HIGHLIGHTED TOPIC | Regulation of the Cerebral Circulation

Influence of sex steroid hormones on cerebrovascular function

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Krause, Diana N., Sue P. Duckles, and Dale A. Pelligrino. Influence of sex steroid hormones on cerebrovascular function. J Appl Physiol 101: 1252–1261, 2006. First published June 22, 2006; doi:10.1152/japplphysiol.01095.2005.—The cerebral vasculature is a target tissue for sex steroid hormones. Estrogens, androgens, and progestins all influence the function and pathophysiology of the cerebral circulation. Estrogen decreases cerebral vascular tone and increases cerebral blood flow by enhancing endothelial-derived nitric oxide and prostacyclin pathways. Testosterone has opposite effects, increasing cerebral artery tone. Cerebrovascular inflammation is suppressed by estrogen but increased by testosterone and progesterone. Evidence suggests that sex steroids also modulate blood-brain barrier permeability. Estrogen has important protective effects on cerebral endothelial cells by increasing mitochondrial efficiency, decreasing free radical production, promoting cell survival, and stimulating angiogenesis. Although much has been learned regarding hormonal effects on brain blood vessels, most studies involve young, healthy animals. It is becoming apparent that hormonal effects may be modified by aging or disease states such as diabetes. Furthermore, effects of testosterone are complicated because this steroid is also converted to estrogen, systemically and possibly within the vessels themselves. Elucidating the impact of sex steroids on the cerebral vasculature is important for understanding male-female differences in stroke and conditions such as menstrual migraine and preeclampsia-related cerebral edema in pregnancy. Cerebrovascular effects of sex steroids also need to be considered in untangling current controversies regarding consequences of hormone replacement therapies and steroid abuse.

estrogen; testosterone; brain arteries; endothelium

CEREBROVASCULATURE AS A TARGET FOR SEX STEROID HORMONES

It is clear that the vasculature in general and cerebral blood vessels in particular are targets for sex steroids (81). As discussed below, cerebrovascular tissues express specific receptors and metabolic enzymes for gonadal steroids, and isolated cerebral vessels and vascular cells respond directly to gonadal hormones. “Classical” sex steroid receptors are nuclear receptors that modulate gene expression in concert with other transcription factors that are often tissue specific (71). Additional “nongenomic” effects of gonadal hormones appear to be mediated via ion channels, membrane receptors, and cellular signaling pathways (53, 81, 106, 127).

Two nuclear receptors for estrogen, ER-α and ER-β, have been described. The presence of ER-α has been demonstrated in rat pial arteries and intracerebral blood vessels (27, 119, 121, 122). Immunoblots of cerebrovascular lysates show the expected ER-α receptor protein (66–67 kDa) as well as Ser118 phosphorylated ER-α and a lower molecular weight form (50–55 kDa). The latter has characteristics of a splice variant lacking the DNA binding portion of the receptor that has been speculated to mediate membrane signaling (27, 121). Confocal images of cerebral arteries show ER-α immunoreactivity in both endothelial and vascular smooth muscle cells (27, 121, Fig. 1). Within these cells, ER-α has been found in the nucleus...
(122), at the membrane (colocalized with caveolin-1 in endothelium) (27, 119), and within the mitochondria (colocalized with mitochondrial protein subunit I of complex IV) (122). In vivo treatment with estrogen increases expression of ER-α protein in cerebral blood vessels (121) but does not appear to alter the subcellular localization of this receptor (27). In contrast to ER-α, little is known regarding ER-β in the cerebral vasculature. ER-β has been detected by immunoblot in cerebral artery lysates (27), suggesting that this receptor subtype may have a role in mediating effects of estrogen on the cerebral circulation.

Androgen receptors (AR) also have been demonstrated in cerebral blood vessels (80). Interestingly, AR gene expression is higher in capillary endothelial cells cultured from rat brain compared with retina, suggesting the possibility of specific androgen regulation of the blood-brain barrier (80). AR protein has been detected in both rat pial arteries and intracerebral vessels, and the level of AR protein is increased in animals treated with androgen (RJ Gonzales, SP Duckles, and DN Krause, unpublished observations). As yet, there have been no reports of progesterone receptors in cerebral vessels. However, these receptors are found in peripheral vascular tissue and cultured vascular cells (77, 131), so it is possible that progesterone receptors are also expressed in cerebral vessels.

Although the emphasis of this review is on the vasculature, it should be noted that gonadal hormones also act on other cells within the neurovascular unit, e.g., astrocytes and neurons, as well as circulating blood elements such as platelets and leukocytes (25, 48, 58, 87, 90, 95, 140). Thus sex steroids can also indirectly impact cerebrovascular function via actions on these other cell types.

**SOURCES OF SEX STEROID HORMONES**

The primary sources of circulating sex steroids are the gonads, which are responsible for the well-known hormonal differences in males and females. Experimentally, endogenous hormones are often depleted by gonadectomy, followed by administration of the specific hormone of interest. Treatment is usually chronic (1–4 wk) with the goal of achieving blood levels in the physiological range for comparison with gonadectomized animals. Of course, the levels of endogenously derived hormones are not constant, resulting in varying impact on the cerebral vasculature over time. For example, hormone levels change with the onset of puberty and with reproductive senescence and aging (49, 96). Striking fluctuations are seen with estrogen and progesterone over the course of the menstrual or estrous cycle and during pregnancy. Testosterone in males fluctuates somewhat over the diurnal cycle and also changes with advancing age (6, 49). Hormone levels also are altered by stress and in various disease conditions. Exogenous hormones, e.g., contraceptive pills, HRT, anabolic steroids, and phytoestrogens in the environment, are another potential influence on the cerebral circulation.

It is becoming apparent that nonreproductive tissues also can synthesize sex steroids (115). Testosterone is converted by aromatase to 17β-estradiol, whereas it is metabolized to the potent androgen dihydrotestosterone by 5α-reductase. Both aromatase P450 and 5α-reductase proteins have been found in rat cerebral blood vessels, suggesting local metabolism of sex steroids by the cerebral vasculature (RJ Gonzales, SP Duckles, DN Krause, unpublished observations). Interestingly, astrocytes, which have intimate contacts with cerebral resistance vessels and capillaries, possess all of the necessary enzymes for synthesis of progesterone, testosterone, and estrogen from...
HORMONAL REGULATION OF ENDOTHELIAL FACTORS

Endothelial NO Synthase. The ability of estrogen to enhance NO production by endothelial NO synthase (eNOS) is the most extensively studied effect of sex steroids on the cerebrovasculature. Estrogen appears to activate several mechanisms that work in concert to increase eNOS function (81). First of all, it is well known that estrogen receptor (ER) activation can stimulate eNOS gene expression (71). Isolated cerebral vessels express more eNOS protein after in vitro incubation with physiological concentrations of 17β-estradiol; this effect is blocked by the ER antagonist ICI 182,780 (70). Levels of eNOS mRNA (120) and protein (61, 69, 91) are also higher in cerebral blood vessels from rats and mice exposed to estrogen in vivo. This effect is seen in both males and females (69), but it does not occur in ER-α knockout mice (40), suggesting that the ER-α receptor is responsible.

In addition to acting genomically to increase eNOS protein, estrogen stimulates a nongenomic ER pathway in cerebral vessels that activates phosphoinositide-3 kinase-Akt signaling to phosphorylate eNOS at serine-1177/1179 (53, 119). Phosphorylation of eNOS increases enzyme activity and renders the enzyme more sensitive to stimulation by calcium. Activation of eNOS is thought to take place within membrane caveolar complexes that, in cerebral vessels, contain ER-α (27, 119). Estrogen also affects other proteins in this complex such as caveolin-1, the endothelial scaffolding protein that both anchors eNOS to the caveolae and inhibits its activity. Pial arteriolar caveolin-1 expression is higher in ovariectomized vs. intact and estrogen-treated, ovariectomized females (91, 109, 135). Estrogen-mediated decreases in caveolin-1 correlate with

EFFECT OF SEX STEROIDS ON CEREBRAL VASCULAR REACTIVITY

One of the major influences of sex steroid hormones on the cerebral vasculature is the ability to alter vascular reactivity and thereby modulate blood flow. Sex-related differences in cerebral arterial tone have been described. Male arteries tend to be more constricted in response to pressure compared with arteries from females (38). Chronic in vivo exposure to estrogen or testosterone alters the reactivity of cerebral arteries in opposite ways (Fig. 2). Estrogen enhances production or sensitivity to vasodilatory factors (22, 37, 38, 83, 84, 91, 116) and may counteract effects of vasoconstrictors (21). Figure 3 shows a block diagram illustrating the mechanisms involved in genomic and nongenomic estrogen influences on cerebrovascular tone, as discussed in subsequent sections. Chronic treatment with selective ER modulators such as tamoxifen also reduces tone in rat cerebral arteries (125, 126). In contrast, androgens increase vascular tone (39, 45, 46). Experimental data regarding progesterone effects on cerebral vascular regulation are virtually absent from the literature. However, one study reports pial arteriolar dilation after acute intraperitoneal administration of progesterone but not estrogen (65, 67). Generally, physiological levels of the hormones affect vascular reactivity through endothelial mechanisms, involving changes in nitric oxide (NO), prostanoids, and/or endothelial-derived hyperpolarizing factor (EDHF).
increased eNOS activity (32, 109, 118, 135). Furthermore, estrogen increases cerebral artery expression of calmodulin, which is necessary for calcium activation of eNOS (118).

What is the evidence that actions of estrogen on eNOS function actually affect cerebrovascular tone? The influence of estrogen has been examined by two approaches, using either chronic hormone administration in vivo or acute estrogen treatment. It is clear that long-term exposure to estrogen affects cerebrovascular tone. Cerebral arteries and arterioles from females and chronically estrogen-treated animals exhibit greater NO-dependent dilation than similar vessels from either males or ovariectomized females (37–39, 91, 116). The NO dilation is endothelium dependent and has been demonstrated using NO synthase (NOS) inhibitors in pial arterioles in vivo (91, 118) as well as isolated cerebral artery segments, pressurized in vitro (37–39, 116). Interestingly, chronic estrogen treatment of ER-α knockout mice does not increase NO-dependent dilation in cerebral arteries (40), suggesting that this effect, like increases in eNOS, is mediated by ER-α. In cerebral microvessels, cyclic GMP levels are increased after chronic estrogen treatment, consistent with an NO-mediated effect on guanylate cyclase in intraparenchymal vessels (85).

Vasodilation elicited by the NO donor SNAP, however, is unaffected by chronic estrogen exposure, suggesting no direct estrogen influence on NO-mediated vascular smooth muscle relaxation (91). Dilator effects of acetylcholine also are greater after prolonged estrogen exposure (34, 74, 130). An exception to the above may be found in the absence of any differences in reactivity to acetylcholine or to NOS inhibitors of basilar arteries examined in vivo in chronically estrogen-treated vs. untreated ovariectomized female rats (16). This could imply the existence of segmental differences in the effects of chronic estrogen treatment on NO-dependent relaxation within the cerebral vascular system.

It is likely that the effect of estrogen to enhance NO-mediated vasodilation involves both the genomic and non-genomic actions described above (Fig. 3). However, attempts to directly demonstrate acute estrogen stimulation of NO-mediated vasodilation have given mixed results. A number of studies, using both in vivo and in vitro preparations, report cerebral vasodilating responses to estrogen, but the concentrations required were in the micromolar (supraphysiological range (41, 62, 106). Acute vasodilating responses to selective ER modulators, such as raloxifene and tamoxifen (34, 128), have also been observed with use of micromolar concentrations. With only one exception (62), the estrogen responses in these reports were found to be ER and endothelium independent. Recently, however, acute effects on cerebrovascular NO production and NO-mediated dilation have been demonstrated by using nanomolar concentrations of estrogen in vitro (34, 119). These effects, which appear to be mediated via ER and phosphoinositide-3 kinase, may be less evident after prior exposure to estrogen (34, 119), but the specific conditions for optimizing this effect are not yet understood.

It appears that other sex steroids do not directly affect the cerebrovascular NO pathway. Testosterone treatment of orchietomized male rats has no effect on either eNOS levels or NO-mediated dilation in cerebral vessels (39, 46). The progesterins, progesterone and medroxyprogesterone, also do not affect levels of cerebrovascular eNOS protein (70).

Prostanoids. Both estrogen and testosterone modulate cerebrovascular tone via prostanoid mechanisms. Estrogen appears to shift the prostanoid balance toward the vasodilator prostacyclin (PGI2), whereas testosterone enhances thromboxane (TXA2)-mediated vasoconstriction (45, 83). Enhancement of endothelial-dependent dilation after estrogen exposure was found to be dependent on cyclooxygenase (COX) as well as NOS (37, 61, 83). Further investigation indicated that estrogen treatment increases PGI2 production by elevating levels of the two key synthetic enzymes, COX-1 and PGI2 synthase, in cerebral blood vessels (83, 84). Interestingly, effects of estrogen on the COX-1 pathway are more apparent in the absence of NOS activity (61), suggesting that PGI2 may serve as a backup vasodilator during NOS dysfunction.

Testosterone also affects cerebrovascular prostanoids, but, in contrast to effects of estrogen, testosterone enhances TXA2-mediated vasoconstriction. Levels of TXA2 synthase are increased in cerebral vessels from testosterone-treated male rats (45). This effect is functionally manifest as greater endothelial-dependent constriction of cerebral arteries and is blocked by inhibitors of either TXA2 synthase or thromboxane receptors. Estrogen does not affect levels of TXA2 synthase (83) or contractile responses to the TXA2 agonist U-46619 in cerebral arteries (126). Interestingly, it was shown that, after forebrain ischemia, contraction of pial arteries to U-46619, monitored by closed cranial window in vivo, was decreased in ovariec-tomized rats but not in animals treated either acutely or chronically with 17β-estradiol (97). The effect of estrogen was blocked by the ER antagonist ICI 182,780, but nothing more is known about the mechanisms involved. This finding suggests that maintenance of normal constriction to vascular agonists, as well as enhanced endothelial-dependent dilation (130), may contribute to the protective effects of estrogen in the cerebral vasculature.

EDHF. Because sex steroids regulate cerebral vascular tone via endothelial mechanisms, some studies have investigated possible effects on dilation mediated by EDHF (17). The identity of EDHF has not yet been established; however, effects of EDHF are operationally defined as an endothelial-dependent dilation that persists in the presence of NOS and COX inhibitors and is inhibited by specific Ca2+-dependent K+ channel blockers. By using these criteria, it was found that testosterone treatment of orchietomized male rats causes a reduction in the EDHF contribution to resting tone in middle cerebral arteries, studied under pressurized conditions in vitro (46). However, EDHF responses stimulated by ATP are actually smaller in cerebral arteries from female compared with male rats (43, 44). Estrogen appears to decrease the role of EDHF in cerebral arteries (43, 44), contrary to what is found in peripheral arteries (17). Interestingly, the mechanism underlying ADP-mediated pial arteriolar dilation in vivo differs between ovariec-tomized and estrogen-exposed female rats. In ovariec-tomized rats, the dilation to local applications of ADP was endothelium dependent, EDHF-like in nature, dependent on connexin-43-related gap junctions, but completely NO independent (137). In intact and estrogen-treated ovariec-tomized females, ADP-induced dilations were quantitatively similar to those seen in ovariec-tomized females; however, the ADP response was not dependent on EDHF or connexin-43 but was dependent on eNOS (137, 139). The apparent suppression of EDHF by estrogen in cerebral vessels is not related to increases
in eNOS (138) or altered endothelial calcium (43), two mechanisms demonstrated for EDHF suppression in peripheral vessels (20, 78). Thus, although the overall effect of estrogen is to enhance cerebral vasodilation, the specific actions of estrogen on endothelial vasoactive factors are complex and not yet fully understood.

OTHER EFFECTS ON CEREBROVASCULAR REACTIVITY

For the most part, endothelial mechanisms appear responsible for sex hormone modulation of cerebrovascular reactivity. When denuded of endothelium, cerebral arteries from intact, gonadectomized, and hormone-treated animals exhibit similar vascular tone (37–39, 46). Vasodilators and constrictors that act directly on vascular smooth muscle are generally unaffected by sex steroid treatment (74). However, after withdrawal of estrogen, cerebral artery reactivity to serotonin was found to be increased (111). Several hormonal effects have been observed in cerebrovascular smooth muscle, which, as noted above, express both ER and AR. In cultured basilar artery smooth muscle cells, intracellular Mg^{2+} was altered after treatment with either estrogen or progesterone but not testosterone. However, basal levels of smooth muscle cell calcium, measured in isolated rat middle cerebral arteries, are similar for male, female, ovariectomized, and estrogen-treated ovariectomized rats (43). Estrogenic compounds have been shown to directly relax cerebral arteries by suppressing smooth muscle Ca^{2+} influx (41, 94, 106); however, these effects require high hormone concentrations and are not ER mediated. Interestingly, estrogen suppresses the activity of an enzyme linked to increased vascular tone, Rho kinase, in the cerebral vasculature (21), and this action may involve ERs (52). Estrogen inhibition of Rho kinase is a likely explanation for the lack of angiotensin II-mediated constriction in female mouse basilar arteries (33).

HORMONAL EFFECTS ON CEREBRAL BLOOD FLOW

On the basis of the hormonal effects on vascular reactivity described above, sex steroids appear capable of influencing cerebral blood flow (CBF). Surprisingly, results from multiple experimental animal studies do not provide evidence for resting CBF being influenced by changes in chronic estrogen status (2, 51, 54, 104), although CBF reactivity might be affected (4). On the other hand, a number of clinical studies (59, 79), but not all (9), indicate that CBF, measured with Doppler ultrasound or tomographic methods, is increased by estrogen. HRT in postmenopausal women has also been reported to increase CBF (10, 92, 117). The latter treatment often contains a progestin, and effects of HRT on cerebral blood flow can vary depending on the nature of the progestin used (10). Cerebral blood flow in women also exhibits changes during pregnancy when estrogen is high (14) and over the course of the menstrual cycle (15, 30, 59, 60). Decreases in cerebrovascular resistance are correlated with increasing estrogen at the end of the follicular phase (59).

On the other hand, estrogen replacement in women with Alzheimer’s disease was not associated with changes in cerebral perfusion (129). More investigation is needed to understand how gonadal hormones affect cerebral blood flow under different experimental and clinical conditions.

Interestingly, testosterone treatment also has been shown to increase cerebral blood flow in hypogonadal men (8); however, because this hormone is metabolized (115), the effect may reflect either androgen or estrogen influences. In contrast, androgen supplementation of HRT in postmenopausal women decreased cerebral blood flow, as assessed by the pulsatility index of the middle cerebral artery (93).

HORMONAL MODULATION OF THE BLOOD-BRAIN BARRIER

Evidence indicates that sex steroid hormones influence the functionally unique capillary bed of the brain and blood-brain barrier permeability. As discussed previously, estrogen targets cerebrovascular endothelium in capillaries (29) as well as in larger vessels (27, 121). The permeability of tracers into various areas of the brain is increased after ovariectomy of female rats (105). Estrogen treatment of young, ovariectomized rats significantly reduces dye extravasation into both the olfactory bulb and hippocampus, indicating a general tightening of the barrier (11). Furthermore, expression of the critical endothelial glucose transporter, GLUT-1, is increased by estrogen in rat cerebral microvessels (13, 112, 113). Indeed, transport of glucose into the brain is elevated after hormone exposure, indicating a potentially important role of estrogen in modulating cerebral glucose homeostasis (112, 113). Androgens also target cerebral capillaries and specifically upregulate the expression and function of the organic anion transporter-3 in rat cerebral capillary endothelial cells via AR.

The blood-brain barrier plays a key role in regulating water permeability and brain edema; these parameters also are affected by the hormonal milieu (100). During pregnancy and the postpartum period in rats, aquaporin 4, a water channel associated with brain edema, is increased around intracerebral blood vessels (98). Also during these states, the endothelial permeability barrier in cerebral arteries is less resistant to acute increases in intraluminal pressure (23). These alterations may contribute to edema and neurological defects seen with pre-eclampsia-associated hypertension. Progesterone has been shown to reduce brain edema, but the mechanisms have not yet been elucidated (102).

ESTROGEN AND VASCULAR PROTECTION

It is clear that estrogen has many positive effects on endothelial function. Although many actions of estrogen can be ascribed to the multitude of effects of NO and PGI2, estrogen promotes cerebrovascular protection through other mechanisms as well. Estrogen helps prevent atherosclerosis by suppressing mechanisms of vascular smooth muscle migration (21). In contrast, androgens are known to increase proliferation of smooth muscle cells (35). Treatment with estrogen, in vivo and in vitro, affects mitochondrial function in cerebral blood vessels (31, 122). Key enzymes of the electron transport chain are increased in cerebrovascular mitochondria, improving the efficiency of energy production (122). At the same time, estrogen decreases mitochondrial production of damaging free radical species. Estrogen also appears to suppress apoptosis mechanisms involving the mitochondria (114) and cerebrovascular Akt (119) and enhance endothelial cell survival (113). Estrogen protects rat cerebral endothelial cells in culture from damage due to Ca^{2+} influx by 3-nitropropionic acid (73) and may also protect against damage from amyloid β-peptides (101). Ischemic protection may be achieved by estrogen acting, via ER-α, to increase vascular expression of molecules such as angiopoietin-1 and thereby stimulating angiogenesis in the...
Suppress induction of COX-2 protein and PGE2 production (29, 36).}

adhesion molecules by cerebral microvascular endothelial cells in pial venules (109). Estrogen also inhibits expression of a number of vascular inflammatory mediators (28).

Recently, progesterone was shown to suppress the inflammatory response and iNOS expression in the brain after cerebral ischemia (42). However, the cellular sites of this action were not established. In contrast, we have found in rat cerebral blood vessels that endotoxin-mediated induction of COX-2 and iNOS is increased when animals are treated chronically with progesterone or medroxyprogesterone (124). Interestingly, the fluctuating balance of endogenous estrogen and progesterone during the estrous cycle also appears to modulate inflammatory responses of cerebral vessels (124). Induction of inflammatory mediators by endotoxin was dramatically enhanced in female cerebral vessels on the day of estrus when the plasma level of 17β-estradiol is low and progesterone is high (Fig. 5).

CLINICAL IMPLICATIONS OF HORMONAL INFLUENCES ON CEREBRAL VESSELS

The influence of sex steroids on the cerebral vasculature has important implications for a variety of disorders, in particular stroke and ischemic brain injury (16, 50, 68, 133, 140). Certain cerebrovascular-related pathologies coincide with changes in circulating ovarian hormones in women, e.g., menstrual migraine (3, 23, 24, 66) and increased stroke risk postpartum and after menopause (16, 68).

Estrogen in particular has vascular and neuroprotective effects, in part, by improving blood flow during and after an ischemic insult (18, 51, 55, 67, 90, 130, 141). Estrogen treatment also has been shown to suppress formation of experi-
tial cerebral aneurysms (56). As discussed above, estrogen enhances protective endothelial mechanisms while decreasing cerebrovascular inflammation and reactive oxygen species production. Testosterone, on the other hand, can exacerbate ischemic stoke injury (50) and increase cerebrovascular inflammation (99). These hormonal effects may underlie well-known differences in stroke-related neuropathologies between males and females and between pre- and postmenopausal women (1, 68, 142). However, it is possible that conversion of testosterone to estrogen may play an important role in protective effects of testosterone in hypogonadal men (8). Some evidence indicates that cerebrovascular function and disease also are influenced by natural progesterone and progestins in various HRT formulations. Progesterone may improve or worsen ischemic brain injury (42, 63, 75, 76), depending on the brain area and hormone preparation used. However, a more complete picture of the effects and mechanisms of progestins is needed, including better understanding of the different actions of the various forms of progestin on cerebrovascular function (10, 88).

Most of the protective effects of estrogen that have been described have been studied in cerebral blood vessels from healthy young animals. Only recently has it been appreciated that certain cerebrovascular effects of sex hormones may be diminished or even reversed in older animals or postmenopausal women (5, 11, 103), or in disease states such as atherosclerosis (26) and diabetes (110, 134). For example, in female rats with experimental diabetes, estrogen treatment ameliorates the effects of diabetes on the cerebral circulation clearly requires more investigation. Answers to these questions will have direct relevance to improving the outcome of hormone replacement in older men and women.

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SEX HORMONES AND THE CEREBRAL VASCULATURE