Effects of estrogens and selective estrogen receptor modulators on vascular reactivity in the perfused mesenteric vascular bed

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Mark CJ, Tatchum-Talom R, Martin DS, Eyster KM. Effects of estrogens and selective estrogen receptor modulators on vascular reactivity in the perfused mesenteric vascular bed. Am J Physiol Regul Integr Comp Physiol 293: R1969–R1975, 2007. First published September 19, 2007; doi:10.1152/ajpregu.00260.2007.—Estrogens and selective estrogen receptor modulators (SERMs), such as raloxifene (RAL) and tamoxifen (TAM), acutely relax arteries, but the long-term effects of estrogens and SERMs on vascular reactivity in the mesenteric vasculature have not been well defined. In this study, we used an isolated, perfused mesenteric vascular bed technique to investigate the effect of chronic treatment of estrogens and SERMs on vascular reactivity of the mesenteric bed. Ovariectomized female Sprague-Dawley rats were treated by gavage with vehicle (control, 2-hydroxypropyl-β-cyclodextrin), ethinyl estradiol, estradiol benzoate, equilin (EQ), TAM, or RAL for 3 wk. EQ and TAM increased vasoconstriction in response to all three vasoconstrictors tested (KCl, norepinephrine, and 5-HT). Ethinyl estradiol increased vasoconstriction in response to KCl and 5-HT, whereas responses to estradiol benzoate and RAL were less consistent. Only EQ (134 ± 4 mmHg) and TAM (104 ± 4 mmHg) changed mean arterial blood pressure compared with control (117 ± 4 mmHg). These data demonstrate that 3-wk gavage treatment with estrogens and SERMs affects vascular reactivity in the mesenteric vascular bed. However, the three formulations of estrogen did not produce equivalent effects, and the effects of the SERMs were different from those of the estrogens.

Cardiovascular disease (CVD) is one of the most common diseases in the industrialized world and is a major cause of human death (10). The incidence of CVDs, such as hypertension and atherosclerosis, is greater in men than in premenopausal women (13). Among women, the incidence of CVD is greater in postmenopausal women than premenopausal women (39). The loss of estrogen at the menopause is hypothesized to underlie the epidemiological link between menopause and increased cardiovascular risk. Thus it is important to continue to gain a better understanding of the action of estrogen on the vascular system.

Alterations in vascular tone play a major role in the control of blood pressure (BP) and thereby the incidence of hypertension and CVD. 17β-Estradiol has been shown to act acutely as a direct vasodilator (27). Conversely, Li and Stallone have shown that chronic 3-wk treatment with estrogen potentiated the contractile responses of the female rat aorta to vasopressin (24a). For this investigation, we chose a chronic 3-wk treatment with three formulations of estrogen and two selective estrogen receptor modulators (SERMs). The estrogens chosen for this study are among the most routinely used for research and therapeutic treatment. Ethinyl estradiol (EE) is used orally, either alone or with a progestin, in oral contraceptives (43). Equilin (EQ) is an example of a conjugated equine estrogen and a common component of postmenopausal hormone replacement therapy (2). Estradiol benzoate (EB) is an estrogen regularly used in research laboratories. Given the widespread use of these agents, it is important to gain further understanding of their effects on the vasculature, as little information is available regarding the effects of different formulations of estrogen on vascular tone.

SERMs are nonsteroidal molecules with tissue-specific estrogen agonist and antagonist effects. These compounds interact with the estrogen receptor at the ligand-binding domain and differentially recruit coactivators or corepressors to produce their effects (28). The SERMs chosen for this study are the most widely prescribed, tamoxifen (TAM) and raloxifene (RAL). TAM is an estrogen antagonist in the breast, but an agonist in the endometrium (30), and is used for the treatment of estrogen-sensitive breast cancer. RAL is an estrogen antagonist in the breast and uterus, but an estrogen agonist in the bone (33), and is widely used for the prevention and treatment of osteoporosis (33). Evidence has shown that RAL, like estrogen, induces endothelium-dependent acute vasodilation (13, 23) and directly activates nitric oxide synthesis in endothelial cells (35), resulting in dilation of blood vessels. Conversely, chronic RAL treatment of pigs was reported to induce changes in expression of components of the nitric oxide system that would be consistent with a reduction in activity of that system (29). RAL and TAM have been shown to acutely relax coronary arteries in vitro by an estrogen receptor-dependent mechanism, suggesting that estrogen agonist effects of SERMs are preserved in coronary arteries (13, 14). Acute perfusion of RAL and TAM has also been shown to induce significant dose-dependent relaxation of the mesenteric vascular bed (37). Recent work in pigs suggests that estrogens and SERMs may exert differential modulatory effects on venous smooth muscle contractile responses and that these effects may also be agonist specific (24). Several questions regarding the vascular actions of SERMs need to be fully addressed. First, the long-term in vivo effects of SERMs in the mesenteric vascular bed are unknown. Second, the effects of different SERMs have not been systematically compared in a rat model. Third, the bulk of current data was acquired using intravenous or in vitro application of SERMs rather than orally, as occurs in human applications.

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This study was designed to determine whether treatment with various formulations of estrogens or SERMs affects vascular reactivity in the isolated mesenteric vascular bed. It was important to perform this study to examine whether different formulations of estrogens and SERMs have the same effects on vascular reactivity. Gavage treatment was used as it mimics clinical usage and because administration of estrogen by different routes results in different biological effects (8). The mesenteric vascular bed represents a resistive network that contributes to peripheral vascular resistance (6). The isolated, perfused mesenteric bed preparation relies on a fixed flow rate moving through the vessels; therefore, changes in perfusion pressure are considered an index of vascular tone. This technique allows a direct comparison of the changes in vascular responses of the mesenteric tree in response to continuous oral treatment for 3 wk with different formulations of estrogens and SERMs.

MATERIALS AND METHODS

Animal treatments. All experiments and protocols were performed in accordance with the regulations established by the National Institutes of Health Council on Animal Care and were approved by the Institutional Animal Care and Use Committee of the University of South Dakota. Prepubertal female Sprague-Dawley rats (Harlan, Indianapolis, IN; n = 8/group) were ovariectomized at 4 wk of age, before the initiation of regular ovarian cycles. The animals were allowed to recover for 2 wk before the experimental protocol was initiated. The rats were randomly divided into six groups and treated by daily gavage for 3 wk with the vehicle (20% 2-hydroxypropyl-β-cyclodextrin, control (CTL); Sigma, St. Louis, MO], EE (0.15 mg/kg; Sigma), EB (0.15 mg/kg; Sigma), EQ (0.15 mg/kg; Sigma), TAM (3 mg/kg; Eli Lilly, Indianapolis, IN). Doses were chosen from published work from other laboratories (33) as well as our own (32) to have physiological effects.

Perfused, isolated mesenteric vascular bed. After 3 wk of treatment, the rats were anesthetized with isoflurane (3% isoflurane in Sigma), EB (0.15 mg/kg; Sigma), EQ (0.15 mg/kg; Sigma), TAM (3 mg/kg; Sigma), or RAL (3 mg/kg; Eli Lilly, Indianapolis, IN). Doses were chosen from published work from other laboratories (33) as well as our own (32) to have physiological effects.

Effect of estrogens and SERMs on vascular reactivity. Gavage treatment was used as it mimics clinical usage and because administration of estrogen by different routes results in different biological effects (8). The isolated, perfused mesenteric bed preparation relies on a fixed flow rate moving through the vessels; therefore, changes in perfusion pressure are considered an index of vascular tone. This technique allows a direct comparison of the changes in vascular responses of the mesenteric tree in response to continuous oral treatment for 3 wk with different formulations of estrogens and SERMs.

RESULTS

Effect of estrogens and SERMs on baseline characteristics. Body weight (g) was significantly decreased in all treatment groups compared with CTL (Table 1). Uterine weight was used as a marker of estrogen action, because the uterus undergoes hypertrophy in response to estrogen (33). Relative uterine weight (uterine wt/body wt, mg/g) was significantly increased in the EE-, EB-, EQ-, and TAM-treated rats compared with CTL (Table 1). Relative uterine weight was not affected by RAL treatment (Table 1). There was no significant effect of treatment on relative heart weight (heart weight/body weight, mg/g, Table 1). Mean arterial BP (mmHg) was significantly decreased in the rats treated with TAM and significantly increased in rats treated with EQ compared with CTL, but there was no significant effect of the other estrogens or SERMs on BP (Table 1).

Effect of estrogens and SERMs on vascular reactivity. Perfusion of the mesenteric vascular beds at a constant flow rate (5 ml/min) induced a steady basal perfusion pressure after an initial stabilization period of 30 min. Infusion of KCl dose-dependently caused vasoconstriction and increased perfusion pressure (Fig. 1). The various estrogen formulations had different effects on the constrictor responses to KCl (Fig. 1A). At the maximum dose of KCl tested (125 mM), there was a

<table>
<thead>
<tr>
<th>Body Weight, g</th>
<th>Relative Uterine Weight, mg/g</th>
<th>Relative Heart Weight, mg/g</th>
<th>BP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>236 ± 7</td>
<td>0.16 ± 0.01</td>
<td>3.70 ± 0.09</td>
<td>123.1 ± 4</td>
</tr>
<tr>
<td>164 ± 6*</td>
<td>0.70 ± 0.06*</td>
<td>3.84 ± 0.01</td>
<td>118.4 ± 4</td>
</tr>
<tr>
<td>202 ± 5*</td>
<td>0.43 ± 0.04*</td>
<td>3.67 ± 0.01</td>
<td>123.1 ± 6</td>
</tr>
<tr>
<td>190 ± 7*</td>
<td>0.55 ± 0.05*</td>
<td>3.91 ± 0.06</td>
<td>140.1 ± 4*</td>
</tr>
<tr>
<td>160 ± 5*</td>
<td>0.36 ± 0.02*</td>
<td>3.65 ± 0.09</td>
<td>104.2 ± 4*</td>
</tr>
<tr>
<td>193 ± 6*</td>
<td>0.21 ± 0.08</td>
<td>3.61 ± 0.09</td>
<td>116.8 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SE. BP, mean arterial blood pressure; CTL, control/vehicle; EE, ethinyl estradiol; EB, estradiol benzoate; EQ, equilin; TAM, tamoxifen; RAL, raloxifene. *Significant difference from CTL (P < 0.05).
significantly greater constriction in the EQ- and EE-treated groups compared with CTL (Table 2). Maximum constriction for the EB treatment group was not significantly different from CTL (Table 2). The ED70 mmHg in the EQ-treated group (93/11006 4 mM) was significantly lower than CTL (110/11006 3 mM). However, the ED70 mmHg values for the other treatment groups were not different (EE, 101/11006 3 mM; EB, 104/11006 4 mM) from CTL.

The SERMs had different effects on the constrictor responses to KCl (Fig. 1B). TAM treatment significantly increased the perfusion pressure response at the maximum dose of KCl compared with CTL, whereas the KCl-induced maximum constriction was not different in RAL-treated rats (Table 2). RAL (92/11006 3 mM) and TAM (94/11006 4 mM) treatment caused a significant decrease in the ED70 mmHg of the KCl response compared with CTL (110/11006 3 mM, Fig. 1B). Thus estrogen and SERM treatments had differential effects on depolarization-induced constriction.

Estrogens and SERMs also affected NE-induced mesenteric constriction (Fig. 2). Bolus injections of NE dose-dependently caused vasoc restriction and increased perfusion pressure. EQ treatment increased the maximum vasoconstrictor response to NE compared with that obtained in CTL (Table 2, Fig. 2A). Maximum constriction for EE and EB treatment groups was not different from CTL (Fig. 2A). The ED125 mmHg of the concentration-response curve for NE demonstrated a significant increase in the EB-treated group (6.2/11003 10/11002 8/11006 1 nmol) and a significant decrease in the EE-treated group (1.0/11003 10/11002 8/11006 0.5 nmol) compared with CTL (4.4/11003 10/11002 8/11006 1 nmol, Fig. 2A).

Table 2. Perfusion pressure of the mesenteric arcade at the maximum dose of KCl, NE, and 5-HT

<table>
<thead>
<tr>
<th></th>
<th>KCl</th>
<th>NE</th>
<th>5-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTL</td>
<td>73±8</td>
<td>158±19</td>
<td>51±5</td>
</tr>
<tr>
<td>EE</td>
<td>98±8*</td>
<td>178±25</td>
<td>78±10*</td>
</tr>
<tr>
<td>EB</td>
<td>91±5</td>
<td>164±11</td>
<td>54±8</td>
</tr>
<tr>
<td>EQ</td>
<td>117±8*</td>
<td>231±19*</td>
<td>95±7*</td>
</tr>
<tr>
<td>TAM</td>
<td>114±8*</td>
<td>213±14*</td>
<td>84±4*</td>
</tr>
<tr>
<td>RAL</td>
<td>94±12</td>
<td>147±7</td>
<td>39±3</td>
</tr>
</tbody>
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Values are means ± SE in mmHg. KCl, potassium chloride; NE, norepinephrine; 5-HT, serotonin. *Significant difference from CTL (P < 0.05).
indicating that estrogen treatment had differential effects on adrenergic receptor-mediated constriction. The ED\textsubscript{125 mmHg} response for EQ (\textit{3.6} \times 10^{-8} \pm 2 \text{nmol}) was not different from CTL.

SERMs also affected the constrictor responses to NE (Fig. 2B). There was a significant increase in the maximum effect of NE on the mesenteric constriction of the TAM-treated animals compared with CTL (Table 2). In contrast, the response to NE was not affected by RAL treatment (Table 2). There were no significant differences in the ED\textsubscript{125 mmHg} of the SERM groups vs. CTL (Fig. 2B).

The various estrogen formulations had differential effects on the constrictor responses to 5-HT (Fig. 3). Bolus injections of 5-HT caused vasoconstriction and increased perfusion pressure in a dose-dependent manner in all groups. In EQ- and EE-treated rats, the mesenteric vascular bed exhibited exaggerated responses to the highest dose of 5-HT compared with vehicle-treated CTL rats in response to the maximum dose of 5-HT (Table 2, Fig. 3A). The maximum response obtained in EB-treated rats was not different from CTL (Table 2). EQ treatment (\textit{7.7} \times 10^{-10} \pm 1 \text{nmol}) caused a significant decrease in the ED\textsubscript{30 mmHg} response to the vasoconstrictor 5-HT compared with CTL (\textit{1.9} \times 10^{-8} \pm 0.9 \text{nmol}, Fig. 3A), while EE (\textit{6.7} \times 10^{-9} \pm 0.2 \text{nmol}) and EB treatment (\textit{1.8} \times 10^{-8} \pm 1 \text{nmol}) had no effect.

SERM treatment influenced mesenteric responses to 5-HT as well (Fig. 3B). There was a significant increase in the maximum contractile response to 5-HT in the TAM-treated group compared with CTL (Table 2). However, there was no effect on the maximum constriction in the RAL-treated group compared with CTL (Table 2). There were no significant differences in ED\textsubscript{30 mmHg} of the SERM groups vs. CTL (Fig. 3B).

**DISCUSSION**

The most important finding of this study is that the three different formulations of estrogen, EE, EQ, and EB, exhibited significantly different effects on vascular reactivity in the mesenteric vascular bed. All three were clearly estrogenic as shown by their effects on uterine weight (31) and body weight (5, 33), yet their effects on responses to vasoconstrictors varied. The responses were not just quantitatively different, but, as discussed in more detail below, were actually qualitatively different. Our data are supported by the recent work of Okano and coworkers (29) that showed differential responses to different formulations of estrogens in the cardiovascular system in cultured aortic endothelial cells from the pig. Since the estrogens were administered by gavage in our study, they were subject to metabolism in the liver. The metabolites of the different formulations of estrogen also differ (9). Moreover, some metabolites of estradiol, such as catecholestradiols and methoxyestradiols, act via mechanisms that are independent of the estrogen receptor (10). Thus the differential effects of different formulations of estrogens that were observed in the vasculature in this study could have been due to direct effects of the different estrogens on the estrogen receptors (21, 25), or to metabolites of the estrogens acting via estrogen receptor-dependent or -independent mechanisms (9).

A second important finding of this study is that the SERMs, TAM and RAL, are not full agonists of estrogen in the mesenteric vasculature, but are indeed selective in their effects. Moreover, TAM and RAL also undergo metabolism to form active metabolites (3, 40). Thus the effects of TAM and RAL may be due to direct effects of the SERMs on estrogen receptors in the mesenteric vasculature, or to effects of their metabolites.

Taken together, these two findings demonstrate that the structural differences among these formulations of estrogens and SERMs result in functional differences in the responses of the mesenteric vasculature to vasoconstrictors. Activation of the estrogen receptor requires formation of a complex containing the ligand, receptor, tissue-specific coactivators or corepressors (20), and in some cases other transcription factors (42). The ability of SERMs to act as agonists of estrogen lies in their ability to form these ligand-protein complexes and the degree to which the final conformation of the complex mimicks that formed by natural 17\textbeta-estradiol (19). The functional agonist vs. antagonist activities that occur in reproductive tissues in response to structurally different SERMs have been well described (17, 26). The current data suggest that the
different formulations of estrogen or their metabolites may also exhibit subtle differences in their interactions with the estrogen receptor and other proteins of the activational complex in the mesenteric vasculature. Accordingly, the functional differences in the mesenteric vasculature in response to different estrogens and SERMs may be mediated by the same mechanisms as those observed for SERMs in reproductive tissues. Indeed, recent studies suggest that some differential vascular responses involve divergent effects on the endothelial nitric oxide system (29) and endothelial products of vasoconstrictor agents (24).

Although all three formulations of estrogen increased constriction in the isolated, perfused mesentery in response to specific vasoconstrictors, only EQ increased BP in the whole animal. Vasoconstriction also increased in response to TAM, and to a lesser extent to RAL. However, TAM treatment resulted in a decrease in BP in the whole animal. The regulation of BP is multifactorial; these data suggest that effects of EE and EB in other systems counter their vasoconstrictor effects in the mesenteric vasculature, but that the vasoconstrictor effects of EQ are not countered by other systems. Similarly, the ability of TAM to potentiate vasoconstrictor effects in the mesenteric vasculature appear to be completely reversed in that BP actually decreased. Conversely, other work has shown TAM and estrogen to have no significant effects on BP in an ovariectomized rat model (38). In contrast to our study, that experiment was performed using a subcutaneous pellet to administer the hormone instead of gavage treatment, which may account for the discrepancies with our study.

KCl-induced constriction is mediated primarily via depolarization-induced opening of voltage-gated calcium channels and influx of extracellular calcium (18). Accordingly, the ability of EQ, RAL, and TAM to increase constrictor responses to KCl is suggestive of an action on voltage-gated calcium channels or distal mechanisms that respond to this influx of calcium. Alternatively, Miller and coworkers showed recently that estrogens and SERMs exert differential effects on endothelial modulation of vascular smooth muscle constriction (24, 29). Since the endothelium was not removed in our preparation, the effects we observed may reflect the combined actions of the estrogen receptor agonists on the endothelium and vascular smooth muscle compartments.

NE-provoked vasoconstriction is primarily mediated by α-adrenergic receptors. Our data indicate that estrogen receptor ligands have differential effects on α-adrenergic-induced vasoconstriction. EQ and EB increased the sensitivity of the mesenteric vascular bed to NE, and EQ increased the maximum constriction of the tissue to NE. In contrast, the SERMs had no effect on the sensitivity of the tissue to NE-induced constriction, but TAM increased the maximum constriction of the tissue to NE. These data support a previous report that estrogens increase the sensitivity of vascular smooth muscle to NE-induced contraction (34). The mechanisms underlying estrogenic modulation of α-adrenergic constriction remain to be fully elucidated. Estrogens may enhance vascular α-adrenergic receptor affinity (4, 7). Other studies have shown that α-adrenergic receptor expression decreased with estrogen treatment in ovariectomized rats (44). The different effects described in these studies may be due to technical differences, such as different formulations of estrogens or different routes of administration. On the other hand, our data show that, even under the same conditions, different estrogen formulations affect vascular function in a differential manner.

The mechanisms underlying the vasoconstrictor effects of 5-HT are unclear (22), although several signal transduction pathways have been implicated (1, 41). In this study, maximum vasoconstrictor responses to 5-HT were highly exaggerated in the EQ-treated group. A less dramatic, but nonetheless significant, modification of maximum 5-HT constriction was also revealed in the EE- and TAM-treated groups. In addition, EQ increased the sensitivity of the mesenteric vascular bed to 5-HT-induced vasoconstriction. Thus activation of estrogenic pathways also potentiated 5-HT-induced vasoconstriction, but with differing levels of response to different formulations of estrogens and SERMs. These data contrast with studies done by Goodrow and coworkers (16) that showed a decrease in responsiveness to 5-HT in cerebral arteries of ovariectomized rats in response to estrogen. Other recent data showed that 17β-estradiol treatment of ovariectomized pigs induced maximum constrictor responses of venous smooth muscle in response to 5-HT, whereas RAL had no effect (24). Taken together, the data suggest possible regional differences in the effects of estrogen on vascular reactivity.

The present study used three different vasoconstrictor agents. Whereas KCl relies primarily on extracellular sources of calcium (18), NE and 5-HT recruit both extracellular and intracellular pools of calcium to elicit contractions. Thus the differential effect of the estrogen receptor agonist treatment in mesenteric constrictor response may reflect differential effects in extracellular and intracellular calcium handling mechanisms. In fact, recent work is in agreement with the view that the modulatory effects of estrogen receptor agonists in vascular smooth muscle contraction may be contractile-agonist specific (24, 29). These very discrete responses are consistent with the possibility of estrogen agonist-selective interactions with specific elements in each of the contractile signaling pathways rather than a general effect, for example, at the level of myosin ATPase.

The present study examined estrogen effects in rats that had been ovariectomized before sexual maturity. Estrogen receptors-α- and -β are expressed in the blood vessels (21, 25), and we have demonstrated the presence of estrogen receptors in the mesenteric arteries of the ovariectomized immature animal model used in this study (32). Thus the appropriate receptors for response to the oral estrogen receptor agonist treatments were present in our model. However, the effects of estrogens and SERMs on vascular reactivity might be different in aged animals that have been exposed to a lifetime of ovarian cycles and multiple pregnancies, which may impact estrogen-receptor expression and coupling mechanisms. This animal model may be more relevant to the peripuberal or premenopausal female than to the postmenopausal woman.

Perspectives and significance. As Jackson’s group has recently pointed out (11), increased understanding of the pharmacology of different estrogens and their metabolites will be essential to rational design of new therapies for hormone replacement therapy and treatment of CVD. The data reported herein, as well as by others (24, 29), clearly show that different formulations of estrogens and SERMs elicit quite varied effects on vascular function and BP. Further elucidation of the mechanisms underlying these dichotomies will provide a foundation...
for the development of new synthetic estrogens or new SERMs that act as highly selective ligands to achieve very specific biological functions in the vasculature.

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