

# Increased Vascular Catecholamine Sensitivity and $\alpha$ -Adrenergic Receptor Affinity in Female and Estrogen-Treated Male Rats

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**SUMMARY.** Clinical and experimental observations suggest that female sex hormones, and in particular estrogens, increase vascular sensitivity to catecholamines. We confirmed these conclusions by demonstrating in vitro that segments of small mesenteric arteries (190–290  $\mu\text{m}$  in inside diameter) from male rats treated with  $17\beta$ -estradiol (10  $\mu\text{g}/100$  g body weight per day for 5–7 days) exhibited a 3.2-fold leftward shift in the contractile dose-response curve for norepinephrine, the concentration resulting in half maximal contraction decreasing from a control value of  $8.4 \times 10^{-7}$  to  $2.6 \times 10^{-7}$  M. The molecular mechanisms which account for this increase in vascular smooth muscle catecholamine sensitivity are unknown. To evaluate directly the possibility that the effects of female sex hormones on the vascular sensitivity to catecholamines are mediated through changes in the  $\alpha$ -adrenergic receptor, radioligand-binding studies using the  $\alpha$ -1 subtype-selective ligand [ $^3\text{H}$ ]-WB-4101 were performed on homogenates of mesenteric arteries in control and estrogen-treated male rats, and in control and oophorectomized female rats. Following estrogen treatment (250  $\mu\text{g}/100$  g body weight), receptor affinity in male rats was increased 1.9-fold for the antagonist [ $^3\text{H}$ ]-WB-4101, and receptor density decreased slightly, from  $102 \pm 4$  to  $85 \pm 5$  fmol/mg protein. Estradiol at doses of 10 and 250  $\mu\text{g}/100$  g body weight increased receptor affinity for the agonist (–)-epinephrine 2.8- and 5.7-fold, respectively. The lower dose of estradiol also increased receptor affinity for (–)-norepinephrine 4-fold. In female rats, the affinities for [ $^3\text{H}$ ]-WB-4101 and (–)-epinephrine were 1.6- and 3.5-fold higher, respectively, than in age-matched males, with no difference in receptor density between the sexes. Following oophorectomy, the affinity for (–)-epinephrine in females was the same as in males. Testosterone treatment did not affect the density or affinity of receptors in females. These observations demonstrate that estrogens increase the affinity of  $\alpha$ -adrenergic receptors in blood vessels, and provide evidence that the estrogen-induced increase in vascular sensitivity to catecholamine-induced contraction is mediated, at least in part, by these affinity changes. (*Circ Res* 50: 805–811, 1982)

**CLINICAL** observations suggest that sex hormones may play a role in certain human diseases characterized by abnormal vascular tone. Thus, Raynaud's disease is far more common in females than in males (Coffman and Davies, 1975). Migraine headaches in women are often worse during the premenstrual period, and may dramatically abate during pregnancy (Pearce, 1969), as may essential hypertension (Finerty, 1977). Experimental data also indicate that sex hormones can alter the sensitivity of blood vessels for  $\alpha$ -adrenergic agonists. Estrogen treatment of male rats results in an increased sensitivity of terminal mesenteric arterioles for  $\alpha$ -adrenergic agonist-induced constriction (Altura, 1975). Similarly, terminal mesenteric arterioles in female rats are more sensitive to catecholamine-induced constriction than arterioles in males rats (Altura, 1972). These findings have led to the suggestion that estrogens may contribute to the increased catecholamine sensitivity of blood vessels

in females through direct effects on the vascular  $\alpha$ -adrenergic receptor (Altura, 1975).

Recently, radioligand-binding techniques have been used to demonstrate directly that sex hormones may regulate  $\alpha$ -adrenergic receptors in certain non-vascular tissues. Using the radioligand [ $^3\text{H}$ ]-dihydroergocryptine, it has been shown that estrogens increase the number of  $\alpha$ -adrenergic receptors in rabbit myometrium (Williams and Lefkowitz, 1977; Roberts et al., 1977), and decrease the number in rabbit platelets (Roberts et al., 1979), without changing receptor affinity in either tissue. Although there are suggestions that estrogens can regulate  $\alpha$ -adrenergic receptors in the uterus and in platelets, no information is available at present concerning possible effects of estrogens on the number or affinity of  $\alpha$ -adrenergic receptors in vascular tissue.

As a general approach to the study of vascular  $\alpha$ -adrenergic receptor physiology, we have developed

techniques for assaying  $\alpha$ -adrenergic receptors, which are predominantly of the  $\alpha$ -1 subtype, in a particulate fraction of the rat mesenteric artery, using [ $^3$ H]-dihydroergocryptine (Colucci et al., 1980). More recently, we have characterized these receptors using the  $\alpha$ -1 subtype selective ligand [ $^3$ H]-WB-4101 (phenoxy-3- $^3$ H(N)-(2-(2,6,-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane)(Colucci et al., 1981).

To study the influence of sex hormones on the vascular  $\alpha$ -adrenergic receptor, we compared [ $^3$ H]-WB-4101 binding in rat mesenteric arteries in normal and estrogen-treated males, age-matched female controls, and testosterone-treated and oophorectomized females. In parallel with the receptor-binding studies, we evaluated the effect of estrogen treatment of male rats on the contractile dose-response curve for norepinephrine in segments of mesenteric resistance arteries (190–290  $\mu$ m) from the rat, using the methods of Halpern et al. (1978). The data indicate that estrogens increase the sensitivity of vascular smooth muscle to catecholamine-induced contraction, and that this increase may be due to enhanced vascular  $\alpha$ -adrenergic receptor affinity.

## Methods

### Reagents

[ $^3$ H]-WB-4101 (specific activity = 24.4 Ci/mmol) was obtained from New England Nuclear. 17 $\beta$ -Estradiol valerate and testosterone were obtained from E. R. Squibb and Sons, Inc. All other compounds used were purchased from standard chemical suppliers.

### Mesenteric Artery Preparation

A membrane-rich particulate fraction of dissected mesenteric arteries from age-matched male and female Sprague-Dawley rats (300–350 g)(Charles River Breederries) was prepared using modifications of the methods of Wei et al. (1976), as previously described in detail (Colucci et al., 1980, 1981). The particulate fraction from six to 12 mesenteric arterial arcades was resuspended in assay buffer (5 mM MgCl<sub>2</sub>, 50 mM Tris-HCl, pH 7.55) to yield a final protein concentration of 1.0–2.0 mg/ml. Protein concentration was determined by the Lowry method with bovine serum albumin as standard (Lowry et al., 1951).

### [ $^3$ H]-WB-4101 Binding Assay

The binding assay mixture consisted of 25  $\mu$ l of [ $^3$ H]-WB-4101, 100  $\mu$ l of mesenteric artery particulate fraction (0.10–0.20 mg of protein), and 25  $\mu$ l of distilled water or (–)-epinephrine dissolved in distilled water. All assays were performed in the presence of 0.1 mM ascorbic acid to prevent oxidation of catecholamines. Under standard assay conditions, particulate fraction protein was incubated with 0.1–3.0 nM [ $^3$ H]-WB-4101 for 20 minutes at 22°C. After incubation, the assay mixture was diluted with 4.5 ml of assay buffer (22°C), immediately filtered through a Whatman GF/C glass fiber filter, and washed with two additional 4.5-ml portions of assay buffer (22°C). Total time for filtration and washing averaged 15 seconds. Filters were dried overnight at room temperature and placed in 10 ml of Liquescent (National Diagnostics) scintillation fluid for counting on a Beckman (model LSC345) liquid scintillation counter with an efficiency of 45%. Specific binding for [ $^3$ H]-WB-4101 was defined as the radioactivity displaceable by

1 mM (–)-epinephrine, and comprised approximately 75% of total binding at a [ $^3$ H]-WB-4101 concentration of 0.8 nM. At 0.8 nM, total binding averaged 10% and assay blank averaged 2% of the radioactivity in the incubation mixture. Data in all figures and tables refer to specific binding. All data points were obtained in duplicate.

### In Vitro Mesenteric Artery Contraction

The myograph and vessel dissection technique used in this study is a modification of the one described by Halpern et al. (1978). Briefly, a segment of the mesentery is removed approximately 10 cm distal to the pylorus. A vessel segment (approximately 1.4 mm in length and 190–290  $\mu$ m in diameter) was excised from connective and adipose tissue and mounted on two 32- $\mu$ m tungsten wires. The wires were fastened to two stainless steel support blocks, one of which was mounted to a force transducer and the other to a displacement device. The internal circumference could therefore be altered while measuring force production. Vessel dissection and mounting were done in physiological saline solution (PSS) on ice and took approximately 1 hour. A vessel then was allowed to equilibrate at 37°C for about 20 minutes in EGTA-PSS (physiologic saline solution with EGTA). After equilibration, a normalized internal circumference ( $L_1$ ) was determined as previously described (Mullvany and Halpern, 1977).  $L_1$  is defined as 0.8  $L_{100}$ ,  $L_{100}$  being an estimate of the internal circumference which a relaxed vessel would have had in vivo when subjected to a transmural pressure of 100 mm Hg. The vessel was set at  $L_1$  and equilibrated in PSS without EGTA for 1 hour prior to determining the dose-response curves.

Contraction experiments were performed in PSS which contained the following (mM): NaCl, 119; KCl, 4.7; CaCl<sub>2</sub>, 1.6; KH<sub>2</sub>PO<sub>4</sub>, 1.18; MgSO<sub>4</sub>, 1.17; NaHCO<sub>3</sub>, 24.0; and glucose, 5.5. For EGTA-PSS, CaCl<sub>2</sub> was replaced by 1 mM EGTA. Solutions were gassed with 95% O<sub>2</sub>-5% CO<sub>2</sub> and warmed to 37°C. The bath pH was 7.3–7.4, the Pco<sub>2</sub> was 35–45 mm Hg, and the Po<sub>2</sub> was >100 mm Hg. A norepinephrine stock solution (1  $\times$  10<sup>–2</sup> M) was made in PSS containing 176 mg/liter ascorbic acid, and appropriate dilutions were made just prior to use in the bath. Only one vessel was tested from each animal. Vessels were exposed to doses ranging from 2  $\times$  10<sup>–5</sup> to 1  $\times$  10<sup>–8</sup> M norepinephrine: vessels were exposed for approximately 5 minutes to each dose, with at least 15 minutes between doses. There was no vessel response to PSS-ascorbic acid alone. The contractile responses for each vessel were normalized to the maximum tension obtained from each vessel and then fitted to the Hill equation (Hill, 1913).

### Animal Treatment Regimens

17 $\beta$ -Estradiol (10 or 250  $\mu$ g/100 g body weight) or testosterone (2.5 mg/100 g) was injected subcutaneously every day for the indicated number of days, and the animals studied 18–24 hours after the last injection. The only exception to this regimen was the long-term (15–30 day) estrogen treatment group, which was injected once weekly and studied at least 1 week after the last injection. Female rats underwent oophorectomy at 2 weeks of age, were allowed to recover, and were utilized for radioligand studies at 10–12 weeks of age.

### Calculations

Saturation radioligand-binding curves were analyzed by the method of Scatchard (1949) to determine the equilibrium dissociation constant ( $K_d$ ) and the amount of radioligand bound at saturation ( $B_{max}$ ). Inhibition of radioligand

binding and contraction of isolated vascular segments by agonists were normalized to maximum inhibition or tension obtained, respectively, for each experiment, and fitted to the Hill equation (Hill, 1913) from which a straight line was generated by linear least squares regression of the data. From this line, the mean inhibitory concentration of agonists for binding experiments ( $IC_{50}$ ); or mean ( $EC_{50}$ ), 10% ( $EC_{10}$ ), and 90% ( $EC_{90}$ ) effective concentrations for contraction experiments were determined. All data were expressed as the geometric mean  $\pm$  SEM, and analyzed by a non-paired *t*-test.

## Results

### In Vitro Contraction of Isolated Mesenteric Artery Segments from Control and Estrogen-Treated Male Rats

The mean (–)-norepinephrine dose-response curves for tension development in isolated segments of mesenteric arteries from five control and six estrogen-treated male rats is depicted in Figure 1. Following pretreatment with low-dose 17 $\beta$ -estradiol (10  $\mu$ g/100 g per day, sc, for 5–7 days), the dose-response curve is shifted to the left of the control curve; at the  $EC_{50}$  this shift was 3.2-fold (control males,  $EC_{50}$  =  $8.4 \times 10^{-7}$  M; estrogen-treated males,  $EC_{50}$  =  $2.6 \times 10^{-7}$  M;  $P$  < 0.001). Complete data for the contraction experiments are shown in Table 1.

### Effect of Estrogen Treatment on the Vascular $\alpha$ -Adrenergic Receptor in Male Rats

Treatment of male rats with high-dose 17 $\beta$ -estradiol (250  $\mu$ g/100 g) resulted in an increase in the affinity of the mesenteric artery  $\alpha$ -adrenergic receptor. By 18–24 hours, the  $IC_{50}$  for (–)-epinephrine had decreased from a control value of  $12.7 \pm 1.6$   $\mu$ M ( $n$  = 12) to 3.4  $\mu$ M ( $n$  = 2). Animals studied after 5–9 days ( $n$  = 8) and 14–28 days ( $n$  = 5) had  $IC_{50}$ 's of  $3.1 \pm 0.8$

TABLE 1  
Effect of Estrogen Pretreatment on the Norepinephrine-Induced Contraction of Isolated Mesenteric Artery Segments from Male Rats

	Control males (M $\times 10^{-7}$ )	Estrogen-treated males (M $\times 10^{-7}$ )	<i>P</i>
$EC_{10}$ *	1.5 (+0.3, –0.3)	0.25 (+0.2, –0.1)	<0.01
$EC_{50}$ *	8.4 (+0.8, –0.8)	2.6 (+0.5, –0.4)	<0.001
$EC_{90}$ *	46 (+6, –5)	26 (+23, –12)	NS

\* Mean concentration ( $\pm$ SE) causing 10% ( $EC_{10}$ ), 50% ( $EC_{50}$ ), and 90% ( $EC_{90}$ ) of maximal vessel contraction as described in Methods.

and  $3.3 \pm 0.4$   $\mu$ M, respectively. Because no temporal progression of the effect of high-dose estradiol treatment was observed, these data were combined for statistical analysis. These effects of high dose estradiol on  $\alpha$ -adrenergic receptor affinity for (–)-epinephrine, as well as the effects of a lower dose (10  $\mu$ g/100 g) administered for 5–7 days, are illustrated in Figure 2 and summarized in Table 2. There is a dose-related leftward shift in the epinephrine concentration-response curve. The lower dose estrogen regimen decreased the  $IC_{50}$  for (–)-epinephrine 2.6-fold from  $12.7 \pm 1.6$  to  $4.6 \pm 0.7$   $\mu$ M,  $P$  < 0.01; and the higher dose regimen decreased the  $IC_{50}$  for (–)-epinephrine 4-fold to  $3.2 \pm 0.4$   $\mu$ M ( $P$  < 0.001). This same regimen resulted in a 1.9-fold decrease in the  $K_d$  of [ $^3$ H]-WB-4101 from  $0.95 \pm 0.15$  to  $0.49 \pm 0.04$  nM ( $P$  < 0.01). There also was a small, but significant ( $P$  < 0.05), decrease in maximum [ $^3$ H]-WB-4101 binding capacity from  $102 \pm 4$  to  $85 \pm 5$  fmol/mg protein. A similar increase in the affinity of the mesenteric artery  $\alpha$ -adrenergic receptor is seen for the agonist (–)-norepinephrine after treatment with 17 $\beta$ -estradiol (10  $\mu$ g/

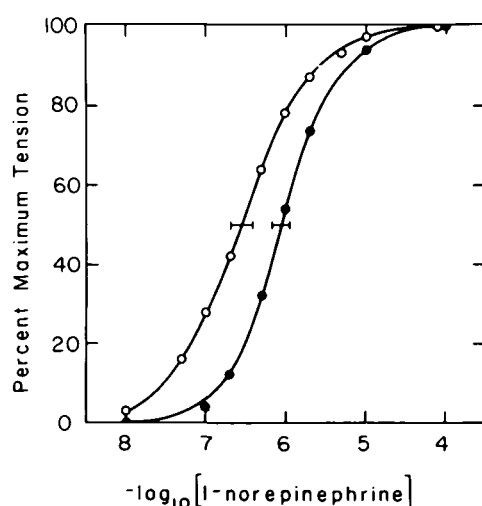


FIGURE 1. Effect of estrogen pretreatment of male rats (10  $\mu$ g/100 g per day for 5–7 days) on contraction of isolated mesenteric artery segments by norepinephrine. Each curve represents the mean of experiments in five control (●) or six estrogen-treated (○) male rats. Bars indicate the mean  $\pm$  SE for the  $EC_{50}$  value of each curve calculated as described in Methods.

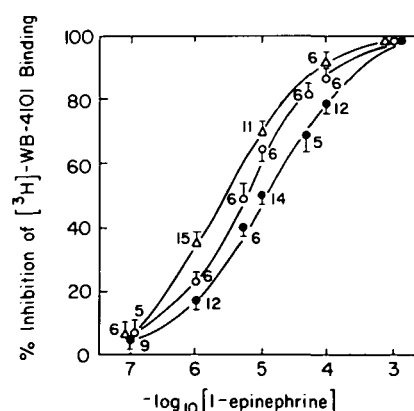


FIGURE 2. Effect of treatment of male rats with two dose levels of 17 $\beta$ -estradiol on the potency of (–)-epinephrine in competing with [ $^3$ H]-WB-4101 binding to mesenteric artery  $\alpha$ -adrenergic receptors. ●, untreated males; ○, low-dose estradiol (10  $\mu$ g/100 g per day); △, high-dose estradiol (250  $\mu$ g/100 g per day). Each binding assay was performed on the pooled particulate fraction obtained from 6–12 animals, using a [ $^3$ H]-WB-4101 concentration of 0.8 nM. Each point represents the mean  $\pm$  SEM. The number of experiments is indicated beside each data point.

TABLE 2  
Mesenteric Artery  $\alpha$ -Adrenergic Receptor Characteristics in Control and Estrogen-Treated Male Rats

	(-)-Epinephrine	[ <sup>3</sup> H]-WB-4101	
	IC <sub>50</sub> ( $\mu$ M)	K <sub>d</sub> (nM)	B <sub>max</sub> (fmol/mg protein)
Untreated	12.7 $\pm$ 1.6 (12)	0.95 $\pm$ 0.15 (6)	102 $\pm$ 4 (6)
Estrogen-treated			
Low-dose (10 $\mu$ g/100 g)	4.6 $\pm$ 0.7 (6)§		
High-dose (250 $\mu$ g/100 g)	3.2 $\pm$ 0.4 (11)§	0.49 $\pm$ 0.04 (10)	85 $\pm$ 5 (10)¶

All values represent mean  $\pm$  SE for the number of experiments indicated in parentheses.

\* The concentration of (-)-epinephrine causing a 50% inhibition of [<sup>3</sup>H]-WB-4101 binding. The IC<sub>50</sub> was calculated as described in Methods.

† The K<sub>d</sub> was obtained by Scatchard analysis of saturation curves for [<sup>3</sup>H]-WB-4101 binding at concentrations between 0.1 and 3.0 nM.

‡ The maximum binding capacity (B<sub>max</sub>) was obtained by Scatchard analysis of saturation curves for [<sup>3</sup>H]-WB-4101 binding.

§  $P < 0.001$  vs. control males.

||  $P < 0.01$  vs. control males.

¶  $P < 0.05$  vs. control males.

100 g per day), the IC<sub>50</sub> for (-)-norepinephrine decreasing from 51  $\pm$  13 to 9.3  $\pm$  1.7  $\mu$ M,  $P < 0.01$  (Fig. 3).

Injection of animals in two experiments with vehicle only (peanut oil) caused no change in maximum [<sup>3</sup>H]-WB-4101-binding capacity, or the affinities for [<sup>3</sup>H]-WB-4101 or (-)-epinephrine. The addition of 17 $\beta$ -estradiol directly to the assay mixture in concentrations from 10<sup>-9</sup> to 10<sup>-3</sup> M also had no effect on (-)-epinephrine displacement curves.

#### Comparison of $\alpha$ -Adrenergic Receptor Properties in Male and Female Rats

In order to gain insight into the significance of the physiological sex hormone milieu for vascular  $\alpha$ -ad-

renergic receptor number and affinity, mesenteric arteries from age-matched males and females were compared. As shown in Figure 4, the (-)-epinephrine concentration response curve in female rats is displaced to the left of the curve for age-matched male rats. As summarized in Table 3, the IC<sub>50</sub> for (-)-epinephrine in females was 4.5  $\pm$  0.8  $\mu$ M, or 2.8-fold lower than for males (IC<sub>50</sub> = 12.7  $\pm$  1.6  $\mu$ M,  $P < 0.001$ ). Similarly, the K<sub>d</sub> for [<sup>3</sup>H]-WB-4101 was lower in female rats (0.58  $\pm$  0.06 nM) than in males (0.95  $\pm$  0.15 nM,  $P < 0.01$ ). There was no significant difference in maximum binding capacity between male and female rats (Table 3). The IC<sub>50</sub> for (-)-epinephrine in oophorectomized females was significantly higher than in control females (oophorectomized females, IC<sub>50</sub> = 11.0  $\pm$  1.1  $\mu$ M; control females IC<sub>50</sub> = 4.5  $\pm$  0.8  $\mu$ M;  $P < 0.001$ ), and was not different from control males 12.7  $\pm$  1.6  $\mu$ M,  $P = \text{NS}$  (Table 3; Fig. 4).

#### Effects of Testosterone on the Vascular $\alpha$ -Adrenergic Receptor in Female Rats

To evaluate the role of testosterone in the sex-related differences in receptor affinity, female rats were treated with testosterone for 48 hours. No change in receptor density (91 fmol/mg protein;  $n = 2$ ), or affinity for (-)-epinephrine (IC<sub>50</sub> = 3.9  $\mu$ M;  $n = 2$ ) or [<sup>3</sup>H]-WB-4101 (K<sub>d</sub> = 0.51 nM;  $n = 2$ ) were noted in these animals.

#### Discussion

We have demonstrated by direct ligand-binding assay that estrogen administration increases the affinity of the vascular  $\alpha$ -adrenergic receptor for the antagonist [<sup>3</sup>H]-WB-4101, and the agonists (-)-epinephrine and (-)-norepinephrine. The receptor in female rats exhibited higher affinity than the receptor in male

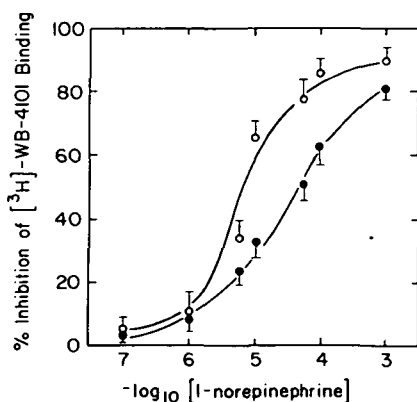


FIGURE 3. Effect of 17 $\beta$ -estradiol administration to male rats (10  $\mu$ g/100 per day) on the potency of (-)-norepinephrine in competing for [<sup>3</sup>H]-WB-4101 binding to  $\alpha$ -adrenergic receptors in mesenteric artery. ●, untreated males; ○, estradiol treated. Each experiment was performed on the pooled particulate fraction obtained from 6–12 animals, using a [<sup>3</sup>H]-WB-4101 concentration of 0.8 nM. Each point represents the mean  $\pm$  SEM of six (untreated) or four (estradiol-treated) separate experiments.

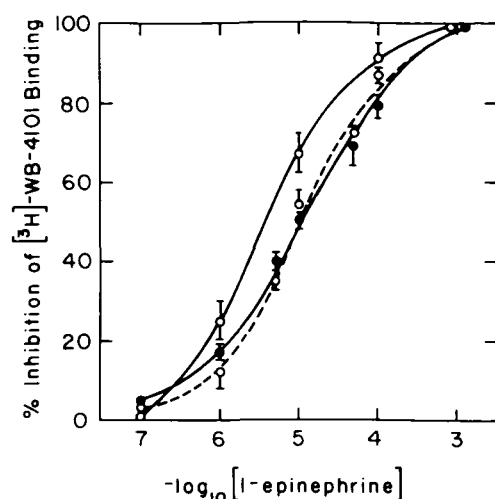


FIGURE 4. Potency of (–)-epinephrine in competing with [ $^3\text{H}$ ]-WB-4101 binding to mesenteric artery  $\alpha$ -adrenergic receptors in male (●), female (○—○), and oophorectomized female (○- -○) rats. Each experiment was performed on the pooled particulate fraction obtained from 6–12 animals, using a [ $^3\text{H}$ ]-WB-4101 concentration of 0.8 nM. Male and female animals were aged-matched; females were used without regard for time of estrous cycle. Each point represents the mean  $\pm$  SEM.

rats, and following oophorectomy, receptor affinity in female rats was no longer different from that in male rats. There was no difference in receptor number between male and female rats, although a small reduction in receptor number was noted following high-dose estrogen treatment of male rats.

These findings are consistent with physiological observations of the effect of estrogen treatment on catecholamine-induced contraction of rat mesenteric arteries in vitro and in situ. Pretreatment of male rats with  $17\beta$ -estradiol in the same dose as used in the binding studies caused a 3.2-fold leftward shift of the dose-response curve for  $\alpha$ -adrenergic receptor-mediated contraction of isolated segments of mesenteric artery. This magnitude of increase in physiological responsiveness is very similar to the magnitude of increases in vascular  $\alpha$ -receptor-binding affinities for (–)-epinephrine (2.8-fold) and (–)-norepinephrine (5.6-fold). The effect of estrogens on vascular physiological responsiveness to catecholamines observed in our study is similar to previous observations by

Altura in the same vascular bed. Using optical techniques to evaluate the contraction of rat terminal mesenteric arterioles in situ, Altura demonstrated that  $17\beta$ -estradiol administered to male rats in the dose used in our binding studies (10  $\mu\text{g}/100$  g) caused a rapid (18–24 hour) 2.2-fold increase in the sensitivity to (–)-epinephrine (Altura, 1975), and that vessels in female rats were 7.5-fold more sensitive to epinephrine-induced contraction than vessels from male rats (Altura, 1972). Thus, the effect of estrogen treatment and the sex of the animal on mesenteric artery catecholamine responsiveness and  $\alpha$ -adrenergic receptor affinity are directionally the same and quantitatively similar.

Interestingly, the  $\text{IC}_{50}$  for inhibition of [ $^3\text{H}$ ]-WB-4101 binding by norepinephrine ( $51 \pm 13$   $\mu\text{M}$ ) is considerably higher than the  $\text{EC}_{50}$  for contraction of isolated mesenteric artery segments by norepinephrine (0.84  $\mu\text{M}$ ). Even after adjustment of the radioligand-derived  $\text{IC}_{50}$  to a dissociation constant ( $K_d$ ) by the methods of Cheng and Prusoff (1973), the  $K_d$  is 26-fold higher than the  $\text{EC}_{50}$ . There are a number of potential explanations for this difference. First, such a discrepancy is consistent with the existence of "spare receptors" in vascular smooth muscle such that the maximum physiological response is obtained by occupation of less than 100% of the receptors (Lefkowitz, 1979). Alternately, the homogenization procedure may alter the natural milieu of the receptor so that the affinity determined in membrane preparations may not reflect in absolute terms the affinity in the intact vessel. Finally, the  $\text{IC}_{50}$  determined from ligand-binding data is a complex function of both the receptor (Lewald and Rodbard, 1970)(Alexander et al., 1975) and ligand (Cheng and Prusoff, 1973) concentration, and may be related to the physiological  $\text{EC}_{50}$  only relatively.

The data suggest that higher endogenous estrogen levels in females contribute to the differences in vascular catecholamine sensitivity and  $\alpha$ -adrenergic receptor affinity. Although further studies will be required to clarify the role of other sex hormones or sex-related factors, in a limited number of observations we found that testosterone, when administered for 2 days, had no effect on  $\alpha$ -adrenergic receptor density or affinity.

The mechanism(s) by which estrogen increases vas-

TABLE 3  
Mesenteric Artery  $\alpha$ -Adrenergic Receptor Characteristics in Male, Female, and Oophorectomized Female Rats

	(–)-Epinephrine	[ $^3\text{H}$ ]-WB-4101	
	$\text{IC}_{50} (\mu\text{M})$	$K_d$	$B_{\text{max}}$
Males	$12.7 \pm 1.6$ (12)	$0.95 \pm 0.15$ (6)	$102 \pm 4$ (6)
Females	$4.5 \pm 0.8$ (6)§	$0.58 \pm 0.06$ (5)	$82 \pm 11$ (5)
Oophorectomized females	$11.0 \pm 1.1$ (4)¶		

All values represent mean  $\pm$  SE for the number of experiments indicated in parentheses.

\*, †, ‡, §, || —As in Table 2.

¶ —  $P < 0.001$  vs. females.

cular  $\alpha$ -adrenergic receptor affinity is unknown. It is possible that synthesis of a new receptor protein is being induced. Alternatively, estrogen-induced changes in vascular smooth muscle cell membranes may alter the micro-environment and, hence, the conformation of the receptor. Effects by estrogens on such general membrane properties as transmembrane potential (Harder and Coulson, 1979) and sterol content (Fuller and Wilson, 1975) have been demonstrated in vascular smooth muscle. Although such changes could be brought about indirectly through general metabolic effects, the demonstration that estrogen receptors exist in vascular smooth muscle (Harder and Coulson, 1979) makes the probability of a direct cellular effect more likely.

Previous studies on the regulation of  $\alpha$ -adrenergic receptors by estrogens have shown an increase in  $\alpha$ -receptor number in rabbit uterus (Williams and Lefkowitz, 1977; Roberts et al., 1977), and a decrease in rabbit platelets (Roberts et al., 1979), with no change in affinity in either case. A recent report indicates that the estrogen-induced increase in rabbit uterine  $\alpha$ -receptor number is due entirely to an increase in the subpopulation of  $\alpha$ -2 receptors, with no change in the number of  $\alpha$ -1 receptors (Hoffman et al., 1981). Under the conditions of our study, it is unlikely that the change in agonist-binding affinity is due to an effect of estrogens on  $\alpha$ -2 receptors. Although [ $^3$ H]-WB-4101 has been reported not to be selective for  $\alpha$ -1 receptors in rabbit uterus (Hoffman and Lefkowitz, 1979), the ligand identifies predominantly  $\alpha$ -1 receptors in the rat mesenteric artery particulate fraction (Colucci et al., 1981). Furthermore, attempts to identify  $\alpha$ -2 adrenergic receptors in this tissue with the  $\alpha$ -2 selective ligands [ $^3$ H]-clonidine, [ $^3$ H]-p-aminoclonidine, and [ $^3$ H]-yohimbine have failed to identify a measurable density of  $\alpha$ -2 receptors (unpublished observations). Whether this effect of estrogens on vascular smooth muscle  $\alpha$ -1 adrenergic receptor affinity is unique remains to be determined. Interestingly, we have found recently that chemical sympathectomy, a maneuver which results in an increase in adrenergic receptor number in several systems (Lefkowitz, 1979), also results in an increase in affinity, but not number, of vascular  $\alpha$ -1 adrenergic receptors (Colucci et al., 1981).

In conclusion, the observations presented in this report provide a potential explanation for the increased vascular reactivity to catecholamines associated with states characterized by elevated estrogen levels. The molecular mechanisms accounting for the differences in receptor affinity that we have observed, and the pathophysiological implications of these differences, require further investigation.

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