Indian J Physiol Pharmacol 2005; 49 (1): 77–82

CNS α1 RECEPTOR IN TONIC PAIN DURING ESTROUS CYCLE IN RATS*

M. TAHERIANFARD*, H. RAJAIAN, A. BAHAODDINI AND A. DABBAGHLOTFI

Department of Physiology,
School of Veterinary Medicine,
Shiraz University, Shiraz-Iran,
P.O. Box : 1731
Email : taherian@shirazu.ac.ir

(Received on December 3, 2003)

Abstract : Estrogen and progesterone are known to affect nociception. The plasma concentrations of these hormones vary during estrous cycle in rodents. The aim of the present study was to investigate the effect of evidence of α1 receptor agonist and antagonist on tonic pain in all phases of estrous cycle in female rats. Phenylephrine (α1 agonist) and prazosin (α1 antagonist) were administered via intracerebroventicular (ICV) injection. Adult female rats weighting 200–220 g were maintained on 12 h light/dark cycle for 10–14 days prior to the experiment. Food and water were made available *ad libitum.* Formalin test was performed in all phases of estrous cycle. Results showed that phenylephrine caused significant ($P<0.05$) reduction in pain sensitivity. This reduction was more pronounced during proestrus phase. Prazosin significantly ($P<0.05$) increased pain sensitivity, particularly during metestrus phase. It is possible that fluctuation in pain sensitivity during estrous cycle is related to the level of sex hormones during estrous cycle.

Key words : formalin test estrous cycle pain phenylephrine prazosin

INTRODUCTION

Gonadal hormones can alter nociception in both the central and peripheral nervous system. It has been reported that progesterone (P), a pregnane precursor of neurosteroids and 4-chlordiazepam (4-CD), a high affinity ligand for mitochondrial diazepam binding inhibitor receptor stimulates neurosteroid synthesis, in both acute (tail flick latency test) and chronic (formalin test), pain models (1). Both P and 4-CD showed analgesic response in these models (1). The effect of P and 4-CD was antagonized by bicuculline on tail flick latency test but not in formalin test. However, naloxone attenuated the antinociceptive response of P and 4-CD in tail flick latency test as well as formalin test. Pretreatment with P and 4-CD potentiated the analgesic effect of morphine and nimodipine in both models of pain

*This research was financially support by Shiraz University
**Corresponding Author

sensitivity. The peripheral nervous system "silent" afferents, which arise form the uterus, appear to be affected by the phase of the estrous cycle, and estrogen alters the receptive field properties of primary afferents (2). More over, pregnancy influences nerve fiber conduction and the susceptibility of somatic and visceral nerves to the effects of local anesthetics (3). In the central nervous system, gonadal hormones influence endogenous opioid system (4), as well as the activity of other neuromodulators, for example substance P (5), and neurotransmitters such as dopamine, serotonin and norepinephrine (6, 7). These neurochemical are involved in nociceptive processing. The aim of this study was to determine the effect of phenylephrine (α1 agonist) and prazosin (α1 antagonist) on tonic pain in all phases of estrous cycle in female rats.

METHODS

Animals

Twenty female Sprague Dawly rats weighing 200–250 g were used. Food and water were made available *ad libitum,* under a 12 h light/dark (lights on at 6 a.m.) and controlled temperature (20–24°C). Before experiment, different phases of estrous cycle were detected by microscopic examination of vaginal smear based on the relative frequency of leukocyte, cornified and nucleated epithelial cells (8). To investigate the effect of phenylephrine and prazosin in pain sensitivity during the estrous cycle, formalin test was performed (9). Animals were divided into 3 groups: first, Control (5 intact animals); second, Sham (5 animals received artificial cerebrospinal fluid (ACSF) via ICV route); and third, Experimental (5 animals received phenylephrine (5 µg/rat via ICV route) and 5 animals received prazosin 20 mg/rat via ICV route).

Surgery

The rats were anesthetized with IP injection of sodium pentobarbital (50 mg/kg) and after mount in sterotaxic instrument (stoelting, USA) cannula (23 guge) implanted unilaterally at the lateral ventricle (AP: 1 mm behind the Bregma, lateral: 2.5 mm and vertical: 4.5 mm form 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 extending just beyond the tip of cannula was inserted and left in place until injection. The animals were allowed to recover for at least 7 days after surgery (10).

Formalin test

Five minutes after ICV injection of ACSF or α1 receptor drugs 50 µl of 2.5% formalin solution was injected subcutaneously into the planar surface of hind paw with a 30 gauge needle. A pain score was determined for each 5 minutes block by measuring the amount of time spent in each of the following four behavioral categories: 0, the injected paw is not favored; 1, the injected paw has little or no weight on it; 2, the injected paw is elevated and is not in contact with any surface; 3, the injected paw is licked, bitten or shaken (11).

Data analysis

Data were analyzed separately for each group with Two way (time × phase) and Two way (time × group) analysis of variance (ANOVA) with repeated measures on one factor (time). Post-hoc analysis was performed with Turkey's test. Significant value was $P<0.05$.

RESULTS

A: Effect of phenylephrine on tonic pain during estrous cycle

Pain score after phenylephrine administration in proestrus phase shows significant time × group interaction [F (3, 16) = 11.11, P<0.0001] in first stage of formalin test and [F (27, 30) = 6.33, P<0.0001] in second stage of formalin test (Fig. 1). Thus, during proestrus phenylephrine significantly (P<0.05) decreased pain sensitivity in two stages of formalin test

Pain score after phenylephrine administration in proestrus phase shows significant time×group interaction [F (3, 16) = 6, P<0.001] in first stage of formalin test and [F (27, 30) = 4.7, P<0.0001] in second stage of formalin test (Fig. 2). Thus, during estrus phenylephrine significantly (P<0.05) decreased pain sensitivity in two stages of formalin test.

Pain score after phenylephrine administration during metestrus phase shows significant time × group interaction [F (27, 30) = 2.66, P<0.01] in second stage of formalin test (Fig. 3). Phenylephrine had no significant effect during metestrus in first stage of formalin test; while, relative to control and sham groups phenylephrine significantly (P<0.05) decreased pain sensitivity during metestrus in second stage of formalin test. Pain score after phenylephrine in diestrus phase showed significant time × group interaction [F (27, 30)

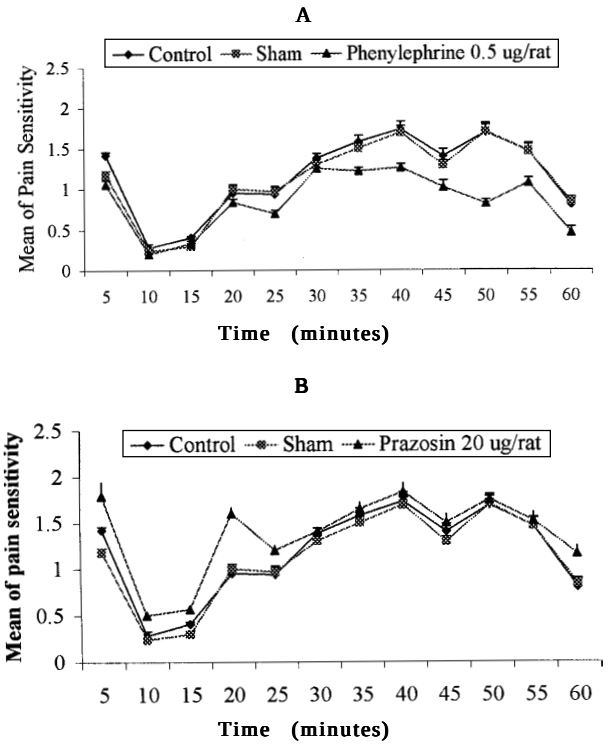

Fig. 1: Effect of phenylephrine 0.5 µg/rat (A) and prazosin 20 µg/rat (B) on pain sensitivity in proestrus phase of estrous cycle during 60 minutes.

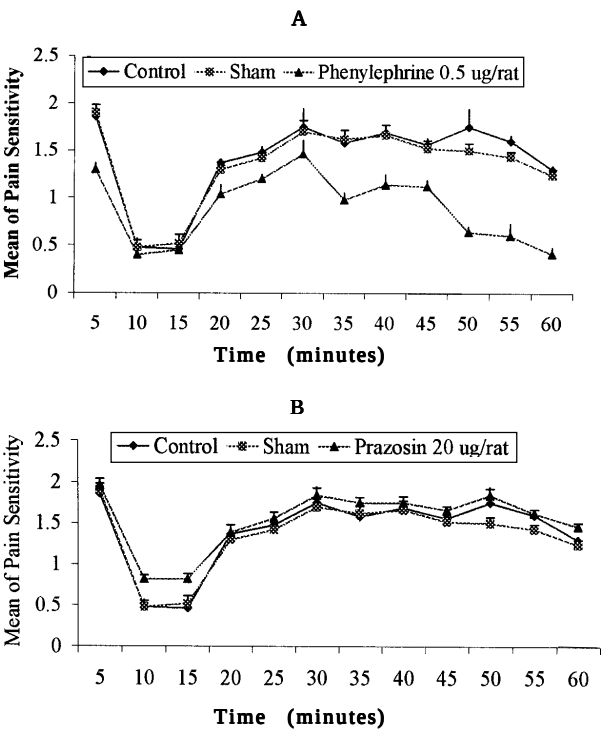

Fig. 2: Effect of phenylephrine 0.5 µg/rat (A) and prazosin 20 µg/rat (B) on pain sensitivity in estrus phase of estrous cycle during 60 minutes.

= 3.54, P<0.001] in second stage of formalin test (Fig. 4). Phenylephrine in diestrus had no significant effect in first stage of formalin test; while, phenylephrine significantly (P<0.05) decreased pain sensitivity during diestrus in second stage of formalin test.

In all phases of estrous cycle phenylephrine showed significant time×phase interaction [F (3, 16) = 22.7, P<0.0001] in first stage of formalin test and [F (27, 30) = 4.5, P<0.0001] in second stage of formalin test. Thus, phenylephrine significantly (P<0.05) decreased pain sensitivity in all phases of estrous cycle, but this analgesia is lower in metestrus and higher in diestrus.

B : Effect of prazosin on tonic pain during estrous cycle

Compared to control and sham groups prazosin had no significant (P>0.05) effect on pain sensitivity in two stages of formalin test (Fig. 1, 2 and 4). Pain score after prazosin in metestrus phases showed significant time × group interaction [F (27, 30) = 2.66, P<0.05] in second stage of formalin test (Fig. 3). Prazosin had no significant effect in metestrus in first stage of formalin test; while, prazosin compared to control and sham groups, significantly (P<0.05) decreased pain sensitivity during metestrus phase of estrous cycle in second stage of formalin test. In all phases of estrous cycle prazosin showed significant

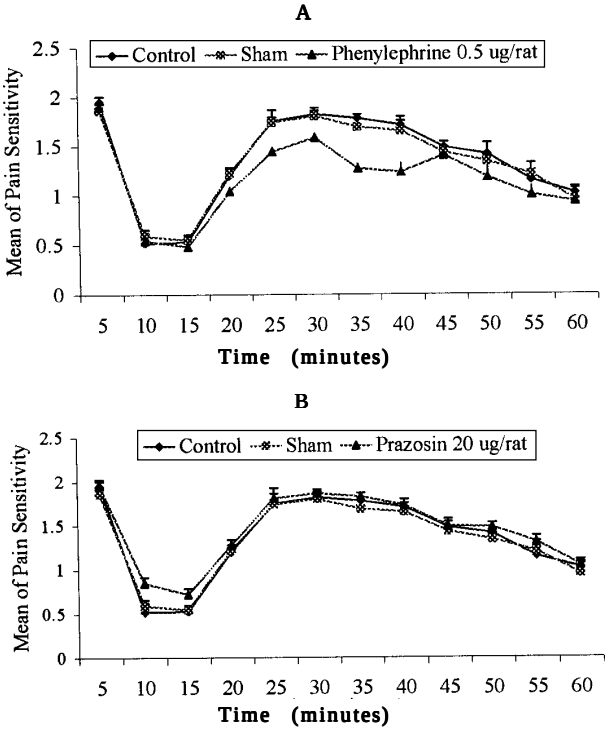

Fig. 3 : Effect of phenylephrine 0.5 µg/rat (A) and prazosin 20 µg/rat (B) on pain sensitivity in metestrus phase of estrous cycle during 60 minutes.

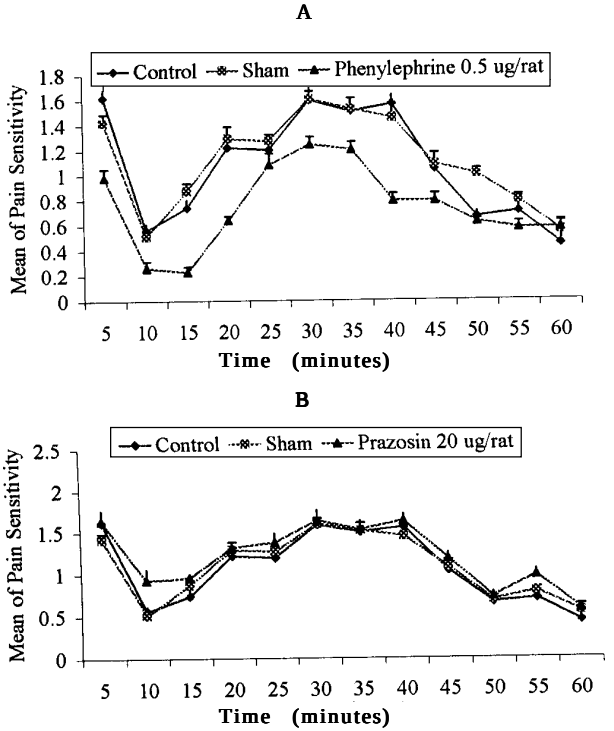

Fig. 4 : Effect of phenylephrine 0.5 µg/rat (A) and prazosin 20 µg/rat (B) on pain sensitivity in diestrus phase of estrous cycle during 60 minutes.

time × phase interaction [F (27, 30) = 7.861, P<0.0001] in second stage of formalin test. Thus, prazosin significantly (P<0.05) increased pain sensitivity during metestrus phase of estrous cycle.

DISCUSSION

The stimulus provided by injection of formalin is tonic, including a behavioral response, with a duration of one hour. The lone-lasting stimulus facilitates the observation of feed-back modulation and the role of endogenous pain-regulatory systems, such as opioid and monoaminergic systems. In rats, the two distinct stages of the test response may be used to address different aspects of nociception; since the first stage seems to be due to direct chemical stimulation of nociceptors, whereas the second stage is dependent on peripheral inflammation and changes in central processing (12).

In this study phenylephrine induced the highest analgesia during diestrus phase. This is in accordance with the earlier report of longer tail flick latencies in rats during diestrus; this effect may be due to general arousal or distractibility associated with hormonal changes (13). Another study shows that baseline hotplate latencies are higher in sham group females tested during diestrus than in those tested during estrous (14). Mitrovic *et al.* (1999) reported that diestrus phase is characterized by basal level of estradiol with small gradual peak of progesterone (15). Progesterone produces an antinociceptive effect which may be mediated by modulation of GABAergic and/ or opiodergic mechanisms (1). Voltage gated calcium channels may also be involved (1). According to present study phenylephrine induced lower analgesia during metestrus phase. Mitrovic *et al* (15) have shown that progesterone and estradiol during metestrus are in high and low levels, respectively. It was shown that persistent high plasma level of progesterone, in the absence of estrogen, produces a consistent antinociceptive effect in a model of persistent inflammatory hyperalgesia. They suggested that antihyperalgesic effect of progesterone includes suppression of NMDA receptor activation at the spinal cord level (16). Several investigation indicate that plasma concentrations of progesterone in ewes (17) and 5 α-pregnan-3 α-ol-20-one (progesterone metabolite) in rat (18) were not different during metestrus and diestrus. So, it seems that the difference in pain sensitivity in these two phases depends on another sex steroid. Rhodes *et al.* (2001) reported that tailflick latencies increased during estrous following the blockade of progesterone metabolism to 5 α-pregnan-3 α-ol-20-one (3 α, 5 α-THP) with finasteride in hippocampus (19). In the present study, however, prazosin hyperalgesia was observed during metestrus phase. According to Vinogradoova *et al.* (20), electrical pain threshold is higher during proestrus and estrus phases in comparison to metestrus and diestrus. Johnson and Berkley (21) reported that micturation threshold after bladder inflammation was significantly lower in proestrus or estrus that in metestrus or diestrus. Giamberardino *et al.* (22) demonstrated an enhancement of urethral pain sensitivity in metestrus/ diestrus. Levin and Taiwo (23) reported that estradiol induces a catecholamine sensitive hyperalgesia. This hyperalgesia was antagonized by yohimbin (α2 receptor antagonist) but not by prazosin (α2 receptor antagonist). According to the results of the

present study, the effect of phenylephrine and prazosin was changed in a complex fashion during estrous cycle. It seems that in addition to the effect of progesterone and estradiol on pain sensitivity their metabolites may have influence on the pain sensitivity and that it deserves future detail study.

REFERENCES

1. Gambhir M, Mediratta PK, Sharma KK. Evaluation of the analgesic effect of neurosteroids and their possible mechanism of action. *Indian J Physiol Pharmacol* 2002; 46: 202–208.
2. Robbins A, Berkley KJ, Sato Y. Estrous cycle variation of afferent fibers supplying reproductive organs in the female rat. *Brain Res* 1992; 596(1–2): 353–363.
3. Kaneko M, Saito Y, Kirihara Y, Kosaka Y. Pregnancy enhances the antinociceptive effects of extradural lignocaine in the rat. *Br J Anaesth* 1994; 72: 657–661.
4. Smith YR, Zubietaii JK, del Carmen MG, et al. Brain opioid receptor measurements by positron emission tomography in normal cycling women : relationship to luteinizing hormone pulsatility and gonadal steroid hormones. *J Clin Endocrinol Metab* 1998; 83: 4498–4505.
5. Duval P, Lenoir V, Moussaoui S, Garret C, Kerdelhue B. Substance P and neurokinin A variations throughout the rat estrous cycle; comparison with ovariectomized and male rats : II. Trigeminal nucleus and cervical spinal cord. *J Neurosci Res* 1996; 45: 610–660.
6. Smith AD, Justice JB. The effect of inhibition of synthesis, release, metabolism and uptake on the microdialysis extraction fraction of dopamine. *J Neurosci Methods* 1994; 54(1): 75–82.
7. Fillingim RB. Sex, gender, and pain : women and men really are different. *Curr Rev Pain* 2000; 4: 24–30.
8. Hafez E.S.E. Rat and mice, in Reproduction and breeding techniques for laboratory animals (Bennet JP and Vickery BH), Raven, New York, 1970 pp.299–315.
9. Kuraishi Y, Harada Y, Takagi H. Noradrenaline regulation of pain-transmission in the spinal cord mediated by alpha-adrenoceptors. *Brain Res* 1979; 174: 333–336.
10. Soignier RD, Vaccarino AL, Brenan AM, Kastin AJ, Zadina JE. Analgesic effects of endomorphine-1 and endomorphin-2 in the formalin test in mice. *Life Science* 2000; 67: 907–912.
11. Corderre TC, Vaccarino AL and Melzack R. Central nervous system plasticity in the tonic pain response to subcutaneous formalin injection. *Brain Res* 1990; 535: 155–158.
12. Tjϕlsen A, Berge OD, Hunskaar S, Rosland JH and Hole K. The formalin test : an evaluation of the method. *Pain* 1992; 51: 5–17.
13. Gomez MM, Cruz Y, Salas M, Hudson R and Pacheco P. Assessing pain threshold in the rat : change with estrous and time of day. *Physiology Behavior* 1994; 55: 651–657.
14. Rhodes ME, Frye CA. Inhibiting progesterone metabolism in the hippocampus of rats in behavioral estrous decreases anxiolytic behaviors and enhances exploratory and antinociceptive behaviors. *Cogn Affect Behav Neurosci* 2001; 1: 287–296.
15. Mitrovic AD, Maddison JE, Johnston GAR. Influence of the estrous cycle on L-glutamate and L-aspartate transport in rat brain synaptosomes. *Neurochemistry International* 1999; 34: 101–108.
16. Ren K, Weil F, Dubner R, Murphy A, Hoffman GE. Progesterone attenuates persistent inflammatory hyperalgesia in female rats : involvement of spinal NMDA receptor mechanisms. *Brain Res* 2000; 26: 272–277.
17. Allison Gray C, Bartol FF, Taylor KM, et al. Ovine uterine gland knock-out model : effects of gland ablation on the estrous cycle. *Biol Reprod* 2000; 62: 448–456.
18. Frye CA, Bayon LE. Cyclic withdrawal from endogenous and exogenous progesterone increases kainic acid and perforant pathway induced seizures. *Pharmacol Biochem Behav* 1999; 62: 315–321.
19. Stock HS, Caldarone B, Abrahamsen G, Mongeluzi D, Wilson MA, Rosellini RA. Sex differences in relation to conditioned fear-induced enhancement of morphine analgesia. *Physiol Behav* 2001; 72: 439–447.
20. Vinogradova EP, Zhukov DA, Batuev AS. The effects of stages of the estrous cycle on pain thresholds in female white rats. *Neurosci Behav Physiol* 2003; 33: 269–272.
21. Johnson OL, Berkely KJ. Estrous influences on micturition thresholds of the female rat before and after bladder inflammation. *Am J Physiol Regul Integr Comp Physiol* 2002; 282: R289–R294.
22. Giamberardino MA, Affaitati G, Valente R, Iezzi S, Vecchiet L. Changes in visceral pain reactivity as a function of estrous cycle in female rats with artificial ureteral calculosis. *Brain Res* 1997; 774(1–2): 234–238.
23. Levine JD, Taiwo YO. Beta-estradiol induced catecholamine-sensitive hyperalgesia : a contribution to pain in Raynaud's phenomenon. *Brain Res* 1989; 15: 143–147.