Estrogen and Tamoxifen Modulate Cerebrovascular Tone in Ovariectomized Female Rats

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Abstract—Postmenopausal estrogen deficiency increases the incidence of cerebrovascular disease. However, hormone replacement therapy is associated with an increased cardiovascular risk. Tamoxifen is a selective estrogen receptor modulator with estrogenic effects on cardiovascular risk factors, but its long-term impacts on cerebral vasculature are unknown. We hypothesized that chronic 17β-estradiol or tamoxifen treatment exerted similar effects in reducing cerebrovascular tension in ovariectomized rats. We therefore determine whether (1) chronic 17β-estradiol treatment could influence vasomotor activities, (2) chronic tamoxifen therapy could exert an estrogen-like or estrogen-antagonistic effect, and (3) acute exposure to estrogen could mimic the effect of 17β-estradiol. Isometric tension was measured in cerebral arteries from female rat groups: control, ovariectomy, ovariectomy plus 17β-estradiol treatment, ovariectomy plus tamoxifen treatment, and ovariectomized rats treated with tamoxifen and 17β-estradiol. Ovariectomy enhanced cerebrovascular contractions to endothelin-1 or CaCl2, but not to U46619 or phenylephrine. 17β-Estradiol therapy reversed these effects. Chronic tamoxifen treatment exerted estrogen-like actions by reversing ovariectomy-induced enhancement of vessel tone without antagonizing the effect of chronic 17β-estradiol treatment. Ovariectomy enhanced the relaxing potency of nicardipine, and 17β-estradiol treatment prevented this effect. Acute exposure to 10^-7 mol/L 17β-estradiol or 10^-8 mol/L tamoxifen did not modulate contractions in rings from nonoperated female rats. In conclusion, ovariectomy differentially enhances agonist-induced cerebrovascular tone, an effect that was reversed by estrogen therapy. Tamoxifen does not act as an estrogen antagonist; instead, it functions as an estrogen agonist during estrogen deficiency. Thus, tamoxifen may confer beneficial effects similar to estrogen in cerebrovascular vessels.

Key Words: cerebral arteries ■ estrogen ■ vasoconstriction ■ rats

There appears to be a significant age dependency of coronary artery disease and stroke among women. The risk of cardiovascular disease in women increases sharply after menopause. In pooled analyses, observational studies suggest significant reductions in cerebrovascular and cardiovascular diseases in postmenopausal women receiving estrogen replacement therapy. However, the first randomized trial of hormone replacement therapy for primary prevention of heart disease found no overall benefit. Thus, use of hormone therapy for even its approved indications probably has limited merit.

Epidemiological studies suggest that the risk of stroke is lower in premenopausal women. In support of clinical observations, experimental studies show that female animals experience less brain injury after stroke than males, and this protection disappears after ovariectomy. Estrogen reduces stroke injury in ovariectomized (Ovx) or estrogen-deficient female animals. However, other studies show little impact of estrogen on nonfatal strokes in women. Estrogen is neuroprotective in different models of cerebral ischemia and ameliorates ischemic damage by improving cerebral perfusion to the ischemic brain, by recruitment of collateral arteries during cerebral artery occlusion, or by perfusion-independent mechanisms. There is also evidence for the induction of heat shock proteins in male and female rat cerebral arteries, which may involve the protective effects of estrogen during ischemic injury. Despite its beneficial effects in experimental models of cerebral ischemia, the role of estrogen replacement therapy in stroke prevention remains to be determined.

Estrogen levels correlate directly with cerebral artery blood flow velocity in women undergoing ovarian hyperstimulation, suggesting an important influence of sex steroids on cerebral circulation. Estrogen enhances carotid and cerebral blood flow and reduces cerebral vascular resistance in postmenopausal women. Estrogen regulates cerebral autoregulation by enhancing basal release of NO and so reducing pressure-induced myogenic constriction. There are many studies on effects of physiological levels of estrogen on systemic vascular beds, but little is known about long-term...
Effects of selective estrogen receptor modulators (SERMs), either alone or in combination with estrogen on cerebrovascular tone.

Most women discontinue estrogen treatment because of its many side effects or the increased risk of breast and uterus cancer. This, in large part, has contributed to development of SERMs, such as tamoxifen and raloxifene, as alternative therapeutic agents. A SERM is a molecule that binds with high affinity to estrogen receptors but has tissue-specific effects distinct from estrogen, acting as an estrogen agonist in some tissues and as an antagonist in others. SERMs, such as tamoxifen, potentially confer cardiovascular benefits in women without increasing estrogen-associated risks of breast cancer. Tamoxifen is used primarily in breast cancer patients. The clinical efficacy of tamoxifen is greatest in premenopausal women who have circulating estrogen. Of interest is the increased use of combined tamoxifen and hormone-replacement therapy for breast cancer risk reduction in postmenopausal women. Tamoxifen exerts an estrogenic action by acutely relaxing blood vessels. Despite long-standing use of tamoxifen, few studies have addressed its long-term impact on cardiovascular function.

In this study, we investigated the effects of chronic administration of tamoxifen (with and without concurrent estrogen replacement) on cerebral artery tone of Ovx rats. Thus, we examined whether (1) 17β-estradiol (E2) replacement therapy could reverse vascular changes produced by ovariectomy, (2) chronic tamoxifen could mimic or antagonize the effect of E2, and (3) acute treatment with E2 or tamoxifen mimics effects of chronic treatment.

Methods

An expanded Methods section can be found in an online supplement available at http://www.hypertensionaha.org.

Force Measurement

A 2-mm segment of the posterior communicating cerebral artery was mounted in a Multi Myograph System (Danish Myo Technology) for measurement of isometric contractions. Each ring was bathed in Krebs solution aerated with 95% O2–5% CO2 at 37°C. The segment was stretched to a previously determined optimal tension of 0.3 mN and allowed to stabilize for 90 minutes.

Cumulative concentration-dependent contractions to endothelin-1, U46619, phenylephrine, and extracellular K⁺ in normal Krebs solution or to CaCl2 in Ca2+-free, 6×10⁻⁵ mol/L K⁺ solution were determined. For acute experiments, 2 concentration-response curves for phenylephrine, U46619, or CaCl2 were obtained. The second concentration-response curve was made with and without incubation (1 hour) with 10⁻⁹ mol/L E2, 10⁻⁸ mol/L tamoxifen, or vehicle. Because arteries developed desensitization to endothelin-1, each experiment began with repeated contraction by high K⁺ until sustained responses were repeatable. Rings were then exposed to E2 or tamoxifen before addition of endothelin-1.

Results

Serum E2 Levels, Uterine Weights, Body Weights, and Mean Arterial Blood Pressure

E2 therapy, but not tamoxifen therapy, increased serum E2 levels in Ovx rats. Ovariectomy reduced uterine weights, and E2 or tamoxifen treatment attenuated the effect of estrogen deficiency. Ovx rats had higher body weights than sham-operated controls, E2-treated, or tamoxifen-treated rats. Mean arterial blood pressure in all groups was similar (Figure 1, available online at http://www.hypertensionaha.org).

Effects of Chronic E2 and Tamoxifen Treatment on Agonist-Induced Contraction

Tracings in Figure 1A show that endothelin-1 produced concentration-dependent increments in cerebrovascular tone. Endothelin-1–induced contractions were increased in Ovx compared with control rats, whereas chronic E2 treatment partially reversed the effect of ovariectomy (Figure 1B). Tamoxifen treatment exerted an estrogen-like effect by fully reversing ovariectomy-induced enhancement of contractions (Figure 1C). To test whether tamoxifen treatment could antagonize the effect of E2, Ovx rats were implanted with tamoxifen and E2 pellets. Figure 1D shows that tamoxifen did not inhibit the effect of chronic E2; instead, tamoxifen appeared to be more effective than E2 in attenuating the enhanced contraction to 3×10⁻⁸ mol/L endothelin-1 in Ovx rats. Emax values for endothelin-1–induced contractions are summarized in Figure 1E.

In contrast, U46619-induced contractions were similar in rings from control, Ovx, and Ovx+E2 rats (Figure 2A). Tamoxifen treatment insignificantly enhanced U46619-induced response (Figure 2B). Similarly, ovariectomy did not alter phenylephrine-induced responses (Figure 2C). Neither E2 nor tamoxifen treatment affected contractions to phenylephrine (Figure 2C and 2D). For contractions induced by
U46619 or phenylephrine, $E_{max}$ values are summarized in Figure 2E and 2F.

**Effects of Chronic E$_2$ and Tamoxifen Treatment on Ca$^{2+}$/H$_{11545}$-Induced Contraction**

Traces in Figure 3A show that CaCl$_2$ produced enhanced contractions in arteries from Ovx rats. Chronic E$_2$ or tamoxifen treatment prevented this effect. E$_2$ and tamoxifen were equally effective (Figure 3B and 3C). Tamoxifen treatment did not antagonize the effect of E$_2$, nor did it produce additive effects (Figure 3D). Maximal contractions to CaCl$_2$ are summarized in Figure 3E.

High K$^+$-induced responses were enhanced in rings from Ovx rats, and chronic treatment with either E$_2$ or tamoxifen prevented this effect (Figure III, available online at http://www.hypertensionaha.org).

**Effects of Chronic Treatment With E$_2$ and Tamoxifen on Vessel Tone in Control Rats**

Contractions to endothelin-1, phenylephrine, or CaCl$_2$ were unaffected in cerebral arteries from control rats chronically treated with E$_2$ or tamoxifen (Figure III, available online at http://www.hypertensionaha.org).

**Effects of Chronic E$_2$ on Nicardipine-Induced Relaxation**

In K$^+$-contracted rings, nicardipine induced relaxations with a $pD_2$ of 9.40±0.035 (n=6) in control arteries. Ovariectomy enhanced the relaxant effect of nicardipine ($pD_2$ 9.64±0.047; n=7; $P<0.05$ versus control; Figure 4A), and this enhancement was prevented by E$_2$ ($pD_2$ 9.43±0.06; n=6; $P<0.05$ versus Ovx; Figure 4B).

**Effects of Acute E$_2$ and Tamoxifen Treatment**

In U46619-contracted cerebral arteries from nonoperated female rats, E$_2$ ($pD_2$ 6.40±0.10; n=6) and tamoxifen ($pD_2$ 5.16±0.04; n=4) induced relaxations. Threshold concentrations for dilation were $>3\times10^{-8}$ mol/L for E$_2$ and $10^{-8}$ mol/L for tamoxifen. Acute exposure to E$_2$ ($10^{-9}$ mol/L) or tamoxifen ($10^{-8}$ mol/L) did not affect agonist-induced contractions (Table I, available online at http://www.hypertensionaha.org).

**Discussion**

This study examined the influence of circulating estrogen receptor ligands on cerebrovascular responses to receptor-dependent and receptor-independent vasoconstrictors in female rats. There are several novel findings of this study using isolated rat cerebral arteries. First, ovariectomy differentially impacts cerebrovascular tone by enhancing contractions to endothelin-1 or CaCl$_2$, but not to U46619 or phenylephrine. Second, E$_2$ replacement therapy reverses the effect of ovariectomy on vasomotor activity, but acute treatment with E$_2$ or tamoxifen did not modulate cerebral artery tone. Third,
chronic tamoxifen treatment mimics E₂ by preventing the effect of ovariectomy. Finally, tamoxifen is not antiestrogenic and fails to antagonize the chronic effects of E₂. To the best of our knowledge, this is the first study that examines long-term effects of tamoxifen compared with estrogen on vascular reactivity.

Cerebral artery contractions to endothelin-1 or CaCl₂ were markedly enhanced in Ovx rats. Ovariectomy also augments endothelin-1-induced contractions in rabbit cerebral arteries.²⁵ Our results show that ovariectomy-induced enhanced contractions are prevented after chronic E₂ treatment. E₂ inhibits endothelin-1-induced vessel tone in Ovx rats and reduces protein and mRNA expression of endothelin A receptors in vascular smooth muscle,²⁶ suggesting that enhanced vascular endothelin receptor expression may partly mediate estrogen deficiency–induced increase in vasoconstrictive responses to endothelin-1. In contrast, ovariectomy did not influence contractions induced by U46619 and phenylephrine. Contractions to U46619 are similar in mesenteric arteries in Ovx rats and Ovx+E₂ rats.²⁷ Estrogen treatment does not affect phenylephrine-induced contraction in rabbit femoral arteries.²⁸ These data indicate that cerebrovascular tone can be modulated differentially by the circulating level of estrogen with an outcome depending on the constrictor or vascular beds and possibly species chosen for study.

Tamoxifen produces acute relaxant effects in rabbit and porcine coronary arteries²³,²⁴ and reduces oxyhemoglobin-induced cerebral vasoconstriction.²⁹ A finding of this study is that tamoxifen exerts an estrogenic effect on cerebrovascular tone without altering circulating E₂ levels in Ovx rats. Enhanced contractions in Ovx rats were attenuated or prevented by tamoxifen therapy. Tamoxifen, like E₂, exerts differential modulatory effects on cerebrovascular responses. It suppressed contractions induced by endothelin-1 or CaCl₂, but not by phenylephrine or U46619.

Tamoxifen, a partial estrogen agonist/antagonist may interact with nuclear estrogen receptors.³⁰ Although our data clearly show similar effects of chronic treatment with tamoxifen and E₂, it is unknown whether tamoxifen antagonizes the vascular action of E₂ in estrogen-deficient animal models. Our results demonstrate that chronic tamoxifen does not inhibit effects of E₂ treatment on cerebrovascular contractions. In addition, the effect of a combined treatment with E₂ and tamoxifen was nonadditive. Therefore, tamoxifen is likely to be an estrogen agonist in rat cerebral circulation.

Enhanced contractile responses to membrane depolarization (induced by elevated extracellular K⁺) in Ovx rats raises a possibility that estrogen deficiency may upregulate vascular voltage-gated Ca²⁺ channel activity. This is partially supported by our observation that nicardipine, an L-type Ca²⁺ channel blocker, produced a greater relaxant effect in rings from Ovx rats. The increased relaxing potency of nicardipine was prevented by E₂ treatment. Ovariectomy increases mRNA expression of the α₁c subunit of L-type Ca²⁺ channels in the rat aorta, and this effect is prevented in Ovx rats treated with E₂ or tamoxifen.³¹

Acute treatment with E₂ (10⁻⁹ mol/L) or tamoxifen (10⁻⁸ mol/L) did not inhibit contractions to the 4 constrictors, indicating that neither estrogen nor tamoxifen acutely modulate cerebral artery tone. In contrast, chronic treatment with both estrogen receptor agonists prevented ovariectomy-induced enhanced vasoconstriction. However, acute treatment with higher concentrations (≥10⁻⁶ mol/L) of E₂ reduces agonist-induced vasoconstrictions.³² Thus, acute and non-genomic effects of estrogen and tamoxifen may differ from their long-term and genomic effects on vascular motor activity.

One limitation of the current model is use of isolated artery ring segments, which do not mimic their physiological settings. In their natural state, cerebral arteries are exposed continuously to flow and sheer stress, both of which can affect vascular reactivity.³³ Isometric contractile responses may be different had they been investigated in perfused and pressurized vessels under isobaric conditions. In addition, it should also be pointed out that there are currently no suitable models of menopause. Most investigators have used relatively young (aged 6 to 12 weeks) female rats to study menopause. This may not be appropriate when examining vascular changes occurring during menopause;³⁴ studies to determine whether tamoxifen and E₂ exert similar vascular benefits in aged Ovx rats are under way.

In conclusion, our study demonstrates that ovariectomy differentially modulates cerebrovascular tone induced by different vasoconstrictors. E₂ prevents ovariectomy-induced enhancement of cerebral contraction. Tamoxifen exerts an estrogen-like effect but does not act as an antagonist of estrogen in rat cerebral arteries. In contrast, chronic treatment with E₂ or tamoxifen did not affect agonist-induced cerebrovascular tone in control rats. These new findings suggest that
estrogen and tamoxifen exert beneficial effects in cerebral circulation during estrogen deficiency.

Perspectives
Adjuvant treatment with tamoxifen is used to treat breast cancer in premenopausal women. This study demonstrates that in the absence of normal circulating estrogen, tamoxifen mimics the effect of estrogen on cerebral artery contractility, and that tamoxifen does not antagonize the vascular effect of exogenous estrogen. Besides, inhibition of expression or function of the voltage-gated Ca\(^{2+}\) channel may, in part, mediate effects of chronic estrogen treatment on cerebrovascular tone. Although we cannot attribute this observation in rats to therapy in humans, it does suggest that tamoxifen for treatment of breast cancer in the absence (postmenopausal women) or presence (premenopausal women) of circulating estrogen may have protective cerebrovascular effects.

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