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Ovarian Hormones Modulate Endothelin-1 Vascular Reactivity and mRNA Expression in DOCA-Salt Hypertensive Rats

Flavia L. David, Maria Helena C. Carvalho, Ana L.N. Cobra, Dorothy Nigro, Zuleica B. Fortes, Nancy A. Rebouças, Rita C.A. Tostes

Abstract—We previously demonstrated a differential activation of the endothelin-1 (ET-1) pathway in male and female deoxycorticosterone (DOCA)-salt hypertensive rats, with the male rats exhibiting marked alterations in vascular and pressor responses to ET-1 and Suc-[Glu,9, Ala,11,15]-ET-1(8-21) (IRL-1620), an ET \textsubscript{B} agonist. Mechanisms underlying these gender differences are unclear, and we hypothesized that the ovarian hormones attenuate vascular ET \textsubscript{B} responses in female DOCA-salt rats. Female Wistar rats were randomized in 3 groups: sham-operated, ovariectomized (OVX), and OVX plus hormone replacement with estradiol (E) or estradiol/progesterone (EP). Two weeks later, rats were uninephrectomized and further randomized in DOCA-salt (subcutaneous injections of deoxycorticosterone and drinking water containing NaCl/KCl) and control normotensive (subcutaneous injections of vehicle and tap water). Blood pressure was evaluated both by direct and standard tail-cuff methods. Responses to IRL-1620 were evaluated in vivo/in situ in the mesenteric microcirculation. mRNA expression of ET-1 and ETA/B receptors was evaluated in mesenteric arteries by reverse transcription–polymerase chain reaction and expressed relative to GAPDH. OVX-DOCA rats developed a more severe form of hypertension than did DOCA rats. Treatment with E or EP restored blood pressure to levels observed in DOCA rats. In the mesentery, IRL-1620 induced vasodilatation in control rats, a mild vasoconstriction in DOCA rats, and marked vasoconstriction in OVX-DOCA rats. Both E and EP decreased IRL-1620–induced vasoconstriction in the DOCA group. In the normotensive group, OVX did not change blood pressure or IRL-1620–induced vasodilation. Removal of the ovaries increased ET-1 mRNA in arteries from DOCA and control rats, although treatment with E or EP reversed these changes. Vascular ET \textsubscript{B} receptor mRNA levels were greatly enhanced in OVX-DOCA but not OVX-control rats. Hormone replacement with E or EP restored ET \textsubscript{B} receptor expression in the DOCA group. A greater blood pressure–lowering effect of bosentan (ET \textsubscript{A}/ET \textsubscript{B} blocker) was observed in OVX-DOCA rats. The observation that OVX worsens hypertension as well as the altered ET \textsubscript{B} receptor–mediated responses and the effects of bosentan in female DOCA rats supports our suggestion that the ovarian hormones modulate ET-1/ET \textsubscript{B} receptor vascular responses/expression in DOCA-salt hypertension. *(Hypertension. 2001;38[part 2]:692-696.)*

**Key Words:** deoxycorticosterone ■ vasoconstriction ■ endothelin ■ estrogen ■ receptors, endothelin

In deoxycorticosterone (DOCA)-salt hypertension and other experimental models of hypertension, male rats develop an earlier and more severe form of hypertension than do female rats.\textsuperscript{1–2} We have recently suggested that differential activation of endothelin-1 (ET-1) pathways, expressed by a functional upregulation of ET \textsubscript{B} receptors, may play a role in the higher blood pressure (BP) levels observed in male DOCA-salt hypertensive rats.\textsuperscript{3–4} We observed that mesenteric arterioles from male but not female DOCA-salt rats display increased ET-1– and ET \textsubscript{B}–mediated responses and also that male DOCA-salt animals exhibit a marked and greater BP-lowering effect in response to bosentan, a mixed ET \textsubscript{A}/ET \textsubscript{B} antagonist, compared with that of female DOCA rats.\textsuperscript{3–4} Mechanisms underlying the attenuated alterations in the vascular ET-1 pathway in female DOCA-salt rats are unclear but may be related to the cardioprotective effects of ovarian hormones. Increasing evidence suggests that ovarian hormones suppress or modulate ET-1 production and its effects: (1) 17\beta-estradiol attenuates ET-1–induced coronary artery constriction both in vitro\textsuperscript{5} and in vivo\textsuperscript{6}; (2) plasma ET-1 levels fluctuate during the menstrual cycle\textsuperscript{7} and are higher in men than in age-matched women;\textsuperscript{8} (3) ET-1 decreases during estrogen replacement therapy, during pregnancy (when estrogen levels are high), and during estrogen administration in transsexual male patients\textsuperscript{9,10}; and (4) in the absence of ovarian hormones, ET-1 mRNA increases, as does the peptide.\textsuperscript{10,11}
Therefore, the aim of the present study was to investigate whether the ovarian hormones modulate ET-1 vascular reactivity and expression in female DOCA-salt rats. We evaluated (1) responses to the ET_B agonist Suc[-Glu, 9 Ala 11,15]-ET-1(8-21) (IRL-1620) in vivo/in situ in the mesenteric microcirculation, (2) the vascular mRNA expression of ET-1 and ET_A receptors, and (3) the BP-lowering effect of bosentan in female normotensive and DOCA-salt rats that were sham-operated, were ovariectomized, or received hormone replacement therapy following the ovariectomy.

Methods

Animals

Female Wistar rats from the Institute of Biomedical Science’s animal facility were used in this study. All animals were fed standard laboratory rat chow, had ad libitum access to both food and water, and were housed individually in a room with a constant temperature (24°C) and a 12-hour/12-hour light/dark cycle. Experimental protocols followed standards and policies of the University of Sao Paulo’s Animal Care and Use Committee.

Surgical Procedures

At 6 weeks of age, rats underwent ovariectomy under chloral hydrate anesthesia (300 mg/kg SC). Briefly, under aseptic conditions, a small incision was performed in the lower abdomen, and both ovaries were removed (OVX rats) or touched with the surgical instruments (sham). On the same day, some of these rats received pellets containing 17β-estradiol (E; 0.1 mg/pellet; 60-day release; Innovagen, J-driven); DOCA and chloral hydrate were from Sigma Chemical Co. Ovariectomy was kindly provided by Dr Martine Clozel (Actelion Ltd, Allschwil, Switzerland).

Table. Ovariectomized rats, both control and DOCA-salt rats, were housed with E or EP groups. Systolic BP values are shown in the Table. Ovariectomized rats, both control and DOCA-salt rats, were housed with E or EP groups. Systolic BP values are shown in the Table.

<table>
<thead>
<tr>
<th>Group</th>
<th>Group (n=25)</th>
<th>SBP at 2 Weeks, mm Hg</th>
<th>SBP at 4 Weeks, mm Hg</th>
<th>Body Weight, g</th>
<th>Uterine Weight, mg/100 g</th>
<th>Body weight</th>
<th>Estradiol, pg/mL</th>
<th>Progesterone, pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>120±2</td>
<td>118±1</td>
<td>219±2</td>
<td>39±2</td>
<td>127±10</td>
<td>511±9</td>
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<td></td>
</tr>
<tr>
<td>Control-OVX</td>
<td>124±2</td>
<td>121±2</td>
<td>239±6*</td>
<td>12±4*</td>
<td>45±11*</td>
<td>268±11*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control-OVX-E (n=18)</td>
<td>121±2</td>
<td>119±3</td>
<td>210±5†</td>
<td>47±5†</td>
<td>137±13†</td>
<td>475±10†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control-OVX-EP (n=17)</td>
<td>122±3</td>
<td>119±5</td>
<td>201±7†</td>
<td>53±5†</td>
<td>172±15†</td>
<td>549±12†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOCA</td>
<td>147±3‡</td>
<td>167±4‡</td>
<td>215±4</td>
<td>43±2</td>
<td>117±12</td>
<td>515±4</td>
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</tr>
<tr>
<td>DOCA-OVX (n=24)</td>
<td>162±4*,‡</td>
<td>185±4*,‡</td>
<td>215±4</td>
<td>43±2</td>
<td>117±12</td>
<td>515±4</td>
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</tr>
<tr>
<td>DOCA-OVX-E (n=20)</td>
<td>149±4‡</td>
<td>162±6†,‡</td>
<td>192±4†,§</td>
<td>64±5†,§</td>
<td>128±12†</td>
<td>471±13†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOCA-OVX-EP (n=18)</td>
<td>150±3‡</td>
<td>167±6†,‡</td>
<td>185±3†,§</td>
<td>61±7†,§</td>
<td>140±12†</td>
<td>565±19†</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are mean±SEM. *P<0.05 OVX vs sham; †P<0.05 OVX-E or OVX-EP vs OVX; ‡P<0.05 DOCA vs control; ††P<0.05 OVX-E or OVX-EP vs sham.

Results

Ovariectomy accelerated the development and severity of hypertension in DOCA-salt rats (P<0.05). Hormone replacement, both with E or EP, restored BP levels in OVX-DOCA rats to values seen in DOCA rats (P<0.05). No differences were observed between the control, OVX-control and E- or EP-control groups. Systolic BP values are shown in Table 1. Ovariectomized rats, both control and DOCA-salt rats, had significantly higher body weights than that of sham-operated rats, and the weight gain was reduced in the groups.
that received hormone treatment ($P<0.05$). To control for the
efficacy of ovariectomy and hormone replacement, both the
uterine weight and hormone plasma levels were evaluated. As
can be observed in the Table, uterine body weight and plasma
levels of E and EP were significantly reduced in the OVX
groups and were restored with the hormone replacement
therapy ($P<0.05$). Because responses in E- and EP-treated
rats were similar at all protocols, only responses in the E-groups will be shown.

IRL-1620 ($10^{-11}$ to $10^{-9}$ mol/L) induced vasodilation in
control (initial diameter, $19.3\pm1.5$ mm; after $10^{-9}$ mol/L
IRL-1620, $20.7\pm1.6$ mm) and OVX-control (initial diameter,
$18.9\pm1.3$ mm; after $10^{-9}$ mol/L IRL-1620, $20.1\pm1.8$ mm)
rats. Treatment with E or EP did not modify IRL-1620–
induced responses (initial diameter, $20.6\pm0.8$ mm; after $10^{-9}$
mmol/L IRL-1620, $21.9\pm1.2$ mm) in the normotensive group.
A mild vasoconstrictor response occurred in DOCA rats
(initial diameter, $18.9\pm2.1$ mm; after $10^{-9}$ mol/L IRL-1620,
$18.2\pm1.6$ mm), but a greater and marked vasoconstriction
was observed in the OVX-DOCA rats on IRL-1620 stimula-
tion (initial diameter, $18.8\pm1.8$ mm; after $10^{-9}$ mol/L IRL-
1620, $17.2\pm1.5$ mm; $P<0.05$) (Figure 1). Treatment with E
or EP significantly attenuated IRL-1620–induced vasocon-
striction in the hypertensive rats (initial diameter, $20.5\pm0.9$
mm; after $10^{-9}$ mol/L IRL-1620, $19.9\pm1.1$ mm; $P<0.05$).

Figure 2 demonstrates results obtained from RT-PCR show-
ing mRNA expression of ET-1 and ETA and Epsilon receptor
genes in mesenteric arteries from sham, OVX, and E-treated
control and DOCA-salt rats. Similar results were observed in
aortas (not shown). DOCA-salt treatment slightly but signif-
ically increased ET-1 expression in female rats ($P<0.05$).
The OVX increased ET-1 mRNA expression in control and
DOCA rats ($P<0.05$), whereas treatment with E or EP
reversed these changes ($P<0.05$). A trend to increased ETA
receptor mRNA expression was observed in the OVX-control
group, but only reached statistical significance in the DOCA-
OVX group ($P<0.05$). Treatment with E or EP normalized
ETA receptor mRNA expression ($P<0.05$). No alterations
were observed in ETA receptor mRNA expression. To further
investigate alterations in the ET-1 pathway, we assessed
effects of the mixed ETA/ETB antagonist bosentan on BP
(Figure 3). Mean arterial BP was higher in DOCA-salt rats
compared with control rats ($P<0.05$). Bosentan (10 mg/kg
IV) slightly but significantly decreased BP in the control
groups. The antagonist lowered BP in DOCA-salt hyperten-
sive rats, but the decrease on BP was significantly greater in
the OVX-DOCA group. In the E-DOCA group, the effects of
bosentan were similar to those observed in the DOCA group.

**Discussion**

Ovarian hormones are believed to possess cardiovascular
protective effects, and they seem to play a role in the
gender-related differences in the development of hyperten-
sion in experimental models.1–7 Because ET-1 plays a role in
the pathogenesis of DOCA-salt hypertension,13–17 it is possi-
ble that the attenuated development of hypertension in female
DOCA-salt rats may be related to a modulation exerted by the
gonadal hormones on ET-1 effects on the cardiovascular
system.5–11 We have recently reported that in DOCA-salt
hypertension, male rats display altered vascular and pressor
responses to ET-1 and to the ETB agonist IRL-1620 and that
these alterations are attenuated in female rats.3,4 We specu-
lulated that the differential and gender-related alterations in ET-1–mediated effects in DOCA-salt hypertension may be important in the higher blood pressure levels observed in male DOCA-salt hypertensive rats. On the basis of these results and considering that ovarian hormones modulate ET-1 responses/production, in the present study we tested the hypothesis that the ovarian hormones influence the attenuated changes in ET-1 actions observed in female DOCA-salt rats.

The major observation in this study is that removal of the ovaries and, consequently, a significant reduction in estradiol and progesterone levels worsens the changes in ET-1–induced effects observed in female DOCA-salt rats: it enhances IRL-1620–stimulated vasoconstriction, it further increases the lack of ovarian hormones increases vascular mRNA expression of ET-1 and ETB receptor subtype as well as vasoconstrictor responses to IRL-1620 and the BP-lowering effects of bosentan, whereas hormone replacement with E or EP restores these responses to a pattern similar to that observed in female DOCA-salt rats.

Acknowledgments

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