Effect of xylazine and yohimbine on the phasic pain during the estrous cycle in the rat

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Summary

The aim of the present study was to investigate the effect of α_2-adrenergic agonist (xylazine) and antagonist (yohimbine) on phasic pain during estrous cycle in female rats. Adult female rats weighing 180-220 g were kept under controlled temperature (21-24°C) and light/dark conditions (light on at 6:00 a.m. and light off at 6:00 p.m.). Animals were divided into four groups: 1) control group which received 0.3 ml of normal saline by intraperitoneal (IP) route; 2) IP experimental group which received 0.3 ml xylazine 3, 4.5 and 6 mg/kg and yohimbine 1, 2 and 4 mg/kg by IP route; 3) sham group which received 2 μl of artificial cerebrospinal fluid by intra cerebral ventricle (ICV) route and 4) ICV experimental which received 2 μl xylazine 10 and 20 μg/rat and yohimbine 5 and 10 μg/rat by ICV route. Cannulae were implanted into the left lateral ventricle using stereotaxic method. Pain sensitivity was measured by tail flick test, which was performed before injection, 15 and 30 min after injection in all groups. Xylazine decreased pain sensitivity significantly ($P<0.05$) during the estrous cycle; while higher analgesia was observed in the proestrus phase for IP and ICV routes. Yohimbine increased pain sensitivity significantly ($P<0.05$) during the estrous cycle; while higher hyperalgesia was observed in the metestrus phase for IP and ICV route groups. There was interaction ($P<0.05$) between endogenous steroids and the α_2-adrenergic system in the modulation of phasic pain sensitivity.

Key words: Xylazine, Yohimbine, Phasic pain, Female rat

Introduction

There is some evidence that male and female subjects differ in their response to painful stimuli during noxious heat stimulation (Paulson *et al*., 1998). Sex differences were observed in morphine-induced analgesia in the rat that may be due to differences in the central nervous system sensitivity to morphine. One reasonable hypothesis is that there are differences between males and females in the number or affinity of those opiate receptors involved in mediating antinociception (Cicero *et al*., 2002). In female rats pain sensitivity is different during stages of life such as pregnancy, parturition and phases of estrous cycle (Gomez *et al*., 1994). These differences may be related to the effect of steroid hormones on pain sensitivity (Bradshaw *et al*., 2000). Data indicate that sex steroids have a positive effect on pain and cluster headaches (Frye and Duncan, 1994).

Although endogenous hormonal fluctuations or experimental steroid manipulations modulate pain threshold, contradictory results on the effect of steroid hormones have been reported. For example, administration of estradiol-17β increases (Frye *et al*., 1992), decreases (Ratka and Simpkins, 1991) or has no effect (Dawson-Basoa and Gintzler, 1993) on tail flick test latency. Similarly, acute progesterone administration has been reported to increase (Frye and Duncan, 1994), and chronic progesterone administration to decrease analgesia (Frye *et al*., 1992) or have no analgesic effect (Dawson-Basoa and Gintzler, 1993). 3α-hydroxy-5α-pregnane-20-one (3α-5α-THP) also increases tail flick latency (Winfree *et al*., 1992).

On the other hand, norepinephrine appears to play an important role in the modulation of pain transmission at the level of the spinal cord (Miller and Proudfit,

1990). Electrical stimulation of locus coeruleus extended the latency of hot plate reaction in rat; this analgesic effect was antagonized by intrathecal administration of the α_2 antagonist yohimbine. Similarly, systemic or intrathecal injection of the α_2 agonist clonidine prolonged the latency to the nociceptive stimuli measured by hot plate reaction or tail flick (Wang *et al*., 1998). Estradiol induces a catecholamine sensitive hyperalgesia. This hyperalgesia is antagonized by yohimbine (an α_2-adrenergic antagonist) but not prazosin (an α-adrenergic antagonist) (Levine and Taiwo, 1989).

The purpose of the present study was to investigate the effects of IP and ICV administration of α_2 agonist (xylazine) and α_2 antagonist (yohimbine) on phasic pain during the estrous cycle in female rats using tail flick test.

Materials and Methods

Twenty female Sprague-Dawley rats weighing 180-220 g were used. Food and water were available ad libitum, under a $^{12}/_{12}$ hrs of light/dark conditions (lights on at 6 a.m. and off at 6 p.m.) and controlled temperature (21-24°C). Phases of estrous cycle were determined by microscopic examination of vaginal smear from animals before each test and looking for the relative frequency of leukocyte, cornified and nucleated epithelial cells (Hafez, 1970).

To investigate the effect of xylazine and yohimbine in mediating changes in pain sensitivity during the estrous cycle, tail flick test was performed. Animal were divided into four groups: 1) control group which received 0.3 ml of normal saline IP; 2) IP experimental group which received 0.3 ml xylazine 3, 4.5 and 6 mg/kg and yohimbine 1, 2 and 4 mg/kg IP; 3) sham group which received 2 µl of artificial cerebrospinal fluid (ACSF) ICV and 4) ICV experimental which received 2 µl xylazine 10 and 20 µg/rat and yohimbine 5 and 10 µg/rat ICV.

Tail flick test

Animals were handled twice a day for 15 days to reach minimum stress before they were subjected to experimental procedures. The restrainer consisted of a Plexiglass tube with a hole in the bottom to allow free movements of the tail during testing. The stimulus consisted of a radiant heat source, focused as 1-cm spot on the third of their tails. The intensity of heat was initially adjusted (current 4.6 A) so that the baseline tail flick latency averaged 2-3 sec in all animals. The heat source and times were activated simultaneously by a pedal. Both were terminated automatically by tail movement, exposing a photocell beneath the tail or by the experimenter at the end of 10 sec cut off time (in order to avoid injury to the tail). Tail flick tests were performed 15 min before IP and ICV injection as "control latency time" and 15 and 30 min after IP and ICV injection as "test latency time". Percentage of analgesic index (AI%) was calculated as follow (James *et al*., 1995):

$$AI\% = \frac{\text{test latency time} - \text{control latency time}}{\text{cut off time} - \text{control latency time}}$$

Surgery

The rats were anaesthetized with IP injection of sodium pentobarbital (50 mg/kg) and implanted unilaterally with a 23-gauge outer cannula aimed at the lateral ventricle (anteroposterior: 1 mm behind the Bregma, lateral: 2.5 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 probe extending just beyond the tip of cannula was inserted during surgery and left in place until time of injection. The animals were allowed to recover for at least 7 days after surgery (Soignier *et al*., 2000).

Statistical analysis

Data were analysed separately for each group with 2 ways (time×phase) and 2 ways (time×group) analysis of variance (ANOVA) with repeated measures for one factor (time). Post-hoc analysis was performed with Tukey's test. The level of statistical significance was set at $p<0.05$.

Results

Effect of xylazine on phasic pain during the estrous cycle

Fifteen and 30 min following IP administration of 4.5 and 6 mg/kg xylazine,

significant (time × group) interaction of percentage of analgesic indices were shown during the proestrus [F(1,20) = 0.297, (P<0.05)], estrus [F(1,20) = 0.292, (P<0.05)], metestrus [F(1,20) = 0.6, (P<0.05)] and diestrus phase of the estrous cycle [F(1,20) = 4.6, (P<0.05)] (Fig. 1). Xylazine at the dose of 3 mg/kg did not show any significant effects.

Fifteen and 30 min following ICV administration of 10 and 20 µg/rat xylazine, significant (time × group) interaction of percentage of analgesic indices were shown during the proestrus [F(1,15) = 0.66, (P<0.05)], estrus [F(1,15) = 0.12, (P<0.05)], metestrus [F(1,15) = 0.132, (P<0.05)] and diestrus phase of the estrous cycle [F(1,15) = 0.6, (P<0.05)] (Fig. 2).

Thus IP and ICV administration of xylazine significantly decreases pain sensitivity (P<0.05) in all phases of estrous cycle (Figs. 1 and 2). Analgesic effect of xylazine in both IP and ICV administration was high in the proestrus and low in the metestrus phase of the estrous cycle.

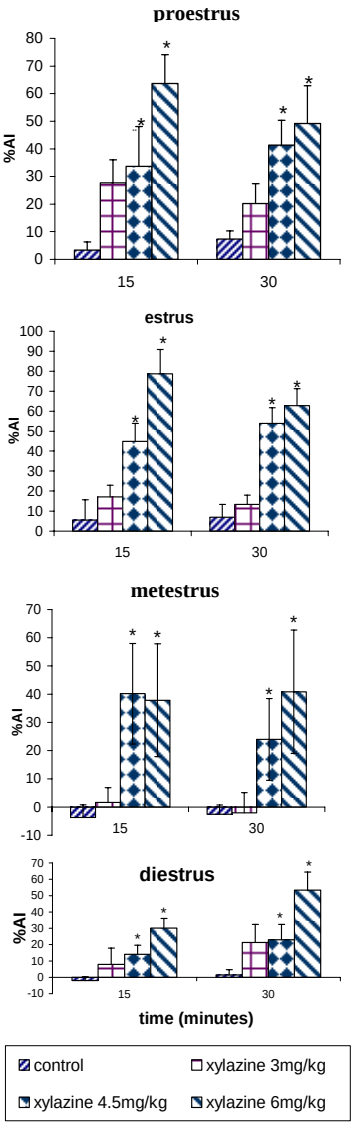

Fig. 1: Effect of IP administration of xylazine on AI% in all phases of the estrous cycle. *Significant difference from the control group (P<0.05)

Effect of yohimbine on the phasic pain during the estrous cycle

Fifteen and 30 min following IP

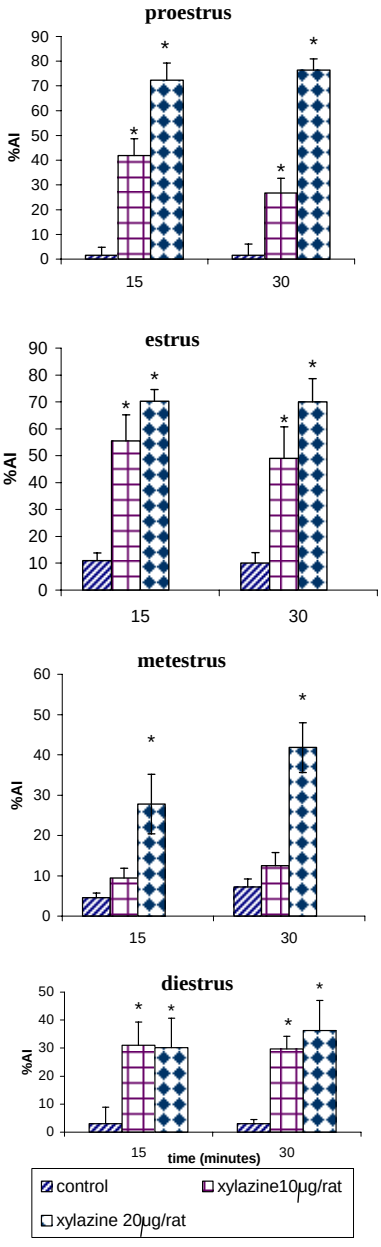

Fig. 2: Effect of ICV administration of xylazine on AI% in all phases of the estrous cycle. *Significant difference from the control group (P<0.05)

administration of 1, 2 and 4 mg/kg yohimbine, significant (time × group) interaction of percentage of analgesic indices were shown during proestrus [F(1,20) = 0.349, (P<0.05)], estrus [F(1,20) = 0.756, (P<0.05)], metestrus [F(1,20) = 9.3, (P<0.05)] and diestrus phase of the estrous cycle [F(1,20) = 0.7, (P<0.05)] (Fig. 3).

Fifteen and 30 min following ICV

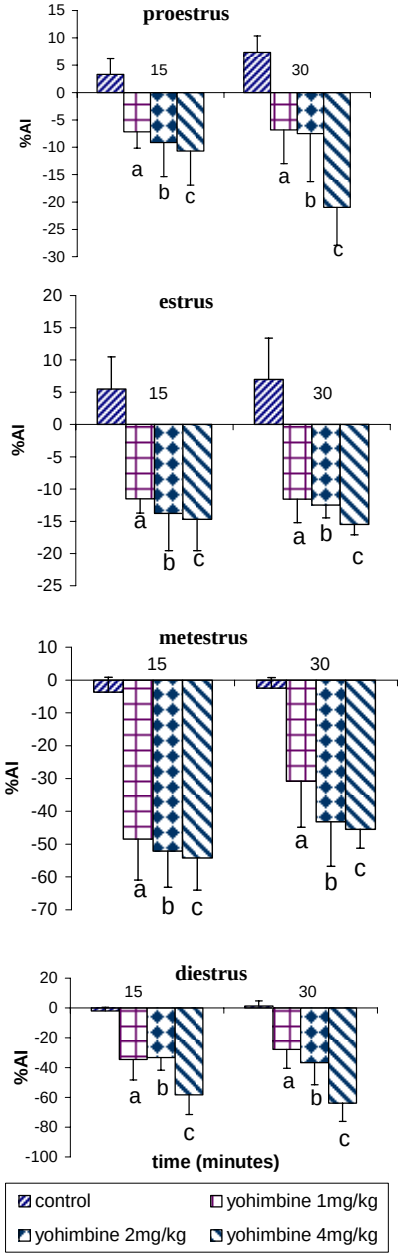

Fig. 3: Effect of IP administration of yohimbine on AI% in all phases of the estrous cycle. a: significant level of yohimbine 1 mg/kg relative to control; b: significant level of yohimbine 2 mg/kg relative to control and c: significant level of yohimbine 4 mg/kg relative to the control group

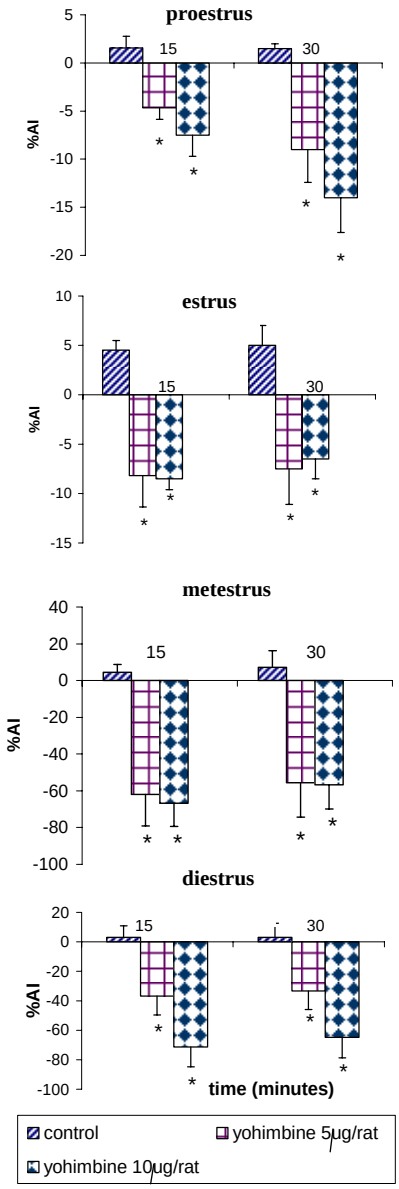

Fig. 4: Effect of ICV administration of yohimbine on AI% in all phases of the estrous cycle. *Significant difference from the control group (P<0.05)

administration of 5 and 10 µg/rat yohimbine, significant (time × group) interaction of percentage of analgesic indices were shown during proestrus [F(1,15) = 2.45, (P<0.05)], the estrus [F(1,15) = 0.131, (P<0.05)], metestrus [F(1,15) = 3.96, (P<0.05)] and the diestrus phases of the estrous cycle [F(1,15) = 1.65, (P<0.05)] (Fig. 3).

Thus IP and ICV administration of yohimbine significantly increases pain sensitivity (P<0.05) in all phases of estrous cycle (Figs. 3 and 4). Hyperalgesic effect of

yohimbine in both IP and ICV administration was high in the metestrus phase and low in the proestrus phase of the estrous cycle.

Discussion

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 role 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 (Stoffel *et al*., 2003). In the present study the effect of α_2-adrenergic agonist and antagonist on phasic pain during estrous cycle was investigated.

Adrenergic α_2 receptors in the brain and spinal cord are known to participate in the pharmacological and physiological modulation of pain transmission. Activation of supraspinal α_2 receptors by clonidine-like drugs results in antinociception which is attenuated by supraspinally-administered α_2 antagonists, demonstrating an analgesic role for brain stem α_2 receptors, although spinal α_2 receptors may also participate in some of these kinds of experiments (Svokos *et al*., 2001).

The results of the present study show that xylazine induced analgesia in all phases of the estrous cycle and this analgesic effect was high in the proestrus phase of the estrous cycle, during which estrogen, progesterone, LH and FSH are in peak levels (Fillingim and Ness, 2000a) and 3α-5α-THP is also elevated (Frye *et al*., 1996). Xylazine analgesic effect is low during the metestrus phase of the estrous cycle when progesterone is elevated while estrogen and LH levels are low. Disparate reports on the effect of the above substances on pain can be found in the literature. Frye and Duncan (1994) reported that progesterone and 3α-5α-THP increased tail flick latency response. Levine and Taiwo (1989) observed that β-estradiol induced a norepinephrine-sensitive cutaneous hyperalgesia and this hyperalgesic property was antagonized by yohimbine but not prazosin. Some investigators have reported that estradiol elevation in the periovulatory stage of the menstrual cycle increases response to painful stimuli (Fillingim and Ness, 2000a, b).

Variations in pain threshold across the estrous cycle have been demonstrated repeatedly in rats (Robbins *et al*., 1992; Kayser *et al*., 1996; Giamberadino *et al*., 1997). In the present study it seems that xylazine improved the analgesic effect of ovarian steroid hormones, such that in the metestrus phase of the estrous cycle, progesterone and estradiol levels are lower than in the proestrus phase of estrous cycle where the analgesic effect of xylazine is low. Our results are consistent with those of Gomez *et al*., (1994) who reported that tail flick latency increases during both proestrus and estrus phases and decreases during the metestrous phases of the estrous cycle. Also Ryan and Maier (1988) reported that flinch thresholds in response to foot shock were lower during both proestrus and estrus phases and elevated during the metestrus phase of the estrous cycle. Studies using different behavioural assays have also produced varying conclusions about which cyclical stage correlates with the lowest nociceptive threshold, although most studies have reported that rats are most sensitive during proestrus and/or estrus (Kayser *et al*., 1996; Giamberadino *et al*., 1997).

In the present study, yohimbine increased pain sensitivity in all phases of the estrous cycle. Our results are consistent with Sagen and Proudfit (1985) who reported that yohimbine produced hyperalgesia and such hyperalgesic effect is probably due to the inhibition of nerves within the nucleus raphe magnus. The hyperalgesic effect of yohimbine is low in the proestrus phase and high in the metestrus phase of the estrous cycle. We postulated that in the presence of high concentrations of progesterone and estradiol (proestrus) the hyperalgesic effect of yohimbine declines and in the presence of low concentrations of progesterone and estradiol (metestrus) yohimbine can induce a significant increased hyperalgesic effect.

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