Original Article

Effect of Estrogen on Pressor Responses to \( \alpha_1 \)-Adrenoreceptor Agonist in Conscious Female Rats

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We examined the effect of estrogen on pressor responses to an \( \alpha_1 \)-adrenoreceptor agonist (phenylephrine) in conscious female Wistar-Kyoto rats. At the age of 11 weeks, rats underwent ovariectomy or a sham procedure. At the age of 15 weeks, ovariectomized (OVX) rats received intramuscular injection of estradiol valerate (EV) 5 \( \mu \)g (OVX + EV 5 \( \mu \)g group; \( n = 6 \)), EV 25 \( \mu \)g (OVX + EV 25 \( \mu \)g group; \( n = 7 \)), or placebo (OVX group; \( n = 8 \)), and sham-operated rats received placebo (sham group; \( n = 8 \)). After 4 days, dose-pressor response curves to phenylephrine were examined under the condition where the renin-angiotensin, vasopressin and autonomic nervous systems were pharmacologically blocked. Ovariectomy shifted the dose-pressor response curve to phenylephrine leftward with a significantly decreased log ED50 (\( \mu \)g/kg) (sham: 0.81 \( \pm \) 0.04; OVX: 0.57 \( \pm \) 0.05; \( p < 0.05 \)). Supplementa- tion with EV 25 \( \mu \)g, but not EV 5 \( \mu \)g, reversed the dose-pressor response curve to phenylephrine in OVX rats to the level of the curve in sham-operated rats with a significantly increased log ED 50 (\( \mu \)g/kg) (OVX + xEV 5 \( \mu \)g: 0.47 \( \pm \) 0.05; OVX + EV 25 \( \mu \)g: 0.75 \( \pm \) 0.08). These results suggest that the physiological level of estrogen seen in intact female rats attenuates pressor responses to \( \alpha_1 \)-adrenoreceptor agonist, while supplementation with a moderate dose of estrogen is needed to restore such effects of physiological-level estrogen within a short-term period after chronic estrogen withdrawal. (Hypertens Res 2002; 25: 609–613)

Key Words: estrogen, \( \alpha_1 \)-adrenoreceptor, pressor responses, rats

Introduction

Estrogen replacement therapy is associated with a reduction in cardiovascular events (1). Although estrogen may protect women from cardiovascular disease through modification of the lipid profile, this mechanism accounts for only 25% to 50% of the observed risk reduction (2). Recent work suggests that estrogen could have a beneficial effect on microcirculation by improving the rheological behavior of erythrocytes (3). Estrogen may also exert its cardioprotective effect by directly acting on the cardiovascular system. Estrogen improves vasomotor function of the coronary artery and peripheral vessels in postmenopausal women (4, 5). It has also been shown that estrogen attenuates vasoconstrictor respons-
Although some studies have demonstrated that long-term replacement of estrogen attenuates the adrenergic vasoconstrictor responses (6–8), other studies do not concur (10–12). This controversy may be in part due to differences in the methods of “ex vivo” measurement of vascular responsiveness and different schemes of estrogen treatment. The purposes of this study were to determine 1) whether physiological levels of estrogen seen in intact female rats contribute to attenuation of the adrenergic vasopressor responses and 2) if so, how the adrenergic vasopressor responses following long-term estrogen withdrawal are modulated within the short-term period after administration of different doses of estrogen. Accordingly, we examined the dose-pressor responses to phenylephrine (α₁-adrenoreceptor agonist) in sham-operated rats, OVX rats and OVX rats administered estradiol valerate 5 µg or 25 µg 4 days before the experiments.

**Methods**

**Animals and Measurement of Blood Pressure**

Female Wistar-Kyoto rats were obtained from Charles River Japan (Kanagawa, Japan). All experiments in the present study were conducted in accordance with the institutional guidelines of the National Research council. At the age of 11 weeks the rats were anesthetized with ether, and a small abdominal incision was made using a sterile technique. Ovariectomy was performed on 21 rats; another 8 rats underwent a sham operation without ovariectomy. At the age of 15 weeks, under ether anesthesia, OVX rats received intramuscular injection of estradiol valerate (EV) 5 µg (OVX + EV 5 µg group; n = 6), EV 25 µg (OVX + EV 25 µg group; n = 7), or placebo (OVX group; n = 8), and sham-operated rats received placebo (sham group; n = 8). Subsequently, arterial and two venous catheters were implanted into the femoral artery, femoral vein and jugular vein, respectively. The surgical procedures and methods used for the measurement of blood pressure have been described previously (13). The free ends of these catheters were brought subcutaneously to the back of the neck. The arterial catheter was connected to a hydraulic cathetering system. To keep the arterial catheter patent, heparinized saline (50 U/ml) was continuously infused at a rate of 0.1 ml/h. The venous catheters were also filled with heparinized saline (50 U/ml). Rats were placed in individual plastic cages and housed at a controlled temperature (23 ± 1°C) under a 12 h light/dark cycle and given free access to food and water. The rats were allowed to recover for 4 days after surgery. The experiments were then performed on fully conscious, unrestrained rats. Sham-operated rats were used without regard for time of estrous cycle.

Blood pressure was recorded from the femoral arterial catheter using a p23ib Statham pressure transducer (Oxford, USA) and straingauge amplifier (Model 1257; NEC-San-ei, Tokyo, Japan).

**Dose-Pressor Responses to Phenylephrine**

Measurement of the dose-pressor responses to phenylephrine has been previously described (13). The renin-angiotensin, vasopressin and autonomic nervous systems were blocked in that order before we estimated the dose-pressor responses to phenylephrine. Each drug was administered intravenously through a jugular vein catheter as follows: captopril, 10 mg/kg; OPC-21268 (vasopressin V₁-receptor antagonist), 3 mg/kg; methyl atropine, 1 mg/kg; atenolol, 1 mg/kg; and pentolinium, 10 mg/kg. Pentolinium was administered 10 min after atenolol was given. The ganglion blocker, atenolol and methyl atropine were administered to eliminate the baroreflex modulation of cardiac output and vascular tone. Captopril and vasopressin V₁-receptor antagonist were administered to prevent the restoration of the blood pressure after ganglion blockade (14). Approximately 2.5 min after the injection of pentolinium, when the blood pressure had reached its lower plateau, we obtained cumulative dose-pressor responses to 10 different doses of phenylephrine in the range of 0.125–64 µg/kg i.v. The mean arterial pressure (MAP)-log dose relationship was fitted by computer to the sigmoidal logistic equation as follows (15):

\[
\text{MAP} = P_{\text{MAP}} + P_{\text{MAP}} / [1 + e^{P_3 \log \text{dose} - P_4}],
\]

where \(P_{\text{MAP}}\) is the upper plateau of the mean arterial pressure, \(P_{\text{MAP}}\) is the range, \(P_{\text{MAP}}\) is a curvature coefficient, and \(P_{\text{MAP}}\) is the log dose at half the mean arterial pressure range (ED₅₀).

After clearance studies, the weight of the uterus body was measured in each rat to evaluate the state of estrogen deficiency as well as the effects of estrogen supplementations.

**Drugs**

Phenylephrine, pentolinium, captopril, methyl atropine, and atenolol were obtained from Sigma Chemical Co. (St. Louis, USA), furosemide from Hoechst (Tokyo, Japan), and OPC-21268 from Otsuka Pharmaceutical Co., Ltd. (Tokushima, Japan). OPC-21268 was dissolved in dimethylformamide (Wako Pure Chemicals, Osaka, Japan) and was injected i.v. in a volume of 100 µl/kg, followed by 100 µl saline. A dose of OPC-21268, 1 mg/kg i.v., lower than that used in the present study, has been shown to completely antagonize the pressor effect of arginine vasopressin 30 mU/kg i.v. (16). The phenylephrine used to construct the dose-pressor response curves was dissolved in saline (0.125 mg/kg/ml). The other drugs were dissolved in saline and were injected intravenously in a volume of 1 ml/kg.

**Data Analysis**

Data are expressed as the means ± SEM. Data analyses were performed by Fisher’s least significant difference test after one way-analysis of variance. A level of \(p < 0.05\) was considered to indicate statistical significance.
Results

Figure 1 shows the dose-pressor response curves to phenylephrine, which were investigated during the blockade of the renin-angiotensin, vasopressin and autonomic nervous systems, in sham, OVX, OVX + E5, estradiol valerate 5 µg-treated OVX rats; OVX + E25, estradiol valerate 25 µg-treated OVX rats; log ED₅₀, log dose at half the mean arterial pressure range. Values are mean ± SE; n, no. of rats; * p < 0.05, vs. Sham. ** p < 0.01, vs. OVX.

Table 1. Parameters of Dose-Pressor Response Curve to Phenylephrine Evaluated during Blockade of Major Pressor Systems and Autonomic Reflex

<table>
<thead>
<tr>
<th>Group</th>
<th>Sham (n = 8)</th>
<th>OVX (n = 8)</th>
<th>OVX + E5 (n = 6)</th>
<th>OVX + E25 (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper plateau (mmHg)</td>
<td>170 ± 3</td>
<td>179 ± 2*</td>
<td>177 ± 5</td>
<td>170 ± 2**</td>
</tr>
<tr>
<td>Lower plateau (mmHg)</td>
<td>46 ± 1</td>
<td>52 ± 2*</td>
<td>54 ± 1*</td>
<td>50 ± 3</td>
</tr>
<tr>
<td>Curvature coefficient</td>
<td>3.5 ± 0.1</td>
<td>4.0 ± 0.4</td>
<td>3.6 ± 0.2</td>
<td>3.8 ± 0.2</td>
</tr>
<tr>
<td>Log ED₅₀ (µg/kg)</td>
<td>0.81 ± 0.04</td>
<td>0.57 ± 0.05*</td>
<td>0.47 ± 0.05*</td>
<td>0.75 ± 0.08**</td>
</tr>
</tbody>
</table>

Sham, sham-operated rats; OVX, ovariectomized rats; OVX + E5, estradiol valerate 5 µg-treated OVX rats; OVX + E25, estradiol valerate 25 µg-treated OVX rats; log ED₅₀, log dose at half the mean arterial pressure range. Values are mean ± SE; n, no. of rats; * p < 0.05, vs. Sham. ** p < 0.01, vs. OVX.

Table 2. Effects of Ovariectomy and Estrogen Supplementation on Body and Uterus Weights

<table>
<thead>
<tr>
<th></th>
<th>11 Weeks</th>
<th>15 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BW (g)</td>
<td>BW (g)</td>
</tr>
<tr>
<td>Sham</td>
<td>214 ± 4</td>
<td>238 ± 3</td>
</tr>
<tr>
<td>OVX</td>
<td>211 ± 4</td>
<td>257 ± 3*</td>
</tr>
<tr>
<td>OVX + E5</td>
<td>216 ± 7</td>
<td>264 ± 7*</td>
</tr>
<tr>
<td>OVX + E25</td>
<td>219 ± 7</td>
<td>265 ± 4*</td>
</tr>
<tr>
<td></td>
<td>UW (g)</td>
<td>UW (g)</td>
</tr>
<tr>
<td>Sham</td>
<td>0.102 ± 0.009</td>
<td>0.431 ± 0.040</td>
</tr>
<tr>
<td>OVX</td>
<td>0.032 ± 0.006</td>
<td>0.124 ± 0.022</td>
</tr>
<tr>
<td>OVX + E5</td>
<td>0.068 ± 0.003</td>
<td>0.257 ± 0.011</td>
</tr>
<tr>
<td>OVX + E25</td>
<td>0.084 ± 0.003</td>
<td>0.314 ± 0.012</td>
</tr>
<tr>
<td></td>
<td>UW/BW (x 10⁻³)</td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>0.431 ± 0.040</td>
<td></td>
</tr>
<tr>
<td>OVX</td>
<td>0.124 ± 0.022</td>
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</tr>
<tr>
<td>OVX + E5</td>
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<tr>
<td>OVX + E25</td>
<td>0.314 ± 0.012</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SE; n, no. of rats; BW, body weight; UW, uterus body weight. * p < 0.05, vs. Sham. ** p < 0.01, vs. Sham.

Discussion

We demonstrated that the pressor responses to phenylephrine

Fig. 1. Cumulative dose-pressor response curves to phenylephrine during the blockade of the renin-angiotensin, vasopressin and autonomic nervous systems in sham ( ), OVX ( ), OVX + EV 5 µg ( ), and OVX + EV 25 µg ( ) rats. MAP, mean arterial pressure. Dashed lines represent the log dose of phenylephrine at half the MAP range.
(an \(\alpha\)-adrenoreceptor agonist) were significantly increased in OVX rats compared with those of sham-operated rats. This finding is in accordance with previous “ex vivo” studies showing that long-term replacement of estrogen significantly blunted the adrenergic sensitivity of mesenteric arteries (6, 7) and aortic rings (8). In their studies, NO synthase inhibition reverted or lessened the attenuating effect of estrogen on the adrenergic vasoconstrictor responses, suggesting that basal NO release is a major determinant of this phenomenon. However, it seems that other mechanisms are also involved. Meyer et al. (7) have shown that blockade of cyclooxygenase reverses the attenuating effect of estrogen on the vasoconstrictor responses to adrenergic agonist, indicating that basal release of vasodilator prostaglandins is also involved. In addition, Zhang and Davidge (6) have recently demonstrated that \(\alpha\)-adrenoreceptor expression is reduced in the mesenteric arteries of estrogen-replaced rats. Thus it is also possible that estrogen modulates adrenergic vasoconstriction by modulating its receptors.

The results of previous studies which examined the effects of estrogen on adrenergic vasoconstrictor or vasopressor responses have been inconsistent. Although this controversy may be in part due to differences in the methods of “ex vivo” measurement of vascular responsiveness, the differences in dose of estrogen may also be involved. It has been shown that a high dose of estrogen decreases basal release of NO and prostacyclin in aortic rings (17), and enhances the adrenergic vasoconstrictor responses (18).

In the present study, EV 25 \(\mu\)g, but not EV 5 \(\mu\)g, administered 4 days before or after (40–60 min) the experiments reversed the vasopressor responses to phenylephrine in OVX rats to the level in sham-operated rats. EV used in the present study exerts its full effect on the 4th day after administration and thereafter maintains its effect for a prolonged period of time (19). According to a previous study which examined the effect of estrogen on bone mass, EV 2 \(\mu\)g/week is an appropriate dose for hormone replacement in OVX rats (20). Thus the doses of EV 5 \(\mu\)g and 25 \(\mu\)g used in the present study might have exerted 2.5 and 12.5 times more potent action than the dose used for hormone replacement, respectively. Paredes-Carbajal et al. (8) have shown that the contractile responses to phenylephrine in aortic rings excised from OVX rats are not altered before or after (40–60 min) addition of a physiological level of 17\(\beta\)-estradiol (10\(^{-9}\) mol/l), while chronic (11–13 days) treatment with a physiological level of estrogen significantly attenuated the adrenergic vasoconstriction. They speculated that acute administration of estrogen does not increase basal NO release enough to attenuate the adrenergic vasocnstriction, because it takes at least several days for estrogen to increase eNOS mRNA and eNOS activity (21, 22). Hishikawa et al. (21) have shown that 17\(\beta\)-estradiol increases eNOS protein and eNOS activity of human aortic endothelial cells in a dose-dependent manner with a maximal effect at a concentration similar to that during pregnancy. Thus it is possible that a higher dose of estrogen than that used for hormone replacement is needed to restore basal NO release sufficiently to attenuate the adrenergic vasoconstriction within a short-term period after long-term withdrawal of estrogen, which would explain the different effect of EV 5 \(\mu\)g and EV 25 \(\mu\)g in the present study.

Alternatively, it is possible that a nongenomic action of estrogen affected the adrenergic vasopressor responses in the present study. Estrogen rapidly increases basal NO release or eNOS activity in cultured endothelial cells without affecting gene transcription (23, 24). Although this response seems to be induced by concentrations of estrogen well below those found in normally cycling women, basal eNOS activity rises in a dose-dependent manner with a maximal effect (100% increase) at 10\(^{-6}\) mol/l 17\(\beta\)-estradiol (25). Thus it is possible that supplementation with EV 25 \(\mu\)g, but not EV 5 \(\mu\)g, increased basal NO release sufficiently to attenuate the adrenergic pressor responses via a nongenomic action in the present study.

In conclusion, we demonstrated that the physiological levels of estrogen seen in estrous cycle contribute to attenuation of the adrenergic vasopressor responses in conscious female rats. However, to normalize the adrenergic vasopressor responses within a short-term period after long-term estrogen withdrawal, supplementation with a moderate dose of estrogen is needed.

References