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

Running head: Mesenteric vascular responses to estrogens and SERMs

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Abstract

Estrogens, and 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 (CTL, 2-hydroxypropyl-β-cyclodextrin), ethinyl estradiol (EE), estradiol benzoate (EB), equilin (EQ), TAM, or RAL for 3 weeks. EQ and TAM increased vasoconstriction in response to all three vasoconstrictors tested (KCl, NE, and 5-HT). EE increased vasoconstriction in response to KCl and 5-HT, whereas responses to EB and RAL were less consistent. Only EQ (134 ± 4 mmHg) and TAM (104 ± 4) changed mean arterial blood pressure compared to control (117 ± 4). These data demonstrate that 3 week 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.

Key terms: equilin, ethinyl estradiol, estradiol benzoate, tamoxifen, raloxifene
Introduction

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 cardiovascular diseases such as hypertension and atherosclerosis is greater in men than in premenopausal women (13). Among women, the incidence of cardiovascular disease 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 and thereby the incidence of hypertension and cardiovascular disease. 17β-estradiol has been shown to act acutely as a direct vasodilator (27). Conversely, Stallone and colleagues have shown that chronic three week treatment with estrogen potentiated the contractile responses of the female rat aorta to vasopressin (36). For this investigation, we chose a chronic three week 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 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 ER-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 were acquired using intravenous or in vitro application of SERMs rather than orally as occurs in human applications.

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 weeks 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 (NIH) 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 weeks of age, prior to the initiation of regular ovarian cycles. The animals were allowed to recover for 2 weeks before the experimental protocol was initiated. The rats were randomly divided into 6 groups and treated by daily gavage for 3 weeks with the vehicle (20% 2-hydroxypropyl-β-cyclodextrin, control, CTL; Sigma, St Louis, MO), ethinyl estradiol (EE, 0.15 mg/kg; Sigma), estradiol benzoate (EB, 0.15 mg/kg; Sigma), equilin (EQ, 0.15 mg/kg; Sigma), tamoxifen (TAM, 3 mg/kg; Sigma), or raloxifene (RAL, 3 mg/kg; Eli Lilly & Co., 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 weeks of treatment, the rats were anesthetized with isoflurane (3% isoflurane in 100% O₂) and the carotid artery was isolated. A polyethylene cannula (PE 50, Clay Adams, Parsippany, NY) containing saline was inserted into the left carotid artery. After 30 minutes of equilibration, blood pressure (BP) and heart rate (HR) were measured using a low-volume pressure transducer (Transpac Abott, Transonic, NY). The pressure transducer was connected to a computer that acquired data for carotid blood pressure using BIOPAC data.
acquisition software (model MP 100A, AcqKnowledge Software 3.1; BIOPAC Systems, Inc., Goleta, CA).

The superior mesenteric artery was then cannulated and the mesenteric arcade removed from the animal as previously described (37). The uterus was also removed and its wet weight was immediately recorded.

The mesenteric vascular bed was constantly perfused (5 ml/min) and superfused (0.2 ml/min) using 2 separate pumps. The perfusate was modified Krebs-Henseleit (118 mM NaCl, 4.7 mM KCl, 1.2 mM MgCl₂·6H₂O, 1.0 mM NaH₂PO₄, 2.6 mM CaCl₂·2H₂O, 25 mM NaHCO₃, 11.1 mM glucose; 37ºC; pH 7.4; chemicals from Sigma), oxygenated with a 95% O₂ - 5% CO₂ gas mixture. Arteriolar constriction or dilation was determined by the change in perfusion pressure (mmHg) recorded from a low pressure transducer (Transpac Abott, Transonic, NY) placed in the perfusion circuit just before the mesenteric vascular bed (37).

**Experimental protocol**

The mesenteric vascular bed was allowed to stabilize for 30 minutes after it was placed in the perfusion circuit. Following the stabilization period, the mesenteric vascular beds (n=8/group) were challenged with a series of vasoconstrictors. Concentration-response curves to potassium chloride (25-125 mM), norepinephrine (0.1-100 nmol), and serotonin (0.1-30 nmol) were constructed by injecting increasing doses of the drugs into the perfusion system at 5-minute intervals. KCl was infused for 30 seconds, whereas NE and 5-HT
were injected as bolus injections of 100 µl. A 15-minute equilibration period was allowed between drugs which was sufficient for basal perfusion to return to control levels. The sequence of drug injection (KCl, NE, 5-HT) was randomized, but lower doses were always given before higher doses.

Data analysis

Results are expressed as means ± SEM. Experiments were performed according to a randomized block design. Animal characteristics and the maximum dose of each vasoconstrictor were examined by analysis of variance followed by Dunnett’s Multiple Comparison test (GraphPad Prism 4 Software, San Diego CA). ED is the effective dose of the vasoconstrictor that produced a particular perfusion pressure in the mesentery. Since the range of responses was different for each agonist, a different ED was chosen for each concentration response curve; each ED was chosen so that it fell in the linear portion of the graph. ED$_{70\text{mmHg}}$ for KCl, ED$_{125\text{mmHg}}$ for NE, and ED$_{30\text{mmHg}}$ for 5HT were derived from non-linear regression analyses of the concentration response curves and were evaluated by analysis of variance followed by Dunnett’s Multiple Comparison test (GraphPad Prism 4 Software, San Diego CA). Differences were considered significant at $p \leq 0.05$.

Results

Effect of estrogens and SERMs on baseline characteristics
Body weight (g) was significantly decreased in all treatment groups compared to 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 to 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 blood pressure (BP, mmHg) was significantly decreased in the rats treated with TAM and significantly increased in rats treated with EQ compared to 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 potassium chloride (KCl) dose-dependently caused vasoconstriction and increased perfusion pressure (Figure 1). The various estrogen formulations had different effects on the constrictor responses to KCl (Figure 1A). At the maximum dose of KCl tested (125 mM), there was a significantly greater constriction in the EQ and EE treated groups compared to CTL (Table 2). Maximum constriction for the EB treatment group was not significantly different from control (Table 2). The ED$_{70}$mmHg in the EQ treated group (93±4 mM) was significantly lower than CTL (110±3 mM). However, the
ED$_{70\text{mmHg}}$ values for the other treatment groups were not different (EE, 101±3 mM; EB, 104±4 mM) from CTL.

The SERMs had different effects on the constrictor responses to KCl (Figure 1B). TAM treatment significantly increased the perfusion pressure response at the maximum dose of KCl in comparison to CTL, whereas the KCl induced maximum constriction was not different in RAL treated rats (Table 2). RAL (92±3 mM) and TAM (94±4 mM) treatment caused a significant decrease in the ED$_{70\text{mmHg}}$ of the KCl response in comparison to CTL (110±3 mM, Figure 1B). Thus estrogen and SERM treatments had differential effects on depolarization induced constriction.

Estrogens and SERMs also affected NE-induced mesenteric constriction (Figure 2). Bolus injections of norepinephrine (NE) dose-dependently caused vasoconstriction and increased perfusion pressure. EQ treatment increased the maximum vasoconstrictor response to NE compared to that obtained in CTL (Table 2, Figure 2A). Maximum constriction for EE and EB treatment groups were not different from CTL (Figure 2A). The ED$_{125\text{mmHg}}$ of the concentration response curve for NE demonstrated a significant increase in the EB treated group (6.2x10$^{-8}$±1 nmol) and a significant decrease in the EE treated group (1.0x10$^{-8}$±0.5 nmol) compared to CTL (4.4x10$^{-8}$±1 nmol, Figure 2), indicating that estrogen treatment had differential effects on adrenergic receptor mediated constriction. The ED$_{125\text{mmHg}}$ response for EQ (3.6x10$^{-8}$±2 nmol) was not different from CTL.
SERMs also affected the constrictor responses to NE (Figure 2B). There was a significant increase in the maximum effect of NE on the mesenteric constriction of the TAM treated animals compared to 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_{125mmHg} of the SERM groups versus CTL (Figure 2B).

The various estrogen formulations had differential effects on the constrictor responses to serotonin (5-HT) (Figure 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 to vehicle treated CTL rats in response to the maximum dose of 5-HT (Table 2, Figure 3A). The maximum response obtained in EB treated rats was not different from CTL (Table 2). EQ treatment (7.7x10^{-10}±1 nmol) caused a significant decrease in the ED_{30mmHg} response to the vasoconstrictor 5-HT compared to CTL (1.9x10^{-8}±0.9 nmol, Figure 3A), while EE (6.7x10^{-9}±0.2 nmol) and EB treatment (1.8x10^{-8}±1 nmol) had no effect.

SERM treatment influenced mesenteric responses to 5-HT as well (Figure 3B). There was a significant increase in the maximum contractile response to 5-HT in the TAM treated group compared to control (Table 2). However, there was no effect on the maximum constriction in the RAL treated group compared to CTL (Table 2). There were no significant differences in ED_{30mmHg} of the SERM groups versus CTL (Figure 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 mimics that formed by natural 17β-estradiol (19). The functional agonist versus 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 blood pressure 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 blood pressure in the whole animal. The regulation of blood pressure 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 blood pressure actually decreased. Conversely, other work has shown TAM and estrogen to have no significant effects on blood pressure 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 that showed a decrease in responsiveness to 5-HT in cerebral arteries of ovariectomized rats in response to estrogen (16). 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 raloxifene 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 current study examined estrogen effects in rats that had been ovariectomized prior to 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 was 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 peripubertal 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 cardiovascular disease. 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 blood pressure. 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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Disclosures

The authors of this manuscript have no conflicts of interest to report.
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FIGURE LEGENDS

**Figure 1:** KCl-induced changes in perfusion pressure in the mesenteric vascular bed of treated animals. Values represent mean + SEM, \( n = 8 \) per group. Differences were compared at the maximum dose of KCl and at \( ED_{70mmHg} \) and were analyzed by ANOVA followed by Dunnett’s Multiple Comparison Test. A) Treatment with control (CTL), ethinyl estradiol (EE), estradiol benzoate (EB), equilin (EQ). The \( ED_{70mmHg} \) was significantly lower for EQ (\( * \), \( P < 0.05 \)) vs. CTL. EQ (\( † \)) and EE (\( ‡ \)) significantly increased the perfusion pressure at the maximal dose of KCl tested compared to CTL (\( P < 0.05 \)). B) Treatment with CTL, tamoxifen (TAM), or raloxifene (RAL). The \( ED_{70mmHg} \) was significantly lower for TAM vs. CTL (\( * \), \( P < 0.05 \)) and RAL vs. CTL (\( # \), \( P < 0.05 \)). TAM significantly increased the perfusion pressure at the maximal dose of KCl tested compared to CTL (\( † \), \( P < 0.05 \)).

**Figure 2:** NE-induced changes in perfusion pressure in the mesenteric vascular bed of treated animals. Values represent mean + SEM, \( n = 8 \) per group. Differences were compared at the maximum dose of NE and at \( ED_{125mmHg} \) and were analyzed by ANOVA followed by Dunnett’s Multiple Comparison Test. A) Treatment with CTL, EE, EB, or EQ. The \( ED_{125mmHg} \) was significantly lower for EE vs. CTL (\( * \), \( P < 0.05 \)) and higher for EB vs. CTL (\( # \), \( P < 0.05 \)). EQ significantly increased the perfusion pressure at the maximal dose of NE tested compared to
CTL (†, P<0.05). B) Treatment with CTL, TAM, or RAL. No significant differences in ED125mmHg were observed among the groups. TAM significantly increased the perfusion pressure at the maximal dose of NE tested compared to CTL (†, P<0.05).

**Figure 3:** 5-HT-induced changes in perfusion pressure in the mesenteric vascular bed of treated animals Values represent mean + SEM, n= 8 per group. Differences were compared at the maximum dose of 5-HT and at ED30mmHg and were analyzed by ANOVA followed by Dunnett’s Multiple Comparison Test. A) Treatment with CTL, EE, EB, or EQ. The ED30mmHg was significantly lower for EQ vs. CTL (*, P<0.05). EQ (†) and EE (‡) significantly increased the perfusion pressure at the maximal dose of 5-HT tested compared to CTL (P<0.05). B) Treatment with CTL, TAM, or RAL. No significant differences in ED30mmHg were observed among the groups. TAM significantly increased the perfusion pressure at the maximal dose of 5-HT tested compared to CTL (†, P<0.05).
Table 1: Characteristics of ovariectomized rats treated with vehicle control, estrogens or SERMs.

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<th>Body weight (g)</th>
<th>Relative uterine weight (mg/g)</th>
<th>Relative heart weight (mg/g)</th>
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<td>236±7</td>
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<td>3.91±0.06</td>
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<td>TAM</td>
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<td>3.61±0.09</td>
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BP=mean arterial blood pressure, CTL = control/vehicle, EE=ethinyl estradiol, EB=estradiol benzoate, EQ=equilin, TAM=tamoxifen, RAL=raloxifene
Values represent means ± SEM.
* =significant difference from CTL (p<0.05)
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CTL=control/vehicle, EE=ethinyl estradiol, EB=estradiol benzoate, EQ=equilin, TAM=tamoxifen, RAL=raloxifene, KCl=potassium chloride, NE=norepinephrine, 5-HT=serotonin
Values represent means ± SEM.
* =significant difference from CTL (p<0.05)
Figure 1

A) 

B)
Figure 2

A)  

B)
Figure 3

A) Control, EE, EB, EQ

B) Control, TAM, RAL

Change in perfusion pressure (mmHg) vs. log of 5-HT (mol)