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EFFECTS OF 17-β-ESTRADIOL ON NOCICEPTION IN IMMATURE FEMALE RATS

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ABSTRACT

PURPOSE: Pain transmission involves dynamic and interactive peripheral and central nervous system events and gonadal hormones have been demonstrated to interact with these systems at multiple levels. Estrogens have widespread effects throughout the CNS. Considerable evidence in the literature indicates that they modulate neural circuits known to participate in pain processes. The aim of our study was to investigate the effects of 17-β-estradiol on the pain latention of female rats during the period of prematuration.

METHODS: The experiments were carried out on immature female Wistar rats (90g). 17-β-estradiol was administered intraperitoneally once daily for 3 days of the experiment. The changes in the pain latency of the rats were evaluated using tail flick test 4 and 24 hours after injections.

RESULTS: The obtained data show that 4 and 24 hours after injection 17-β-estradiol increased significantly TF latency during the whole period of the experiment.

CONCLUSIONS: According to our data 17-β-estradiol takes part in the mechanisms of regulation of pain reaction of female immature rats. The effect of the gonadal steroid on pain perception can not be fully explained by the known mechanisms at present. Further studies are needed to clarify the primary site and mechanism of these actions.

Key words: 17-β-estradiol, pain latention, tail flick, immature female rats

INTRODUCTION

Estrogens are gonadal hormones present in both males and females. They have widespread effects throughout the central nervous system. In addition to regulating the reproductive function, estrogen has multiple actions in the brain to modulate homeostasis, synaptic plasticity/cognition, neuroprotection, as well as motor coordination and pain sensitivity (1). Human and animal studies suggest that estrogens is involved in the processing of nociceptive sensory information and analgesic responses in the nervous system. (2). For instance they modulate enkephalinergic neurons in the spinal cord, catecholaminergic neurons in the brain stem and midbrain, midbrain serotoninergic pathways and the basal forebrain cholinergic neurons (3). There are data also, that estrogens appears to be neuroprotective against NMDA-induced seizure in female rats (4).

Intracerebroventricular injection of estradiol in male rats induces a female-like behavioral response, which suggests a pronociceptive role for female gonadal hormones (5). However, other studies demonstrate that estrogens have

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acute antinociceptive effects when administered i.p. or s.c. (6). During pregnancy in both humans and rodents, elevated levels of estrogen are accompanied by an increase in the pain threshold (7). It is known that the sex specificity of the behavior at the early ontogenesis develops under the action of hormonal inductors and has as final result an organization at specific, for the given sex level of sensitivity and responses of the central nervous system to hormonal stimulation at mature age. They might be decreased or increased by endogenous or exogenous estrogenization during brain development. In this connection it would be interesting to investigate the effect of 17-β-estradiol on the pain latention of female rats during the period of prematuration.

MATERIALS AND METHODS

Animals: The experiments were carried out on immature female Wistar rats (body weight 90g; age 6 weeks). The animals were housed in groups of 6 per cage and kept under a normal 12 h light/dark cycle and 22 ± 2°C temperature. Food and water were available *ad libitum.*

Nociceptive test: Antinociceptive effects were evaluated using tail flick (TF) test. The TF response was elicited by applying radiant heat (infrared rays source 56°C) to the dorsal surface of the tail. A cut-off tail flick latency of 20s. was used to avoid injury to the tail. The intensity of heat stimulus in the tail-flick test was adjusted so that the animal flicked its tail within 3 to 5 sec.

Drugs and treatment: 17-β-estradiol (40 μg/100g, i.p.) was purchased from Sigma Chemical Co. (St. Louis, MO). The hormone was dissolved in propyleneglycol. Animals with similar pain latention were selected in advance. Rats from experimental groups were injected intraperitoneally (i.p.) once daily for 3 days with 17-β-estradiol. The control groups were injected i.p. with the same volume of propyleneglicol. TF latency was determined four and 24 hours after drug administration.

In all experiments, attention was paid to the Ethical Guidelines for investigation of experimental pain in conscious animals issued by the ad-hoc Committee of the International Association for the Study of Pain.

Data analysis: The results were statistically assessed by analysis of variance (ANOVA.). Values are mean ± S.E.M. Values of $P < 0.05$ were considered to indicate statistical significance.

RESULTS

The obtained data show that 4 hs after administration of 17-β-estradiol (40 μg/100 g) in immature female rats, TF latency was significantly increased compared to the control group during three days of the experiments ($P<0.001$). This effect was more pronounced on the second day. The same pattern was followed by pain sensitivity 24 hs after estradiol injection **(Figure 1).**

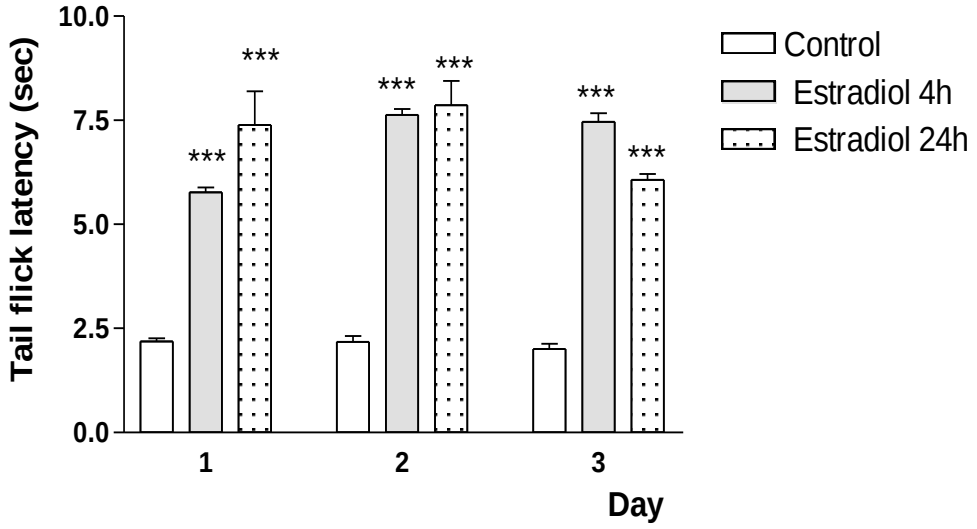

Figure 1. Tail flick latency in female immature rats estimated by tail-flick test 4 and 24 hours after i.p. injection of 17-β-estradiol (40 μg/l00 g b.w.). Data are presented as mean + S.E.M.; *** $P<0.01$ vs. control.

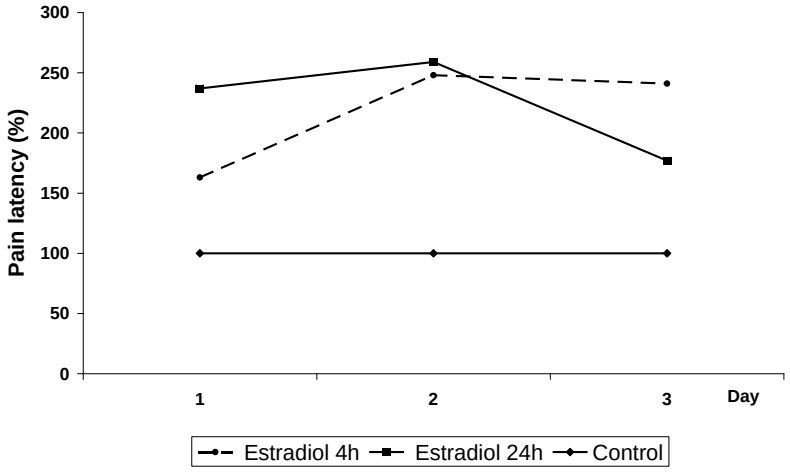

Figure 2. The relative changes of pain latention by exogenous estrogenization compared to the control during the three days of the experiment. Data are presented as percentage % = [test/control] x 100 -100%.

The increase of pain latention by estrogen treatment of immature female rats was 166% 4 hours after the injection and approximately 230 % 24 hrs after it (compared to controls) at the first day of the experiment. At the second day increase of pain latency was just about equal - 228 % four hours after the estrogen injection and 259 % 24 hours after it. At the third experimental day the effect of applied gonadal hormone was measurable to the previous day hormonal effect – about 241 %. Although the TF latency was remained significantly high 24 hrs after injection, the effect of hormone showed tendency of reduction – 177% **(Figure 2).**

DISCUSSION

In animals, estradiol modulation of acute nociception has been studied by innumerable investigators, with highly equivocal results. Although there are studies demonstrating longer latencies to respond to acute nociceptive stimuli in ovariectomized, estradiol-treated female rodents compared to hormone-depleted controls (8-10), there are many more studies reporting no effect of estradiol on acute nociceptive responses (though few report hyperalgesia (11). Some authors suggest that 17β-estradiol plays an anti-hyperalgesic role in physiological pain (12).

Studies examining changes in nociceptive response latencies across the estrous cycle in rodents demonstrate a similar lack of agreement regarding which stage, if any, is associated with the lowest or highest acute nociceptive threshold (13).

Our studies showed that 17-β-estradiol, either 4 or 24h after application, inhibited thermal nociceptive responses in immature female rats. The mechanisms by which estrogen regulate nociception are complex, including direct action on the central and peripheral nervous systems, as well as indirect actions via their modulation of the skeletal and immune systems (13). It is well known that the classical estrogen action occurs through the entry of estrogen into the cell, interaction with the nuclear estrogen receptors (ER) - ERa and ERb, and transcriptional activation of estrogen-responsive genes. This cell signaling mechanism can take several hours or more to achieve its final downstream effects. Besides the nucleus localization, ER a and ER b were recently reported to localize at the plasma membrane to affect cellular physiology or traffic to the plasma membrane, in which they associate with G-proteins and mediate activation of multiple membrane signaling cascades (1).

Additional non-genomic estrogen-induced rapid cell signaling pathways via membrane bound receptors have been recognized as important contributors to the overall biological response. Wang et al., (2013) demonstrated for the first time that testradiol could affect cell excitability, firing properties through the modulation of sodium currents (2). According to their study estradiol inhibited voltage-gated Na^+ channels in mouse dorsal root ganglion neurons in a concentration-dependent manner. The suggested mechanism is linkage of membrane ERs to protein kinase C and protein kinase A. In the

central nervous system, 17β-estradiol decreased pain sensitivity via opioid receptors in the spinal cord and periaqueductal gray - areas involved in the transmission of nociceptive information (12). The observed effects of estradiol on pain latention in our experiments could have been also mediated by the adrenergic, cholinergic and serotoninergic systems (as the estrogens increase the number of serotonin receptor sites in the brain) and by the decreased neurons lability as result (14). Changes in activity of both monoaminoergic and amino acid transmitters may contribute to mechanisms, participating in regulation of pain latention.

In conclusion, the obtained data about the effects of 17-β-estradiol on pain perception of immature female rats during prematurity, confirm the important role of estrogens in the mechanisms of regulation of pain sensitivity in female organism.

REFERENCES

1. Gintzler, A.R. and Liu, N.J., Importance of sex to pain and its amelioration; relevance of spinal estrogens and its membrane receptors, *Front. Neuroendocrinol*, 33: 412-424, 2012.
2. Wang, Q., Cao, J., Hu, F., Lu, R., Wang, J., Ding, H., Gao, R. and Xiao, H., Effects of estradiol on voltage-gated sodium channels in mouse dorsal root ganglion neurons, *Brain Res*., 1512:1-8, 2013.
3. McEwen, B.S., Alves, S.E., Bulloch, K. and Weiland, N.G., Ovarian steroids and the brain: implications for cognition and aging, *Neurology*, 48:88-15, 1997.
4. Reddy, D.S., Role of hormones and neurosteroids in epileptogenesis, *Front Cell Neurosci*, 7:115. doi: 10.3389/fncel.2013.00115. 2013.
5. Aloisi, A.M. and Ceccarelli, I., Role of gonadal hormones in formalin-induced pain responses of male rats: modulation by estradiol and naloxone administration, *Neuroscience*, 95:559-566, 2000.
6. Liu, N.J. and Gintzler, A.R., Prolonged ovarian sex steroid treatment of male rats produces antinociception: identification of sex-based divergent analgesic mechanisms, *Pain*, 85:273-281, 2000.
7. Cogan, R. and Spinnato, J.A., Pain and discomfort thresholds in late pregnancy, *Pain*, 27:63-68, 1986.
8. Craft, R.M., Ulibarri, C., Leitl, M.D. and Sumner, J.E., Dose- and time-dependent estradiol modulation of morphine antinociception in adult female rats, *Eur J Pain*, 12(4):472-479, 2008.
9. Stoffel, E.C., Ulibarri, C.M. and Craft, R.M., Gonadal steroid hormone modulation of nociception, morphine antinociception and reproductive indices in male and female rats, *Pain*, 103:285-302, 2003.
10. Walf, A.A., and Frye, C.A., Anti-nociception following exposure to trimethylthiazoline, peripheral or intra-amygdala estrogen and/or progesterone, *Behav Brain Res*, 144:77-85, 2003.
11. Ji, Y., Murphy, A.Z. and Traub, R.J., Estrogen modulation of morphine analgesia of visceral pain in female rats is supraspinally and peripherally mediated, *J Pain*, 8:494-502, 2007.
12. Lu, Y., Li, Z., Li, H.J., Du, D., Wang, L.P., Yu, L.H., Burnstock, G., Chen, A. and Ma, B., A comparative study of the effect of 17β-estradiol and estriol on peripheral pain behavior in rats, *Steroids*, 77(3):241-249, 2012.
13. Craft, R.M., Modulation of pain by estrogens, *Pain*, 132 (Suppl. 1):S3–S12, 2007.
14. Kalat, J.W., Biological Psychology, Cengage Learning; 11 edition, 2012.