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# Supersensitivity to serotonin- and histamine-induced arterial contraction following ovariectomy

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**Abstract**

The modulating role of estrogens and ovariectomy on coronary artery and thoracic aortic rings was examined in female rabbits. Three treatment groups were studied: (1) control, (2) ovariectomy, and (3) ovariectomy + 17β-estradiol acetate (40 μg/kg per day, i.m. for 7 days). Coronary artery reactivity was studied in the isolated retrogradely perfused heart. Aortic reactivity was studied using endothelium intact and denuded aortic rings. Concentration–response curves were performed to serotonin (5-HT) and histamine. A 21-fold, a 4.7-fold, and a 5.2-fold increase in sensitivity to 5-HT-induced contraction were observed in the ovariectomy group compared to the control group for coronary artery, intact aortic, and denuded aortic preparations, respectively ($P < 0.05$ for each comparison). Similarly, 34-fold, 4.9-fold, and 5.0-fold increases in sensitivity to histamine-induced contraction were observed in the ovariectomy group compared to control group for coronary artery, intact aortic, and denuded aortic preparations, respectively ($P < 0.05$ for each comparison). 17β-Estradiol administration reversed the supersensitivity to serotonin- and histamine-induced vascular contraction observed following ovariectomy. No differences in $EC_{50}$ or maximal contraction were noted between control and ovariectomy + estrogen groups. Baseline nitric oxide release and maximal 5-HT- and histamine-induced nitric oxide release from the perfused heart were decreased ($P < 0.05$) in ovariectomy rabbits compared to control and ovariectomy + estrogen treatment groups. The data demonstrate that (1) reduced autacoid-induced nitrous oxide release following ovariectomy and (2) direct effects upon the vascular smooth muscle contractility, which are probably mediated by altered receptor sensitivity by ovariectomy and estrogen replacement therapy. The information obtained from this study provides additional information regarding possible beneficial actions of estrogen replacement therapy in post-menopausal women. 

*Keywords:* Coronary artery; Coronary artery spasm; Supersensitivity; Histamine; 5-HT (5-hydroxytryptamine, serotonin)

## 1. Introduction

Bilateral ovariectomy is associated with accelerated atherosclerosis and an increased incidence of angina pectoris (Wuest et al., 1953). A less severe syndrome of angina characterized by a positive exercise tolerance test and minimal atherosclerosis in the large coronary arteries (syndrome X) has been described with increased frequency in women following hysterectomy or menopause (Sarrel et al., 1992), conditions reducing plasma estrogen concentration (Siddle et al., 1987; Rosano et al., 1987). Women with exercise-induced angina and angiographically normal coronary arteries (syndrome X) have a reduced incidence and severity of anginal symptoms following 17β-estradiol replacement therapy (Sarrel et al., 1992). The pharmacologic basis for an amelioration of angina pectoris by estrogen replacement therapy in syndrome X is not clear. Although coronary artery spasm has been proposed as the mechanism responsible for myocardial ischemia and chest pain in patients with syndrome X (Tofler et al., 1987), the independent roles for (1) endothelial dysfunction, (2) vascular smooth muscle hyperactivity, or (3) increased autacoid sensitivity have not been systematically evaluated.

Both acute and chronic estrogen therapy has been shown to increase endothelial derived relaxant factor (EDRF)/

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nitric oxide (NO) release in both the human (Collins et al., 1992; Gilligan et al., 1994) and animal models (Gisclard et al., 1988; Ma et al., 1997). Collins et al. (1992) reported an acute increase in acetylcholine-induced relaxation to acetylcholine in coronary arteries of women (but not men) following intracoronary 17β-estradiol. A similar potentiation of acetylcholine-induced forearm vasodilation has been reported by Gilligan et al. (1994) following 17β-estradiol administration in post-menopausal women. In female baboons with documented coronary atherosclerosis, acute estrogen administration reversed an abnormal vasoconstriction induced by intracoronary acetylcholine and restored a normal, vasodilatory response to the drug (Williams et al., 1990). Both acute 17β-estradiol (Ma et al., 1997) and chronic 17β-estradiol (Gorodeski et al., 1995) have been reported to facilitate NO release in perfused female rabbit hearts. With chronic 17β-estradiol administration, both NO synthase mRNA and $Ca^{2+}$-dependent NO synthase are increased in the porcine uterine artery, heart, and skeletal muscle tissues (Weiner et al., 1994). Conversely, ovariectomy may decrease NO synthase activity.

Alternative hypotheses suggest that low serum estrogen concentrations may (1) alter the sensitivity of vascular smooth muscle to autacoids by increasing inherent vascular smooth muscle sensitivity to calcium or (2) alter receptor sensitivity or post-receptor coupling for individual autacoids. Both mechanisms could provide for increased rabbit basilar artery reactivity to serotonin in ovariectomized rabbits, with a reversal following subsequent 17β-estradiol administration (Futo et al., 1992; Shay et al., 1994).

Vascular smooth muscle responsiveness to serotonin and histamine differs in the same vascular bed from different animal species and, within the same species, among blood vessels of different anatomical origin (Vanhoutte, 1978; Konishi et al., 1981; Kong and Stephens, 1981; Toshimitsu et al., 1983). Augmented contractions to serotonin have been also observed in the aorta of hereditary hyperlipidemic rabbits (Yokoyama et al., 1983) and in the hind limb of atherosclerotic monkeys (Heistad et al., 1984). Intravenous injections of histamine provoke coronary vasospasm in patients with variant angina (Ginsburg et al., 1981) while an increased contractile response to histamine is noted in the human epicardial coronary arteries with atherosclerosis (Ginsburg et al., 1984).

In the present study we compared nitric oxide release and vascular contractility in coronary arteries and aortic rings in age-matched normal female rabbits, ovariectomized rabbits, and ovariectomized rabbits treated with 17β-estradiol. The protocol allowed us to study the influence of ovariectomy and estrogen replacement on intact and denuded aortic rings and on the coronary vasculature, in response to vasoconstrictors and vasodilators. In the perfused heart, we measured directly, concentration-dependent NO production in response to histamine and serotonin in control, ovariectomy, and ovariectomy + 17β-estradiol treatment groups.

## 2. Materials and methods

### 2.1. Animal preparation

The present experiments were performed in adult female New Zealand white rabbits weighing between 2.0 and 3.0 kg. Three groups were studied: (1) Rabbits not subjected to ovariectomy, and receiving 0.05 ml/kg per day sunflower oil by intramuscular injection (i.m.) for 7 days, (2) Rabbits subjected to bilateral ovariectomy, and receiving 0.05 ml/kg per day sunflower oil i.m. on days 7 to 13 following bilateral ovariectomy, and (3) Rabbits subjected to bilateral ovariectomy, and receiving 40 μg/kg per day 17β-estradiol in 0.05 ml/kg per day i.m. sunflower oil injections on days 7 to 13 following bilateral ovariectomy. According to our previous study (Patterson et al., 1998), estrogen treatment for 7 days is enough for observation of estrogen effects in this study. Aseptic bilateral ovariectomy was performed using anesthesia induction with 75 mg/kg of ketamine and 0.75 mg/kg of acepromazine i.m., and maintained by 3% isoflurane inhalation. Nalbuphine (0.15 mg/kg i.m.) was used for post-surgical analgesia. On day 14, the experiment was performed and rabbits were anesthetized with sodium pentobarbital (70 mg/kg) via a marginal ear vein. A total of 200 U Sodium heparin/kg were injected intravenously (i.v.) to prevent blood coagulation. The heart and aorta were removed via a thoracotomy. All experimental procedures were performed as approved by the University of Oklahoma Health Sciences Center Institutional Animal Care and Use Committee.

### 2.2. Studies with isolated aortic rings

The upper two-thirds of the thoracic aorta was removed, cut into ring segments (5 mm wide), and placed into cold

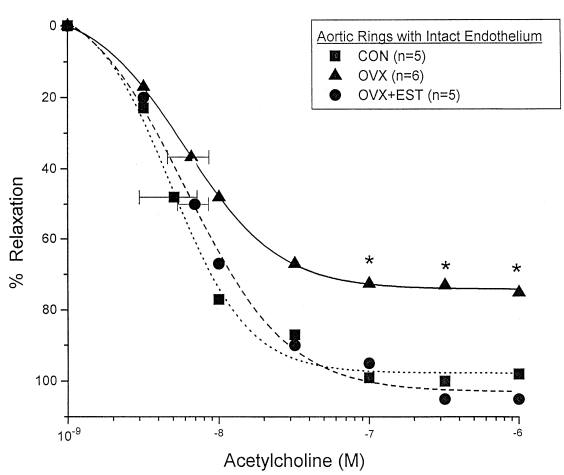


Fig. 1. Acetylcholine-induced relaxation (in norepinephrine-contracted aortic rings, $10^{-7}$ M) was reduced in ovariectomized (OVX) vs. control (CON) or ovariectomized + estrogen (OVX + EST) treated groups. Data are presented as mean ± S.E.M. for the $EC_{50}$ value of each curve. * $P < 0.05$, ovariectomy vs. control and ovariectomy + estrogen.

Table 1
The EC50s for relaxation and maximal relaxation of norepinephrine-contracted intact aortas from female rabbits

| Agent | Control ($n = 5$) | Ovariectomy ($n = 6$) | Ovariectomy + estrogen ($n = 5$) |
|---|---|---|---|
| $EC_{50}$ (mean ± S.E.M. × $10^{-8}$ M) | | | |
| Acetylcholine | 0.54 ± 0.16 | 0.66 ± 0.6 | 0.71 ± 0.16 |
| Adenosine | 350 ± 30 | 280 ± 50 | 390 ± 60 |
| Pinacidil | 73 ± 12 | 98 ± 43 | 62 ± 12 |
| Maximal relaxation (%) | | | |
| Acetylcholine | 91 ± 6 | 73 ± 8[a] | 96 ± 7 |
| Adenosine | 97 ± 5 | 92 ± 5 | 93 ± 3 |
| Pinacidil | 73 ± 6 | 77 ± 5 | 73 ± 7 |

[a] $P < 0.05$, ovariectomy group vs. control or ovariectomy + estrogen groups.

(10–15°C) Krebs solution containing (mM): 118.5 NaCl, 4.7 KCl, 1.2 $MgCl_2$, 23.8 $NaHCO_3$, 1.2 $KH_2PO_4$, 11 D-glucose, 2.5 $CaCl_2$ and 0.01 EDTA, aerated with 95% $O_2$/5% $CO_2$, at a pH of 7.4. In some rings the endothelium was removed by gently rubbing the inner surface with a 3 mm diameter cotton swab. Denudation of endothelium was then confirmed by acetylcholine administration. The rings were mounted onto two triangular wire supports and suspended in 6 ml organ baths containing 5 ml of Krebs solution at 37°C. A resting tension of 2 g was applied to all aortic rings. The aortic rings were equilibrated for 1.5 h with the bathing fluid changed every 15 min. In order to obtain a stable contracting state the rings were then exposed with to norepinephrine ($10^{-7}$ M) for 4 min twice at 15 min intervals. Tension was measured using Metrigram isometric force transducers and recorded by a Gould RS 3800 Recorder.

### 2.3. Measurement of contractions of rabbit aorta rings

After equilibration, dose–response curves were performed for serotonin, histamine, potassium chloride, and norepinephrine. The agents were added to the tissue bath in a cumulative fashion in half-log increments and each ring segment was allowed to contract maximally. When the next concentration failed to increase force development, maximum contraction was assumed.

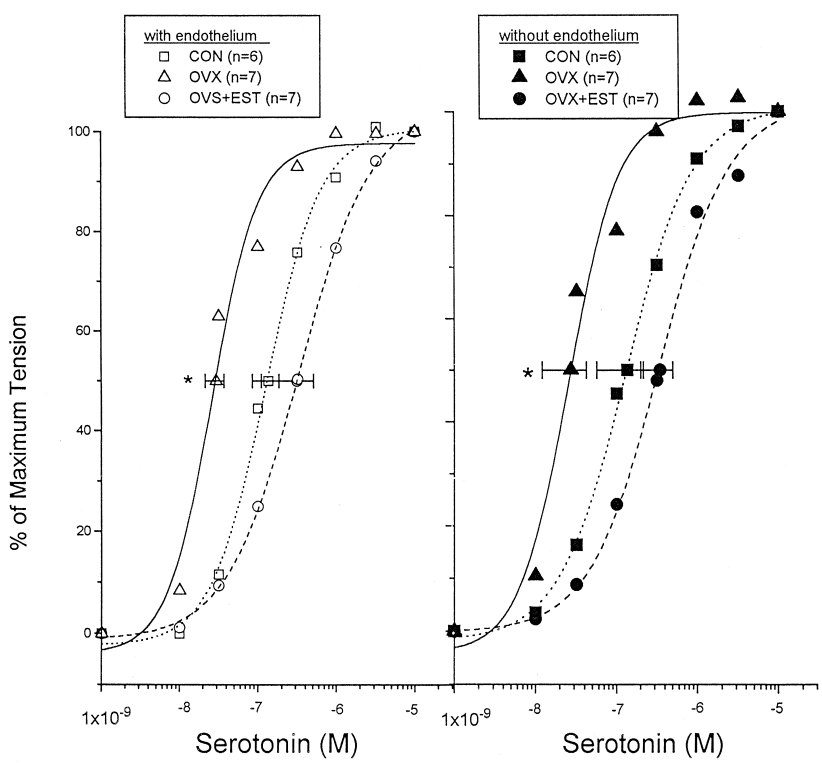


Fig. 2. Concentration–response curve to serotonin (5-HT) are shown for the intact and denuded aortic rings from control (CON), ovariectomized (OVX), and ovariectomy + estrogen (OVX + EST) treated rabbits. Data are presented as mean ± S.E.M. for the $EC_{50}$ value of each curve. * $P < 0.05$, ovariectomy vs. control and ovariectomy + estrogen.

Table 2
$EC_{50}$ values for agonists initiating contraction of aortic rings

| Agent | Control ($n = 6$) | Ovariectomy ($n = 7$) | Ovariectomy + estrogen ($n = 7$) |
|---|---|---|---|
| Rings with endothelium | | | |
| Serotonin | 1.4 ± 0.5 | 0.3 ± 0.06[a] | 3.1 ± 0.20 |
| Histamine | 27 ± 8.0 | 5.5 ± 2.0[a] | 27 ± 1.0 |
| Norepinephrine | 0.29 ± 0.12 | 0.35 ± 0.07 | 0.48 ± 0.21 |
| KCl | 28.8 ± 4.7 (mM) | 20.9 ± 8.3 (mM) | 21.9 ± 4.9 (mM) |
| Rings without endothelium | | | |
| Serotonin | 1.4 ± 0.8 | 0.27 ± 0.15[a] | 3.5 ± 1.0 |
| Histamine | 19 ± 8.0 | 3.8 ± 1.5[a] | 22 ± 9.0 |
| Norepinephrine | 0.20 ± 0.12 | 0.34 ± 0.13 | 0.28 ± 0.11 |
| KCl | 23.6 ± 4.30 (mM) | 24.1 ± 3.40 (mM) | 19.5 ± 4.30 (mM) |

Values are mean ± S.E.M. × $10^{-7}$ M, unless indicated otherwise.
[a] $P < 0.05$, ovariectomy group vs. control or ovariectomy + estrogen groups.

### 2.4. Measurement of relaxations of rabbit aorta rings

Relaxation of contracted aortic rings to acetylcholine, pinacidil, nitroglycerin, and adenosine were performed using intact and denuded rings. After stable precontraction with $10^{-7}$ M norepinephrine was obtained, the relaxation was recorded following drug administration in half-log increments. $10^{-7}$ M norepinephrine produced approximately 70 to 85% of maximal force development. Relaxation was expressed as a percentage of the initial norepinephrine contraction.

### 2.5. Studies with isolated rabbit hearts

After thoracotomy, the heart was removed and perfused retrogradely through the aorta at 37°C. The perfusate solution (in mM) 140 NaCl; 2.0 $CaCl_2$; 1.0 $MgCl_2$, 1.0 $Na_2HPO_4$, 5.0 KCl, 5.0 HEPES, 5 NaOH, 10.0 glucose, and 0.5 aspartic acid (pH 7.4) was oxygenated with 100% $O_2$. An apical puncture was performed to drain the perfusate from the left ventricle. A latex balloon (10 × 25 mm, approximately) connected to a short polyethylene tube (100 mm long) was inserted into the left ventricle

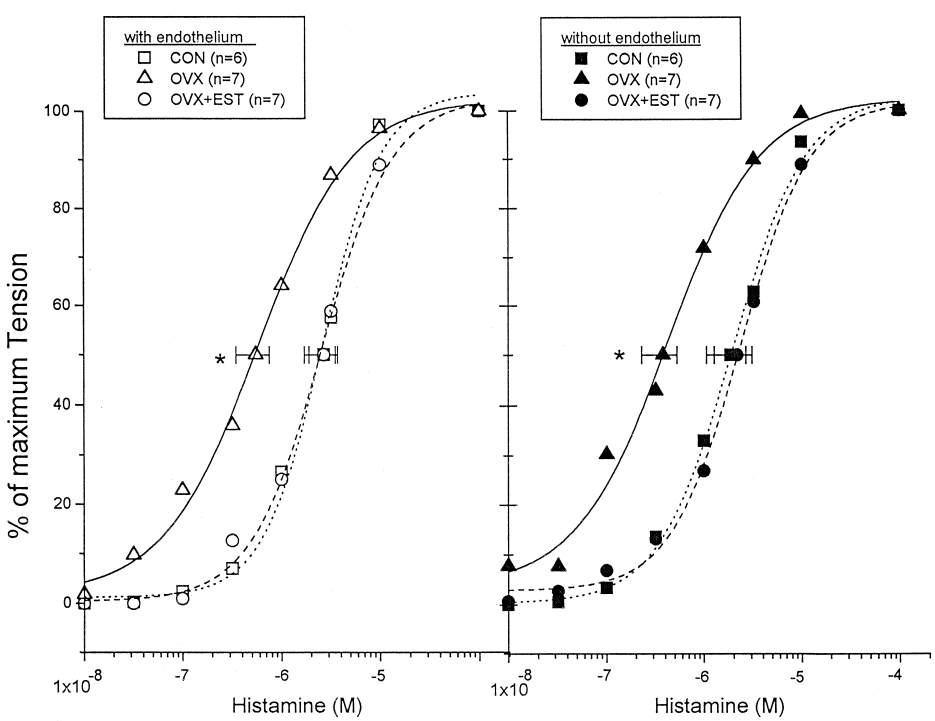


Fig. 3. Concentration–response curve to histamine for the intact and denuded aortic rings from control (CON), ovariectomized (OVX), and ovariectomy + estrogen (OVX + EST) treated rabbits are shown. Data are presented as mean ± S.E.M. for the $EC_{50}$ value of each curve. * $P < 0.05$, ovariectomy vs. control and ovariectomy + estrogen.

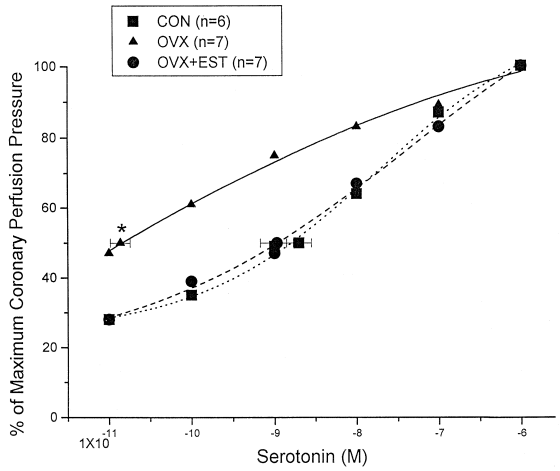


Fig. 4. Effects of ovariectomy on coronary perfusion pressure (CPP) in isolated rabbit hearts perfused with serotonin (5-HT). Data are presented as mean ± S.E.M. for the $EC_{50}$ value of each curve. * $P < 0.05$, ovariectomy vs. control and ovariectomy + estrogen.

through the left atrium. The left atrial appendage was tied around the polyethylene tube to secure the balloon in the left ventricle. The balloon was filled with water, and connected to a Statham P23 ID transducer (Statham Instruments, Oxnard, CA, USA). The balloon volume was adjusted to produce a left ventricular end-diastolic pressure of 10 mmHg. Coronary flow was fixed to maintain the coronary perfusion pressure at 90–100 mmHg (Masterflex, Cole-Parmer Instruments, Chicago, IL, USA).

Nitric oxide release was measured with an amperometric microsensor (ISO-NOP, World Precision Instruments, Sarasota, FL, USA) as previously described (Shibuki, 1990; Malinski and Taha, 1992; Engelman et al., 1995). The probe measures the concentration of NO gas in aqueous solution. After a stabilization period of 30 min, the amperometric microsensor was inserted into the right atrium of the rabbit heart. The heart was maintained in a 25-ml tissue baths to maintain a constant temperature at 36°C. This experimental arrangement permits the determination of newly generated NO from the coronary sinus efferent. Electrode calibration was performed daily using NO generated by the reaction of nitrite with iodide in an acidic medium as previously described (Ma et al., 1997).

Baseline measurements of NO generation, coronary perfusion pressure and coronary flow were performed following a 30 min stabilization period. Equilibration at each drug concentration was performed for 10 min. Concentration–response curves to serotonin ($10^{-11}$–$10^{-6}$ M) and histamine ($10^{-11}$–$10^{-6}$ M) were determined and the nitric oxide generation was measured.

### 2.6. Blood analyses

Blood was taken at the time of euthanasia for measurement of estradiol content. Serum estradiol was measured with a radioimmunoassay procedure using Coat-A-Count estradiol kits (Diagnostic Products, Los Angeles, CA, USA).

### 2.7. Statistical analysis

Unless otherwise stated, data were expressed as the mean ± S.E.M. When the same experiments were performed on more than one artery ring from the same rabbit, the mean values were reported. The concentration–response curves were analyzed using a two-way analysis of variance for repeated measures. Comparisons within a treatment group were made using Dunnett's test. $P < 0.05$ was criterion for statistical significance. Concentration–response curves were fitted by non-linear regression and the $EC_{50}$ values were calculated from the fitted curves (ORIGIN, Cal Micro Products).

## 3. Results

### 3.1. Serum estradiol concentrations

Serum estradiol concentrations in control, ovariectomy, and ovariectomy + estrogen treatment groups were measured in randomly selected animals. Ovariectomy reduced serum estradiol from 34.7 ± 1.3 pg/ml ($n = 5$) to 12.6 ± 2.8 pg/ml ($P < 0.05$, $n = 5$). 17β-estradiol acetate replacement yielded plasma estradiol concentrations of 66.2 ± 8.2 pg/ml (24 h after the last dosage) ($P < 0.05$, $n = 4$, compared to ovariectomy and ovariectomy + estrogen groups).

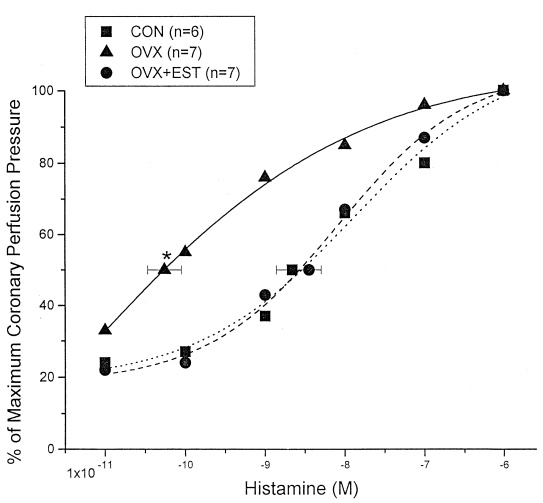


Fig. 5. Effects of ovariectomy on coronary perfusion pressure (CPP) in isolated rabbit hearts perfused with histamine. Data are presented as mean ± S.E.M. for the $EC_{50}$ value of each curve. * $P < 0.05$, ovariectomy vs. control and ovariectomy + estrogen.

Table 3
$EC_{50}$ (mean ± S.E.M. × $10^{-8}$ M) for serotonin and histamine coronary perfusion pressure

| Agent | Control ($n = 6$) | Ovariectomy ($n = 7$) | Ovariectomy + estrogen ($n = 7$) |
|---|---|---|---|
| Serotonin | 1.03 ± 0.19 | 0.04 ± 0.01[a] | 1.67 ± 0.10 |
| Histamine | 1.02 ± 0.19 | 0.03 ± 0.02[a] | 1.40 ± 0.14 |

[a] $P < 0.05$, ovariectomy group vs. control or ovariectomy + estrogen groups.

### 3.2. Effects of acetylcholine, pinacidil, adenosine, nitroglycerin, 5-HT, histamine, potassium chloride, and norepinephrine on control, ovariectomy, and ovariectomy + estrogen-treated rabbit aortic rings

Acetylcholine ($10^{-9}$ to $10^{-6}$ M, in 1/2 log intervals) induced relaxation in aortic rings with an intact endothelium (Fig. 1). Maximal relaxation obtained with acetylcholine was reduced in the ovariectomy vs. control and ovariectomy + estrogen treatment groups, with no significant differences in $EC_{50}$ between the three treatment groups (Table 1). Estrogen treatment restored acetylcholine-induced aortic relaxation in ovariectomized rabbits. Vessels devoid of endothelium showed no significant relaxation to acetylcholine.

The maximum relaxation induced with nitroglycerin ($10^{-5}$ M) was not significantly different between control (89 ± 7%), ovariectomy (86 ± 6%) and ovariectomy + estrogen (85 ± 11%) treatment groups for intact aortic rings. There was no difference in relaxation induced with nitroglycerin between intact and denuded aortic rings in the three treatment groups. No differences in maximal relaxation or $EC_{50}$ (Table 1) were present for pinacidil and adenosine for the three treatment groups, for both intact and denuded preparations.

An 11.9-fold and 12.8-fold decrease in the $EC_{50}$ for serotonin was observed in the ovariectomy group compared to the ovariectomy + estrogen group for both intact aortic and denuded aortic preparations (Fig. 2, Table 2). Similarly, 4.8-fold and 4.9-fold decreases in the $EC_{50}$ to histamine were observed in the ovariectomy group compared to ovariectomy + estrogen group for both intact aortic and denuded aortic preparations (Fig. 3, Table 2). No differences in $EC_{50}$ were noted between control and ovariectomy + estrogen groups. Unlike the response to 5-HT and histamine, no difference was observed between the three treatment groups in the maximum contractile response or $EC_{50}$ for aortic preparations in response to norepinephrine and potassium chloride (Table 2).

### 3.3. Concentration-dependent effects of 5-HT and histamine on coronary perfusion pressure and NO formation

Serotonin and histamine produced concentration-dependent increases in coronary perfusion pressure in isolated, perfused hearts from control, ovariectomy, and ovariectomy + estrogen treated rabbits (Figs. 4 and 5, respectively). A 27-fold and 18-fold decrease in the $EC_{50}$ to 5-HT and histamine, respectively, were observed in the ovariectomy group compared to the ovariectomy + estrogen treatment group ($P < 0.05$, Table 3). No differences were noted between control and ovariectomy + estrogen groups for both serotonin- and histamine-induced increases in coronary perfusion pressure.

The baseline value of NO concentration of ovariectomy group (34 ± 15 nM) was lower compared to those of control (47 ± 16 nM, $P < 0.05$) and ovariectomy + estrogen groups (71 ± 13 nM, $P < 0.05$). Nitric oxide formation induced by serotonin and histamine administration in isolated heart is reported as changes in NO concentrations from baseline. Maximal NO release during serotonin and histamine perfusion is shown in Table 4. Decreased NO formation was seen in ovariectomized rabbits compared to the ovariectomy + estrogen treatment group for both serotonin and histamine administration ($P < 0.05$).

Table 4
Maximal nitric oxide release (in nM) stimulated by serotonin and histamine

| Rabbits | Control ($n = 6$) | Ovariectomy ($n = 7$) | Ovariectomy + estrogen ($n = 7$) |
|---|---|---|---|
| Stimulated by serotonin | | | |
| Baseline | 37 ± 4.4 | 31 ± 4.7 | 47 ± 5.9[a] |
| Serotonin ($1 \times 10^{-7}$ M) | 48 ± 5.6 | 35 ± 4.5 | 57 ± 6.6[a] |
| Stimulated by histamine | | | |
| Baseline | 35 ± 3.9 | 24 ± 9.9 | 68 ± 6.9[b] |
| Histamine ($1 \times 10^{-6}$ M) | 98 ± 7.3[c] | 54 ± 9.0 | 140 ± 13[b] |

[a] $P < 0.05$, ovariectomy + estrogen group vs. ovariectomy group.
[b] $P < 0.05$, ovariectomy + estrogen group vs. control or ovariectomy groups.
[c] $P < 0.05$, control group vs. ovariectomy group.

## 4. Discussion

Coronary vasospasm has been proposed to result from an intrinsic hyperactivity of vascular smooth muscle and/or a reduced release of endothelial derived relaxant factors (Maseri et al., 1978; Shepherd and Vanhoutte, 1986). Typical coronary artery spasm accompanying coronary atherosclerosis is associated with the major coronary arteries. In the less common, variant form of exercise-induced angina in post-menopausal women termed syndrome X, a reduction in coronary artery flow with recurrent myocardial ischemia has been reported in the absence of a critical stenosis (Tofler et al., 1987). This may be due to an increase in resistance in smaller coronary arteries. An association between syndrome X and estrogen-status has been made (Tofler et al., 1987), but the mechanisms responsible remain unknown.

Our discussion examines three possible hypotheses to provide explanation for our experimental findings and the pathogenesis of syndrome X: (1) ovariectomy decreases autacoid-induced endothelial NO formation and prolonged 17β-estradiol administration restores endothelial NO formation; (2) ovariectomy increases intrinsic vascular smooth muscle reactivity by altering post-receptor coupling and prolonged 17β-estradiol administration restores normal vascular smooth muscle reactivity; and (3) ovariectomy increases 5-HT and histamine receptor agonist sensitivity in vascular smooth muscle while prolonged 17β-estradiol administration restores normal receptor function.

### *4.1. Hypothesis 1: ovariectomy decreases autacoid-induced NO formation and prolonged 17β-estradiol administration restores endothelial NO formation*

Endothelial-dependent relaxation to acetylcholine in aortic rings from ovariectomized rabbits was decreased compared to control and ovariectomy + estrogen treatment groups. In perfused heart ovariectomy lowed the maximal NO release during histamine and serotonin perfusion. Relaxation to pinacidil, nitroglycerin, and adenosine, agents acting directly upon vascular smooth muscle intact or denuded, was not different between control, ovariectomy, and ovariectomy + estrogen treatment groups. A narrow view including only the present observations would suggest that impaired vascular smooth muscle relaxation with ovariectomy and restoration by estrogen is endothelium-dependent.

Weiner et al. (1994) have reported that estrogen regulates nitric oxide synthetase formation. Estradiol increases the activity of the $Ca^{2+}$-dependent NO synthase enzyme in the porcine uterine artery, heart and skeletal muscle (Weiner et al., 1994). The amount of mRNA for the NO synthase isozymes was increased by 17β-estradiol administration, suggesting that the observed increase in NO synthase activity may be due to enzyme induction. Both serotonin (Sanders-Bush and Mayer, 1995) and histamine (Toda et al., 1982; Toda, 1983) increase NO release from vascular endothelium by stimulation of endothelial 5-$HT_1$ or histamine $H_1$ receptors, respectively. Although the increased sensitivity of aortic rings and coronary artery to contraction with 5-HT and histamine following ovariectomy could be mediated by decreased intrinsic NO synthase and decreased NO release in vascular endothelium, this mechanism cannot provide the explanation for the increased sensitivity of both endothelium-intact and endothelium-denuded aortic preparations to serotonin and histamine in the present studies.

Both serotonin and histamine have a double effect: direct contraction of vascular smooth muscle and release of a relaxing factor from the endothelium (Dorigo et al., 1992). Indeed, a wide-rage of literature suggests that the removal of the endothelium makes the arterial tissue more sensitive to vasoconstrictors. However, it has also been reported in some studies that removal of the endothelium did not make difference in vascular response to serotonin and histamine. Like acetylcholine, in arteries of the rat, histamine induces the release of a relaxant factor and this histamine-induced relaxation is transient (Chen and Suzuki, 1989). Histamine produced much less relaxation than induced by acetylcholine (Nishio et al., 1988). Schoeffter and Cogfraind (1991) reported that histamine reduced contraction of rat aorta rings was probably mediated by $\alpha_1$-adrenoceptors and histamine-stimulated release of EDRF (involving histamine $H_1$ receptors) plays a minor role, if any, in the endothelium-dependent modulation of histamine-induced contraction.

Holecyova et al. (1993) reported that a significant enhancement of contraction to noradrenaline and serotonin was found in aortas 4 days after endothelium denudation as compared with controls with endothelium. The enhancement, however, did not differ from that found already in acutely denuded vessels (immediate denudation). In a study of determination of thrombin effects on arterial sensitivity to serotonin, Consigny et al. (1992) observed that removal of the endothelium had no effect on maximal serotonin-induced contractions in rabbit abdominal aorta. Purdy and Milburn (1991) also reported that endothelium removal of bovine coronary artery rings had no effect on serotonin concentration contractile response curves in normal Krebs' solution, but enhanced the response to serotonin in artery rings precontracted with 25 mM K + Krebs' solution.

We detected ligand-induced NO release in both perfused aortic ring and Langendorff preparations. We were unable to find a comparison of normal and denuded rabbit aortic rings in the literatures. The relaxation produced by NO could have been masked by an overwhelming serotonin- or histamine-mediated vascular smooth muscle contraction. The capacity of serotonin and histamine to induce a relaxation in precontracted endothelium-intact aortas may differ between species. The response in the rabbit may be species specific, different from the rat and guinea pig.

*4.2. Hypothesis 2: ovariectomy increases vascular smooth muscle reactivity and prolonged 17β-estradiol administration restores normal vascular smooth muscle reactivity*

In the present studies, ovariectomy showed no effect on potassium-activated vascular smooth muscle contraction. Unlike observation with histamine and serotonin, no differences were noted between the control, ovariectomy, and ovariectomy + 17β-estradiol treatment groups for contraction induced by norepinephrine in either intact or denuded aortic ring preparations. Serotonin, histamine, and norepinephrine each initiate vasoconstriction via stimulation of G protein-coupled receptors, activation of phospholipase C (De Chaffoy de Courcelles et al., 1985), increased formation of inositol-1,4,5-triphosphate and diacylglycerol, and ultimately, cytosolic free $Ca^{2+}$ concentrations (Hathaway et al., 1991). Our data suggests that ovariectomy does not produce an overall increase in vascular smooth muscle reactivity in as much as the response to all agonists initiating vascular smooth muscle contraction was not increased, even when a common mechanism, activation of phospholipase C, was exploited by serotonin, histamine, and norepinephrine.

The influence of prolonged estrogen administration on vascular smooth muscle responsiveness to norepinephrine differs between individual animal species. In rats, prolonged estrogen administration increases the sensitivity of aorta to norepinephrine-induced contraction (Altura, 1972; Colucci et al., 1982). In guinea pigs, norepinephrine-induced vascular smooth muscle contraction is markedly reduced by 17β-estradiol pretreatment (McCaffrey and Czaja, 1989). In ewes, 17β-estradiol attenuates norepinephrine-induced increases in systemic vascular resistance (Naden and Rosenfeld, 1985). In the present studies, no differences in the response to norepinephrine were observed between treatment groups. The differing results observed in the present studies may reflect differences in the species (rabbit) and the individual vascular bed (thoracic aorta) studied.

*4.3. Hypothesis 3: ovariectomy increases serotonin and histamine sensitivity in vascular smooth muscle by altered receptor sensitivity and 17β-estradiol administration restores normal receptor sensitivity / normal receptor coupling*

In the present studies, we observed that ovariectomy selectively increased the sensitivity of the rabbit coronary arteries and aorta to contraction by 5-HT and histamine but did not increase the response of intact or denuded aorta to potassium chloride and norepinephrine. The increased vascular reactivity was observed in both intact and denuded aortic rings, and was observed in the coronary vasculature of the retrogradely perfused rabbit heart. These findings agree with a previously described increased serotonergic sensitivity of intact rabbit basilar arterial rings in ovariectomized rabbits (Shay et al., 1994), but fail to suggest a specific pharmacologic mechanism. The changes observed in the present studies were specific to serotonin and histamine and suggest an increased intrinsic smooth muscle sensitivity to 5-HT and histamine, rather than decreased vascular endothelial responsiveness.

Prolonged alterations in NO formation have been noted to alter the response of porcine coronary arteries to serotonin. Following a 4 week period of nitro-L-arginine methyl ester (L-NAME) administration, enhanced vasoconstriction of porcine coronary arteries to intracoronary serotonin has been observed. In coronary microvessels only (not in large epicardial vessels), prolonged L-NAME administration produces an irregular as opposed to a normal smooth pattern of endothelialization (Ito et al., 1995) and a possible shift in serotonin receptor types mediating vasoconstriction (Kadokami et al., 1996), as serotonin-induced vasoconstriction was completely suppressed in control pigs by ketanserin (a $5\text{-}HT_2$ receptor antagonist) while both ketanserin and methiothepin were needed to block the increased reactivity observed in the L-NAME treatment group. The studies of Ito et al. (1995) and Kadokami et al. (1996), however, made no attempt to separate endothelial mediated and vascular smooth muscle mediated responses of intracoronary serotonin injections. The altered responses to histamine and 5-HT following ovariectomy and estrogen replacement may reflect changes in NO release from vascular endothelium rather from direct effects of estrogen on histamine or serotonin receptors.

*4.4. Clinical implications*

Estrogen-specific binding sites have been demonstrated in the intima, endothelium, and adventitia of normal and atheromatous arteries using radiolabeled estradiol (Malinow et al., 1963). Cytosolic receptors for estrogens are present in the aorta of the dog (Horowitz and Horowitz, 1982), rat (Lin and Shain, 1985; Orimo et al., 1993; Bayard et al., 1995), rabbit (Perrot-Applanat et al., 1988), baboon (McGill and Sheridan, 1981; Lin et al., 1986) and in human (Losordo et al., 1994) coronary arteries. Estrogen binding sites are also reported in cytosol isolated from rabbit endothelium (Colburn and Buonassisi, 1978). The presence of cytosolic estrogen receptors in vascular smooth muscle cells and endothelium suggests that the tissues may be targets for altered transcription or translation of autacoid receptors by estrogen.

The present studies demonstrate that ovariectomy and estrogen replacement therapy modulate vascular responses to autacoids. This modulation was manifested by (1) reduced autacoid-induced NO release following ovariectomy and (2) direct effects upon the vascular smooth muscle contractility, which are probably mediated by altered receptor sensitivity by ovariectomy and estrogen replacement therapy. The information obtained from this study provides additional information regarding possible benefi-

cial actions of estrogen replacement therapy in postmenopausal women.

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