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Short communication

Estradiol administration mediates the inflammatory response to formalin in female rats

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Abstract

Female rats demonstrate higher pain sensitivity than do males in various nociceptive assays of inflammation. In the present study, we found that estradiol (20%) replacement in ovariectomized rats attenuated the chronic phase of the formalin response but only at high formalin concentrations thought to rely on peripheral inflammation. An inactive isomer of estradiol, α-estradiol, failed to result in the same attenuation ($P > 0.05$). Our results suggest that estradiol's actions in inflammatory responses are mediated through genomic estrogen receptor-mediated mechanisms.

Theme: Sensory systems
Topic: Pain modulation

Keywords: Inflammatory; Chronic; Pain; Estrogen; Female

Epidemiological pain studies have found that women are more likely than men to report a variety of temporary and persistent pains with more frequency and longer duration [2,3,6,7,11,12,19,21,22,34,36,39,44,47,48]. Similarly, in rats, females display higher pain scores in chronic and inflammatory pain models than do males [1,4,9,16,42,43]. Ovarian hormones have been postulated to be the basis for this health disparity between the sexes. For example, during proestrus (when estradiol serum levels are at their highest), painful episodes in an artificial ureteral calculosis model decreased, whereas hyperalgesic responses increased after either complete Freund's adjuvant or carrageenan [9,17,42]. Estradiol replacement reduced vaginal hyperalgesia in a menopause-associated dyspareunia model of pain and lowered autotomy scores after nerve injury [8,46]. These findings suggest that circulating estradiol may, in part, mediate some inflammatory responses in rats.

Formalin administration in the hind paw of rats is a commonly used model to study inflammatory and persistent pain responses [35]. Although persistent nociceptive responses to formalin may partly be due to central sensitization, responses to high concentrations of formalin may rely to a greater extent on peripheral inflammation [53,55,56]. In view of these different mechanisms regulating formalin responses, this study aimed to determine the role of estradiol in peripheral versus central inflammatory responses in ovariectomized rats.

Eight-week-old ovariectomized Sprague–Dawley rats (Taconic, Germantown, NY) were double-housed under a 12-h light/12-h dark cycle (lights on at 8:00 a.m.) with food and water available ad libitum. Two weeks after ovariectomy, SILASTIC capsules (1 cm, 0.058 in. ID × 0.077 in. OD, Dow Corning) were inserted into the nape of the animal's neck and contained either vehicle (100% cholesterol), estradiol (20% β-estradiol 3-benzoate:80% cholesterol), or α-estradiol (an inactive estradiol isomer; 20% α-estradiol:80% cholesterol). These doses have been shown to fall within the range of serum levels during the reproductive

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cycle [33]. Observed levels of serum estradiol fall within those observed in approximate days 15 to 22 during pregnancy [10]. Mannino et al. [31] have shown that 20% estradiol implants are maximally effective in attenuating the formalin response during Phase II. Furthermore, that group showed the time course of this effect remained stable from day 7 through day 21 after the implant [31].

One week after hormone replacement, a soft metal band was placed on the right hind paw with the opening positioned at the plantar surface of the paw. To minimize the novelty of the testing environment and band, rats were placed inside the testing chamber for a total of 30 min prior to the formalin injection. One- or five-percent formalin, at a volume of 50 μL, was injected intra-plantar on the banded right hind paw. Rats were then placed in the testing chamber, and behavioral activity data were collected at 1-min intervals for a total of 60 min after the formalin injection. An automated flinch detecting system was used in the formalin nociceptive assay [54]. All parameters of the program were set to default values [54]. Behavioral testing was conducted between 9:00 a.m. and 3:00 p.m.

Sixty minutes after formalin injection, rats were sacrificed by decapitation, following a brief exposure to CO_2 (20 s), and trunk blood was collected. Blood was then centrifuged at 3000 RPM for 30 min at 4 °C. Serum was collected and stored at −80 °C until analyzed with Coat-A-Count radioimmunoassay kits for estradiol (Diagnostic Products Corporation, Los Angeles, CA). Intra-assay coefficients of variation averaged 10.0% ± 1.0%. Results for these assays were determined via a log-logit analysis within GraphPad Prism Software (San Diego, CA). Estradiol serum levels were expressed as pg/mL.

One-way ANOVAs were used to test for significant differences in the sum of the flinching response across treatments for Phase I (0–9 min) and Phase II (10–60 min) after formalin administration. Statistical significance in estradiol serum levels across treatment groups was also analyzed with use of one-way ANOVAs. Fisher's least significant difference post hoc testing was done when appropriate. For all analyses, significance was at the level of $P < 0.05$.

The serum estradiol levels of rats receiving the same hormone replacement paradigm did not significantly differ from one another across formalin concentrations (Vehicle, $t(21) = 1.99$, $P > 0.05$; Estradiol, $t(21) = -0.17$, $P > 0.05$). Therefore, for purposes of analysis, these values were combined. Serum levels of estradiol significantly increased after estradiol replacement; animals receiving 20% estradiol had significantly higher levels of estradiol than vehicle-treated groups [$F(3,52) = 18.545$; $P < 0.00$); Table 1]. No significant differences between vehicle- and α-estradiol-treated groups were observed [$F(2,52) = 18.545$; $P = 0.80$)]. After injection of 5% formalin, estradiol-treated animals displayed a significant attenuation in the flinching response during Phase II as compared with vehicle-treated animals [$F(2,35) = 3.252$; $P = 0.01$; Fig. 1]. However, animals receiving α-estradiol had flinching responses similar to those in vehicle-treated animals [$F(2,35) = 3.252$; $P > 0.05$; Fig. 1; Table 2]. After injection of 1% formalin, there were no significant differences between vehicle- and estradiol-treated animals in the number of flinches during Phase I and II [$F(2,35) = 2.52$; $P > 0.05$); Table 2]. In estradiol-treated animals, no significant effect was observed during Phase I after either 1% or 5% formalin administration (Table 2).

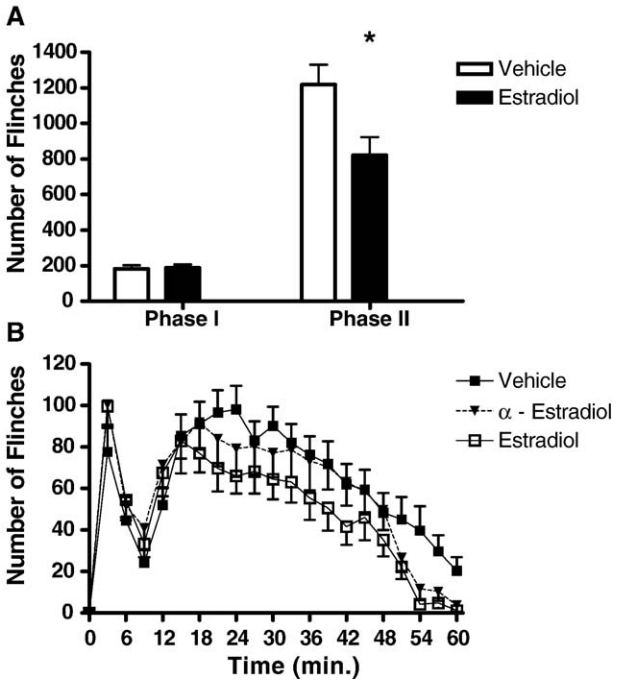

Fig. 1. (A) Total sum of flinches (±SEM) across Phase I (0–9 min) and Phase II (10–60 min) in vehicle- and estradiol-treated animals after 5% formalin administration. (B) α-estradiol and estradiol replacement effects across time ($N = 8$/group). * Denotes significant difference ($P < 0.05$).

Table 1
Estradiol serum concentration in female rats receiving estradiol or α-estradiol

Treatment	Estradiol serum levels
Vehicle	22.82 ± 10.35
Estradiol	216.65 ± 36.83*
α-Estradiol	11.73 ± 5.01

Levels represented as mean ± SEM pg/mL ($N = 8$/group). * Denotes significant difference between vehicle- and estradiol-treated group.

Table 2
Flinching activity after 1% and 5% formalin administration

Treatment	Phase I	Phase II
1% formalin		
Vehicle	152.58 ± 12.98	673.00 ± 64.09
Estradiol	204.81 ± 20.52	583.60 ± 41.51
5% formalin		
Vehicle	181.72 ± 20.83	1219.00 ± 110.63
α-Estradiol	196.54 ± 25.72	1096.90 ± 142.55

Levels represented as mean number of flinches ± SEM ($N = 8$/group).