Hemodynamic effect of 17β-estradiol in absence of NO in ovariectomized rats: role of angiotensin II

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Hernández, Isabel, Juan L. Delgado, Luis F. Carbonell, M. Carmen Pérez, and Tomas Quesada. Hemodynamic effect of 17β-estradiol in absence of NO in ovariectomized rats: role of angiotensin II. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R970–R978, 1998.—Previous reports correlate plasma levels of estrogen with increased nitric oxide (NO) production. To investigate whether the hemodynamic effects of estrogens are mediated by NO, we compared the hemodynamic changes induced by 17β-estradiol (100 µg/kg) in the absence and presence of the NO synthesis inhibitor Nω-nitro-L-arginine methyl ester (L-NAME). All protocols were performed in ovariectomized, conscious rats. Estradiol alone resulted in no significant changes in cardiac index (CI) or mean arterial pressure (MAP). However, in the presence of L-NAME, estradiol induced a significant increase in total peripheral resistance (TPR) of 37.3 ± 11.7% and a decrease in CI of 27 ± 4.9%, without changes in MAP. Previous blockade of angiotensin II AT1 receptors with losartan prevented any change in CI and TPR induced by 17β-estradiol in the presence of L-NAME. These observations suggest that NO is necessary to offset a vasoconstrictor action of angiotensin II, which is stimulated by estradiol administration.

cardiac output; vascular resistance; estrogen; Nω-nitro-L-arginine methyl ester

A SIGNIFICANT ROLE for estrogen has been demonstrated in cardiovascular regulation. Emerging data suggest that estrogen may account for the reduced incidence of cardiovascular disease in premenopausal women. Estrogen therapy is associated with a reduction in the incidence of coronary heart disease (10a, 41) and reduction of blood pressure in postmenopausal women (32). In addition, estradiol treatment attenuated the development of hypertension in female spontaneously hypertensive rats (40) and transgenic hypertensive rats expressing the mouse Ren-2 gene (6). Although the estrogen-dependent mechanisms contributing to the regulation of arterial pressure remain unclear, some evidence indicates a possible relationship between estrogen, endothelial function, and the renin-angiotensin system.

Estrogen receptors were demonstrated in smooth muscle cells of the aorta of dog, rat, and human coronary arteries (25, 26), and high-affinity binding sites for estrogen were also reported in cytosols isolated from endothelium of rabbit (10) and bovine aortas (3), suggesting that the vascular endothelium is also estrogen sensitive. According to these data, local and systemic responses of estradiol administration are interpreted to mean that there is local production of one or more vasodilator substances such as prostaglandin and nitric oxide (NO) (9, 46). Thus Sudhir et al. (43) reported that in perimenopausal women, estrogen supplementation enhances basal NO release in forearm resistance arteries. Furthermore, studies performed by Van Buren et al. (46) in animals reported that local uterine artery injection of Nω-nitro-L-arginine methyl ester (L-NAME) suppressed, in a dose-dependent manner, the estrogen-induced increase in uterine blood flow, suggesting that such a vasodilatory effect is mediated mainly by NO. In addition, pregnancy and estradiol treatment increase the amount of mRNA for nitric oxide synthase (NOS) isozymes in the skeletal muscle and heart of female guinea pigs (47).

On the other hand, during pregnancy, decreased vascular reactivity to angiotensin II (1, 8) is associated with increased activity of the renin-angiotensin system (18). In addition, after estradiol administration to chronically instrumented sheep, there are significant increases in plasma renin activity (PRA) and cardiac output and a decrease in vascular resistance (27, 29) associated with a reduced pressor effect of angiotensin II (39). This attenuated pressor response to angiotensin II seen in pregnancy may simply reflect downregulation of the vascular AT receptor, as suggested by Bellin et al. (5). This explanation, however, is not supported by recent studies of angiotensin II binding characteristics and AT receptor density in arteries from pregnant animals (13, 37).

Bearing in mind the above data, we hypothesized that NO plays an important role in countermodulating the angiotensin II vasoconstrictor effect induced by estrogen administration. Therefore, the purpose of this study was to determine the role of NO in modulating the actions of 17β-estradiol on systemic hemodynamic effects and the implication of angiotensin II on these hemodynamic effects. To achieve this goal, we compared the hemodynamic effect of estradiol in the presence and absence of the NO synthesis inhibitor L-NAME and evaluated whether or not angiotensin II is implicated in the hemodynamic effect of estrogen when NO synthesis is blocked. These last experiments were also performed in the presence of AT1 angiotensin receptor blockade.

METHODS

Experiments were performed on female ovariectomized Sprague-Dawley rats (330–400 g). Castration was done under anesthesia with Thalamonal (mixture of 16 mg/kg fentanyl and 0.83 mg/kg droperidol), and rats were placed in their cages for 2 mo until the day of the experiment. All the
Experimental protocols were carried out in previously intrumentized conscious rats.

Surgical procedures. Catheters were placed into the left femoral artery for measurement of mean arterial pressure (MAP) and heart rate (HR) and into the left femoral vein for infusion. A right atrial catheter and a thoracic aortic thermocouple were implanted via the right external jugular vein and right carotid artery, respectively. The catheters were brought out through the skin on the dorsal side of the neck. Finally, the distal ends of these lines were threaded through a lightweight flexible spring connected to a swivel. All surgical procedures were performed under aseptic conditions. Rats were placed in plastic cages with the swivels mounted above, allowing complete freedom of movement and free access to chow and tap water. Two full days were permitted for recovery from surgery.

Cardiac output was measured by thermodilution as previously described in our laboratory (23). The thermodilution curve and the pressure signal were processed with a microcomputer system (Cardiomax II R, Columbus Instruments). Hemodynamic values were the mean of three determinations. Cardiac output was measured by rapid injection of 200 µl 0.9% saline at room temperature (20°C) through the jugular catheter, using a spring-loaded, constant-rate, constant-volume syringe (Hamilton CR 700–200). Cardiac index (CI) was calculated by dividing cardiac output by animal weight (100 g), and total peripheral resistance (TPR) was calculated by dividing MAP by CI.

Experimental protocols. Four protocols were designed to evaluate systemic hemodynamic interactions among estrogens, NO, and angiotensin II (Fig. 1).

Protocol I was designed to determine the cardiovascular responses to estrogen alone (100 µg 17β-estradiol/kg in 25 µl of absolute ethyl alcohol) in group I (n = 9). Vehicle for estrogen was infused in another group of rats (group II, n = 5). Hemodynamic parameters were measured before the administration of 17β-estradiol or vehicle (time 0) and 1, 2, and 3 h after the administration of 17β-estradiol.

Protocol II was designed to evaluate how NO synthesis blockade modified the hemodynamic responses to 17β-estradiol. In group III (n = 7), L-NAME (a bolus of 3 mg/kg plus continuous infusion of 50 µg·kg\(^{-1}\)·min\(^{-1}\) in a 0.5% bovine albumin solution) was administered 30 min before 17β-estradiol. In group IV (n = 5), the same dose of L-NAME was supplied and vehicle was administered instead of 17β-estradiol. Basal hemodynamic measurements were performed 30 min after L-NAME administration (time 0), and hemodynamic measurements were performed again 1, 2, and 3 h after administration of 17β-estradiol or vehicle.

Protocol III was designed to determine the hemodynamic effects of estradiol in the presence of a vasconstrictor other than L-NAME, such as phenylephrine. In group V (n = 5), phenylephrine (15 µg·kg\(^{-1}\)·min\(^{-1}\)) was continuously infused, and 1 h was necessary to achieve a steady state; 17β-estradiol was then administered as described in protocols I and II. In group VI (n = 5), the same dose of phenylephrine was given and vehicle instead of estradiol was administered. Basal hemodynamic measurements were performed 60 min after phenylephrine administration (time 0), and hemodynamic measurements were performed again 1, 2, and 3 h after administration of 17β-estradiol (group V) or vehicle (group VI).

Protocol IV was designed to determine the role of angiotensin II on the cardiovascular effects of 17β-estradiol administration in rats given L-NAME (group VII, n = 7). In this group, losartan (10 mg/kg) was administered 30 min before the onset of L-NAME infusion. After another 30-min equilibration period, hemodynamic measurements were taken before the administration of 17β-estradiol (time 0) and 1, 2, and 3 h after 17β-estradiol. To investigate the role of angiotensin II on the hemodynamic effects of 17β-estradiol in normal conditions, losartan was administered to another group of rats (group VIII, n = 5) 30 min before estradiol infusion. The rest of protocol was the same as in group VII.

Hematocrit was measured in all protocols before any infusion (control time), after L-NAME or losartan + L-NAME (time 0), and 3 h after 17β-estradiol or vehicle administration.

To test inhibition of the angiotensin II receptor in the presence of losartan, angiotensin II (50 ng/kg; Sigma, St. Louis, MO) was administered as a bolus 10 min before and 1 h after losartan administration and also at the end of the experiment. Evidence of the magnitude and persistence of receptor blockade is presented in Table 1.

![Fig. 1. Experimental protocols. Time period at -60 min, before infusion of phenylephrine, represents control for protocol III and before infusion of losartan represents control for protocol IV. Time period at -30 min, before infusion of N-nitro-l-arginine methyl ester (L-NAME), represents control for protocol II and steady-state response to AT1 receptor blockade with losartan for protocol IV. Time period marked 0, before 17β-estradiol (E2β), represents basal values for protocols I–IV. Furthermore, this time represents the steady-state response to inhibition of NO synthesis with L-NAME to phenylephrine and AT1 receptor blockade plus inhibition of NO synthesis with L-NAME for protocols II, III, and IV, respectively. Time periods at 60, 120, and 180 min are the responses to E2β in absence (protocol I) and presence of 1 or 2 antagonists (protocols II, III, and IV, respectively).](http://ajpregu.physiology.org/attachment/figs/1.jpg)
Blood sample collection and analysis. One milliliter of whole blood was obtained at the end of the experiment in groups I and II. PRA was determined by using an angiotensin I radioimmunoassay kit (RENCTC, P2721). Plasma level of estradiol was measured by microparticle enzyme immunoassay (IMx estradiol assay; Abbott Laboratories, North Chicago, IL).

Data analysis. Analysis of variance for repeated measures was used to examine changes over time within and between groups at each time period, and Bonferroni’s multiple-range test was used to determine differences between means. Changes were considered significant at $P < 0.05$. All values are reported as means ± SE.

RESULTS

Hemodynamic responses to systemic $17\beta$-estradiol administration in protocol I are illustrated in Fig. 2. Baseline values at time 0 for all cardiovascular measurements did not differ among two groups treated with $17\beta$-estradiol or vehicle and were similar to those previously reported in our laboratory (23, 24). In group I, estradiol alone had no significant effect on hemodynamic parameters such as MAP, CI, stroke volume index (SVI), and TPR. However, HR achieved a significant increase (10 ± 5%) at the third hour of $17\beta$-estradiol administration. Similar results were obtained in group II, in which vehicle was administered instead of $17\beta$-estradiol, although HR remained unchanged in these animals. Baseline hematocrit in groups I and II was 39.2 ± 1.4 and 37.7 ± 1.1%, respectively. Neither $17\beta$-estradiol nor vehicle had a significant effect (38.7 ± 1.5 and 35.3 ± 1.8% 3 h after $17\beta$-estradiol and vehicle, respectively).

Control data obtained before infusion of phenylephrine in protocol III and of antagonists (L-NAME and/or losartan) in protocols II and IV, as well as the systemic responses after their administration and before $17\beta$-estradiol infusion, are presented in Table 2. A significant increase in MAP was observed after L-NAME, accompanied by a significant fall in CI and HR and a rise in TPR ($P < 0.01$). AT1 receptor blockade with losartan resulted in slight but significant hemodynamic changes, a decrease in MAP and TPR associated with increases in CI and HR. However, L-NAME in the presence of losartan (group VII) produced hemodynamic changes similar to those of L-NAME alone (group III). Infusion of phenylephrine resulted in a similar increase in blood pressure and TPR as observed after L-NAME. CI and HR significantly decreased with phenylephrine to values that were not different from the L-NAME-treated group.

Data obtained in protocol II are shown in Fig. 3, in which the systemic responses to $17\beta$-estradiol or vehicle in presence of L-NAME are compared (groups III...
and IV, respectively). Vehicle did not produce any hemodynamic changes after L-NAME. When we examined the response to \(17\beta\)-estradiol in presence of NO synthesis inhibition, we found that MAP was unchanged over the 3-h period of the experiment. However, 1 h after systemic \(17\beta\)-estradiol administration, CI significantly decreased by \(37.3 \pm 4.9\%\) and TPR increased by \(37.3 \pm 11.7\%\) (P < 0.01), and these changes were maintained throughout the 3 h of the experiment (P < 0.01). This fall in CI and rise in TPR were also significantly different from vehicle administration in presence of L-NAME (Fig. 3). Furthermore, the hemodynamic changes induced by \(17\beta\)-estradiol in the presence of L-NAME were significantly different from those seen with \(17\beta\)-estradiol alone (group I, Fig. 2) (P < 0.05). Of note, the increase in TPR by \(17\beta\)-estradiol in the presence of L-NAME were associated with alterations in HR throughout the study. HR increased 1 h after systemic administration of \(17\beta\)-estradiol and continued to increase, reaching \(353 \pm 17\) beats/min at 3 h (P < 0.001). In the presence of L-NAME, SVI significantly decreased from \(82.1 \pm 5.3\) to \(48.4 \pm 7.3\), \(51.7 \pm 7.2\), and \(44.4 \pm 3.8\) \(\mu\)l \(\cdot\) beat\(^{-1}\) \(\cdot\) 100 g\(^{-1}\) 1, 2, and 3 h after \(17\beta\)-estradiol administration, respectively. In addition, hematocrit increased from \(42.5 \pm 6\%\) at time 0 after L-NAME to 45.2 \pm 0.9\% after \(17\beta\)-estradiol (P < 0.05). In contrast, in group IV, vehicle administration in the presence of L-NAME did not induce changes in SVI or hematocrit (SVI was \(74.3 \pm 5.1\) \(\mu\)l \(\cdot\) beat\(^{-1}\) \(\cdot\) 100 g\(^{-1}\) after L-NAME and \(73.5 \pm 5.1\), \(59.7 \pm 6.4\), and \(66.1 \pm 3.1\) \(\mu\)l \(\cdot\) beat\(^{-1}\) \(\cdot\) 100 g\(^{-1}\) 1, 2, and 3 h after vehicle administration, respectively; hematocrit was 40.4 \pm 1.4\% before and 40.1 \pm 1.9\% 3 h after injection of vehicle).

Data obtained in protocol III are shown in Fig. 4. Neither estradiol nor vehicle administration during phenylephrine infusion induced any significant changes in hemodynamic parameters. When group III (L-NAME + estradiol) and group V (phenylephrine + estradiol) were compared, no differences between basal values were seen. However, CI and TPR were significantly different 1 and 3 h after estradiol administration, between the L-NAME- and phenylephrine-pretreated groups.

When the effects of both AT1 receptor and NO synthesis blockade on the \(17\beta\)-estradiol response were exam-

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>L-NAME</th>
<th>Control</th>
<th>Phenylephrine</th>
<th>Control</th>
<th>Los</th>
<th>Los + L-NAME</th>
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<td>MAP, mmHg</td>
<td>117±5</td>
<td>152±5*</td>
<td>111±4</td>
<td>147±3*</td>
<td>105±2</td>
<td>95±3*</td>
<td>128±1*†</td>
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<td>CI, ml·min(^{-1})·100 g(^{-1})</td>
<td>34.6±2</td>
<td>20.9±2*</td>
<td>40.4±4</td>
<td>26.3±1.9*</td>
<td>36.9±1.4</td>
<td>42.2±3.0*</td>
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<tr>
<td>TPR, mmHg·ml·min(^{-1})·100 g</td>
<td>3.5±0.4</td>
<td>7.3±0.3*</td>
<td>2.9±0.2</td>
<td>5.7±0.5*</td>
<td>2.9±0.1</td>
<td>2.3±0.2*</td>
<td>5.1±0.2†</td>
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<tr>
<td>HR, beats/min</td>
<td>386±21</td>
<td>262±18*</td>
<td>362±14</td>
<td>291±41*</td>
<td>363±7</td>
<td>445±22*</td>
<td>293±9†</td>
</tr>
<tr>
<td>Hct, %</td>
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<td>42.5±0.7</td>
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<td>41.7±0.6</td>
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Values are means ± SE. Control values were obtained before infusion of either or both antagonist N\(^{\text{--}}\)nitro-L-arginine methyl ester (L-NAME) and phenylephrine. CI, cardiac index; TPR, total peripheral resistance; HR, heart rate; Hct, hematocrit; Los, losartan. *P < 0.01 vs. respective control values; †P < 0.05 vs. losartan.
ined in protocol IV, a significant inhibition of the hemorrhagic effects induced by 17β-estradiol in presence of L-NAME was observed. As shown in Fig. 5, in group VII (losartan + L-NAME + estradiol), losartan markedly prevented the decrease in CI and the rise in TPR. Accordingly, losartan also significantly attenuated the fall in SVI seen after L-NAME plus 17β-estradiol administration (SVI decreased from 87.7 ± 3.4 to 80.2 ± 7.6, 64.1 ± 5.6, and 65.5 ± 2.9 µl/beat 1, 2, and 3 h after estradiol administration, respectively; values were significantly higher than in untreated animals of group III (L-NAME + estradiol)). Further-

Fig. 4. Effect of systemic administration of 17β-estradiol (■, 100 µg/kg) or vehicle (□, ethanol) in presence of phenylephrine at dose of 20 µg·kg⁻¹·min⁻¹ in ovariectomized conscious rats on cardiac index, mean arterial pressure, heart rate, and TPR. Values are means, and bars show SE.

Fig. 5. Effect of systemic administration of 17β-estradiol (100 µg/kg) in presence of L-NAME (■) at dose of 3 mg/kg + 50 µg·kg⁻¹·min⁻¹ and in presence of losartan (Los; 10 mg/kg) + L-NAME (△) in ovariectomized conscious rats on cardiac index, mean arterial pressure, heart rate, and TPR. Values are means, and bars show SE. *P < 0.05 vs. time 0.
more, pretreatment with losartan prevented the hemodynamic changes induced by 17β-estradiol administration in the presence of L-NAME (hematocrit was 42.5 ± 1.1 and 42.4 ± 1.1% in animals that received losartan + L-NAME before and after 17β-estradiol administration, respectively). However, pretreatment with losartan did not prevent the increase in HR seen in group III (L-NAME + 17β-estradiol; Fig. 5).

Hemodynamic responses to 17β-estradiol administration in the presence of losartan (group VIII) did not differ from those seen with 17β-estradiol alone (group I). After losartan, the changes in MAP were not significant: from a basal value of 87 ± 2 to 87 ± 3, 86 ± 3, and 82 ± 5 mmHg 1, 2, and 3 h after estradiol administration, respectively. Similarly, the changes in HR were not significant: from 386 ± 22 beats/min before to 381 ± 17, 409 ± 20, and 400 ± 23 beats/min after 1, 2, and 3 h of estradiol infusion, respectively. CI after losartan was 30.8 ± 1.5 ml·min⁻¹·100 g⁻¹ and was 29.9 ± 1.7, 31.1 ± 1.3, and 33 ± 1.9 ml·min⁻¹·100 g⁻¹ 1, 2, and 3 h after estradiol administration, respectively. After losartan, TPR values were 2.9 ± 0.2, 2.9 ± 0.2, and 2.8 ± 0.2 mmHg·ml⁻¹·min⁻1·100 g 1, 2, and 3 h after estradiol administration, respectively. In the presence of losartan, estradiol administration again did not change hematocrit (41.6 ± 0.8 and 40.7 ± 0.9% before and after estradiol, respectively).

As expected, PRA was unchanged by estradiol administration (PRA was 1.34 ± 0.28 ng ANG l·ml⁻¹·h⁻¹ in the estradiol-treated group and 1.84 ± 0.43 ng ANG l·ml⁻¹·h⁻¹ in the group treated with vehicle). These data are in agreement with data from group VIII, in which losartan failed to unmask a vasodilatory action of estradiol.

Plasma 17β-estradiol concentration 3 h after intravenous administration (100 µg/kg) was 103.3 ± 21.6 pg/ml.

DISCUSSION

Because estrogen replacement therapy appears to be beneficial for the prevention of cardiovascular disease in postmenopausal women (17), the cardiovascular response to estrogen administration is of substantial interest. Furthermore, increased estrogen levels during pregnancy play a key role in cardiovascular adaptation (44), but other vasoregulatory systems as NO and the renin-angiotensin system are also activated (11, 18, 48).

The present study was designed to evaluate the role of NO in modulating the actions of estradiol on systemic hemodynamics and the implication of angiotensin II in these hemodynamic effects. We found that acute 17β-estradiol (100 µg/kg) administration in rats given L-NAME results in substantial systemic vasoconstriction, as evidenced by increases in TPR and decreases in CI. Moreover, these hemodynamic changes appear to be mediated by potentiation of angiotensin II, because they were abolished in the absence of an angiotensin AT1 receptor antagonist (losartan).

Acute estrogen infusion to ovariectomized rats resulted in no significant changes in TPR and MAP. This differs from other studies performed in ovariectomized ewes in which estrogen administration increased CO and decreased TPR with little change in MAP (29). These discrepancies may be due to species differences. In our study, the light vasodilator effect of estrogens could have been modulated by other compensatory systems, such as enhanced activation of the autonomic system. In view of this, it might be interesting to investigate whether or not pretreatment with a ganglionic blocker or an α-receptor antagonist might unmask a vasodilatory action of 17β-estradiol.

However, one major new finding in the present study is that 17β-estradiol administration to rats pretreated with L-NAME induced an unexpected further increase of TPR and decrease of CI without changes in MAP. These hemodynamic changes are due to estradiol administration, because hemodynamic variables did not change after the administration of L-NAME plus vehicle. Infusion of L-NAME alone resulted in a rise of MAP and TPR and a decrease in CI and HR, as expected after inhibition of NO synthesis. The further vasoconstriction observed after estradiol in the presence of L-NAME seems to be due to the suppression of NO and not to the increased peripheral resistance, because estradiol did not induce further vasoconstriction in the presence of a continuous phenylephrine infusion that increased TPR to similar levels as those of L-NAME. On the other hand, from the results of protocol II (group III, L-NAME + estradiol), a mechanism other than vasoconstriction seems to contribute to produce the decrease in CI seen after administration of estradiol in the absence of NO. One possibility is a decrease in plasma volume and stroke volume, because estradiol administration after L-NAME significantly increased hematocrit. It is well accepted that in the face of a constant red cell volume (RCV), rising hematocrit signifies a fall in plasma volume. Van Beaumont (45), in an elegant mathematical derivation based on actual and theoretical data, illustrates the relationship between hematocrit and plasma volume in a nomogram. Because the hematocrit is actually the ratio of RCV and total blood volume (RCV + plasma volume), the change in plasma volume must always be larger than the change reflected by hematocrit. Thus, from that nomogram, an increase of 2.7 points in the hematocrit represents an 11% drop in plasma volume, and such a fall could be responsible at least in part for the decrease in stroke volume observed after estradiol administration in the presence of L-NAME.

The underlying mechanism by which estrogen-induced vasoconstriction when NO synthesis was inhibited by L-NAME is difficult to explain. It appears that 17β-estradiol, either directly or mediated by a vasoconstrictor substance, increased vascular tone and TPR. One candidate is angiotensin II, because angiotensin II AT1 receptor blockade with losartan suppressed the vasoconstrictor effect of 17β-estradiol in these circumstances. Nevertheless, the role of angiotensin II in maintaining hemodynamics in the presence of 17β-estradiol is controversial. On one hand, several studies observed greater decreases in blood pressure in response to an angiotensin II receptor antagonist, sarala-
which estradiol was administered, independent of because venous return was maintained constant. Ventricular filling time as a result of the rise in HR, in hematocrit and the fall in stroke volume observed after estradiol administration in the group treated with L-NAME plus 17β-estradiol and the group treated with 17β-estradiol alone. This may be because under resting conditions in conscious, sodium-replete animals and humans, the renin-angiotensin system does not play a very important role in the maintenance of normal blood pressure and vascular tone. This explanation is consistent with our measurement of PRA data from group I, treated with estradiol alone, suggesting a minor role of the renin-angiotensin system. On the other hand, the administration of an angiotensin AT1 receptor antagonist to rats treated with estradiol in presence of L-NAME prevented the estradiol-induced vasoconstriction and the rise in hematocrit and decreased the fall in stroke volume observed in group III (L-NAME + estradiol), suggesting a key role of angiotensin II in mediating these effects in the absence of NO. Schricker et al. (42) have recently reported that inhibition of NO synthesis led to an attenuation of basal renin secretion and to an increase in blood pressure. Therefore, because administration of L-NAME increased blood pressure in this study, we can expect a decrease or no change in PRA. In this case, one may speculate that the hemodynamic effects of estradiol in the absence of NO would more likely be due to a potentiation of angiotensin II than to an increase in renin secretion. Thus our results showing that administration of 17β-estradiol induced a further increase of TPR and decrease of CI in the presence of L-NAME indicate that NO is necessary to offset a vasoconstrictor action of angiotensin II enhanced by estradiol administration. Furthermore, losartan inhibited the increase in hematocrit and the fall in stroke volume observed after L-NAME plus estradiol, indicating that angiotensin II may contribute to the decrease in stroke volume and CI seen in the group treated with L-NAME plus estradiol. These data are in agreement with other reports that support a role for angiotensin II in the pathogenesis of vascular injury and permeability via mechanisms that are independent of its pressor activity (38, 48). The remaining fall in stroke volume seen after estradiol administration in the group treated with losartan plus L-NAME may be due to a decrease in ventricular filling time as a result of the rise in HR, because venous return was maintained constant.

An increase in HR was observed in all groups in which estradiol was administered, independent of changes in other hemodynamic variables. The tachycardia seen in rats given estradiol alone probably reflects a baroreceptor-mediated tachycardia, because MAP tended to fall. Another possibility is that estrogen may have a direct effect on the sinoatrial node, as has been proposed by others. Estradiol has been shown to accumulate in the nuclei of atrial myocytes (31) and to cause positive chronotropism in isolated perfused rabbit heart (2); thus estrogen may have a direct effect on the sinoatrial node. On the other hand, estrogen may also induce positive chronotropism via activation of the renin-angiotensin system (34), but it is unlikely that this last mechanism may explain the rise in HR by estradiol, because HR still increased in the presence of AT1 receptor blockade. Furthermore, these hemodynamic differences were seen between the group treated with AT1 receptor antagonist plus 17β-estradiol and the group treated with 17β-estradiol alone. This may be because under resting conditions in conscious, sodium-replete animals and humans, the renin-angiotensin system does not play a very important role in the maintenance of normal blood pressure and vascular tone. This explanation is consistent with our measurement of PRA data from group I, treated with estradiol alone, suggesting a minor role of the renin-angiotensin system. On the other hand, the administration of an angiotensin AT1 receptor antagonist to rats treated with estradiol in presence of L-NAME prevented the estradiol-induced vasoconstriction and the rise in hematocrit and decreased the fall in stroke volume observed in group III (L-NAME + estradiol), suggesting a key role of angiotensin II in mediating these effects in the absence of NO. Schricker et al. (42) have recently reported that inhibition of NO synthesis led to an attenuation of basal renin secretion and to an increase in blood pressure. Therefore, because administration of L-NAME increased blood pressure in this study, we can expect a decrease or no change in PRA. In this case, one may speculate that the hemodynamic effects of estradiol in the absence of NO would more likely be due to a potentiation of angiotensin II than to an increase in renin secretion. Thus our results showing that administration of 17β-estradiol induced a further increase of TPR and decrease of CI in the presence of L-NAME indicate that NO is necessary to offset a vasoconstrictor action of angiotensin II enhanced by estradiol administration. Furthermore, losartan inhibited the increase in hematocrit and the fall in stroke volume observed after L-NAME plus estradiol, indicating that angiotensin II may contribute to the decrease in stroke volume and CI seen in the group treated with L-NAME plus estradiol. These data are in agreement with other reports that support a role for angiotensin II in the pathogenesis of vascular injury and permeability via mechanisms that are independent of its pressor activity (38, 48). The remaining fall in stroke volume seen after estradiol administration in the group treated with losartan plus L-NAME may be due to a decrease in ventricular filling time as a result of the rise in HR, because venous return was maintained constant.

An increase in HR was observed in all groups in which estradiol was administered, independent of
estrogen is reported to be mediated indirectly by an effect on lipoprotein metabolism and directly by an effect on the blood vessel wall itself (7, 17, 20, 22). This effect appears to be mediated by enhancing the endothelial function, as indicated in several studies in which physiological levels of estrogen increased the endothelium-dependent vasorelaxation in the coronary vasculature in postmenopausal women (20). Estrogen supplementation has also been shown to augment endothelial NOS activity and mRNA for NOS isoenzymes in the uterine artery, heart, and skeletal muscle in female guinea pigs (47). On the other hand, preeclampsia is a disease characterized by hypertension in which the normal vascular adaptations of pregnancy are compromised (19). The attenuated response to angiotensin II seen in normal pregnancy is lost in women destined to develop preeclampsia. Then, an abnormal endothelial function, as has been described in the arteries of women with preeclampsia (30), may cause a decrease in urinary nitrite/nitrate (14), and the resulting imbalance between vasodilator and vasoconstrictor systems may be responsible for this characteristic hypertensive state with augmented sensitivity to angiotensin II (16). Thus it is of interest to determine the quantitative importance of NO in protecting the systemic hemodynamics in these physiological and pathophysiological states, and more studies will be necessary in the future.

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