Estrogen reduces myogenic tone through a nitric oxide-dependent mechanism in rat cerebral arteries

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Geary, Greg G., Diana N. Krause, and Sue P. Duckles. Estrogen reduces myogenic tone through a nitric oxide-dependent mechanism in rat cerebral arteries. *Am. J. Physiol.* 275 (*Heart Circ. Physiol.* 44): H292–H300, 1998.—Gender differences in the incidence of stroke and migraine appear to be related to circulating levels of estrogen; however, the underlying mechanisms are not yet understood. Using resistance-sized arteries pressurized in vitro, we have found that myogenic tone of rat cerebral arteries differs between males and females. This difference appears to result from estrogen enhancement of endothelial nitric oxide (NO) production. Luminal diameter was measured in middle cerebral artery segments from males and from females that were either untreated, ovariectomized (Ovx), or ovariectomized with estrogen replacement (Ovx + Est). The maximal passive diameters (0 Ca^{2+} + 1 mM EDTA) of arteries from all four groups were identical. In response to a series of 10-mmHg step increases in transmural pressure (20–80 mmHg), myogenic tone was greater and vascular distensibility less in arteries from males and Ovx females compared with arteries from either untreated or Ovx + Est females. In the presence of N^G-nitro-L-arginine methyl ester (L-NAME; 1 μM), an NO synthase inhibitor, myogenic tone was increased in all arteries, but the differences among arteries from the various groups were abolished. Addition of L-arginine (1 mM) in the presence of L-NAME restored the differences in myogenic tone, suggesting that estrogen works through an NO-dependent mechanism in cerebral arteries. To determine the target of NO-dependent modulation of myogenic tone, we used tetraethylammonium (TEA; 1 mM) to inhibit large-conductance, calcium-activated K^+ (BK_{Ca}) channels. In the presence of TEA, the myogenic tone of arteries from all groups increased significantly; however, myogenic tone in arteries from males and Ovx females remained significantly greater than in arteries from either untreated or Ovx + Est females. This suggests that activity of BK_{Ca} channels influences myogenic tone but does not directly mediate the effects of estrogen. Estrogen appears to alter myogenic tone by increasing cerebrovascular NO production and/or action.

estrogen; cerebral arteries; nitric oxide; myogenic tone

FOR WOMEN in the childbearing years, the incidence of stroke and cerebrovascular disease is significantly less than for males (28). After the onset of menopause, the incidence of cerebrovascular disease in women rises to that of males within a few years (9, 31, 32). Postmenopausal women undergoing estrogen replacement have significantly reduced cerebrovascular mortality rates compared with their untreated counterparts (9, 31). Cerebral artery blood flow velocity also has been shown to be significantly increased after ovarian hyperstimulation (37). These observations suggest that ovarian hormones have an important influence on the cerebral circulation. However, few studies exist that address the question of how gonadal hormones affect cerebral blood vessels.

One possibility is that gonadal hormones may influence autoregulation of the cerebral circulation (18). This is an important protective mechanism whereby cerebral blood flow and capillary perfusion pressure are kept relatively constant during changes in systemic blood pressure (6). Thus the brain is protected against hypoxia during low arterial pressures and against brain edema and stroke at high pressures. Cerebral autoregulation is thought to result from intrinsic myogenic and metabolic factors but is also known to be modified by extrinsic factors such as perivascular nerves, hormones, and pathological conditions (6).

The myogenic response, which is one component of autoregulation, is defined as the constriction or dilation of vascular smooth muscle cells in response to increases or decreases in the transmural pressure, respectively (4, 25). Cerebral arterioles and small arteries are known to be especially responsive to changes in transmural pressure. The mechanisms by which an active contractile response is initiated by application of force to the blood vessel wall are not well understood, although changes in membrane potential and Ca^{2+} and K^+ conductances appear to be involved (1). Endothelial factors also may play a role in the myogenic response (13).

Interestingly, in peripheral arteries estrogen has been shown to alter vascular smooth muscle reactivity through either increased endothelial nitric oxide (NO) production (15, 39), inhibition of voltage-dependent Ca^{2+} channels (12, 20), activation of Ca^{2+}-sensitive K^+ channels (14, 42), and/or inhibition of cell proliferation (3). If these effects of estrogen also occur in cerebral arteries, they could alter autoregulatory responses. Currently, the influence of naturally circulating gonadal steroids on cerebrovascular myogenic reactivity is unknown. However, a recent study using arteries from the rat coronary circulation showed an increase in myogenic tone after ovariectomy that could be reversed with chronic estrogen replacement. Lower myogenic tone was related to greater basal NO release (40).

Therefore, the aim of our study was to investigate the influence of gender and estrogen status on myogenic reactivity in pressurized rat middle cerebral arteries. Myogenic reactivity was determined in vitro by altering transmural pressure in proximal segments of middle cerebral arteries from males, cycling females, ovariectomized females, and ovariectomized females with estrogen replacement. The myogenic response was further investigated during inhibition of either NO synthase (NOS) or large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels. Measurements also were made after removal

of extracellular Ca^{2+} to provide an indication of the passive response of the arterial segment to changes in pressure.

METHODS

Animals. Animal procedures were approved by the Animal Care and Use Committee of the University of California, Irvine. Male and female 3-mo-old Fisher 344 rats were purchased from Harlan Sprague Dawley (Indianapolis, IN) and housed under a 12:12-h light-dark cycle with food and water available ad libitum. Four groups of rats were used in the present study: males (n = 11), untreated cycling females (n = 12), ovariectomized females (Ovx; n = 12) and Ovx females with physiological levels of estrogen replacement (Ovx + Est; n = 12). Ovariectomy and ovariectomy with estrogen replacement were performed under anesthesia [ketamine (90 mg/kg) and xylazine (10 mg/kg)] (3). Estrogen was replaced at the time of ovariectomy by subcutaneous insertion of 10-mm silicone elastomer capsules made from Dow Corning Silastic medical-grade tubing (1.57 mm ID × 3.18 mm OD, Dow Corning), sealed with silicone elastomer adhesive type A (Dow Corning) and packed with 17β-estradiol. Both Ovx and Ovx + Est animals were euthanized 1 mo after surgery.

Tissue preparation. At 4 mo of age, rats were decapitated in the middle of the day, and trunk blood was collected for determination of 17β-estradiol by radioimmunoassay. Brains were rapidly removed from the cranial cavity and placed in a dissecting dish with cold oxygenated physiological salt solution (PSS) containing (in mM) 118 NaCl, 4.8 KCl, 1.6 $CaCl_2$, 1.2 KH_2PO_4, 25 $NaHCO_3$, 1.2 $MgSO_4$, 0.3 ascorbic acid, and 11.5 glucose. A 1- to 2-mm segment of the middle cerebral artery, taken about 2 mm from the circle of Willis, was carefully dissected and mounted in an arteriograph (Living Systems, Burlington, VT). Micropipettes were inserted into each end of the artery and secured in place with nylon ties. The proximal cannula was connected through a pressure transducer and windkessel to a reservoir of PSS equilibrated with 95% O_2-5% CO_2. The distal cannula was connected to a Luer Lok that was open during the initial equilibration to gently flush the luminal contents. After the equilibration period, the Luer-Lok remained closed so that all experiments were conducted under no-flow conditions. A constant-flow peristaltic pump continuously superfused (30 ml/min) the artery with PSS. During a 60-min equilibration period, a pressure servosystem maintained transmural pressure at 40 mmHg. The artery was viewed with an inverted microscope equipped with a videocamera and monitor. A videoelectronic dimension analyzer was used to measure luminal diameter. Changes in transmural pressure and lumen diameter were digitized by a MacLab analog-to-digital converter and recorded on a Macintosh computer. All drugs, individually or in combination, were added to the superfusate in their final concentration.

Experimental protocols. In all protocols the changes in artery diameter in response to increased transmural pressure (no flow) were measured. Only those vessels that developed spontaneous tone were used. After the 60-min equilibration period, pressure was reduced to 20 mmHg. Pressure was then increased to 80 mmHg with a single 60-mmHg pressure step, maintained for 10 min, and then returned to 20 mmHg. Three such cycles were performed on each vessel to remove mechanical hysteresis. After the three initial cycling periods, four separate series of pressure steps (each from 20 to 80 mmHg in 10-mmHg steps) were performed. The first series of pressure steps was in PSS, the second in the presence of N^G-nitro-L-arginine methyl ester (L-NAME; 1 μM), the third in L-NAME + L-arginine (100 μM), and the fourth in 0 Ca^{2+}-EDTA (1 mM). All drugs were perfused for 20 min before the first pressure step, and each pressure step was maintained for 5–10 min to allow the vessel to reach a stable condition before diameter was measured. Control arteries showed consistent responses to four series of pressure steps. In a separate series of similar experiments, three series of pressure steps were carried out: first in PSS, second in tetraethylammonium (TEA, 1 mM), and third in 0 Ca^{2+} + EDTA.

Myogenic tone was determined by subtracting the steady-state diameter at any given pressure in PSS from the passive diameter (0 Ca^{2+} + 1 mM EDTA) at that same pressure. Constriction to L-NAME was determined by subtracting steady-state diameter at any given pressure in the presence of L-NAME from the steady-state diameter in PSS at that same pressure. Distensibility of the arterial wall (in μm) was determined by measuring the transient change in diameter that occurred immediately after a 10-mmHg step increase in pressure.

Radioimmunoassay for serum levels of estradiol. Immediately after rats were decapitated, trunk blood was collected in plain tubes and centrifuged at 1,000 rpm for 5 min. The supernatant was decanted and frozen at −70°C until time of radioimmunoassay determination of 17β-estradiol. Double-antibody estradiol ^{125}I-radioimmunoassay kits (Diagnostic Products, Los Angeles, CA) were used. Serum samples were defrosted at room temperature, and the estrogens were extracted using diethyl ether. The organic phase was evaporated to dryness, and the extract was then reconstituted with anti-estradiol antiserum from the kit. ^{125}I-labeled estradiol was then added, and bound ^{125}I-estradiol was separated using the PEG-accelerated double-antibody method according to the manufacturer's instruction. The antiserum is highly specific for 17β-estradiol with extremely low cross reactivity to other naturally occurring steroids. The detection limit of the assay is 1.4 pg/ml.

All drugs were purchased from Sigma Chemical (St. Louis, MO). Data are expressed as means ± SE. Statistical significance was determined using paired Student's t-test or ANOVA with Scheffé's test. Acceptable level of significance was defined as $P < 0.05$.

RESULTS

Animal weight and serum estrogen concentrations. Animal weights and serum estrogen levels are shown in Table 1. Age-matched males weighed more than each of the female groups. The body weights of Ovx females were significantly ($P < 0.05$) greater than those of either untreated females or Ovx + Est females. The average concentration of serum estradiol in untreated, cycling females (taken randomly throughout the estrous cycle) was slightly higher than, but not significantly different from, that in males and Ovx females.

Table 1. *Effect of Ovx and Ovx + Est on body weight and serum concentrations of estradiol*

Rats	Body Wt, g	n	Estradiol, pg/ml	n
Male	318 ± 7*	11	10 ± 2	10
Untreated female	170 ± 2	12	14 ± 2	9
Ovx	192 ± 4*	12	9 ± 2	5
Ovx + Est	174 ± 2	12	35 ± 4*	5

Values are means ± SE; n, no. of rats. Ovx, ovariectomy; Ovx + Est, Ovx with estrogen replacement. *Significantly different from 3 other groups ($P < 0.05$).

Estrogen replacement resulted in serum levels in the physiological range (2, 22, 36), significantly higher ($P < 0.05$) than Ovx females. Ranges of serum estradiol in normal cycling, Ovx, and Ovx + Est females were 10–34, 3.4–16.2, and 24–52 pg/ml, respectively.

Effect of gender and estrogen. Middle cerebral arteries from males and females responded passively to each step increase in pressure in the absence of Ca^{2+} (with EDTA, 1 mM) (Fig. 1, *A* and *B*). Maximal passive artery diameters (80 mmHg) were not significantly different between males (294 ± 2 μm) and females (290 ± 4 μm). As shown in Figs. 1 and 2, male arteries in PSS were smaller at 20 mmHg than arteries from females ($P < 0.05$). After each pressure step, the steady-state myogenic tone (difference between passive and PSS) was significantly greater in males than females (Figs. 1 and 2; $P < 0.01$). These gender differences in myogenic tone were seen consistently after either repeated single (not shown) or multiple step changes in pressure. Male arteries remained constricted after each pressure step, whereas diameters of arteries from the females changed passively in response to step increases at lower pressures but increased myogenic tone at the higher pressures (Figs. 1*B* and 2*B*). When the diameter-pressure relationship was expressed as diameter change (difference between passive and PSS; Fig. 3), as pressure was increased diameters of arteries from males decreased significantly more than arteries from females (Fig. 3).

To determine if circulating estradiol contributes to the lower myogenic tone in female arteries, we performed a series of experiments on arteries from Ovx and Ovx + Est female rats (Fig. 1, *C* and *D*, and Fig. 2). When studied 1 mo after ovariectomy, cerebral artery diameters were significantly smaller and possessed greater myogenic tone than arteries from untreated, cycling females ($P < 0.01$). No significant difference was observed between males and Ovx females (Fig. 3). When serum estrogen concentrations were raised by estrogen replacement, myogenic tone of arteries was significantly reduced ($P < 0.01$). Most importantly, gender differences in myogenic tone were completely restored after estrogen replacement in Ovx females.

Effect of inhibition of NOS. To determine whether NO contributes to steady-state myogenic tone, we inhibited NOS with L-NAME (1 μM). In the presence of L-NAME, arteries from all groups gained significantly greater

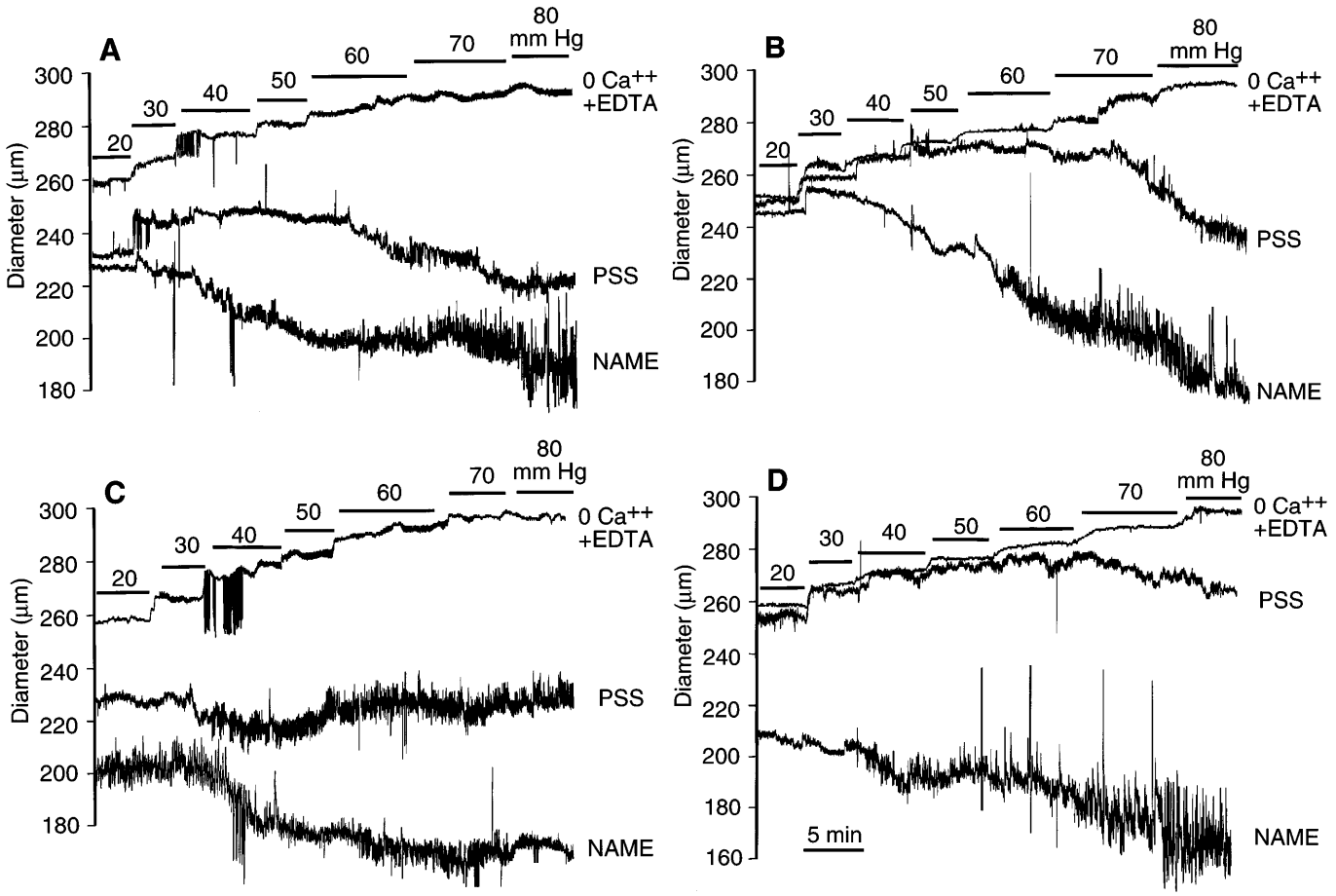

Fig. 1. Representative tracings of the effect of increasing transmural pressure (20–80 mmHg) on lumen diameter of rat middle cerebral arteries from male rats (*A*), untreated female rats (*B*), ovariectomized rats (Ovx; *C*), and Ovx rats with estrogen replacement (Ovx + Est) (*D*). Step increases in pressure (10 mmHg) were achieved in <1 s, and each pressure was maintained for 5–10 min. Arteries were exposed to either normal PSS, N^G-nitro-L-arginine methyl ester (L-NAME; 1 μM), or 0 Ca^{2+} PSS + 1 mM EDTA (passive response). Passive diameters at 80 mmHg were 292 μm (male), 301 μm (untreated female), 295 μm (Ovx), and 300 μm (Ovx + Est).

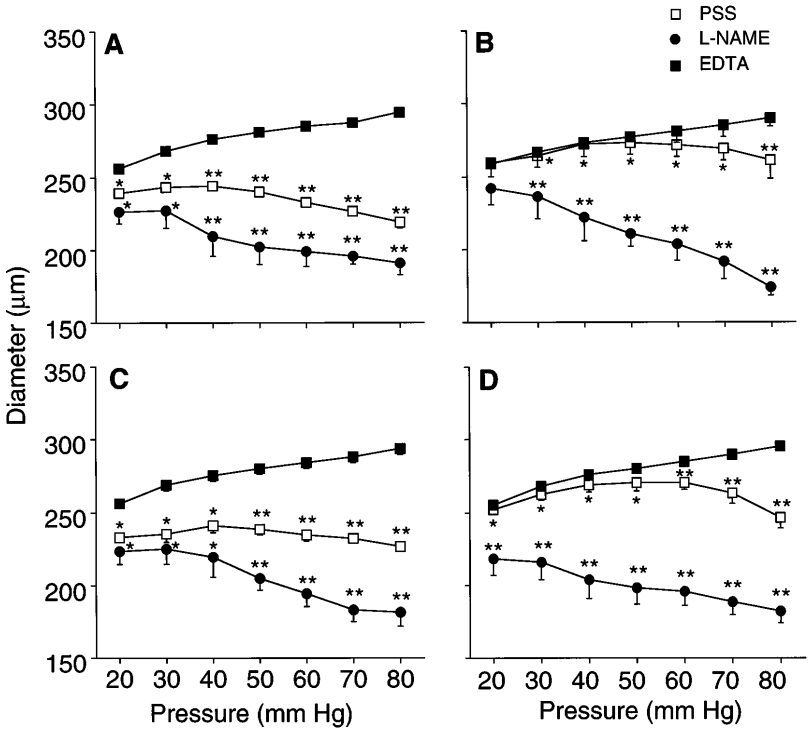

Fig. 2. Mean steady-state diameter is plotted as a function of pressure for rat middle cerebral arteries from 4 groups of rats: male (*A*), untreated female (*B*), Ovx (*C*), and Ovx + Est (*D*). Luminal diameters were measured either in PSS, in the presence of L-NAME (1 μM), or in 0 Ca^{2+} + 1 mM EDTA (passive response). Solutions of either L-NAME or 0 Ca^{2+}-EDTA were superfused for 20 min before the step increases in pressure were performed. Values are means ± SE; $n = 6$. *$P < 0.05$, different from 1 other group. **$P < 0.05$, different from 2 other groups. Comparisons were among PSS, L-NAME, and EDTA groups.

myogenic tone after each pressure step than in PSS alone (Figs. 1 and 2). However, in the presence of L-NAME, the myogenic tone that developed in arteries from untreated and Ovx + Est females was significantly greater than that in arteries from either the males or Ovx females. Furthermore, and most importantly, the differences in steady-state myogenic tone originally observed among the groups in PSS were abolished after NOS inhibition (Figs. 1 and 2; $P > 0.05$).

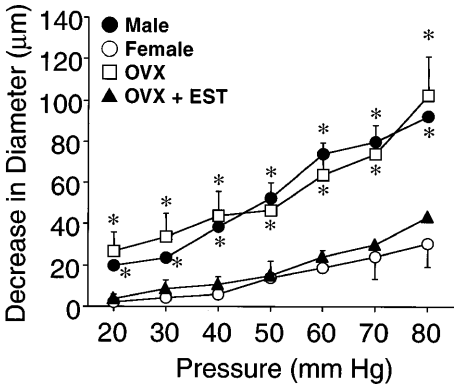

Fig. 3. Comparison of myogenic tone in rat middle cerebral arteries from male, untreated female, Ovx, or Ovx + Est rats. Myogenic tone was calculated as the difference between diameter in PSS and passive diameter (0 Ca^{2+} + EDTA) and was plotted as a function of pressure. Values are means ± SE; $n = 6$. *$P < 0.05$ compared with untreated female and Ovx + Est rats.

To quantitate the effect of L-NAME treatment in the four groups, we calculated the constriction to L-NAME (difference between PSS and L-NAME) after each pressure increase (Fig. 4). The constriction to L-NAME (decrease in diameter) of arteries from untreated and

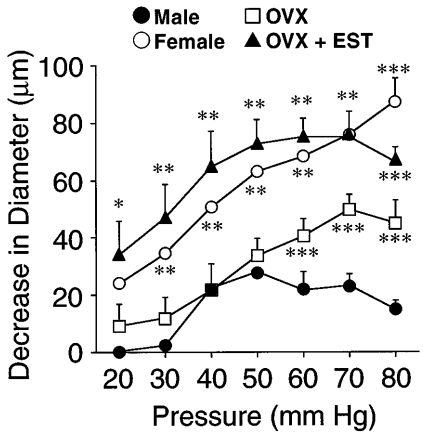

Fig. 4. Effect of L-NAME to constrict rat middle cerebral arteries from male, untreated female, Ovx, or Ovx + Est rats. Difference in luminal diameter between diameter in PSS and that in the presence of L-NAME is plotted as a function of transmural pressure. After the initial series of pressure steps in PSS, L-NAME (1 μM) was superfused for 20 min before and during the next series of pressure steps. Values are means ± SE; $n = 6$. *$P < 0.05$, different from 1 other group. **$P < 0.05$, different from 2 other groups. ***$P < 0.05$, different from 3 other groups.

Ovx + Est females was significantly greater than in arteries from either males or Ovx females. Interestingly, though, beyond 50 mmHg the decrease in diameter of Ovx arteries to each subsequent pressure step was significantly greater than in arteries from males, although the absolute diameters attained (μm) were not significantly different (181 ± 9 vs. 191 ± 8 μm, respectively).

Because L-arginine and L-NAME act as competitive substrates for NOS, we sought to determine if L-arginine could reverse the effects of L-NAME (Fig. 5). In all groups, myogenic tone of L-NAME-treated arteries at 80 mmHg was restored toward control (PSS) levels after exposure to L-arginine (100 μM; 20 min). At pressures <80 mmHg, the reversal by L-arginine was complete in arteries from males and Ovx females; however, slight but significant differences between PSS and L-arginine plus L-NAME persisted in arteries from untreated and Ovx + Est females. These differences were most evident in arteries from Ovx + Est animals, where, except for the response at 80 mmHg, L-arginine was unable to completely restore diameters to those found in PSS.

Effect of Ca^{2+}-activated K^+ channel blocker. BK_{Ca} channels have been shown to modulate myogenic tone in cerebral arteries (1). To assess the extent to which BK_{Ca} channels contribute to myogenic tone in the different groups, we conducted a separate series of experiments with TEA (1 mM) to inhibit BK_{Ca} channels. Differences in control responses to pressure steps among arteries from the various groups (Fig. 6) were very similar to those seen previously (Fig. 2). Addition of TEA caused a significant increase in myogenic tone of arteries from males and from Ovx and Ovx + Est females at 20 mmHg (Fig. 6). After each pressure step in the presence of TEA, myogenic tone remained significantly larger in arteries from males and Ovx females compared with arteries from untreated females (Fig. 6; $P < 0.05$). Myogenic tone in the presence of TEA was also significantly larger in arteries from male and Ovx females compared with Ovx + Est females, although significant differences did not occur at the lower pressures (20 and 30 mmHg).

A comparison of artery diameters from males and Ovx females, in the presence of either TEA or L-NAME, showed similar levels of constriction at 80 mmHg. However, arteries from both untreated and Ovx + Est females attained a significantly different level of constriction in the presence of TEA compared with in the presence of L-NAME (Figs. 2 and 6). For example, at 80 mmHg, arteries from untreated females had diameters of 174 ± 6 μm in L-NAME and 237 ± 9 μm in TEA, whereas arteries from Ovx + Est females had diameters of 182 ± 8 μm in L-NAME and 224 ± 15 μm in TEA ($P < 0.05$). Thus effects of inhibition of NOS and BK_{Ca} channels were not identical in arteries from untreated and Ovx + Est females.

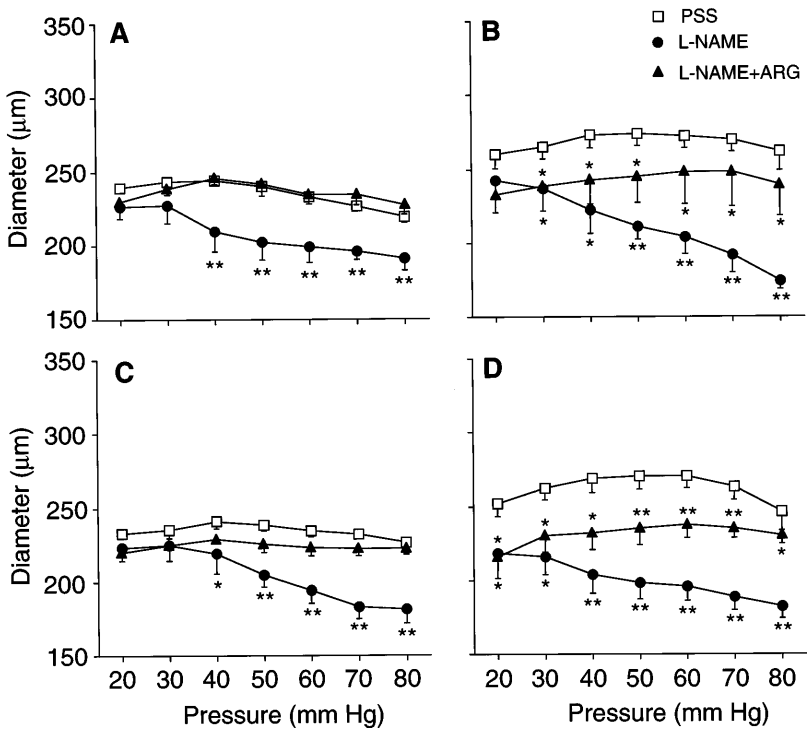

Fig. 5. Effect of L-arginine (L-Arg) on diameter of rat middle cerebral arteries from male (*A*), untreated female (*B*), Ovx (*C*), or Ovx + Est rats (*D*) in presence of L-NAME. Luminal diameter is plotted as a function of transmural pressure. After 2 series of pressure steps in PSS and L-NAME, L-NAME (1 μM) and L-Arg (100 μM) were superfused for 20 min before and during the next series of pressure steps. Values are means ± SE; $n = 6$. *$P < 0.05$, different from 1 other group. **$P < 0.05$, different from 2 other groups. Comparisons were among PSS, L-NAME, and L-NAME + L-Arg groups.

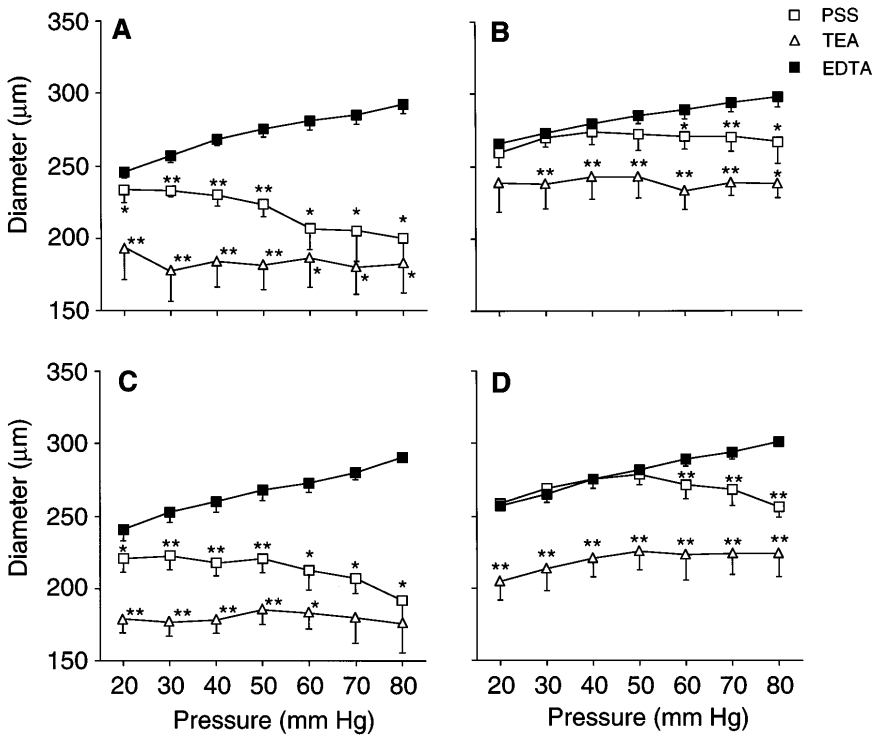

Fig. 6. Effect of tetraethylammonium (TEA) on diameter of rat middle cerebral arteries from male (*A*), untreated female (*B*), Ovx (*C*), or Ovx + Est rats (*D*). Mean steady-state luminal diameter in either PSS, TEA (1 mM), or 0 Ca^{2+} + 1 mM EDTA (passive response) is plotted as a function of pressure. TEA was superfused for 20 min before increases in pressure were performed. Maximum passive diameters (at 80 mmHg) were not significantly different among groups (male, 292 ± 6 μm; untreated female, 297 ± 6 μm; Ovx, 290 ± 5 μm; Ovx + Est, 301 ± 3 μm). Values are means ± SE; $n = 6$. *$P < 0.05$, different from 1 other group. **$P < 0.05$, different from 2 other groups. Comparisons were among PSS, TEA, and EDTA groups.

Effects on arterial distensibility. To assess the initial state of myogenic activation of arteries in each group, we determined arterial distensibility in PSS by measuring the initial expansion of the artery wall immediately after each pressure elevation (Fig. 1). As summarized in Fig. 7, arterial distensibility in arteries from untreated and Ovx + Est females was significantly greater than arteries from either males or Ovx females. The distensibility of arteries from untreated females was significantly smaller than distensibility of arteries from Ovx + Est females at the 40- to 50-mmHg pressure step.

DISCUSSION

The present study suggests that estrogen is an important modulator of myogenic tone in rat resistance-sized cerebral arteries. The modulatory effect of estrogen appears to be mediated by an action on NO, and part, but not all, of this effect may involve regulation of

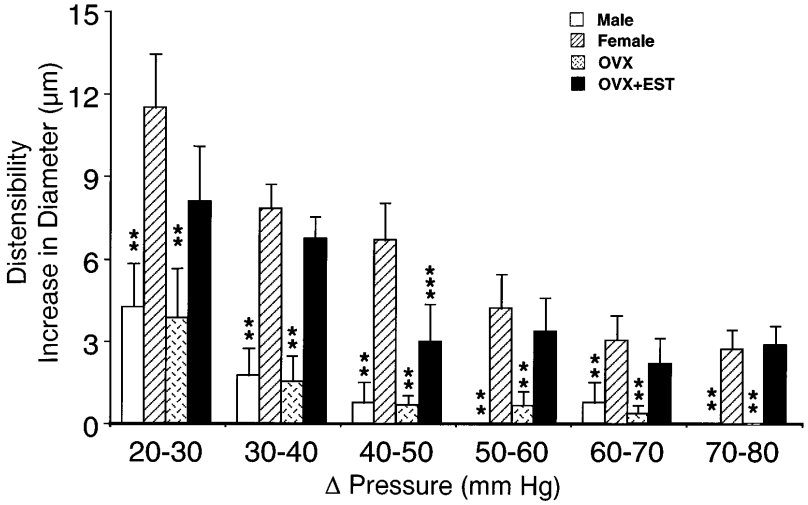

Fig. 7. Effect of pressure steps on distensibility in rat middle cerebral arteries from male, untreated female, Ovx, or Ovx + Est rats. Initial increases in luminal diameter are plotted as a function of 10-mmHg changes in transmural pressure. Values are means ± SE; $n = 6$. *$P < 0.05$, different from 1 other group. **$P < 0.05$, different from 2 other groups. ***$P < 0.05$, different from 3 other groups.

BK_{Ca} channels. Because the level of myogenic tone is a critically important determinant of cerebral autoregulation, estrogen may have important effects on modulatory mechanisms that maintain cerebral blood flow.

Autoregulation is a protective process that maintains cerebral blood flow and capillary perfusion pressure during changes in systemic blood pressure (6). Cerebral autoregulation is the net result of myogenic, metabolic, and neural influences on the blood vessels. Myogenic activity is defined as the intrinsic ability of vascular smooth muscle to maintain a partial state of contraction and to alter its state of contraction spontaneously or in response to transmural pressure changes (3, 20). Although it has been reported that myogenic behavior requires an intact endothelium (13), most evidence in the rat cerebral vasculature suggests that the endothelium merely modulates the magnitude of myogenic tone (4).

In the present study, myogenic tone was significantly lower in female cerebral arteries compared with the same sized artery in the male. The occurrence of a significant difference in myogenic tone between males and untreated females despite the lack of significant differences between these groups in mean serum concentration of estradiol can be explained by the cyclic fluctuation of estradiol in the untreated female rat (2, 36). Estradiol concentrations were shown to range from 17 ± 2 to 88 ± 2 pg/ml during the normal 4-day rat estrous cycle (2). On the day of proestrus, circulating levels of estradiol have been found to be elevated between 1030 and 1500 h (37–38 pg/ml) but significantly decreased between 1800 and 1930 h (9 pg/ml) (36). In the present study, the serum estradiol concentrations measured in untreated females fall within the reported physiological range. Thus the differences in myogenic tone between females and males could result from cyclic exposure to elevated estrogen concentrations in the female. The role of estrogen was further explored using Ovx and Ovx + Est females. After ovariectomy (mean serum estradiol levels <10 pg/ml), female arteries attained levels of myogenic tone similar to arteries from males. After 4 wk of estrogen replacement (mean serum estradiol levels ≥ 35 pg/ml) in ovariectomized females, arteries maintained myogenic tone at levels similar to those of arteries from normal, cycling females. Together, these data suggest that either cyclic or sustained exposure to circulating estrogen modulates myogenic tone in the rat cerebral vasculature.

One target of the effect of estrogen is the vascular endothelium. In a variety of experimental models, the effect of gender and estrogen status on endothelial function appears related to NO formation. For example, basal release of NO from endothelium-intact aortic rings from both rats and rabbits was greater in females than males (15, 19). In another study estrogen was shown to modulate myogenic tone in skeletal and coronary vascular beds through endothelium- and NOS-dependent mechanisms. Studies in humans indicate that when postmenopausal women received 17β-estradiol, the circulating levels of NO metabolites, nitrite and nitrate, were increased (34, 35).

Estrogen may act through a genomic mechanism to upregulate NOS-III, the constitutively expressed NOS in endothelial cells. In fact, upregulation of NOS-III mRNA by estrogen has been shown in cultured endothelial cells (24). Estrogen could also augment the activity of NOS-III by increasing cofactor or substrate availability necessary for the synthesis of NO (19). Alternatively, estrogen could decrease the rate of inactivation of NO by inhibiting superoxide anion formation or by increasing the concentration and/or activity of NO-protective free radical scavengers like superoxide dismutase or glutathione (19).

If, in the present study, the primary site of action of estrogen is to modulate production of NO from vascular endothelium, then inhibition of NOS should increase myogenic tone in arteries from untreated and Ovx + Est females to levels found in arteries from males and Ovx females. Indeed, when NOS was inhibited with L-NAME, all arteries developed significantly greater myogenic tone after each pressure step compared with arteries with active NOS. Furthermore, arteries from untreated and Ovx + Est females showed a significantly greater effect of inhibiting NOS than arteries from either males or Ovx females. Thus, after inhibition of NOS, original differences in myogenic tone with gender and estrogen status were abolished. These findings suggest that estrogen has no effect on fundamental mechanisms of the myogenic response. Rather, estrogen modulates NO production and/or action, resulting in modulation of myogenic tone.

Because an arginine derivative such as L-NAME is a competitive inhibitor of NOS, these effects of L-NAME should be reversed by L-arginine (27, 33). Indeed, addition of L-arginine restored the original level of myogenic tone in arteries from males and Ovx females. L-Arginine also significantly reversed effects of L-NAME in arteries from untreated and Ovx + Est females, although these effects were incomplete under the conditions tested. Recently published studies show similar effects of NOS inhibition on gender differences in myogenic tone in coronary and skeletal muscle arteries (17, 40). These data are consistent with the hypothesis that estrogen targets the vascular endothelium and, by increasing either the expression and/or activity of NOS, modulates myogenic tone through enhanced synthesis of NO.

The role of estrogen in modulating vascular reactivity was further characterized in the present study by investigating the role of one possible target of the action of NO. NO signal transduction involves activation of soluble guanylate cyclase in vascular smooth muscle, leading to the subsequent activation of cGMP-dependent protein kinase (PKG) (23). The NO-cGMP-PKG signal transduction axis has been shown to activate multiple modulatory and vasodilatory mechanisms (23). However, a recently published study suggests that BK_{Ca} channels are primary targets of NO (40). We therefore hypothesized that inhibition of BK_{Ca} channels should cause significantly greater constriction in

arteries from untreated and Ovx + Est females than arteries from either males or Ovx females. Furthermore, if this hypothesis were correct, the overall level of myogenic tone after inhibition of BK_{Ca} channels should be identical to the level of myogenic tone after inhibition of NOS. When TEA was used to inhibit BK_{Ca} channels, all vessels from each group developed greater myogenic tone (passive minus TEA), demonstrating that activation of BK_{Ca} channels does indeed modulate the development of myogenic tone (29, 30). However, in the presence of TEA, arteries from males and Ovx females still possessed significantly greater myogenic tone than arteries from untreated or Ovx + Est females. Thus, although the BK_{Ca} channel is thought to be modulated by NO, it may be only one of multiple mechanisms that are modulated by the NO-cGMP-PKG cascade.

Modulatory effects of estrogen on cerebral blood vessels appear to be more dependent on alterations in NO than on changes in smooth muscle characteristics. Distensibility, or the transient expansion of the vessel wall after increased pressure, is dependent on both the activation state (contractility) of the muscle (29, 30) and physical properties (collagen, elastin) of the vessel wall (3, 10). The passive mechanical properties (maximal diameter in 0 Ca^{2+}-EDTA) of arteries from all four groups of animals in the present study were not significantly different. Thus we do not believe that estrogen status changed the structural composition of the vessel wall, even though estrogen has been shown to inhibit proliferation of vascular smooth muscle cells (3, 16).

Because distensibility is also directly related to the activation state of the vascular smooth muscle (41), arteries from males and Ovx females, which possess greater myogenic tone, should be less distensible than arteries from untreated or Ovx + Est females. Indeed, the transient expansion of the artery wall after each step increase in pressure was significantly smaller in arteries from males and Ovx females than arteries from either untreated or Ovx + Est females. These data support the hypothesis that reduced function of the NOS system places arteries from males and Ovx females in a greater state of activation than arteries from either untreated or Ovx + Est females. This finding is consistent with previous work showing that estrogen, working through an NO-dependent mechanism, modulates the initial activation state of vascular smooth muscle in peripheral arteries (26).

The diameter and myogenic tone differences attributed to gender and estrogen status in the present study of arteries 280–300 μm in diameter were not seen in a recently published study of smaller rat cerebral arteries (approximate diameter 200 μm) despite apparent greater basal release of NO in arteries from normal and Ovx + Est females (38). Interestingly, the lack of gender- or estrogen-dependent difference in myogenic tone of smaller cerebral vessels (≤200 μm) is supported by preliminary results from our laboratory using rat cerebral arteries with passive diameters of ~200 μm (11). The precise mechanisms responsible for these disparate findings are unknown. However, both myogenic reactivity and basal production of NO in rat cerebral arteries have been shown to vary with vessel size (5, 7, 11). These findings may suggest that the effects of estrogen on vascular reactivity and basal NO production may operate within a response gradient dependent on artery diameter.

The effect of estrogen on NOS is certainly not restricted to modulation of myogenic tone of cerebral blood vessels but probably reflects a general increase in arterial NOS throughout the body. This modulatory hypothesis is supported by recent findings in two other vascular beds, coronary and skeletal, where estrogen reduced myogenic tone through an endothelium- and NOS-dependent mechanism (17, 40). Although the role of estrogen-induced modulation is unknown, a general broadening of the range of myogenic control of vascular resistance would benefit organ autoregulation and capillary perfusion during changes in systemic pressure (8, 21).

In conclusion, male-female differences were found in the myogenic response of rat cerebral arteries to changes in transmural pressure. It appears that circulating estrogen decreases myogenic tone indirectly by increasing endothelial production of NO. Ovariectomy resulted in increased myogenic tone and decreased sensitivity to NOS inhibition, effects that were reversed by chronic estrogen replacement. The physiological implications of the effects of estrogen on the cerebral circulation remain to be determined. However, myogenic tone is an important fundamental property of cerebral arteries relevant to the autoregulation of cerebral blood flow. Thus the current findings may help to explain differences in incidence of cerebrovascular disease such as stroke and migraine that are associated with gender and estrogen status.

We thank Jonnie Sephus for technical assistance with the radioimmunoassay.

This work was supported by National Heart, Lung, and Blood Institute Grant RO1-HL-50775.

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Received 22 December 1997; accepted in final form 1 April 1998.

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