17β-Estradiol decreases vascular tone in cerebral arteries by shifting COX-dependent vasoconstriction to vasodilation

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Ospina, Jose A., Sue P. Duckles, and Diana N. Krause. 17β-Estradiol decreases vascular tone in cerebral arteries by shifting COX-dependent vasoconstriction to vasodilation. Am J Physiol Heart Circ Physiol 285: H241–H250, 2003.—We have previously shown that estrogen treatment increases cerebrovascular cyclooxygenase-1, prostacyclin synthase, and production of prostacyclin. Therefore, vascular tone and prostanoïd production were measured to investigate functional consequences of estrogen exposure. Middle cerebral arteries were isolated from ovariectomized female Fischer-344 rats with or without chronic in vivo 17β-estradiol treatment. In vivo 17β-estradiol treatment increased cerebral artery diameter; functional endothelium was required for expression of these differences. The nonspecific cyclooxygenase inhibitor indomethacin constricted, whereas arachidonic acid dilated, cerebral arteries from estrogen-treated animals. Estrogen exposure increased production of prostacyclin by cerebral arteries. Conversely, in estrogen-deficient animals, indomethacin dilated and arachidonic acid constricted cerebral blood vessels. This correlated with vasorelaxation following inhibition of the thromboxane-endoperoxide receptor with SQ-29548 but not after selective blockade of thromboxane synthase with furegrelate, suggesting prostaglandin endoperoxide (i.e., PGH2) activity. Removal of the endothelium or selective blockade of cyclooxygenase-1 with SC-560 abolished estrogen-mediated differences in the effects of arachidonate on vessel diameter and on prostacyclin production by cerebral arteries. These data suggest 17β-estradiol decreases cerebrovascular tone by shifting the primary end product of the endothelial cyclooxygenase-1 pathway from the constrictor prostaglandin PGH2 to the vasodilator prostacyclin. These effects of estrogen may contribute to the heightened thromboresistance and enhanced cerebral blood flow documented in pre- versus postmenopausal women.

cyclooxygenase; prostacyclin; prostaglandin endoperoxide

CEREBRAL VASCULAR FUNCTION is affected by variations in circulating estrogen levels. This influence is apparent whether hormone levels are altered physiologically due to sex, menstrual cycle, and menopause or by exogenous manipulations such as ovariectomy and hormone replacement therapy. Overall, estrogen appears to enhance cerebral blood flow (CBF). For example, young women exhibit higher CBF than men; this sex difference dissipates by the sixth decade of life, a period that coincides with declining ovarian function and the onset of menopause (6, 35, 39). CBF varies over the menstrual cycle (2, 7) and is increased by estrogen administration in postmenopausal women (24, 30).

Cerebrovascular effects of estrogen may afford some protection against stroke, ischemia-reperfusion injury, brain trauma, and menstrual migraine (1, 19, 46). For example, numerous studies have found that premenopausal women are less likely to suffer from cerebrovascular disease compared with age-matched men (43); however, with the onset of menopause, sex differences in stroke incidence are lost (47). These observations have led to clinical trials of hormone replacement therapy for the reduction of stroke risk, morbidity, and mortality; however, the results so far have been controversial (9, 15, 36). In contrast, studies in animal models of stroke consistently support the contention that estrogen is neuroprotective (19, 38). Certainly, a better understanding of the mechanisms underlying the beneficial effects of estrogen on the vasculature is needed to optimize possible therapeutic applications.

A growing body of evidence indicates that estrogen promotes the release of endothelium-derived factors that would augment cerebral perfusion and confer resistance against thrombotic events. Nitric oxide (NO) production by cerebrovascular endothelium is clearly enhanced by estrogen (11, 25, 26). Recently, we found evidence that estrogen also upregulates production of the prostanoïd vasodilator prostacyclin (PGI2) in cerebral blood vessels (32). Protein levels of both cyclooxygenase-1 (COX-1) and PGI2 synthase were elevated following chronic estrogen treatment of ovariectomized (Ovx) rats (32). At that time, however, we did not assess functional consequences of these estrogen-mediated changes. We hypothesized that increases in COX-1 and PGI2 synthase would result in greater release of PGI2 to dilate cerebral arteries. Therefore, in the current study, we measured vascular tone in pressurized, small caliber branches of middle cerebral arteries isolated from Ovx female rats with and without chronic in vivo 17β-estradiol treatment. We investigated the vascular response to blockers of prostanoïd pathways and exogenously applied arachidonic acid and measured the production of vasoactive prostanoïds. Estrogen did indeed enhance prostanoïd-medi...
ated vasodilation; however, in the absence of estrogen, arachidonic acid was actively converted to a COX-1-dependent constrictor. Thus estrogen-mediated elevation in COX-1 and PGI2 synthase appears to shift the balance of prostanooid products from constrictor to dilator.

MATERIALS AND METHODS

In vivo 17β-estradiol treatment. All protocols involving the use of animals were approved by the Institutional Animal Care and Use Committee at the University of California-Irvine. Ovx rats and Ovx rats treated with estrogen (Ovx + E) were prepared as described (11, 25). Briefly, 3-mo-old female Fischer-344 rats (Charles Rivers-SASCO Laboratories) were anesthetized (46 mg/kg ketamine and 4.6 mg/kg xylazine ip) and then ovariecromized. At the time of ovarietomy, some rats were also implanted subcutaneously with a 17β-estradiol-filled silicone capsule. Approximately 1 mo postsurgery, animals were anesthetized by CO2 and blood samples were obtained by cardiac puncture for measurement of serum 17β-estradiol levels by radioimmunoassay (Diagnostic Products). Rats were then euthanized by decapitation, and blood remaining in the vessel lumen. The distal end of the arteries were subsequently pressurized to 60 mmHg and vessel diameters were recorded following exposure to increasing concentrations of inhibitors-antagonists of the prostanooid cascade to define the role of vessel-derived prostaglandins in vascular tone modulation. The concentrations of inhibitors-antagonists used were chosen based on those reported in the literature. Indomethacin (10 μmol/l; Sigma; St. Louis, MO) was used to inhibit COX, furegrelate (5 μmol/l; Cayman Chemical; Ann Arbor, MI) was used to selectively inhibit thromboxane A2 (TXA2) synthase (18), and SQ-25548 (1 μmol/l; Cayman Chemical) was used to selectively block the TXA2-PGH2 receptor (5, 18). After a 30-min exposure to the drugs, pressure was returned to 60 mmHg. Arteries were then superfused with PSS containing various inhibitors-antagonists and vessel diameters were recorded. In some experiments, the endothelium was removed by passing air through the vessel lumen as previously described (10–12). Once a stable diameter was reached after endothelial denuding, diameters at various pressure steps were again recorded. At the conclusion of all experiments, vessels were superfused with Ca2+-free PSS containing 3 mmol/l EDTA for 10–15 min, and the passive intraluminal diameter and wall thickness were measured at each pressure step. Vascular tone at any given pressure was defined as the difference between the diameters in EDTA (passive) and PSS. The effects of prostanooid pathway inhibitors-antagonists were expressed as the change in diameter (in μm) relative to the PSS baseline.

Table 1. Effect of treatment on 17β-estradiol serum concentration, body weight, and uterine weight

<table>
<thead>
<tr>
<th>Rat Group</th>
<th>17β-Estradiol, pg/ml</th>
<th>Body Weight, g</th>
<th>Uterine Weight, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovariectomized</td>
<td>9 ± 1</td>
<td>196 ± 2</td>
<td>20 ± 1</td>
</tr>
<tr>
<td>Ovariectomized + 17β-estradiol</td>
<td>68 ± 8*</td>
<td>172 ± 2*</td>
<td>93 ± 3*</td>
</tr>
</tbody>
</table>

Values represent means ± SE; n = 39–52 rats. Statistical differences were determined by Student’s t-test. *Significantly different from ovariectomized rats (P < 0.05).

Prostanoid assays. To examine the effects of chronic estrogen treatment on pial arterial prostanooid production, vessels from Ovx and Ovx+E animals were incubated in vitro, and the medium was analyzed for PG12, TXA2, PGF2α, PGE2, and 8-isoprostane. Briefly, freshly isolated brains were placed in ice-cold physiological saline solution containing 122 NaCl, 1.6 CaCl2, 5.6 NaHCO3, 5.2 KCl, 1.2 MgSO4, 1.2 KH2PO4, 0.03 EDTA, and 11.5 dextrose (all from Fisher Scientific; Pittsburgh, PA) and bubbled with 95% O2-5% CO2. With the use of a dissecting microscope, a 2- to 3-mm segment of a small caliber branch of the middle cerebral artery (MCA) from the area overlying the parietal cortex was isolated. Arteries with a passive intraluminal diameter of ~130–140 μm at a pressure of 80 mmHg were chosen for this study. Once isolated, arteries were cleaned of any adhering tissue and placed into an arteriograph containing PSS (Living Systems Instrumentation; Burlington, VT). One end of the artery was mounted to the selective COX-1 inhibitor SC-560 (1 μmol/l; Sigma; St. Louis, MO) was used to inhibit COX, furegrelate (5 μmol/l; Cayman Chemical; Ann Arbor, MI) was used to selectively inhibit thromboxane A2 (TXA2) synthase (18), and SQ-25548 (1 μmol/l; Cayman Chemical) was used to selectively block the TXA2-PGH2 receptor (5, 18). After a 30-min exposure to the drugs, pressure was returned to 60 mmHg. Arteries were then superfused with PSS containing various inhibitors-antagonists and vessel diameters were recorded. In some experiments, the endothelium was removed by passing air through the vessel lumen as previously described (10–12). Once a stable diameter was reached after endothelial denuding, diameters at various pressure steps were again recorded. At the conclusion of all experiments, vessels were superfused with Ca2+-free PSS containing 3 mmol/l EDTA for 10–15 min, and the passive intraluminal diameter and wall thickness were measured at each pressure step. Vascular tone at any given pressure was defined as the difference between the diameters in EDTA (passive) and PSS. The effects of prostanooid pathway inhibitors-antagonists were expressed as the change in diameter (in μm) relative to the PSS baseline.

In a separate set of experiments, the effects of the exogenous prostanooid precursor arachidonic acid were determined in pressurized, isolated arteries from Ovx and Ovx+E animals. After the initial 1-h equilibration period, transmural pressure was maintained at 60 mmHg, and vessel diameters were recorded following exposure to increasing concentrations of arachidonic acid (10−8 to 10−6 mol/l; Cayman Chemical). Control experiments were performed in the presence of equivalent volumes of vehicle (<0.05% ethanol by volume) alone. In addition, the effects of arachidonic acid were determined after endothelium removal or after a 30-min exposure to the selective COX-1 inhibitor SC-560 (1 μmol/l; Cayman Chemical). This concentration of SC-560 was chosen to achieve selective inhibition of COX-1, without affecting COX-2, based on the reported IC50 values for SC-560 with respect to these enzymes (41). Concentration-response experiments were also performed with the TXA2-PGH2 receptor agonist U-46619 (10−10 to 10−6 mol/l; Cayman Chemical). For these studies, endothelium-intact vessels from Ovx and Ovx+E rats were pressurized at 20 mmHg to lower resting vascular tone without the use of vasodilating agents. Concentration-response data were expressed as the change in diameter (in μm) induced by drug exposure. EC50 values for U-46619 concentration-response curves were determined by nonlinear regression analysis (GraphPad Prism 2.0 software; San Diego, CA).
into ice-cold 0.01 mol/l PBS under a dissecting microscope, and 8-mm-long segments of pial arteries, beginning about 1–2 mm proximal to the first main branch of the MCA, were isolated from each cortical hemisphere. Two arterial segments from the same animal were placed into a well of a 96-well plate containing incubation buffer and equilibrated in a tissue culture incubator (37°C and 95% O₂-5% CO₂) for 1 h. Arterial samples were then placed into fresh incubation buffer and maintained for 6 h at 37°C (95% O₂-5% CO₂). In some cases, 1 μmol/l SC-560 was added to assess the contribution of COX-1 to the prostanoid production. Arteries denuded by passing air through the lumen at the time of isolation were similarly incubated to determine whether vascular PGI2 and TXA2 production was endothelial dependent. After incubation, the medium was collected and immediately stored frozen at −20°C until analysis. PGI2 and TXA2 in the medium were detected as their respective stable metabolites 6-keto-PGF1α, or TXB2 by using commercially available enzyme immunoassay kits (Amersham; Piscataway, NJ). PGF2α, PGE2, and 8-isoprostane were directly measured by enzyme immunoassay (Assay Designs; Ann Arbor, MI). Assays and data analyses were performed according to the protocols provided by the manufacturer.

Statistical analysis. Data are expressed as means ± SE. Statistical analysis was performed with GraphPad Prism 2.0 software. Differences were assessed by one-way ANOVA followed by Newman-Keuls post hoc analysis. Alternatively, Student’s t-test was used where appropriate. For all comparisons, statistical significance was set at P ≤ 0.05.

RESULTS

In this study, we used a rat model to investigate the effects of chronic in vivo 17β-estradiol treatment on prostanoid production and modulation of contractile function in small caliber cerebral arteries. Vascular tone in pial arteries, pressurized in vitro, was compared in Ovx and Ovx+E animals (Fig. 1). The passive diameters of vessels used in this study were not statistically different between Ovx and Ovx+E (Fig. 1A). The cerebrovascular autoregulatory response to transmural pressure also was unaffected by estrogen treatment because total net constriction achieved over the range of pressures examined (i.e., 20–80 mmHg) was of similar magnitude between Ovx (23 ± 4 μm; n = 13) and Ovx+E (22 ± 2 μm; n = 9) vessels. However, at 40–80 mmHg, the absolute vessel diameter in PSS was significantly greater in arteries from estrogen-treated animals compared with those from the Ovx rats (Fig. 1A). Thus, as shown in Fig. 1B, estrogen treatment resulted in significantly lower vascular tone. After endothelial removal, all vessels became substantially constricted, and the estrogen-related differences in diameter were abolished (Fig. 1A). Artery wall thickness was unaffected by estrogen treatment (for example, at 80 mmHg, Ovx+E = 8 ± 1 μm, n = 10, vs. Ovx = 9 ± 1 μm, n = 12).

Considering the importance of endothelium-derived prostanooids in maintaining CBF, we examined whether COX pathways contribute to the functional differences between Ovx and Ovx+E vessels. In the presence of the COX-1-COX-2 inhibitor indomethacin, the difference between the diameters of Ovx and Ovx+E arteries in PSS was abolished (for example, diameter at 40 mmHg, Ovx+E = 84 ± 9 μm, n = 3, vs. Ovx = 93 ± 9 μm, n = 4). On closer inspection, it was evident that indomethacin had a significant effect on vessels from both Ovx and Ovx+E animals, but the nature of the effect was statistically different (Fig. 2). In cerebral arteries from Ovx rats, the diameters were significantly increased by indomethacin, and this vasodilatory response was thus consistent with the blockade of constrictor prostanooid activity. Conversely, in Ovx+E arteries, indomethacin caused a significant constriction indicating a predominance of dilator prostanooid activity following estrogen treatment.

To determine whether estrogen caused a shift in the balance of cerebrovascular prostanooid pathways, we measured the responses to the exogenous precursor arachidonic acid (Fig. 3). Vehicle (ethanol) alone had no

*Fig. 1. Effect of chronic estrogen treatment on diameter and vascular tone of small caliber cerebral arteries. A: diameters were recorded in physiological saline solution (PSS) following changes in transmural pressure (20–80 mmHg) in endothelium-intact and -denuded (−Endo) vessels from ovariectomized (Ovx) rats and Ovx rats given estrogen (Ovx+E). Passive diameters were determined in the presence of 3 mmol/l EDTA/Ca²⁺-free PSS. Mean data for passive, intact (PSS), and −Endo diameters are plotted together to facilitate comparisons. B: vascular tone was calculated for each experiment as the difference between passive and PSS diameters. Values represent means ± SE. *Significantly different from Ovx (P ≤ 0.05; n = 6–14).*
effect on the diameter of vessels from either Ovx or Ovx+E animals. In arteries from estrogen-treated animals, arachidonic acid caused a concentration-dependent dilation. However, in arteries from Ovx animals, arachidonic acid caused a concentration-dependent constriction (Fig. 3A). Vascular responses to arachidonic acid were significantly different between Ovx and Ovx+E at all concentrations beyond 0.01 μmol/l (Fig. 3A). If, however, the endothelium was removed or the vessels were exposed to the selective COX-1 inhibitor SC-560, then all the effects of arachidonic acid were eliminated in vessels from Ovx and Ovx+E animals (Fig. 3B).

To determine whether estrogen treatment altered the cerebrovascular production of dilator and/or constrictor eicosanoids, the release of various prostaglandins was quantified in vitro using pial arterial segments similar to those used in the contractile studies. Compared with the other substances measured (TXB2, PGF2α, PGE2, and 8-isoprostane), prostacyclin (measured as 6-keto-PGF1α) was the predominant prostanoid released by cerebral arteries (Fig. 4). Arteries from 17β-estradiol-treated rats produced approximately twofold more 6-keto-PGF1α compared with arteries from Ovx animals. This finding suggests that prostacyclin is responsible for the increased dilator prostanoid activity observed in cerebral arteries from estrogen-treated animals. Surprisingly, there was also a slight, yet significantly greater thromboxane (i.e., TXB2) production in vessels from Ovx+E compared with Ovx (Fig. 4B). When the endothelium was removed or the vessels incubated in the presence of the selective COX-1 inhibitor SC-560, the levels of 6-keto-PGF1α and TXB2 were reduced by ~90%, and the differences between vessels from Ovx and Ovx+E animals were no longer detected (Fig. 4, A and B). Low levels of PGF2α, PGE2, and 8-isoprostane were also produced by cerebral arteries; however, their production was unaffected by estrogen (Fig. 4C). From these results, it appears that increased COX-dependent constriction in Ovx arteries is not due to an increased production of thromboxane, PGF2α, PGE2, or 8-isoprostane.

Using a pharmacological approach, we sought to identify the prostanoid constrictor being elaborated by Ovx arteries. As shown in Fig. 5A, the selective TXA2-PGH2 receptor antagonist SQ-29548 caused a significant increase in the diameter of cerebral arteries from Ovx animals at every pressure examined. In contrast, SQ-29548 had no effect on the diameter of arteries from estrogen-treated Ovx animals. These results suggest that, in the absence of estrogen, female cerebral arteries produce a substance that causes constriction by activating the TXA2-PGH2 receptor.

In vessels of Ovx animals, removal of the endothelium abolished the effect of SQ-29548, supporting the endothelial derivation of a constrictor prostanoid (Fig. 5A). In contrast to SQ-29548, the selective TXA2 synthase inhibitor furegrelate did not alter the diameter of endothelium-intact vessels from Ovx animals (Fig. 5B), although it substantially reduced TXB2 production by blood vessels in vitro (Gonzales RJ, Krause DN, and...
Duckles SP; unpublished observations). The TXA2-PGH2 receptor agonist U-46619 was used to examine whether estrogen treatment altered the pharmacological properties of cerebral artery TXA2-PGH2 receptors. U-46619 caused a concentration-dependent constriction of arteries from both Ovx and Ovx+E animals (Fig. 6). However, there was no significant effect of estrogen on either of the EC50 values for U-46619 (Ovx = 37 ± 11 nmol/l vs. Ovx+E = 24 ± 8 nmol/l) or the maximal response (Emax, ∼61 ± 3 μm in Ovx vs. 58 ± 3 μm in Ovx+E).

DISCUSSION

The most striking finding of this study is that prostanoid-mediated constriction predominates in cerebral arteries from estrogen-deficient animals, whereas dilator prostanoids dominate after estrogen treatment (Fig. 7). The data suggest that 17β-estradiol decreases cerebrovascular tone by shifting the primary end-prod-

Fig. 4. Levels of prostanoid production by small-caliber cerebral arteries from Ovx and Ovx+E animals. A: PGI2 (measured as 6-keto-PGF1α). B: thromboxane A2 (TXA2) (measured as TXB2) from either intact, endothelium-denuded, or endothelium-intact vessels incubated with 1 μmol/l SC-560 was determined by ELISA (n = 3–5). C: PGE2, PGF2α, and 8-isoprostane production by endothelium-intact vessels from Ovx and Ovx+E rats was assessed (n = 7–10). Values represent means ± SE. *Significantly different from Ovx. #Significantly different from endothelium intact of same treatment group (P ≤ 0.05).

Fig. 5. Effect of estrogen treatment on cerebrovascular response to blockade of the TXA2-PGH2 pathway. A: diameter change in response to the TXA2-PGH2 receptor antagonist SQ-29548 (1 μmol/l) was determined in endothelium-intact Ovx and Ovx+E and endothelium-denuded Ovx cerebral arteries. Data are expressed as the change in diameter versus that in PSS alone at each pressure examined (20–80 mmHg). B: effect of the thromboxane synthase inhibitor furegrelate (5 μmol/l) on diameter of endothelium-intact Ovx cerebral arteries. Values represent means ± SE. #Significant change in diameter from PSS (P ≤ 0.05; n = 3).
uct of the endothelial COX-1 pathway from the constrictor prostaglandin endoperoxide H2 (i.e., PGH2) to the vasodilator PGI2. These observations were made in pressurized segments of small caliber branches of the rat MCA where endothelium-derived prostanoids had significant influences on arterial diameter. Similar effects in human arteries could contribute to changes in cerebrovascular function as a result of alterations in circulating estrogen levels.

We used an Ovx female rat model to explore the consequences of chronic 17β-estradiol treatment on the contractile function of small caliber cerebral arteries. Pressurized arteries from estrogen-treated rats were more dilated than arteries from the Ovx group. These differences were dependent on the presence of the endothelium, suggesting a role for endothelial vasodilators. In similar studies with female mice (10) and male rats (12), estrogen treatment also enhanced endothelium-dependent dilation of cerebral arteries. This effect was sensitive to N-nitro-L-arginine methyl ester (L-NAME) and indomethacin, inhibitors of NO synthase and COX, respectively. In contrast to the current study, in the main branch of MCA from female rats, estrogen treatment increased endothelial NO production, and there was no apparent contribution of COX pathways (11). However, the relative contributions of other endothelium-derived factors, NO and endothelium-derived hyperpolarizing factor (EDHF), have been shown to vary with cerebral vessel size (8, 49). Therefore, we hypothesized that, in smaller cerebral arteries, such as those used in the current study, prostanoids may play a more prominent role. Indeed, in the present study, the functional effects of COX inhibitors indicate a significant role for endothelial prostaglandins in smaller branches of the rat MCA (≤140 µm intraluminal passive diameter). In these arteries, COX-dependent mechanisms appear to be a major contributor to estrogen-mediated differences in vascular tone.

Currently, there are two known isoforms of COX (PGH synthase), and both are inhibited by indomethacin. In blood vessels, COX-1 is constitutively expressed, whereas COX-2 is not normally present but rather is induced during inflammation or infection (5). Both COX isoforms are rate-limiting enzymes that convert arachidonic acid to the prostaglandin intermediate PGH2. PGH2 is subsequently isomerized by prostanoid-specific synthases to a variety of prostaglandins. PGI2 and PGE2 are generally considered vasodilators in the cerebral circulation, whereas TXA2 and PGF2α are vasoconstrictors (3). We were able to measure production of all of these products in isolated segments of rat cerebral arteries, but clearly PGI2 was the predominant prostaglandin, as reported by others (3).

Of the prostaglandins measured, only PGI2 and TXA2 were altered in cerebral arteries after chronic in vivo estrogen treatment. Endothelial denuding or incubation with SC-560 at a concentration sufficient to inhibit COX-1 (1 µmol/l), but not COX-2, nearly abolished production of these prostanoids. This further supports the conclusion that endothelial COX-1 is the

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Fig. 6. Effect of estrogen treatment on U-46619 concentration-response curves in small-caliber arteries pressurized to 20 mmHg. Data are expressed as the change in diameter after exposure to 0.1 nmol/l U-46619. Values represent means ± SE (n = 5–6).

Fig. 7. Schematic representation of cerebrovascular prostaglandin pathways in the absence (A) and presence (B) of estrogen. COX-1 cyclooxygenase-1; cPLA2, cytosolic phospholipase A2; S, synthase; R, receptor.
primary source of these vasoactive substances. These data correlate with previous findings that estrogen increases the level of COX-1 protein in cerebral vessels (13, 32) and cultured endothelial cells (21). PGI₂ synthase levels also are elevated by estrogen in cerebral (32) and peripheral vessels (37). The latter alteration, in particular, would lead to an estrogen-mediated increase in PGI₂ production as observed in this study in the MCA, in our previous study of cerebral blood vessels (32), and in the uterine artery (14) and peripheral vascular endothelial cells (21).

In this study, we examined the effects of inhibiting COX-1 and COX-2 nonselectively with indomethacin or COX-1 selectively with SC-560. However, we did not examine the effects of selective inhibition of COX-2 on prostanoid production or the functional arterial response to pressure. Although it has been suggested that COX-2 can be a large source of PGI₂ in vascular tissues, it seems unlikely that COX-2-derived PGI₂ contributed to the effects of estrogen documented here. Our observation that incubation of cerebral arteries with a selective COX-1 inhibitor (SC-560) nearly abolishes PGI₂ production suggests that COX-1 is responsible for nearly all of the PGI₂ produced by these vessels. Moreover, the functional effects of arachidonic acid were also eliminated when arteries were pretreated with SC-560. These findings, combined with our observation that COX-2 is not detected in rat cerebral vessels under normal conditions (Ospina JA, Krause DN, and Duckles SP, unpublished observation) and published evidence that COX-2 is not normally expressed in the vasculature under noninflammatory conditions (4), indicate that the contribution of COX-2 is negligible in the vessels we have studied.

The possibility also exists that reactive oxygen species (ROS), derived from increased COX activity, contributed to the change in cerebral vascular tone seen in estrogen-treated rats. Because free radical scavengers were not used in this study, it is difficult to comment on the role of ROS in mediating the effects documented here. However, several lines of evidence suggest COX-derived ROS do not contribute to the estrogen-mediated decrease in vascular tone that we have observed. Niwa et al. (29) demonstrated in rodents that resting CBF is unaffected by treatment with superoxide dismutase, whereas it was reduced by treatment with the selective COX-1 inhibitor SC-560, supporting the contention that COX-1-derived prostanoids, but not ROS, regulate resting cerebrovascular tone. Furthermore, ROS derived from COX activity have also been associated with the release of 8-isoprostane from arachidonic acid, a process that appears to occur under conditions of oxidative stress (4). However, in our study, estrogen treatment did not affect levels of 8-isoprostane, further discounting the contribution of COX-derived ROS. Considering that prostacyclin release from cerebral arteries was increased by estrogen treatment, but 8-isoprostane levels were not, it seems plausible that prostanoids rather than ROS contribute to the estrogen-mediated decrease in resting cerebrovascular tone.

Thus increased endothelial PGI₂ production is the simplest explanation for how estrogen treatment decreases tone in small caliber branches of the MCA. In peripheral resistance arteries, estrogen also attenuates adrenergic constriction by upregulating PGI₂ (27). In the present study, the effect of estrogen on arterial diameter was both indomethacin sensitive and endothelial dependent. Furthermore, arachidonic acid-dilated arteries from estrogen-treated animals, apparently through synthesis of vasodilator prostaglandins because the effect required endothelial COX-1. The lack of available PGI₂ receptor antagonists or specific PGI₂ synthase inhibitors, however, precludes us from definitively confirming the role of PGI₂ through physiological experiments. Interestingly, we recently found that the stable PGI₂ receptor agonist iloprost has a greater dilator response in rat cerebral arteries following estrogen treatment (Ospina JA, Duckles SP, and Krause DN, unpublished observations). Together the evidence suggests that estrogen decreases cerebrovascular tone through increasing both the endothelial production and vascular smooth muscle response to PGI₂.

In addition to a simple decrease of vasodilator prostanoids, estrogen deficiency resulted in active vasoconstriction mediated via the COX pathway. We were surprised to find that cerebral arteries from ovariectomized rats dilated in response to indomethacin and constricted in response to arachidonic acid. This response was dependent on endothelial COX-1 as well as TXA₂-PGH₂ receptors. At the same concentration as used in other tissue bath studies (5, 18), the TXA₂-PGH₂ receptor blocker SQ-29548 caused an endothelium-dependent dilation in arteries from ovariectomized rats but had no effect in estrogen-treated animals. The sensitivity and maximal response of TXA₂-PGH₂ receptors in cerebral arteries, however, did not change with estrogen status, as demonstrated with the agonist U-46619. In contrast, mesenteric artery constriction to U-46619 was suppressed by estrogen (5). We first hypothesized that the prostanoid vasoconstrictor was endothelium-derived TXA₂, which could be measured, albeit in relatively low levels, from isolated cerebral arteries. Estrogen has been shown to reduce TXA₂ production in cultured coronary endothelial cells (42). However, TXA₂ production in cerebral arteries was actually increased following estrogen treatment, which may be due to increased COX-1 levels (23, 32), although platelet contamination cannot be ruled out. Furthermore, inhibiting TXA₂ synthase with furegrelate did not alter the diameter of pressurized arteries from ovariectomized animals. As mentioned previously, others in our laboratory (Gonzales RJ, Krause DN, and Duckles SP; unpublished observations) have demonstrated that this concentration of furegrelate reduces TXA₂ production by over 75% attesting to its effectiveness. Together, these data rule out TXA₂ as the substance responsible for vasoconstriction in Ovx arteries.

We considered other vasoconstrictors that can act on TXA₂-PGH₂ receptors such as PGF₂α, high concentra-
tions of PGE₂ (45), and isoprostanes, which are generated from arachidonic acid by free radicals (17). Although these substances were released by cerebral arteries, their production was unaffected by estrogen treatment. In cultured umbilical vein endothelial cells, estrogen did suppress F₂α-isoprostane production; however, this process is COX independent (16). The intermediate product in the COX pathway, PGH₂, also acts on TXA₂ receptors to induce vasoconstriction (4). After other prostanoids were ruled out, PGH₂ appears to be the best candidate for the constrictor in estrogen-deficient cerebral arteries, because it fits the criteria of sensitivity to TXA₂-PGF₂α receptor blockade but not TXA₂ synthase inhibition. Davidge and Zhang (5) came to a similar conclusion, i.e., PGH₂ likely caused constriction in resistance-sized mesenteric arteries from Ovx rats. However, PGH₂ is a very labile molecule and not easily quantitated; therefore, this hypothesis is difficult to confirm definitively. The prostanoid vasoconstrictor acting in estrogen-deficient animals does not appear to contribute to cerebrovascular tone following estrogen exposure because SQ-29548 had no effect in the latter condition. These results suggest that estrogen treatment suppresses levels of PGH₂.

Although we have not directly addressed the mechanism by which estrogen shifts prostanoid production from vasoconstriction to vasodilation, this effect can be explained by our previous finding that estrogen increases levels of COX-1 and PGI₂ synthase in cerebral vessels (32). As illustrated in Fig. 7, we postulate that, in vessels from Ovx animals, low levels of PGI₂ synthase result in an accumulation of PGH₂ that subsequently activates smooth muscle TXA₂-PGHA₂ receptors and results in vasoconstriction. In addition, reduction of prostacyclin production in the Ovx vessels would also contribute to a net constriction. Exposure to estrogen, however, upregulates PGI₂ synthase, allowing accumulated PGH₂ to be shunted into PGI₂ production, resulting in dilation. Recently, this shunting concept has been confirmed in a series of studies using adenovirus-mediated gene transfer to upregulate COX-1 and/or PGI₂ synthase in cultured endothelial cells (40), the balloon-injured carotid artery (48), and the brain (23). In all cases, there was a selective increase in PGI₂ production, whereas other prostanoids were decreased or unchanged. The importance of PGH₂ shunting to PGI₂ also was demonstrated by nitration inactivation of PGI₂ synthase, which reduced vasorelaxation of atherosclerotic bovine coronary arteries as a result of PGH₂ accumulation and TXA₂-PGHA₂ receptor activation (50).

Prostaglandins play a significant role in regulating the cerebral circulation in animals and humans as shown by use of COX inhibitors (28, 34) or COX-1 gene deletion (29). Both manipulations reduced CBF, indicating that vasodilator prostanoids are usually predominant. Alteration of endothelium-derived prostanoids due to changes in circulating estrogen would likely impact both CBF and thrombosis. In our study, we found that estrogen decreases cerebral vascular tone by enhancing vasodilator prostanoids and suppressing vasoconstrictor prostanoids under conditions of no flow. Considering that shear stress and flow can induce prostacyclin release from the blood vessel wall (5), it seems plausible that the effects of estrogen on the prostanoid cascade may underlie estrogen-induced increases in cerebral perfusion in vivo. In support of this, gender studies on CO₂ reactivity indicate that cerebral vasodilation in response to hypercapnia was inhibited by indomethacin to a greater degree in females than males (22). CBF was higher in premenopausal women compared with men, a difference that dissipated by the sixth decade of life (6, 39). In studies of postmenopausal women, users of estrogen replacement therapy exhibited higher CBF in various brain regions compared with nonusers (24, 30). Increased CBF also has been found in animal models of estrogen replacement and when estrogen is elevated physiologically during the estrus cycle (31, 44).

Prostanoid mechanisms are probably also involved in the effects of estrogen on pathophysiological conditions such as stroke and migraine (1, 19, 46). Estrogen decreases the thrombotic tendency of pial vessels (31), which is consistent with increased PGI₂ and its ability to potently inhibit platelet aggregation. Inhibition of COX-1 by either SC-560 (23) or gene knockout (20) leads to greater infarct volume in the MCA occlusion model of stroke. Conversely, elevation of COX-1 and PGI₂ synthase by gene transfer into the brain augments PGI₂ levels and significantly reduces infarct volume following MCA occlusion (23). Estrogen acts in a similar fashion to elevate cerebrovascular COX-1 and PGI₂ synthase (32), and the resultant increase in PGI₂ could decrease ischemic neuronal damage by modulating CBF and platelet aggregation. Arachidonic acid accumulates during ischemia; during subsequent reperfusion, there is marked increase in cyclooxygenase products as arachidonic acid is metabolized (33). Our studies suggest estrogen status could determine whether there is a preponderance of vasodilator or vasoconstrictor prostanoids produced. Prostanoid vasoconstriction with estrogen deficiency could exacerbate the shift to COX-dependent vasoconstrictors seen with aging, hypertension, and diabetes (4). The shift in prostanoid products may also contribute to cerebrovascular responses associated with the menstrual cycle, such as changes in flow (2, 7) and migraine (1). The results from the current study suggest that the COX-1 pathway in cerebral artery endothelium can lead to either vasodilation or vasoconstriction, depending on the presence or absence of estrogen.

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