EFFECTS OF GENDER AND ESTROGEN
ON MYOSIN C-TERMINAL ISOFORMS AND
CONTRACTILITY IN RAT AORTA

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Running Head: Gender & Estrogen: Myosin Isoforms and Vascular Contractility

Key Words: estrogen, vascular sensitivity, isometric force, myosin isoforms, ovariectomy

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ABSTRACT

We reported that estrogen treatment of ovariectomized rats increased uterine smooth muscle contractility and the C-terminal myosin heavy chain isoform SM1:SM2 ratio (19). We extended this model to study gender and estrogen effects on vascular contractility. Experimental groups included 10-14 wk male (M), female (F), ovariectomized (OF) and OF treated with estrogen (OF&E) for 7 days with a subcutaneous pellet delivery system, resulting in 17β-estradiol of 85 (OF&E) vs. 5 pg/ml (OF or M). The SM1:SM2 ratio increased from 1.8 to 2.6 in thoracic aorta, similar to uterine muscle. Isometric force was measured in 5 mm segments of intact and endothelium-denuded (-endo) aorta. KCl: maximum forces, OF ≈ M > OF&E; and ED₅₀, OF&E > OF ≈ M. Differences in ED₅₀ with estrogen persisted after endothelial denudation. The decreased force in -endo OF aorta was not seen in OF&E, suggesting that estrogen altered an endothelium-dependent effect. Norepinephrine: no differences in maximum forces; and ED₅₀, OF > OF&E > M. Estrogen treatment, in contrast to KCl, increased sensitivity. Endothelial denudation increased sensitivity but reduced the differences between groups. Acetylcholine relaxation: males were more sensitive than females and estrogen had no effect. In the abdominal aorta, there were no changes in SM1:SM2 with 17β-estradiol and differences in contractility were blunted. In summary, estrogen treatment decreased responses to KCl but increased sensitivity to norepinephrine; male rats always demonstrated the highest contractility. An increase in the C-terminal myosin heavy chain isoform SM1:SM2 ratio with 17β-estradiol treatment may underlie the changes observed in contractility.
INTRODUCTION

The occurrence of cardiovascular diseases is greater in men aged 30–50 yr compared with women of similar age (15, 16). The incidence of cardiovascular diseases is also greater in postmenopausal compared with premenopausal women and vascular protective effects of female sex hormones have been proposed (15, 16, 18, 31). Currently estrogen replacement therapy to postmenopausal women is in widespread use but its validity is questioned (14, 17, 23, 27, 34, 36). The mechanism by which gender and/or estrogen may affect the heart is unknown and likely to be multifactorial and has been reviewed in detail (30, 40). Estrogen receptors have been identified on both vascular endothelial (4, 10) and smooth muscle cells (5, 21, 22, 29) providing two potential pathways by which estrogen could affect vascular function. In addition, non-receptor mediated mechanisms may play a role (35). Estrogen treatment has been reported to increase production of the vasodilator nitric oxide (8), but to decrease production of the inducible nitric oxide synthetase (38). It is also reported to decrease production of the powerful vasoconstrictor endothelin (13).

Using a rat model involving ovariectomy and estrogen replacement, we characterized major changes in uterine smooth muscle function (19). Changes in both maximum isometric force and shortening velocity were associated with estrogen replacement. Interestingly, the changes in shortening velocity were correlated with a shift in the distribution of myosin isoforms. Smooth muscle contains both C- and N-terminal isoforms produced by alternate splicing. With estrogen treatment, no changes in the N-terminal isoforms were noted (6), while the SM1 variant of the C-terminal isoforms was increased (19).

The purpose of this study was to assess whether similar functional changes occurred in aorta in these ovariectomized and estrogen-replaced rats, and their relations, if any, to contractility in the aorta of the male rat. Isometric force in the aorta of male rats was the most
sensitive to all stimuli. Interestingly, estrogen-replacement decreased the sensitivity of aorta from the female rats to KCl depolarization but increased sensitivity to receptor-mediated stimulation with norepinephrine.
METHODS

**Rat Experimental Groups:** Female Sprague Dawley rats (230-250 g) were bilaterally ovariectomized under ether anesthesia. Two weeks after ovariectomy a timed release pellet (Innovative Research) containing 0.05 mg 17β-estradiol was implanted subcutaneously in the abdomen in half of the ovariectomized rats. Animals were sacrificed 7 days after pellet implantation. Randomly cycling female and male rats of similar weight were also studied. Blood was collected for the preparation of serum. Six rats in each class were used so the n-values (ranging from 4-6 depending on the technical success of the experiment) represent one aorta per rat; an intact and endothelium-denuded ring from each thoracic aorta was used.

**Serum 17β-estradiol:** 17β-estradiol levels in serum were measured by a double antibody radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA) and expressed as pg of 17β-estradiol/ ml of serum.

**Myosin Isoforms:** Rats were sacrificed using ether anesthesia, and the aortae were dissected. Adhering fat and connective tissue were removed and the aortae were frozen in liquid N₂ and stored at -80° until use. Frozen tissue samples were pulverized under liquid N₂ and homogenized in 1% sodium dodecyl sulfate (SDS), 50 mM Tris, pH 6.8, and 25 mM dithiothreitol. The C-terminal myosin heavy chain isoforms, SM1 (204 kDa) and SM2 (200 kDa) were separated by electrophoresis on 6% polyacrylamide gels as previously described (19). The gels were stained with Coomassie Blue R and scanned on a LKB Ultrascan XL Laser Densitometer. The relative proportions of SM1 and SM2 were calculated from the area under each peak and expressed as mean ± standard error of the mean (SEM).

**Isometric Force:** Rats were euthanized in a precharged CO₂ chamber with subsequent cervical dislocation; blood was drawn by cardiac puncture for later analysis. The aorta was
dissected and cleaned of loose fat and connective tissue. Two 5 mm rings were cut from the aorta; blot weights were between 4-5 mg. One of each pair of rings was denuded of endothelium by gently everting with forceps to expose the endothelium, then rolling on filter paper. Rings were mounted between a fixed stainless steel post and one connected to a Kistler-Morse force transducer whose output was monitored with a computer-based data collection system (Biopac Systems, Goleta, CA). The length between the wires could be adjusted by a micrometer driven device. The rings were mounted in a 15 ml organ bath and allowed to equilibrate at 37 °C for 1 hr in physiological saline solution (PSS), during which the tension was adjusted to 50 mN. This force was chosen for it places the ring at a length within the range for optimal active force generation (26). Our physiological saline solution (PSS) contained (mM): 118 NaCl, 4.73 KCl, 1.2 MgCl₂, 0.026 EDTA, 1.2 KH₂PO₄, 2.5 CaCl₂, 5.5 glucose and was buffered with 25 NaH₂CO₃; pH, when bubbled with 95%O₂ / 5% CO₂ was 7.4 at 37 °C. The aorta was stimulated by addition of KCl (3 M) to bring the bath concentration to 50 mM. The arteries were then relaxed by exchanging the bathing solution; this contraction/relaxation cycle was repeated until reproducible forces were generated. Then a cumulative KCl concentration-force curve was generated. After returning to the PSS, at least two contraction/relaxation cycles using norepinephrine (NE, 1 µM) were undertaken. Subsequently, a cumulative NE concentration-force relation was generated. After the experiment, the ring dimensions and blot weight were measured. Aortic wall thickness (t) was estimated using the formula t = blot weight/(1.05×length×circumference), and the cross sectional area (CSA) for force normalization taken as CSA = 2×t×length. Cumulative concentration (c) -isometric force (F) data were averaged at each concentration; n is used to denote the number of rats. These data for each class were fitted (Origin software) to the power logistic relation:
\[ F = F_o \{1 - 1/[1+(c/ED_{50})^p]\} , \]

where ED_{50} is the concentration at which 50% of F_o is reached, p is the Hill coefficient and F_o the maximum isometric force.

**Statistical Analysis:** Standard ANOVA was used to assess statistical significance; a P<0.05 was taken as indicative of a significant difference. Newman-Keul’s post hoc test was used for the MHC isoforms and the serum estrogen levels, Bonferroni for the appropriate contractile data. Student’s t-test was used to compare intact to endothelium-denuded arteries.
RESULTS

Effects of Estrogen. We examined relations between the serum 17β-estradiol levels and body weight. We first measured these parameters during the ovulatory cycle in female rats. We combined rats into three groups according to their serum 17β-estradiol levels corresponding to estrous, proestrous, and metestrous, ranging from 11 to 44 pg/ml, and these results are summarized in Table 1.

We chose an experimental model of ovariectomy followed two weeks later by implantation of a pellet containing 17β-estradiol. In preliminary experiments we compared the % SM1 and SM2 present in aorta from 250 g ovariectomized rats after implantation of a 0.05 mg pellet to daily I.P injection of 2 µg 17β-estradiol per day (24) for a period of 7 days. We found a similar increase in % SM1 in the thoracic aorta in groups given the 0.05 mg pellet or injected with 2 µg 17β-estradiol per day. The serum level of 17β-estradiol 7 days after implantation of the 0.05 mg pellet was 87.3 ± 9.8 pg/ml (n=10); moderately elevated relative to physiological levels (11-44). For these reasons corresponding contractility measurements were made after 7 days of 17β-estradiol treatment. Pellets containing 17β-estradiol from Innovative Research of America (Sarasota, FL) are designed to release 17β-estradiol at a constant rate over a period of 21 days. Our results indicate that this is not the case. Serum levels of 17β-estradiol were highest 3 days after pellet implantation and decreased to within the normal range after 15 days. Thus we observed no change in % SM1 expression 15 days after implantation.

We used three experimental groups in this study, randomly cycling females (RCF), ovariectomized females (OF), ovariectomized females treated with estrogen (OF&E), and for comparison, male (M) rats. The average body weights were RCF, 238 ± 6 g; OF, 290 ± 16; OF&E, 241 ± 2; and M, 481 ± 8 g (n=4-6). Body weight is increased (P<0.001) in
ovariectomized compared to randomly cycling female rats and this increase is reversed by 17β-estradiol. Males were the heaviest but the difference was only marginal (P<0.06) with respect to the OF rats.

Thoracic aortic weight per 5 mm rings was similar among these classes averaging approximately 5 mg. The calculated aortic thickness was not different in OF and OF&E rats, averaging 0.137 ± 0.004 mm (n=12); but were somewhat smaller than aorta from M rats, 0.156 ± 0.006 (n=8, P=0.01). The calculated cross section areas showed a similar pattern. There were no differences between OF and OF&E averaging, 0.68 ± 0.02 mm² (n=12), while those of M rats were somewhat larger, 0.80 ± 0.03 mm² (n=8; P<0.01). These variations in dimensions of aorta from M and F rats paralleled the differences in body weights (Table 1).

**Myosin Heavy Chain Isoforms.** Changes in myosin isoforms lagged behind those of serum 17β-estradiol levels, with the estrogen peak occurring at 3 days or earlier and statistically significant changes in % SM1 at 7 days (Table 1). The turnover rate or t½ of myosin heavy chain is 5.4 days in the rat heart (25) and is likely to be of the same order in smooth muscle. Thus it is unlikely that our methods could detect a shift in SM1 relative to SM2 after only 3 days. All measurements of aortic contractile function and C-terminal myosin heavy chain isoforms were made 7 days after implantation of the 17β-estradiol pellet. The % SM1 expressed in the various experimental groups used is summarized in Table 1. There were no significant differences in the SM1:SM2 composition in the aorta amongst the three stages of the estrous cycle. As shown in Fig. 1, there was a significant shift (1.8 to 2.6) in the SM1:SM2 ratio in the aorta after a 7 day exposure to 17β-estradiol. A placebo pellet implanted for 7 days had no effect on the isomyosin composition of the aorta (data not shown).
**Contractility Studies.** Fig. 2 presents graphically relations between developed force and cumulative additions of KCl and NE for aorta. The averaged ED$_{50}$ data are given in Table 2. KCl stimulation is primarily associated with an increase in intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]$_i$) attributable to Ca$^{2+}$ influx through voltage-dependent Ca$^{2+}$-channels. In general, removal of the endothelium enhanced the sensitivity of each rat experimental group, but the relative differences between groups remained. This is reflected in the changes in ED$_{50}$ (Table 2). Males were the more sensitive to KCl than the ovariectomized females. Estrogen treatment of the ovariectomized females reduced the sensitivity to KCl. Since the differences in contractility were largely preserved in the de-endothelialized preparations, these changes appear to reside largely in the smooth muscle component.

Norepinephrine receptor-mediated activation is generally thought to involve both extracellular Ca$^{2+}$ entry and Ca$^{2+}$ release from intracellular stores and is associated with a higher Ca$^{2+}$ sensitivity of isometric force. As for KCl stimulation, endothelial denudation was associated with a large increase in sensitivity to NE for each experimental group. Differences between the rat experimental groups were greater in the intact tissues than in the denuded aorta, suggesting a larger role of the endothelium in these differences. Aorta from male rats again showed the highest sensitivity. In contrast to KCl stimulation, estrogen treatment enhanced, rather than decreased, sensitivity to NE.

An alternative assessment of the “Ca$^{2+}$-sensitivity” of force (26) is to place the arteries in a Ca$^{2+}$-free PSS (+0.5 mM EGTA) and depolarize arteries with KCl (50 mM), then add Ca$^{2+}$ cumulatively to the PSS. Results from this type of protocol are shown in Fig 3. In de-endothelialized preparations, there are little differences between the 3 classes of aorta. However, in endothelium intact vessels, aorta from males were more sensitive to addition of Ca$^{2+}$ (Fig. 3B).
In addition to the sensitivity of force, the absolute value (mN/mm²) of the maximum isometric force is also indicative of changes in contractility. These data are graphically summarized in Fig. 4. To assess the level of basal tone, i.e., actin-myosin interaction leading to active force under unstimulated conditions, the aortae were incubated in Ca²⁺-free PSS. There were no significant decreases in the unstimulated levels of force, indicating that the differences in the stimulated force generation were not due to differing levels of tone (data not shown).

In response to KCl, males and ovariectomized animals developed similar maximum forces (Fig. 4A). These absolute forces were significantly reduced (~30%) by removal of the endothelium. Interestingly, estrogen treated rats had a lower maximum force which was not significantly different after denudation (Fig. 4A). The loss of force upon denudation cannot be attributed to damage of the force generation capacity of the smooth muscle, for with endothelial denudation, force generated with NE stimulation was equivalent to that in the intact aorta with KCl stimulation. In fact, the denuded preparations developed greater force in response to NE than that in the intact preparation (see below; Fig. 4B). Interestingly, these data can be interpreted as indicative of an endothelial-dependent contraction factor (EDCF) which is suppressed in the estrogen treated animals. In the absence of endothelium, maximal forces were similar for all classes (M, OF, OF&E).

For NE stimulation, endothelium-denudation increased force for ovariectomized and estrogen-treated animals, but no apparent effects in males were noted. No differences in maximum forces were observed in denuded vessels.

**Endothelium-Dependent Relaxation.** We studied the effects of estrogen replacement on the endothelium-dependent relaxation of a NE contraction (3 µM) in response to acetylcholine. The concentration-relaxation relations are graphically summarized in Fig. 5. The sensitivity to acetylcholine showed moderate differences, but were statistically different (P<0.01) with ED₅₀’s
being 0.47, 0.82, and $1.20 \times 10^{-7}$ M for M, OF, and OF&E, respectively. The maximum relaxation, normalized to the magnitude of the contraction prior to addition of acetylcholine, was slightly though significantly (P<0.02) greater in males (74%) than the 65% relaxation for females; there was little difference with estrogen treatment.

**Abdominal Aorta.** All studies to this point refer to the thoracic aorta. However in initial studies, we were surprised that the SM1:SM2 isoform ratio did not change with 17β-estradiol treatment; 1.92 ± 0.06 (OF) vs. 2.08 ± 0.08 (OF&E) (P=0.14; n=7, 10, respectively), in the abdominal aorta. Corresponding contractility studies are presented in Fig. 6. Interestingly, there was little difference in the sensitivity of the de-endothelialized abdominal aorta to either KCl or NE, in contrast to that seen in the thoracic aorta (Fig. 2). Changes in the sensitivity of the intact abdominal aorta with 17β-estradiol treatment were in similar directions as the thoracic, but significantly smaller (Fig. 2 vs. Fig. 6).
DISCUSSION

In these studies, we compared aortic contractility in male rats to that of ovariectomized female and ovariectomized female rats with estrogen replacement. For KCl contractures, estrogen treatment reduced both the maximum force/cross section area and sensitivity in aorta. Male rats in comparison demonstrated the highest contractility levels. The decrease in sensitivity to KCl with estrogen remained after endothelial denudation indicating that it was largely an effect on smooth muscle. This decrease in sensitivity is consistent with the shift in myosin heavy chain distribution to the “faster” SM1 isoform that we observed in the aorta (Table 1, Fig. 1), as proposed by Morano (28). Theoretically, the maximum isometric force would not be changed in this model, which is consistent with what was observed for the de-endothelialized preparations, which is assumed to reflect the smooth muscle component. The relative population of the SM1 isoform in males was also higher than that of the averaged ovariectomized females (64.5% vs 62.9%), but was not statistically significant (P=0.12). It is also worth pointing out that in the abdominal aorta the SM1 population did not change with estrogen treatment (65.6% ± 0.7 vs. 67.3% ± 0.8) which was matched by the lack of change in sensitivity of force to KCl, Fig. 6. Thus changes in myosin isoform distribution are consistent with the increased sensitivity of force.

On the other hand, in the protocol in which Ca\(^{2+}\) was added to depolarized preparations (Fig. 3), no differences in sensitivity were measured, except for a somewhat lower sensitivity of endothelium-intact aorta from male rats. These data suggest that the differences in the sensitivity to KCl may lie in the voltage-sensitivity, in that when completely depolarized the differences disappear. L-type Ca\(^{2+}\) channels (33) or K\(_v\) channels (32) immediately come to mind as potential sites for these differences. The higher sensitivity to extracellular Ca\(^{2+}\) in aorta from males is consistent with a lower degree of basal EDRF production, but could not be attributed to a
hyperpolarization factor, under these depolarized conditions. Whether ion channels or myosin isoforms are the dominant factor requires further experimentation, such as with permeabilized fibers (26).

Estrogen treatment also reduced contractility by decreasing the maximum force in response to KCl (Fig. 4A). In ovariectomized rats, endothelial denudation was associated with a decrease in force compared to the endothelium-intact aorta. In contrast, NE-stimulation of de-endothelialized aorta leads to an increase in force (Fig. 4B) relative to the endothelium-intact aorta. This is often attributable to a loss of a relaxing factor, an EDRF such as nitric oxide. Thus we hypothesize that the decrease in force with endothelial denudation in OF aorta, by analogy to EDRF, is attributable to the loss of a contractile factor, an EDCF. This decrease of force is unlikely due to non-specific reduction of the smooth muscle force generating capacity. In NE contractions, maximum force is larger in the de-endothelialized aorta than in the intact aorta and in absolute terms, is of similar magnitude to that in KCl contractures in the intact aorta. These observations are not consistent with some generalized loss of contractility due to damage during removal of the endothelium.

In the estrogen treated ovariectomized rats, the maximum force in the aorta was not affected by removing the endothelium, suggesting that the effects of estrogen were to suppress an EDCF which was observed in ovariectomized rats. Importantly, the behavior of aorta from male rats in this regard was identical to the ovariectomized females (Fig. 4). There is some evidence suggesting that this EDCF might be endothelin (13) and that estrogen may attenuate endothelin-1 in endothelial cells (1). Alternatively, loss of a contraction factor is equivalent to an enhanced EDRF in terms of contractility.

Estrogen treatment led to an increased sensitivity of isometric force to NE relative to that observed for aorta from ovariectomized rats. Interestingly, this is the opposite of that observed
for stimulation with KCl, for which estrogen treatment lead to a relative decrease in sensitivity. The difference in sensitivity for NE stimulation was reduced in de-endothelialized aorta suggesting a significant endothelial contribution. There is evidence that norepinephrine itself is capable of releasing vasodilators from the endothelium and there is enhanced sensitivity to NE when the endothelium is removed (9). However, this effect does not appear to be altered with pregnancy (20). Our observed increase in NE sensitivity with estrogen is consistent with a previous study indicating that adrenergic receptors increased with estrogen treatment (11). Again aorta from male rats showed the greatest sensitivity to NE (Fig. 2), consistent with other studies (12, 37). It would appear that alteration by estrogen of the response to NE is complex, and cannot be explained in terms of myosin isoform shifts.

While a number of differences in estrogen effects were attributable to the presence of endothelium, we found surprisingly little differences in the endothelium-dependent relaxation to acetylcholine. This is in agreement with previous studies in a rat model (7, 41) which also used norepinephrine as the contractile agonist. Interestingly, with KCl as the contractile agent, relaxation to acetylcholine was reported to be blunted in ovariectomized females, which in turn was enhanced by estrogen treatment (42). Acute administration of estrogen in organ bath experiments indicated that endothelium-dependent relaxation of phenylephrine contractures was enhanced (39). There is also some indication that in humans, endothelium-dependent vasodilatation to acetylcholine is enhanced by chronic estrogen treatment (2). The bases for these differences are likely related to widely different experimental conditions.

In summary, estrogen treatment of ovariectomized rats increases vascular sensitivity to NE receptor-mediated stimulation but decreases sensitivity to KCl depolarization. These responses have both endothelial and smooth muscle components. On the other hand male rats always demonstrated the highest contractility of any experimental group. The shift of myosin
isoforms to the SM1 isoform may underlie some of the changes observed in the smooth muscle component.

ACKNOWLEDGEMENTS

Supported by NIH HL64942 and HL52460 (AFM, RJP)
REFERENCES


Table 1. Rat serum β-estradiol body weight and % SM1 MHC in aorta.

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Body weight g</th>
<th>n</th>
<th>Serum β-estradiol pg/ml</th>
<th>% SM1 MHC</th>
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<tbody>
<tr>
<td>Male</td>
<td>481 ± 7.9</td>
<td>4</td>
<td>4.4 ± 0.5‡</td>
<td>62.9 ± 0.6</td>
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<tr>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Estrous</td>
<td>239.0 ± 6.8</td>
<td>6</td>
<td>19.3 ± 1.1</td>
<td>61.3 ± 0.7</td>
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<tr>
<td>Proestrous</td>
<td>245.5 ± 5.8</td>
<td>4</td>
<td>44.0 ± 5.3</td>
<td>62.5 ± 1.3</td>
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<tr>
<td>Metestrous</td>
<td>249.9 ± 6.7</td>
<td>7</td>
<td>11.0 ± 1.0</td>
<td>60.0 ± 0.6</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Random Cycling</td>
<td>269 ± 6</td>
<td>5</td>
<td>38.9 ± 11.4</td>
<td>66.8 ± 1.1</td>
</tr>
<tr>
<td>Ovariectomized</td>
<td>317 ± 5†</td>
<td>5</td>
<td>2.5 ± 0.8</td>
<td>65.0 ± 1.0</td>
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<tr>
<td>β-estradiol 3 days</td>
<td>284 ± 2</td>
<td>5</td>
<td>178.3 ± 17.8</td>
<td>64.1 ± 0.8</td>
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<tr>
<td>Random Cycling</td>
<td>238 ± 6</td>
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<td>17.7 ± 2.9</td>
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<tr>
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<td>4.4 ± 0.5</td>
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<td>β-estradiol 7 days</td>
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<td>11.5 ± 4.6</td>
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<tr>
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<td>336 ± 4†</td>
<td>5</td>
<td>3.3 ± 0.6</td>
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<td>β-estradiol 15 days</td>
<td>277 ± 6</td>
<td>5</td>
<td>42.7 ± 5.4</td>
<td>66.5 ± 0.5</td>
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- † P ≤ 0.01 compared to both normal and ovariectomized groups.
- ‡ Adapted from Barron et al. (3)
Table 2. ED₅₀ values for stimulus concentration-isometric force relations

<table>
<thead>
<tr>
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<th>+Endo</th>
<th>-Endo</th>
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<tr>
<td><strong>AORTA</strong></td>
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<tr>
<td>KCl (mM)</td>
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<tr>
<td>Ovariectomized</td>
<td>23.05 ± 0.25</td>
<td>15.23 ± 0.11</td>
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<tr>
<td>Estrogen Replaced</td>
<td>29.04 ± 0.32</td>
<td>18.95 ± 0.28</td>
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<tr>
<td>Males</td>
<td>20.60 ± 0.22</td>
<td>11.65 ± 0.20</td>
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<tr>
<td>NE (nM)</td>
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<tr>
<td>Ovariectomized</td>
<td>201.50 ± 12.70</td>
<td>18.96 ± 1.55</td>
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<tr>
<td>Estrogen Replaced</td>
<td>80.20 ± 4.13</td>
<td>9.77 ± 0.84</td>
</tr>
<tr>
<td>Males</td>
<td>24.62 ± 0.70</td>
<td>5.99 ± 0.77</td>
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Values are mean ± SEM, n=4-6 for each class. All differences between means within each class are significant (P<0.05) in all cases.
FIGURE CAPTIONS

Figure 1. The effect of exposure to 17β-estradiol for 7 days on SM1:SM2 myosin heavy chain isoform ratio in aorta (left panel) and serum 17β-estradiol levels (right panel) from male (n=8; M, cross hatched), randomly cycling female (n=8; RCF, open bars), ovariectomized (n=7; OF, dotted bars), and ovariectomized rats implanted with a pellet containing 0.05 mg 17β-estradiol 7 days before sacrifice (n=10; OF&E, filled bars). Bars represent means ± SEM. Asterisk indicates P<0.01.

Figure 2. Concentration-isometric force relations for intact (left panels) and endothelium-denuded (right panels) aorta for KCl (upper panels) and NE (lower panels). Males are designated by squares, ovariectomized females by circles and ovariectomized females treated with estrogen for 7 days by triangles. The ED50 values are presented in Table 2. Values represent means ± SEM.

Figure 3. Dependence of KCl-depolarized aorta on extracellular [Ca2+] in A) Endothelium-denuded aorta and B) Intact aorta. Aorta were first Ca2+-depleted by repeated stimulation with KCl and NE in a Ca2+-free solution (containing 0.5 mM EGTA) until no significant isometric force response was noted. The aortas were depolarized by addition of KCl (50 mM) and Ca2+ added cumulatively. Males are designated by squares, ovariectomized females by circles and ovariectomized females treated with estrogen for 7 days by triangles. Differences in ED50 values are statistically significant (P<0.05) only for aorta rings from male rats (Table 2).

Figure 4. Maximum active isometric force per cross section area with A) KCl stimulation and B) Norepinephrine. Aorta from male rats are designated by M, from
ovariectomized females by OF and from ovariectomized females treated with estrogen for 7 days by OF&E. Bars represent means ± SEM. Asterisk indicates P<0.01.

**Figure 5. Concentration dependence of endothelium-dependent relaxation to acetylcholine.**

Contraction in intact aorta was elicited by 1 μM norepinephrine, the amplitude of which was taken as 100%. Males are designated by squares, ovariectomized females by circles and ovariectomized females treated with estrogen for 7 days by triangles. Differences amongst the ED$_{50}$ values (0.47 ± 0.06, 0.82 ± 0.02, and 1.20 ± 0.04 x 10$^{-7}$ M for M, OF, and OF&E, respectively) were statistically significant (P<0.01; n = minimum of 5 aorta for each type).

**Figure 6. Concentration-isometric force relations** for intact (left panels) and endothelium-denuded (right panels) abdominal aorta for KCl (upper panels) and NE (lower panels). Ovariectomized females are designated by circles and ovariectomized females treated with estrogen for 7 days by triangles. Values represent means ± SEM, n=4-6.
Figure 1

A

B

SM1:SM2

$17\beta$-Estradiol

M NC O OF&E

M NC O OF&E

*
Figure 2
Figure 3
Thoracic Aorta

**Figure 4**

**Thoracic Artery**

**KCl (70 mM)**

![Graph showing the effect of KCl on isometric force in thoracic artery with and without endothelium.](image)

**Norepinephrine (3x10^{-6}M)**

![Graph showing the effect of norepinephrine on isometric force in thoracic artery with and without endothelium.](image)
Figure 5

Thoracic Aorta

Relaxation (%NE$_{\text{max}}$) vs. ACh [M] for All plus endothelium.
Figure 6