A comparison of ovariectomy models for estrogen studies

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Ovariectomy is a ubiquitously used model to reduce the production and influence of estrogen. However, it induces hyperphagia that, in turn, results in substantial weight gain (5). This increase in body mass constitutes a considerable source of estrogen, because peripheral tissue is capable of converting adrenal-synthesized androgen precursors into estrogen (3, 13). Consequently, it is difficult to make conclusions regarding the effects of estrogen when the apparent control conditions, which are intended to represent a state of estrogen withdrawal, actually contain substantial levels of estrogen. Indeed, ovariectomized animals are still capable of estrogen synthesis in peripheral tissues because aromatase, the terminal enzyme in the estrogen biosynthetic pathway, is expressed in many tissues (3, 6).

Treatment with tamoxifen is another method of reducing the influence of estrogen. However, tamoxifen is also known to function as a partial agonist in certain physiological conditions and cell types (11, 12). The effect of SERMs is determined by a complex interaction between factors including estrogen receptor (ER) subtypes, adaptor protein expression, ligand identity, and transcription target sites, which vary with cell type (11). Thus, similar to ovariectomy, tamoxifen may not completely abolish the influence of estrogen in vivo.

Therefore, the purpose of this study was to evaluate the validity of various models of estrogen inhibition that are used as control conditions in estrogen studies. The extensive investigation and established impact of estrogen on the vasculature suggest that evaluating the effectiveness of methods of estrogen antagonism is important to the study of vascular physiology. With the use of rat mesenteric arteries, we analyzed four groups of ovariectomized rats: ovariectomized and ovariectomized with concomitant treatment of either tamoxifen, aromatase inhibitor 4-hydroxyandrostene-3,17-dione (4-OHA), or a calorie-controlled diet. These groups were then compared with a control group of intact rats.

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cycling rats in the diestrus phase. Measurements of these groups included body and uterine weights, plasma estrogen levels, vasorelaxation to the endothelium-dependent muscarinic agonist methacholine, and vasorelaxation to the endothelium-independent exogenous nitric oxide (NO) donor sodium nitroprusside (SNP).

**METHODS**

**General animal model.** Female Sprague-Dawley rats (2 mo of age) were obtained from Charles River and housed in the facilities at the University of Alberta. Four groups of rats were ovariectomized (n = 5, each group) and treated with one of the following: a subcutaneous implant of a placebo pellet, a tamoxifen pellet (5 mg/pellet, 60-day release), an aromatase inhibitor, 4-OHA (350 mg/pellet, 28-day release) pellet, or a calorie-controlled (15 g of food/day) diet. Doses for the antagonist effects of the drugs were chosen based on previous reports (2, 4). Calorie-controlled ovariectomized rats were fed a quantity of food that was similar to the quantity of food consumed by the cycling animals, thereby preventing overeating. Four weeks after ovariectomy and treatment, these groups were compared with age-matched cycling rats in the diestrus phase. On the day of experiment, they were killed under light anesthesia with methohexital sodium (50 mg/kg body wt). Blood samples were collected from the right atrium, allowed to clot, and centrifuged at 3,000 rpm for 10 min. 17β-estradiol was measured by use of a radioimmunoassay kit (Diagnostic Products) The animal protocols were examined by the University of Alberta Animal Welfare Committee and found to be in compliance with the guidelines issued by the Canada Council on Animal Care.

**Experimental design.** Mesenteric arteries averaging 250 μm in diameter were chosen as a model for systemic resistance regulation as the splanchnic bed receives 30% of the cardiac output, making this vasculature an important contributor to cardiovascular homeostasis. In addition, pregnancy (a state of elevated estrogen that causes reduced vascular tone) increases mesenteric blood flow by 75% (7). Arteries were mounted in an isometric myograph system containing HEPES-buffered physiological saline solution (in mM: 142 sodium chloride, 4.7 potassium chloride, 1.17 magnesium sulfate, 1.56 calcium chloride, 1.18 potassium phosphate, 10 HEPES, and 5.5 glucose, pH 7.4). Four separate baths were used to study arterial segments simultaneously. Cumulative doses of phenylephrine (1–50 μmol/l) were administered to measure sensitivity and determine the dose that would give a 50% constriction (EC50) for each individual artery. The EC50 of phenylephrine was used to constrict the baths were changed every 10 min with fresh HEPES-buffered physiological saline solution.

**RESULTS**

**Animal model.** Animals in the ovariectomized group had significantly greater (P < 0.05) body weights than those in the three ovariectomy-plus treatment groups or the cycling group. Plasma 17β-estradiol levels were significantly higher (P < 0.05) in the cycling rats than the four ovariectomized groups that were all similar. However, uterine weight, which provides a biological marker of estrogen, was significantly lower in the 4-OHA and calorie-controlled ovariectomized rats (Table 1).

**Vascular responses.** Vasodilatory dose-response curves to methacholine were conducted on mesenteric arteries preconstricted with phenylephrine. Arteries from the cycling, ovariectomized, and tamoxifen-treated ovariectomized groups relaxed similar to the endothelium-dependent muscarinic agonist methacholine (Fig. 1). These groups demonstrated greater sensitivity to methacholine by relaxing more readily than arteries from the 4-OHA-treated ovariectomized and calorie-controlled ovariectomized groups (Fig. 1). Thus, compared with intact cycling rats, only the 4-OHA-treated ovariectomized and calorie-controlled ovariectomized groups demonstrated blunted relaxation in response to estrogen removal.

Finally, differences in vessel responsiveness to methacholine do not appear to be due to smooth muscle sensitivity, because all of the groups relaxed similarly to the exogenous NO donor SNP (Fig. 2).

**DISCUSSION**

In the present study, we measured vascular function to evaluate the efficacy of various methods of estrogen suppression. On the basis of vessel function as well as

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cycling</th>
<th>OVX</th>
<th>OVX-T</th>
<th>OVX-A</th>
<th>OVX-F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>255 ± 4.1a</td>
<td>316 ± 9.5b</td>
<td>260 ± 6.1a</td>
<td>224 ± 6.1a</td>
<td>251 ± 4.0a</td>
</tr>
<tr>
<td>E2, pg/ml</td>
<td>7.9 ± 0.3b</td>
<td>5.0 ± 0.2b</td>
<td>4.7 ± 0.4b</td>
<td>6.0 ± 0.7b</td>
<td>5.8 ± 0.8b</td>
</tr>
<tr>
<td>Uterine wt, g</td>
<td>0.33 ± 0.02b</td>
<td>0.26 ± 0.04b</td>
<td>0.21 ± 0.01b</td>
<td>0.13 ± 0.02b</td>
<td>0.11 ± 0.008b</td>
</tr>
</tbody>
</table>

Values are means ± SE. Different symbols indicate P < 0.05. Groups are cycling (diestrus rats), ovariectomized (OVX), OVX + tamoxifen (OVX-T), OVX + aromatase (OVX-A), and OVX + control-fed (OVX-F). E2, 17β-estradiol.
uterine and body weights, we conclude that ovariecto-
mized rats treated with either an aromatase inhibitor
or calorie-controlled diet constitute an effective control
condition for estrogen studies. However, ovariectomy
models without a calorie-controlled diet or with tamox-
ifen treatment appear to preclude an accurate deter-
mination of the effects of estrogen.

The responses to methacholine-induced relaxation
clearly demonstrate the characteristics of different es-
tram Withdrawal models. The 4-OHA-treated and
and non-calorie-controlled ovariectomized rats relax similarly
to the control group of cycling rats. A likely expla-
nation for the discrepancy in vessel function among
ovariectomized groups is that some of the treatments
did not sufficiently suppress the estrogen pathway.
Indeed, compared with the cycling (diestrus) rats, the
tamoxifen-treated and non-calorie-controlled ovariecto-
mized groups had similar uterine weights, whereas
the uterine weights of the 4-OHA-treated and the ca-
loric-controlled ovariectomized animals were signifi-
cantly smaller, suggesting a greater inhibition of bio-
logically active estrogens in the latter two groups.

Animals that were ovariectomized but not fed a con-
trolled diet were significantly larger than those in the
other groups. Considering that peripheral tissues, such as adipose (8) and skeletal (1, 10) muscle, are capable of synthesizing estrogen, the animals in this group likely experienced elevated estrogen levels as a consequence of the greater tissue mass. Although plasma levels of 17β-estradiol in this group were not greater than other ovariectomized groups, alternative factors could account for the proposed increase in estrogen exposure. Because our assay is specific to 17β-estradiol, several other undetected forms of estrogen could

have been elevated by peripheral hormone synthesis. However, it may be that production of estrogen from peripheral tissues (i.e., from sources other than ova-
ries) may alter vascular function through paracrine
mechanisms that may not be reflected in the plasma.

The partial agonistic effects of tamoxifen on the
estrogen pathway are most likely responsible for main-
taining methacholine-induced relaxation similar to
that of intact cycling animals. Although tamoxifen in-
hibits many estrogen-mediated processes, in certain
conditions such as states of low estrogen concentration
(9), it is capable of binding to ERs and initiating ago-
nostic effects. Hence, tamoxifen treatment in ovariec-
tomized animals is not an effective method of attenu-
at ing the influence of estrogen. It is important to note
that there are many SERMs available that we did not
test in our studies, because it was not our intention to
specifically evaluate efficacy of various ER blockers.

The effect of SERMs is determined by a complex inter-
action between factors including ER subtypes, adaptor
protein expression, ligand identity, and transcription

target sites (11). Indeed, ICI-182,780, a commonly used
estrogen antagonist, acts as an agonist through ERβ
via the human retinoic acid receptor-alpha-1 promotor,
whereas tamoxifen is a more potent antagonist via
ERβ than ERα (14). These data further exemplify the
importance of appropriate control groups for the stud-
ies of estrogen effects.

In summary, we delineated the efficacy of various
models of estrogen suppression by comparing vessel
function among ovariectomized groups. Relaxation
curves to methacholine effectively demonstrated the
differences between treatment conditions and indi-
cated that estrogenic influences may still exist in some
models of ovariectomy. Inhibiting aromatase activity
with 4-OHA and controlling caloric consumption in
ovariectomized animals are the two most effective par-
adigms for estrogen inhibition that we tested. Due to
the potentially prohibitive cost of 4-OHA, controlling

Fig. 1. EC50 values for methacholine in mesenteric arteries from
cycling, ovariectomized (OVX), tamoxifen-treated OVX, 4-hy-
droxyandrostene-3,17-dione (4-OHA)-treated OVX, and control-fed
OVX rats (n = 5, each group). Data represent means ± SE. Letters
represent P < 0.05 vs. other groups.

Fig. 2. EC50 values for sodium nitroprusside in mesenteric arteries
from cycling, OVX, tamoxifen-treated OVX, 4-OHA-treated OVX,
and control-fed OVX rats (n = 5, each group). Data represent
means ± SE.
the diet of ovariectomized rats may be one of the most efficient control conditions for estrogen studies.

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REFERENCES