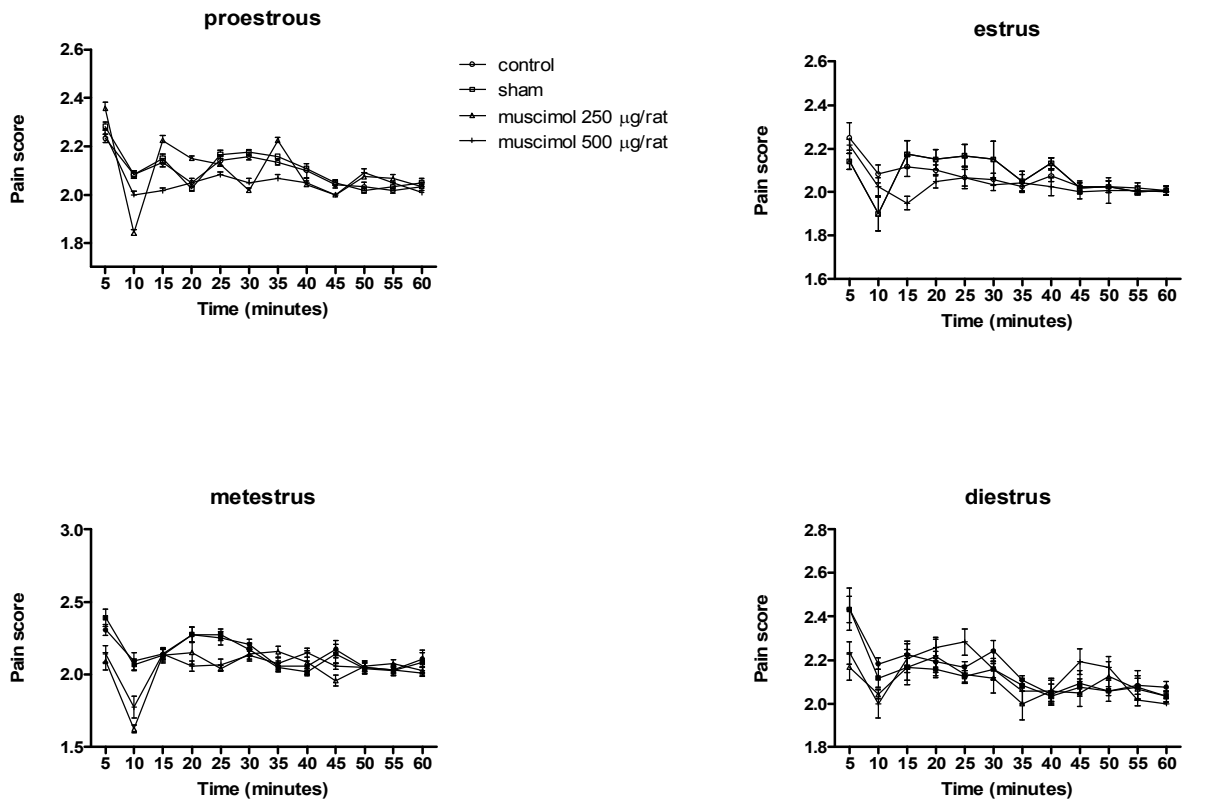

Figure 1: Effects of muscimol on pain score in formalin test during different stages of estrous cycle in the rat.

(P=0.000) stage of estrous cycle (figure 2). Intrahippocampal injection of picrotoxin (20 and 30 µg/rat) significantly increased the level of pain in phase 2 of formalin test during proestrus (P=0.000), estrus (P=0.000) and diestrus (P=0.000) stages of estrous cycle (figure 2), whereas no significant changes in the level of pain was seen during metestrus (P=1.000) stage of estrous cycle (figure 2). Hyperalgesic effect of picrotoxin was significantly (P=0.004) higher during metestrus and diestrus than during proestrus and estrus.

Discussion

Formalin test is a valuable method to study nociception. In rats, responses in two distinct stages of the formalin test may be used to address different aspects of nociception. The first stage of the test seems to be due to direct chemical stimulation of nociceptors, whereas the second stage is dependent on peripheral inflammation and changes in central processing.[18] Da Silva and co-workers,[19] demonstrated that the antinociceptive effect of the opioids in the rostral ventromedial medulla could be mediated by disinhibition of tonically active GABAergic interneurons in the downstream projection neurons of the descending pain control system. This indicates an interaction between the opioidergic and GABAergic pathways of pain modulation.[19] On the other hand, Griffiths and Levick reported that the fall of progesterone levels during estrous cycle induces changes in the expression of $GABA_A$ receptor subunit, which may lead to an increase in the excitability of neuronal circuitry in periaqueductal gray matter.[20]

In the present investigation, muscimol decreased the levels of pain in all stages of estrous cycle. Lee and Lim reported that muscimol had anti-allodynic and anti-thermal hyperalgesic effects.[21] Naik and colleagues reported that two $GABA_A$ receptor agonists, muscimol and 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol, applied to the L_5 dorsal root ganglion at the time of a sciatic nerve crush injury, caused long-lasting alleviation of thermal hyperalgesia in a dose-dependent manner. When muscimol was applied to the dorsal root ganglion of trauma-injured peripheral nerves after neuropathic pain was being fully developed, its pain-alleviating effects, although significant, were short-lived. These findings indicate that exoge-

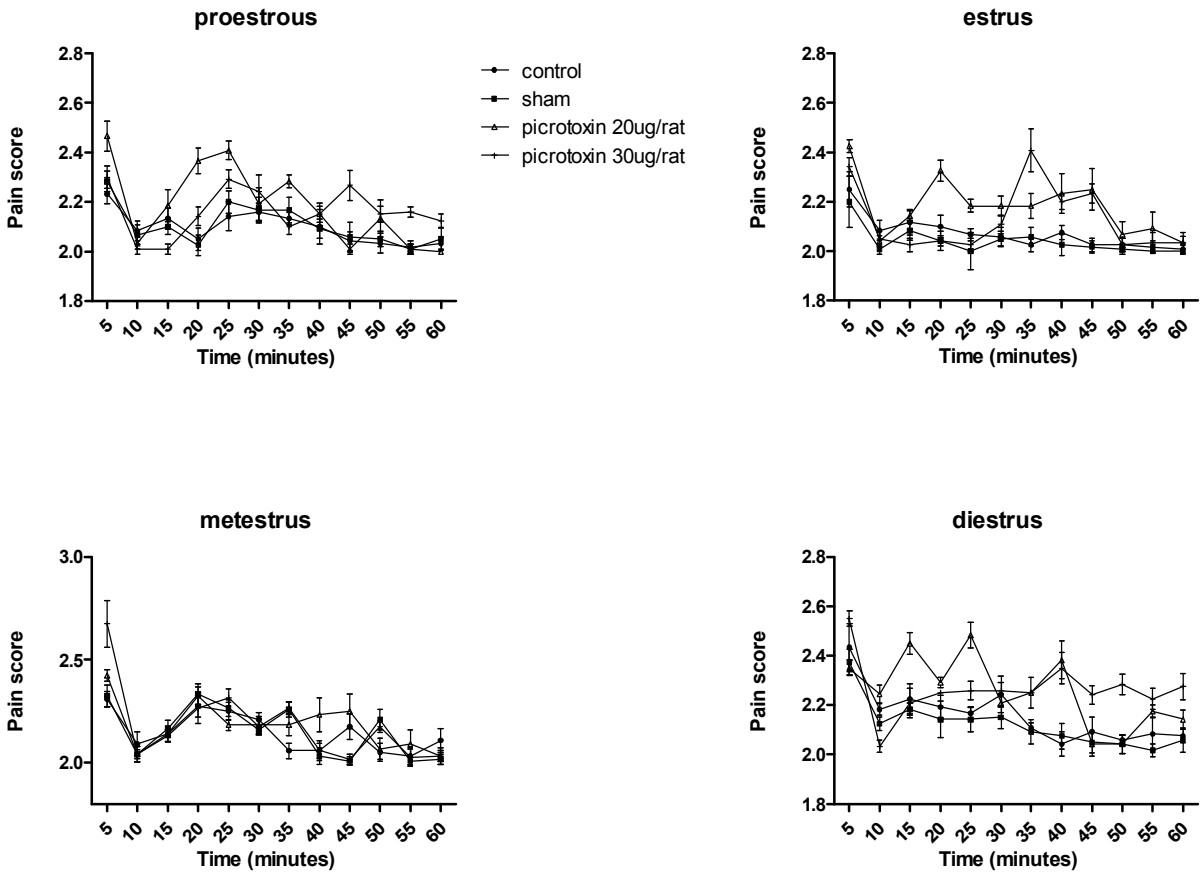

Figure 2: Effect of picrotoxin on pain score in formalin test during different stages of estrous cycle in the rat.

nous $GABA_A$ receptor modulation of the dorsal root ganglion is important in the development and maintenance of chronic pain. Under normal conditions, tonic GABA-mediated inhibition of the afferent inputs modulates sensory processing. By acting both pre- and postsynaptically, GABA exerts tonic modulation of nociceptive neurotransmission between primary afferents and second-order spino-thalamic tract neurons.[22] Sheng et al found that ventrolateral orbital cortex application of the $GABA_A$ receptor agonist muscimol (250 ng) or 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (1.0 µg) significantly attenuated the quinpirole-induced tail flick reflex inhibition.[23]

In the present study picrotoxin increased pain sensitivity in all stages of estrous cycle. Naik et al reported that two $GABA_A$ receptor antagonists, bicuculline and picrotoxin, applied to the lumbar 5 of the dorsal root ganglion at the time of a sciatic nerve crush injury, caused long-lasting exacerbation of thermal hyperalgesia in a dose-dependent manner. Furthermore, muscimol-induced alleviation of thermal and mechanical hyperalgesia, and 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol -induced alleviation of thermal hyperalgesia were completely reversed by bicuculline.[22] Sheng et al found that ventrolateral orbital cortex application of the $GABA_A$ receptor antagonist bicuculline or picrotoxin (100 ng) enhanced the quinpirole-induced inhibition of the tail flick reflex. Oral administration of chrysin (75 mg/kg) also produced a hyperalgesic effect in the tail-immersion test.[24]

In the present investigation, analgesic effect of muscimol was higher in proestrus and estrus than that in metestrus and diestrus. Favaro-Moreira et al have reported that high physiological estradiol level during the proestrus phase of the estrous cycle, or systemic estradiol administration in ovariectomized rats decreases formalin-induced temporomandibular joint nociception. These findings suggest that estradiol decreases temporomandibular joint nociception in female rats through a peripheral non-genomic activation of the nitric oxide-cyclic guanosine monophosphate signaling pathway.[25]

Hyperalgesic effect of picrotoxin was more intense in metestrus and diestrus than in proestrus and estrus. Decreasing levels of progesterone during late diestrus may, therefore, be a predisposing factor for the development of stress-induced hyperalgesia in females.[26]

Watanabe et al suggest that GABA depolarizes neurons of gonadotropin releasing hormone (GnRH) by activating $GABA_A$ receptors, thereby activating voltage-gated Ca^{2+} channels and facilitating Ca^{2+} influx. In addition, the response to GABA is modulated according to the estrous cycle stage, diurnal rhythm, and sex.[27] Akema et al supported the hypothesis that diminution of the GABAergic suppressive activity in the medial preoptic area permited the LH surge to be induced.[28]

Torres-Reveron et al demonstrated that estrogen levels positively regulated the availability of Mu opioid receptors on GABAergic interneurons in the dentate gyros, suggesting a cooperative interaction between opioids and estrogens in modulating principal cell excitability.[29] These results indicated that estrogen status differentially affected morphine modulation of temporomandibular joint unit activity in superficial, but not deep laminae at the trigeminal subnucleus caudalis junction in female rats. The site(s) for estrogen influence on morphine-induced modulation of temporomandibular unit activity was probably outside the medullary dorsal horn.[30] These results show that ovariectomy induces a hyperalgesic state of slow onset and long duration that can be reversed by estrogen. Also, Sanoja and Cervero have observed no modulation of pain sensitivity at different stages of estrous cycle in normal animals.[31]

Although gonadectomy and steroid replacement are frequently used to examine the role of gonadal steroids in nociception and antinociception, it is important to note that steroid replacement does not actually mimic the hormonal milieu of the intact female. Thus, the roles of various steroid hormones deduced from studies of steroid-replaced females do not necessarily tell us what these hormones do in a normal, gonadally intact female whose steroid levels are constantly changing.[32]

The analgesic effect of Muscimol was high in proestrus and estrus stage of the estrous cycle, during which estrogen, progesterone, LH and FSH are in peak levels,[33] and 3α- hydroxyl-5α-pregnan-20-one (3α-5α- THP) is also elevated.[34]

Conclusion

The findings of the present study demonstrate that the analgesic effects of muscimol is low during the metestrus and diestrus stage of the estrous cycle, when progesterone is elevated and estrogen and LH levels are low. They also show that hyperalgesic effect of picrotoxin is low in the proestrus and estrus stages, when concentrations of progesterone and estradiol are high, and high in the metestrus and diestrus stages, when concentrations of progester-

one and estradiol are low. The findings might suggest that estrogen and progesterone might somehow have the ability to reduce the sensitivity to pain.

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Conflict of Interest: None declared

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