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Modulatory role of estradiol in nicotinic antinociception in adult female rats

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ABSTRACT

Aims: Differences in the response to nicotinic analgesia in males and females have been suggested by recent studies, and such differences are presumed to be due to the regulatory effects of gonadal hormones. The aim of this study was to investigate nicotinic antinociception and the effect of estradiol (E2) on this response in female rats.
Main methods: Ovariectomized female rats were implanted with subcutaneous silastic tubes containing E2. On day 28 after implantation, epibatidine, a high-potency nicotinic acetylcholine receptor (nAChR) agonist, was administered intrathecally, and antinociception at the spinal level was assessed by the tail-flick test. In addition, immunohistochemical staining for nAChRα4 was performed in spinal cord sections.
Key findings: We found that female rats showed shorter nociceptive latencies than males, but there was no effect of ovarian status. However, OVX significantly increased epibatidine-induced antinociception compared to that in intact females, and this increase was attenuated by E2 treatment. In addition, OVX resulted in increased nAChRα4 immunostaining in the dorsal horn compared to that in intact females, and this increase was also attenuated by E2 treatment.
Significance: Results of this study provide new evidence that E2 modulates epibatidine-induced antinociception at the spinal level in female rats.

Introduction

Sex differences in nicotinic agonist-induced analgesia have been reported, and it has been postulated that these differences are due to the regulatory effects of gonadal hormones (Fillingim and Ness 2000; Damaj 2001; Craft 2003). Several studies have reported differences in antinociception induced by nicotinic acetylcholine receptor (nAChR) agonists, such as nicotine, epibatidine, and RJR-2403, in male and female rodents (Craft and Milholland 1998; Chiari et al. 1999; Damaj 2001). However, results have been inconsistent; it has been reported that nAChR agonists produce greater antinociception in females (Chiari et al. 1999), in males (Damaj 2001), or are equally effective in both sexes (Carstens et al. 2001). In human studies, women have been found to be less sensitive to nAChR agonists than men (Jamner et al. 1998). These inconsistencies may be due to species or genetic differences or to differences among nicotinic agonists or methods used.

At present, the effects of estradiol (E2) on nicotinic agonist-induced antinociception are poorly understood. In the only published study, nicotine-induced analgesia was found to be attenuated by E2 treatment in gonad-intact female mice (Damaj 2001). The effects of ovariectomy (OVX) and chronic E2 treatment on the nicotinic response remain unknown.

The aim of the present study was to fill gaps in the understanding of nicotinic antinociception in female rats. We examined baseline nociception in male and female rats, as well as antinociception induced by epibatidine administration. We also investigated the effect of E2 on epibatidine-induced antinociception in female rats. Epibatidine was administered intrathecally to induce local stimulation of spinal nAChRs.

Materials and methods

Animals

All experiments were performed in accordance with National Institutes of Health guidelines and approved by the Animal Care and Use Committee of Sun Yat-sen University (Guangzhou, China). Eight-week-old age-matched male (230–250 g) and female (180–210 g) Sprague–Dawley rats were housed individually under a 12-h light–dark cycle with free access to food and water. The experimental groups in this study are listed in Table 1 and included male rats and four groups of female rats (intact, ovariectomized [OVX], OVX + E2[low; 4 mg/mL], and OVX + E2[high; 40 mg/mL]). Estrous stage in intact females was not sampled, therefore it was not known whether intact females were cycling normally.

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Table 1
Summary of experimental groups in this study.

Group	Sample size	Surgery	E2 replacement
Males	40	No surgery	None
Intact	40	Sham surgery	None
OVX females	40	OVX	Vehicle
OVX + E2(low)	40	OVX	E2 (4 mg/mL)
OVX + E2(high)	40	OVX	E2 (40 mg/mL)

E2, estradiol; OVX, ovariectomy.

Drugs

Epibatidine and 17β-estradiol were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Ovariectomy and estradiol replacement

To achieve chronic E2 replacement, female rats were ovariectomized and implanted subcutaneously with a silastic tube (1.5-mm ID; 1.0-cm tube/100 g body weight) filled with a solution of 17β-estradiol in sesame oil, as described previously (Liu and Gintzler 2000). Levels of E2 were altered by changing the concentration of 17β-estradiol (4 mg/mL or 40 mg/mL) in the tube (Mannino et al. 2005).

Intrathecal epibatidine administration

To deliver epibatidine, rats were implanted with an intrathecal catheter, according to our previously described method (Cui et al. 2006). On day 28 after OVX, the baseline threshold for tail-flick latency was determined (0 min). Vehicle (saline) or epibatidine was then administered intrathecally, and tail-flick latencies were recorded at 5, 10, 15, 20, 30, 40, 50, and 60 min after administration.

Measurement of tail-flick latency

Antinociception was assessed by a 50 ± 0.2 °C hot-water tail-flick test (Cui et al. 2006). A cut-off time of 15 s was set to avoid tissue damage. Tail-flick latencies were converted to the percentage of maximal possible antinociceptive effect (%MPE) according to the following equation: %MPE = [(drug-induced latency − baseline latency) / (cut-off time − baseline latency)] × 100.

Evaluation of spontaneous behavior

Spontaneous agitation/spontaneous vocalization (SA/SV) was used to evaluate adverse effects and was scored according to the following scale, as described previously (Khan et al. 1998): 1, slight ambulation; 2, moderate ambulation, tail switching, and touch-evoked vocalization; 3, intense ambulation, whole-body switching, limited escape behavior, and spontaneous vocalization; 4, intense ambulation, severe switching, escape behavior, and spontaneous vocalization; 5, frantic ambulation, seizure, and spontaneous high-pitched vocalization.

Immunohistochemical staining and analysis

Rats were killed immediately after the behavioral test by an overdose of phenobarbital and perfused with 4% paraformaldehyde (pH 7.4). The spinal cord lumbar enlargement was collected and fixed in 4% paraformaldehyde (pH 7.4) at 4 °C for 2 h. Tissues were transferred to 15% and then to 30% sucrose solutions (4 °C) for cryoprotection. Tissues were embedded in optimal cutting temperature compound (OCT) and placed in a −22 °C cryostat for 15 min before sectioning at a thickness of 20 μm. Sections were placed onto poly-D-lysine-coated slides, air-dried, and stored at −20 °C.

For immunostaining, slides were washed with Tris-buffered saline/Triton X-100, and nonspecific staining was blocked with 3% normal goat serum for 1 h. Primary antibody cocktail (hamster anti-rat AChRα4 polyclonal antibody [1:1000; Chemicon, Temecula, CA, USA] and mouse anti-rat NeuN monoclonal antibody [neuronal marker; 1:400; Chemicon]) were added, and the slides were incubated overnight at 4 °C. Secondary antibody cocktail (1:400; Cy3-labeled goat anti-hamster antibody [Jackson ImmunoResearch, West Grove, PA, USA] and FITC-labeled goat anti-mouse antibody [1:400; Jackson ImmunoResearch]) were then added and incubated for 2 h at room temperature in the dark. Staining was visualized and images were obtained with an Olympus fluorescence microscope (BX51, Tokyo, Japan) at magnifications of ×20 and ×40.

Spinal cord tissue sections were selected randomly, and dorsal horn laminae I–IV were identified. nAChRα4-immunoreactive cells were confirmed by cell morphology and NeuN double staining. The ratio of the nAChRα4-immunoreactive area to the dorsal horn laminae I–IV area (positive area ratio [%]) was analyzed with an image analysis system (Image-Pro Plus, Silver Spring, MD, USA) as described previously (George et al. 2000). In brief, the density threshold was set above the background level to identify positively stained structures, the area occupied by these structures was measured as the positive area, and the ratio of the positive area to the dorsal horn laminae I–IV area was measured. Ratios for nAChRα4 positivity obtained from four randomly selected tissue sections were averaged for each rat, and five rats from each group were analyzed.

Radioimmunoassay for serum estradiol

Immediately after the behavioral test on day 28, rats were killed by an overdose of phenobarbital. Aortic blood was collected and centrifuged to separate the serum. Serum E2 concentration was determined with a ^{125}I-labeled radioimmunoassay kit (Laerwen Bioengineering Co., Shen Zhen, China).

Statistical analysis

Data are presented as the mean ± standard error (SE). Differences in tail-flick latency among male, intact, OVX, OVX + E2(low), and OVX + E2(high) groups were examined by one-way analysis of variance (ANOVA). One-way ANOVA was also used for analysis of E2 level and immunohistochemical staining (positive area ratio [%]).When unequal sample sizes in groups were observed, the Scheffé method was implemented; otherwise, Fisher least significant difference (LSD) was used as a post-hoc test. To determine the dose of epibatidine at which the significant difference between the male and intact groups was observed, an independent *t*-test was executed to identify differences at each dose. Two-way ANOVA was performed to inspect the effects of group and epibatidine dose on antinociception (%MPE) and SA/SV; the interaction between group and epibatidine dose was tested by two-way ANOVA as well. We claimed that the effect of group may be different between two epibatidine doses if the interaction between group and epibatidine dose was significant. If the interaction was not significant, the interaction term was removed from the analyzed model, and then analysis of covariance (ANCOVA) was used instead. By ANCOVA analysis, we could also determine the effects of epibatidine dose and group after these two variables were controlled for each other. The LSD test was used as a post-hoc test between any two of the four female groups in both two-way ANOVA and ANCOVA. All statistical analyses were performed with SPSS software version 15.0 (SPSS Inc., Chicago, IL, USA). A P value < 0.05 was considered statistically significant.

Results

Baseline thermal nociception

The baseline nociceptive threshold was examined by assessing tail-flick latency (Fig. 1). The tail-flick latency in intact female rats was