Brain Infusion of Lipopolysaccharide Increases Uterine Growth as a Function of Estrogen Replacement Regimen: Suppression of Uterine Estrogen Receptor-α by Constant, But Not Pulsed, Estrogen Replacement


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The effects of estrogen therapy can differ depending on the regimen of estrogen administration. In addition, estrogen can modulate the effects of stressors. To examine the interaction between these systems, we infused adult female rats with lipopolysaccharide (LPS) into the fourth ventricle of the brain for 6 d and compared the effects of constant and pulsed estrogen replacement. Constant, but not pulsed, estrogen treatment reduced estrogen receptor-α (ERα) protein by 90% in the uterus and increased heat-shock proteins 70 and 90 by 74 and 48%, respectively, whereas progesterone receptor levels increased in all ovariectomized rats receiving estrogen replacement. In contrast to the uterine decline in ERα, no changes in ERα were observed in the hypothalamus or hippocampus, and ERβ levels were unchanged in all regions tested. Brain infusion of LPS did not alter these proteins but increased the number of activated microglia in the thalamus and reduced body weight in all rats as well as activated the hypothalamic-pituitary-adrenal axis in ovariectomized rats, as determined by elevations in circulating corticosterone and progesterone. Estrogen treatments did not alter these markers, and no differences were observed in cortical choline acetyltransferase activity or nitrotyrosine for any of the treatment groups. The current study found an unexpected increase in uterine weight in lipopolysaccharide-infused rats treated with constant, but not pulsed, estrogen. This report suggests that constant and pulsed regimens of estrogen administration produce different effects and that stress may be an important factor in the postmenopausal intervention with estrogen. (Endocrinology 148: 232–240, 2007)

Materials and Methods

Subjects

Ninety-three virgin female F-344 rats (Harlan Sprague Dawley, Indianapolis, IN), aged 3 months, were housed in a temperature-controlled room (21 C) and maintained on a 12-h dark, 12-h light cycle with lights on at 0700 h. Food and water was available ad libitum, and rats were allowed to adjust to their new environment for 1 wk after arrival. Rats
were randomly assigned to one of 10 treatment groups (see Table 1) and housed in triplicate with other rats undergoing the same experimental treatment to reduce the impact of housing on estrous cycle (37). All procedures were in accordance with Institutional Animal Care and Use Committee regulations.

Brain infusion of lipopolysaccharide (LPS)

Vaginal cytology was assessed, and only rats in diestrus were prepared for surgery. Rats were anesthetized using isoflurane gas (2.5% isoflurane by volume in 2 liters/min compressed oxygen; Medical Distributing Co., Phoenix, AZ) and underwent surgery as previously described (26). Briefly, artificial cerebrospinal fluid (CSF) or LPS (1.0 μg/ml dissolved in CSF; Escherichia coli, serotype 055:B5, trichloroacetic acid extraction; Sigma Chemical Co., St. Louis, MO) were slowly infused into the fourth ventricle via an osmotic minipump (0.25 μl/h). All tubing was prefilled with CSF to delay LPS introduction until estrogen was present (25). Body weights were monitored daily, and nutritional supplemental was provided as necessary during the infusion period. Rats whose body weights dropped more than 20% were syringe fed with 1.5 ml animal Stat (PRN, Pensacola, FL).

The effectiveness of the LPS infusion was verified after 6 d of treatment (see Table 2; methods detailed below). Activated microglia in the thalamus were increased by LPS (F(1,7) = 15.552; P < 0.001), as measured by [3H]PK11195 binding to peripheral benzodiazepine receptors (methods detailed below). The increase in activated microglia correlated to a decrease in body weight (r = -0.409; P < 0.0003) such that LPS-infused rats weighed significantly less (109.3 ± 1 g) than rats infused with CSF (132.4 ± 1.1 g; F(1,93) = 218.894; P < 0.001). Activation of the hypothalamic-pituitary-adrenal axis was also observed, as indicated by increased serum corticosterone levels after 6 d of treatment (699.9 ± 75.9 ng/ml and 922.6 ± 79.1 ng/ml for CSF- and LPS-infused rats, respectively; F(1,93) = 4.124; P < 0.05; methods detailed below). Neither ovariectomy nor any of the estrogen replacement regimens had any effect on these markers.

Estrogen regimens

Immediately after implantation of the cannula, rats were either bilaterally OVX or left intact. OVX rats were assigned to either a constant or pulsed estrogen replacement regimen. Rats receiving the constant replacement regimen were implanted (sc) with 5-mm silastic capsules containing estrogen (25% 17β-estradiol and 75% cholesterol) or oil (100% cholesterol) at the time of surgery, as previously described (26). Rats receiving the pulsed estrogen replacement regimen received one injection the day after surgery and another injection 4 d later, 24 h before being killed. The term pulsed estrogen is used as shorthand in the text to convey this regimen of repeated, separate estrogen injections. Injections were given in the morning between 1000 and 1200 h and consisted of either estrogen (10 μg 17β-estradiol dissolved in 100 μl sesame oil; Sigma) or oil vehicle (100 μl sesame oil). The injection paradigm was selected to mimic the proestrus peak seen in the normal rat estrous cycle and has previously been used to induce hippocampal plasticity (38) and enhance learning and memory (17).

Estrogens levels were verified in a separate subset of rats that were OVX, cannulated with a jugular vein catheter, and administered constant or pulsed estrogen replacement regimens for 6 d as described above. Five days after surgery, animals underwent 24 h of serial blood sampling. Eight samples were collected, one 30 min before the estrogen injection and then 5 min, 30 min, and 1, 2, 4, 8, and 24 h after the injection. Each sample removed 300 μl blood (∼3% of total blood volume per sample for a 150-g female rat) that was replaced with the equivalent volume of sterile saline. Rats were immediately anesthetized with isoflurane after the last sample and killed by decapitation, and trunk blood was collected. Circulating estradiol levels were quantified by a double-antibody ultrasensitive 125I RIA (Diagnostic Systems Laboratories, Webster, TX; range, 2.2–750 pg/ml). Rats that were administered constant estrogen by silastic capsules (n = 4) had stable, low physiological levels of circulating estradiol (56.1 pg/ml) that did not change across 24 h (F(3,20) = 1.999; P = 0.151; data not shown). On 5 of 7 rats in the pulsed-estrogen treatment group (n = 7) had basal estradiol levels of 14.8 pg/ml, indicating estradiol levels had declined from the previous injection 4 d before. There was a rapid but nonsignificant rise in estradiol levels within 5 min of the injection. Estradiol levels peaked at 483.2 pg/ml within 1 h of the injection and declined to 83 pg/ml within 8 h of the injection, thereby producing fluctuating circulating estradiol levels similar to previous reports demonstrating 50 pg/ml within 5 h of injection that then decay to baseline within 24 h (39).

Experimental design

Rats were exposed to experimental conditions described above for 6 d. The time point was selected because constant estrogen reduces ER concentrations between 4 and 8 d (23) and enhances cholinergic function between 3 and 10 d of treatment (39–42). Therefore, 6 d was within these time points and allowed for administration of two estrogen injections. After 6 d, each rat was weighed, assessed for vaginal cytology, anesthetized with isoflurane, and killed by decapitation. Trunk blood was collected, and serum was isolated and stored (−70 C) until analysis. All results were analyzed by two-way ANOVA (Sigmastat) unless described otherwise.

Brain dissections

The brain was quickly removed and placed on an ice-cold (4 C) metal plate for dissection. An ice-cold brain matrix was used to section the brain at the level of the optic chiasm. The first blade was inserted just posterior of the optic chiasm (∼2.12 mm from Bregma, according to the atlas of Pellegrino and colleagues) (43). The second blade was inserted approximately −0.8 mm from Bregma, and the hypothalamus was subsequently isolated with two cuts each approximately 1.5 mm lateral of the third ventricle, one cut ventral to the anterior commissure and one cut to dissociate the optic chiasm. The hypothalamic sample included several hypothalamic nuclei including the medial preoptic and anterior hypothalamic areas, suprachiasmatic, periventricular, and lateral anterior and paraventricular nuclei as well as the tuber cinerum. The divided anterior and posterior portions of brain were then turned such that the cortices were face up. The cingulate cortex was separated from the cortex by making two longitudinal cuts on each piece of cortex. A sample of anterior and posterior cortex was then taken, excluding the cingulate cortex. Bilateral hippocampi were removed in their entirety ventral to the posterior cortex sample. Lastly, the thalamus was isolated from the posterior region of the brain between approximately −2.12 and −6.5 mm from Bregma and included the entirety of the thalamic nuclei as well as some regions of the posterior hypothalamus, including the arcuate nucleus, ventral medial, lateral, dorsal, and posterior hypothalamic areas. All samples were placed into labeled microcentrifuge tubes and put immediately on dry ice until storage (−70 C).

Determination of uterine weight

Uterine weights measured 24 h after an estrogen injection indicate changes in gene expression and cell proliferation (44–49). Uteri were dissected, weighed (wet weight), and stored (−70 C). Uteri from a subset of rats were drained and weighed; values were highly correlated to uterine wet weights (r = 0.928; P < 7 × 10−11), suggesting that cell proliferation rather than gross fluid accumulation within the uterine horns contributed to increases in uterine weight. All killing occurred 24 h after the last injection to allow estrogen levels to return to baseline (see Estrogen regimens above).

Brain biochemistry

[3H]PK11195 binding. Activated microglia were quantified in triplicate by [3H]PK11195 filtration binding (1 nM; specific activity, 85.5 Ci/nmol) according to the protocol of Rao and colleagues (50). The thalamus was chosen because of its robust activation of microglia in female rats (26) and correlation to behavioral impairments on a Morris water maze task.

<table>
<thead>
<tr>
<th>TABLE 1. Experimental groups and numbers</th>
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<tbody>
<tr>
<td>Inflammation</td>
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<tr>
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<tr>
<td>CSF</td>
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<td>LPS</td>
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(Marriott, L. K., unpublished observations). Membranes were isolated by repeated centrifugation (40,000 × g at 4 °C) and freeze-thawing. Specific binding was defined by addition of 20 μmol/liter diazepam to the incubation solution before vacuum filtration (Whatman GF/B glass microfiber filters, presoaked in cold 0.3% polyethylenimine solution, pH 7.0) and liquid scintillation spectrometry. Protein content was quantified by the method of Lowry et al. (51) unless otherwise described.

**Choline acetyltransferase (ChAT) activity.** To confirm cholinergic integrity (52), ChAT activity was analyzed in the left anterior cortex in triplicate by the formation of [14C]acetylcholine from [14C]acetyl-coenzyme-A (52), ChAT activity was analyzed in the left anterior cortex in triplicate without dithiothreitol, pH 7.4 at 4 °C) before detection by liquid scintillation spectrometry. Protein content was quantified by the method of Lowry et al. (51) unless otherwise described.

**Verification of LPS effectiveness**

<table>
<thead>
<tr>
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<th>CSF Mean ± SEM</th>
<th>LPS Mean ± SEM</th>
<th>Significance</th>
</tr>
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<tbody>
<tr>
<td>Increased activation of thalamic microglia (nmol/mg protein)</td>
<td>75.4 ± 14.5</td>
<td>164.6 ± 16.2</td>
<td>F&lt;sub&gt;1,75&lt;/sub&gt; = 15.552; P &lt; 0.001</td>
</tr>
<tr>
<td>Reduction in body weight (g)</td>
<td>132.4 ± 1.1</td>
<td>109.3 ± 1.1</td>
<td>F&lt;sub&gt;1,93&lt;/sub&gt; = 218.894; P &lt; 0.001</td>
</tr>
<tr>
<td>Increased circulating corticosterone (ng/ml)</td>
<td>699.9 ± 75.9</td>
<td>922.5 ± 79.1</td>
<td>F&lt;sub&gt;1,93&lt;/sub&gt; = 4.124; P &lt; 0.05</td>
</tr>
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</table>

O VX and estrogen replacement regimens did not significantly alter these markers; therefore, all intact and OVX groups were collapsed into rats receiving CSF or LPS.

(Nitrotyrosine levels. A solid-phase ELISA was used to determine levels of nitrotyrosine, a marker of inflammation produced in the presence of nitric oxide (58). Samples of right posterior cortex were homogenized (20 mg Tris-HCl, 1.5 mmol/L EDTA, 10 mmol/L TEGD buffer containing 10 mmol/L Tris-HCl, 1.5 mmol/L disodium EDTA, 10% vol/vol glycerol, 1 mmol/L dithiothreitol added on the day of use, pH 7.4 at 4 °C) and centrifuged (10,000 × g for 20 min at 4 °C) to isolate supernatants (cytosols). Seven microliters of cytosol were diluted in 68 μL TEGD buffer to minimize background signaling (54). Vacuum filtration using filter papers (25-mm cellulose ester membranes with a pore size of 0.45 μm; Millipore, Burlington, MA) presoaked in cold 0.3% polyethylenimine solution (pH 7.0) and liquid scintillation spectrometry. Protein content was quantified by the method of Lowry et al. (51) unless otherwise described.

**Blood chemistry**

Levels of circulating hormones were quantified using 125I RIA kits (Diagnostic Systems Laboratories). Serum samples were quantified in duplicate for estradiol (200-μL samples, range of 2.2–750 pg/ml), progesterone (25 μL, range of 0.3–60 ng/ml), and corticosterone (25 μL, range of 0–2000 ng/ml).

**Immunoblotting**

Hypothalamic, uterine, and left hippocampal tissues were homogenized in immunoprecipitation buffer, sonicated, centrifuged, and electrophoresed onto precast 4–12% Bis-Tris NuPage gels (Invitrogen, Carlsbad, CA) as previously described (59). Equal amounts of protein (55 μg for hypothalamus, 30 μg for uterus, and 10 μg for hippocampus) were loaded as determined by bicinchoninic acid assay (Pierce Biotechnology Inc., Rockford, IL). One animal per treatment group was included on each gel, and at least four gels were run per region. Protein was transferred to polyvinylidene difluoride membranes and blocked in 5% non-fat milk (NFDM) as previously described (59). Primary antibodies [ERα (AB15) at 1:500 from NeoMarkers, Fremont, CA; ERβ (PA1310B) at 1:1000 from Affinity Bioreagents, Golden, CO; heat-shock protein (Hsp90 and Hsp70 at 1:2000 from Becton Dickinson, Franklin Lakes, NJ); progesterone receptor (PR) (AB13) at 1:1000 from NeoMarkers; and actin at 1:5000 from Sigma] were incubated (overnight at 4 °C) in 5% NFDM/Tris-buffered saline with Tween 20 containing 0.02% sodium azide to prevent bacterial growth. Appropriate secondary antibodies conjugated to horseradish peroxidase were used (1:2000 for 1 h in 5% NFDM; Santa Cruz Biotechnology Inc., Santa Cruz, CA). Bands were visualized and quantified as described (59). A background band of approximately the same weight as ERα (~64 kDa) was picked up by secondary antibody alone (data not shown); therefore, a biotinylated antimuscle secondary antibody (1:5000 for 1 h in 5% NFDM; Maker) and streptavidin-horse-radish peroxidase tertiary antibody (1:5000 for 1 h in 5% NFDM; Vector Laboratories, Burlingame, CA) was used instead for ERα detection. Recombinant human ERα (10 pg) or ERβ (100 pg) proteins (Affinity Bioreagents) were loaded as positive controls.

**Results**

**Brain infusion of LPS increased uterine weight under conditions of constant, but not pulsed, estrogen replacement**

The current study compared the effects of constant and pulsed estrogen replacement on ER levels in the brain and periphery. The relationship between inflammatory stressors and estrogen replacement regimen was also examined. An unexpected response to LPS was observed in rats treated with constant estrogen that increased uterine weight. Specifically, all OVX rats receiving oil had low uterine weights (0.17 ± 0.05 g) that increased in response to both pulsed (0.51 ± 0.05 g) and constant (1.06 ± 0.05 g) estrogen treatment (F<sub>1,93</sub> = 66.546; P < 0.001; see Fig. 1A). Constant estrogen administration produced the largest increase in uterine weight that was further increased by coadministration of LPS (1.32 ± 0.07 g; F<sub>1,93</sub> = 5.84; P < 0.001). CSF-infused rats receiving constant estrogen had a mean uterine weight of 0.79 ± 0.06 g. The synergistic effect of LPS was observed only in the constant estrogen regimen. No LPS-induced changes in uterine weight were seen in intact rats (0.27 ± 0.06 and 0.25 ± 0.07 g for CSF and LPS, respectively) or rats receiving pulsed estrogen (0.47 ± 0.07 and 0.54 ± 0.07 g for CSF and LPS, respectively).
Uterine weights and diestrous vaginal cytology, intact and OVX rats receiving oil vehicle had low estradiol levels (9.6 ± 4.8 and 12.2 ± 3.7 pg/ml, respectively).

Although LPS had no effect on circulating estradiol levels in rats treated with constant estrogen, rats receiving pulsed estrogen had 4-fold higher estradiol levels when infused with LPS (41.3 ± 6.4 pg/ml) than CSF (10.5 ± 0.6 pg/ml; *P < 0.01). The increase was sufficient for LPS to significantly elevate overall circulating estradiol levels (F1,85 = 10.475; *P = 0.002), possibly because of suppression of the cytochrome p450 system (60–62).

Brain infusion of LPS increased circulating progesterone in OVX rats

The ovaries are the major source of circulating progesterone (63); their removal by ovariectomy in the current study reduced circulating progesterone levels as expected (12.8 ± 1.3 ng/ml) compared with intact rats (21.3 ± 2.5; F1,93 = 8.945; *P = 0.004; see Fig. 3). Neither constant nor pulsed estrogen replacement regimens restored progesterone to intact levels; however, progesterone levels increased in OVX rats after brain infusion of LPS (17.6 ± 1.9 ng/ml) but not CSF (7.9 ± 0.714; *P = 0.004). Brain infusion of LPS increased circulating progesterone in OVX rats (P = 0.001) to levels that were comparable to intact controls. Post hoc t tests revealed that all OVX treatment groups showed elevations in circulating progesterone when infused with LPS (+, all P < 0.04), except pulsed oil-treated rats (*P = 0.33). Progesterone levels of intact rats were also not affected by LPS (*P = 0.99). Values reflect group mean ± SEM.

Effect of central LPS on circulating estradiol levels

To determine whether the LPS-induced increase in uterine weight at 6 d was attributable to increased circulating estrogen or progesterone levels, blood serum collected at the time of killing was assayed. Rats were killed 24 h after the last estrogen injection to allow estradiol levels to return to baseline (see Materials and Methods). Circulating estradiol levels strongly correlated with uterine weight (r = 0.714; P = 1.25 × 10⁻¹⁴). Constant estrogen administration increased circulating estradiol levels (68.8 ± 4.1 pg/ml; F4,85 = 37.349; P < 0.001; see Fig. 2); however, there was no difference in estradiol levels between CSF- and LPS-infused rats (61.7 ± 7.0 and 75.9 ± 8.0 pg/ml, respectively, t15 = 1.317; *P = 0.21), suggesting the synergistic increase in uterine weight seen in LPS-infused rats treated with constant estrogen cannot be explained by estradiol levels alone. Constant with the low
1.8 ng/ml; $F_{1,93} = 10.979; P = 0.001$). LPS-induced elevations in circulating progesterone were observed in all OVX treatment groups (all $P < 0.04$) except pulsed oil-treated rats ($t_{17} = 1.003; P = 0.33$). The LPS-induced increase in circulating progesterone was specific for OVX rats; no change was observed in intact rats after 6 d of treatment (21.3 $\pm$ 3.8 ng/ml), possibly because of normal diestrus-related elevations in progesterone (64). No correlation was observed between circulating progesterone and uterine weight; however, progesterone levels strongly correlated to circulating corticosterone ($r = 0.741; P < 2 \times 10^{-15}$), consistent with findings that progesterone is produced by the adrenal gland as a biosynthetic precursor to corticosterone (65). All treatment groups showed significant positive correlations between progesterone and corticosterone (all $P < 0.02$) except CSF-infused intact rats ($r = 0.485; P = 0.131$).

**Constant, but not pulsed, estrogen replacement suppresses ERα in the uterus**

To determine whether the LPS-induced increase in uterine weight seen in rats receiving constant estrogen may reflect altered ER levels, we measured ERα and ERβ levels in the uterus and several brain regions (see Fig. 4). OVX rats exhibited higher ERα levels in the uterus than intact rats (103.4 $\pm$ 0.7 and 62.7 $\pm$ 15.3% for CSF controls, respectively), which were restored by pulsed estrogen treatment (59.1 $\pm$ 5.4%; $P < 0.05$). In contrast, constant estrogen administration suppressed ERα protein levels (9.6 $\pm$ 2.4%) compared with all other treatment groups ($F_{4,62} = 23.26; P < 0.0001$). LPS alone did not alter uterine ERα levels ($P = 0.89$). ERα negatively correlated to both uterine weight ($r = -0.727; P = 0.0000008$) and circulating estradiol ($r = -0.626; P < 0.0007$). No changes in ERα levels were observed in either the hypothalamus or hippocampus; ERβ levels were unchanged in all regions studied, consistent with the $[^{3}H]17\beta$-estradiol binding data measured in the contralateral hippocampus ($F_{4,93} = 0.717; P = 0.583$; see Table 3).

**Both estrogen replacement regimens increased uterine PRs**

The PR gene is ER regulated (66) and increases in the uterus in response to exogenous estrogen administration (63). To determine whether the LPS-induced increase in uterine weight may be related to a potentiation of PR levels by the constant estrogen regimen, we measured PRα and PRβ levels in the uterus after 6 d of treatment. As expected, exogenous estrogen administration increased both PRα (214.6 $\pm$ 10.2%; $F_{2,29} = 26.92; P < 0.001$) and PRβ (343.8 $\pm$ 38.4%; $F_{2,29} = 8.49; P < 0.002$) levels in the uterus compared with OVX+Oil controls (see Fig. 5). However, the regimen of estrogen administration (i.e. constant vs. pulsed estrogen) did not differentially alter uterine PR levels nor did brain infusion of LPS. Both PRα and PRβ were strongly correlated to uterine weight ($r = 0.66, P < 0.0008$; and $r = 0.853, P < 3 \times 10^{-9}$ for PRα and PRβ, respectively), circulating estradiol ($r = 0.551, P < 0.003$; and $r = 0.478, P < 0.02$, respectively), uterine ERα ($r = -0.584, P < 0.0009$; and $r = -0.661, P < 0.0001$, respectively), and each other ($r = 0.694; P < 0.00003$), although neither PR correlated to circulating progesterone.

**Constant, but not pulsed, estrogen replacement increased Hsp70 and Hsp90 in the uterus**

Estrogen administration can increase Hsp70 and Hsp90, which correlate with an increase in uterine weight (67). Equal protein concentrations were loaded and verified using actin to ensure Hsp levels did not reflect the uterine weight differences between groups. As expected, constant estrogen increased both Hsp70 (73.6 $\pm$ 10.3%; $F_{4,93} = 8.068; P < 0.001$) and Hsp90 (47.9 $\pm$ 9.3%; $F_{4,93} = 3.444; P < 0.02$; see Fig. 6) over control levels; however, no increase was observed in rats treated with pulsed estrogen. Uterine weight correlated to increases in both Hsp70 ($r = 0.569; P < 0.001$) and Hsp90 ($r = 0.263; P < 0.03$); however, neither of these proteins were altered by brain infusion of LPS.

There was no significant change in the hypothalamus for either Hsp, although both significantly correlated to uterine PRα ($r = 0.543, P < 0.04$; and $r = 0.704, P < 0.004$), and PRβ ($r = 0.660, P < 0.008$; and $r = 0.751, P < 0.002$).

**Brain chemistry**

There were no significant differences between treatment groups in cortical ChAT activity ($F_{4,93} = 0.186; P = 0.945$), the number of $[^{3}H]17\beta$-estradiol binding sites in the hippocampus ($F_{4,93} = 0.717; P = 0.583$), or nitrotyrosine levels in the posterior cortex ($F_{4,38} = 1.806; P = 0.155$). All values are shown in Table 2.

**Discussion**

ERT given alone or when combined with progesterone (HRT) in a constant manner may be ineffective or detrimental to postmenopausal women (1, 3–9). A pulsed estrogen replacement regimen could be more efficacious in postmenopausal women (1, 11–15, 68–73), in part because of its ability to better activate rapid signaling pathways important for neuroprotection and cognition (20). Estrogen receptors can activate many of these signaling pathways and enable the proper immune response to insults (36). Therefore, the ability...
of estrogen regimens to regulate ER levels may have important consequences in disease states that are characterized by inflammatory or stress-related processes.

The current study found an unexpected uterine response to brain-infused LPS in rats treated with constant estrogen. No uterine effect of LPS was observed in intact or pulsed estrogen-treated rats. Although circulating estradiol levels correlated with uterine weight, there was no difference in estradiol levels between control and LPS-infused rats treated with constant estrogen. In contrast, progesterone levels were elevated by brain infusion of LPS in OVX rats; however, circulating progesterone levels did not correlate to the in-

### Table 3. Biochemical markers after 6 d of treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>ChAT activity in the anterior cortex (nmol/mg protein)</th>
<th>Estrogen binding sites in the hippocampus (fmol/mg protein)</th>
<th>Nitrotyrosine levels in posterior cortex (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact CSF</td>
<td>33.5 ± 1.1</td>
<td>27.1 ± 3.4</td>
<td>3.8 ± 0.2</td>
</tr>
<tr>
<td>LPS</td>
<td>34.1 ± 1.3</td>
<td>22.1 ± 3.8</td>
<td>3.7 ± 0.2</td>
</tr>
<tr>
<td>OVX Pulsed oil CSF</td>
<td>33.9 ± 1.2</td>
<td>22.0 ± 3.6</td>
<td>4.0 ± 0.2</td>
</tr>
<tr>
<td>LPS</td>
<td>34.3 ± 1.3</td>
<td>25.5 ± 3.8</td>
<td>3.8 ± 0.2</td>
</tr>
<tr>
<td>Constant oil CSF</td>
<td>35.2 ± 1.3</td>
<td>23.0 ± 3.8</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>LPS</td>
<td>36.2 ± 1.3</td>
<td>30.0 ± 3.8</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>Pulsed estrogen CSF</td>
<td>34.4 ± 1.3</td>
<td>24.4 ± 3.8</td>
<td>3.4 ± 0.2</td>
</tr>
<tr>
<td>LPS</td>
<td>35.7 ± 1.3</td>
<td>26.2 ± 3.8</td>
<td>4.1 ± 0.2</td>
</tr>
<tr>
<td>Constant estrogen CSF</td>
<td>35.8 ± 1.2</td>
<td>27.1 ± 3.6</td>
<td>4.2 ± 0.2</td>
</tr>
<tr>
<td>LPS</td>
<td>36.3 ± 1.3</td>
<td>27.5 ± 3.8</td>
<td>3.9 ± 0.2</td>
</tr>
</tbody>
</table>

ChAT in the anterior cortex, hippocampal ERs, and nitrotyrosine levels in posterior cortex were unchanged by estrogen regimen and neuroinflammation. Values reflect group mean ± SEM.

No uterine effect of LPS was observed in intact or pulsed estrogen-treated rats. Although circulating estradiol levels correlated with uterine weight, there was no difference in estradiol levels between control and LPS-infused rats treated with constant estrogen. In contrast, progesterone levels were elevated by brain infusion of LPS in OVX rats; however, circulating progesterone levels did not correlate to the in-

![Fig. 5.](https://example.com/fig5.png)

**Fig. 5.** Constant and pulsed estrogen administration were equally effective in increasing PR levels in the uterus (*, PRb, P < 0.001; PRb, P < 0.002). Brain infusion of LPS did not alter uterine PR levels. Values were normalized to OVX constant oil controls and expressed as group mean ± SEM.

![Fig. 6.](https://example.com/fig6.png)

**Fig. 6.** Uterine Hsp90 and Hsp70 protein levels were increased by constant, but not pulsed, estrogen administration (*, Hsp90, P < 0.02; Hsp70, P < 0.0001). Values were normalized to OVX constant oil controls and expressed as group mean ± SEM.
crease in uterine weight. Thus, elevations in estrogen and progesterone cannot fully explain the LPS-induced increase in uterine weight.

In this study, constant estrogen treatment reduced ERα protein by more than 90% in the uterus, an effect concomitant with the LPS-induced increase in uterine growth. ERα can mediate trophic effects of estrogen in the uterus (74–78). Most interestingly, trauma increased uterine growth in rats genetically lacking ERα, suggesting ERα may be a negative regulator of uterine growth (76, 79, 80). It has been suggested that elevations in progesterone, rather than the absence of ERα, mediate uterine growth in response to trauma (81). In the current study, LPS increased circulating progesterone levels in all Ovx rats; however, only rats with reduced ERα levels showed an LPS-induced increase in uterine weight. These data are consistent with the notion that ERα may prevent uterine growth in response to stress; however, additional studies would be needed to support this observation. In contrast to the uterine decline in ERα by constant estrogen, ERβ levels were unchanged and uterine PR levels increased in response to both pulsed and constant estrogen treatments, suggesting that the classical transcription of PR was intact after both estrogen regimens. The regulation of ERα is tissue and time specific; ERα levels were unchanged in the hypothalamus and hippocampus after 6 d of treatment in the current study (Table 4). A regional decline in hypothalamic ERα levels by constant estrogen has been reported by some (23, 82–84) but not others (84, 85). It is possible that a longer duration of constant estrogen administration would be required to detect down-regulation of ERα in other regions.

Estrogen administration can increase Hsp levels in the uterus, which correlate with an increase in uterine weight (67). Although LPS had no effect on Hsp levels in the current study, uterine Hsp70 and Hsp90 levels were increased by constant, but not pulsed, estrogen replacement. Hsp70 can stimulate antigen-presenting cells (86–89) and CD4+ T cell proliferation (89, 90), suggested to play a role in endometriosis, a disease state characterized by aberrant uterine growth (91–94). Likewise, reductions in ERα can enhance both antigen-presenting cell stimulation (95) and CD4+ proliferation (96). LPS stimulation of uterine macrophages and peripheral blood monocytes are capable of increasing levels of the inflammatory cytokine IL-1β, an effect enhanced by coadministration of estrogen in an ER-dependent manner (97). Thus, elevations in Hsp s and reductions in ERα may play a role in the recruitment of inflammatory mediators capable of stimulating uterine growth, although additional research is needed to understand the mechanism by which constant estrogen and brain infusion of LPS increased uterine weight in the current study.

The uterine response to stressors plays an important role in reproductive phenomena such as uterine growth, endometrial receptivity, pregnancy, premature parturition, and defense against sexually transmitted pathogens (98). The peripheral effects observed in the current study after brain infusion of LPS could be mediated by the hypothalamus and hypothalamic-pituitary-adrenal axis, the sympathetic nervous system, or LPS leakage from the brain. Higher doses and rates of infusion can result in LPS leakage from the brain (99); however, previous work from our laboratory in male rats found no evidence of inflammatory cytokines in the blood after brain infusion of LPS (Wenk, G. L., unpublished observations). Estrogen can alter blood-brain barrier and vascular permeability (100), although it is unclear whether permeability to LPS would be affected.

The data presented here suggest that constant and pulsed regimens of estrogen administration have different consequences, despite both being capable of increasing PR and uterine weight. Estrogen administration in a constant vs. pulsed manner differentially alters signaling pathways, uterine physiology, ER regulation, and aspects of learning and memory as well as trophic and other neurochemical markers in the brain (22). The timing of HRT after menopause is being increasingly recognized as an important factor in the success of the therapy (101). This report suggests that the regimen of estrogen replacement also plays a role, because constant estrogen treatment altered protein levels and was influenced by stressors, despite being administered immediately after ovariectomy. Stress may be an important factor in the postmenopausal intervention with estrogen; our results suggest that a pulsed estrogen replacement regimen may be more effective.

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