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Local estrogenic/androgenic balance in the cerebral vasculature

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Abstract

Reproductive effects of sex steroids are well-known, however it is increasingly apparent that these hormones have important actions on non-reproductive tissues such as the vasculature. The latter effects can be relevant throughout the lifespan, not just limited to reproductive years, and are not necessarily restricted to one sex or the other. Our work has established that cerebral blood vessels are a non-reproductive target tissue for sex steroids. We have found that estrogen and androgens alter vascular tone, endothelial function, oxidative stress and inflammatory responses in cerebral vessels. Often the actions of estrogen and androgens oppose each other. Moreover, it is clear that cerebral vessels are directly targeted by sex steroids as they express specific receptors for these hormones. Interestingly, cerebral blood vessels also express enzymes that metabolize sex steroids. These findings suggest that local synthesis of 17 β -estradiol and dihydrotestosterone can occur within the vessel wall. One of the enzymes present, aromatase, converts testosterone to 17 β -estradiol, which would alter the local balance of androgenic and estrogenic influences. Thus cerebral vessels are affected by circulating sex hormones as well as locally synthesized sex steroids. The presence of vascular endocrine effector mechanisms has important implications for male-female differences in cerebrovascular function and disease. Moreover, the cerebral circulation is a target for gonadal hormones as well as anabolic steroids and therapeutic drugs used to manipulate sex steroid actions. The long-term consequences of these influences have yet to be determined.

Keywords

estrogen; androgen; cerebral circulation; endothelium

Introduction

There is increasing interest in the non-reproductive actions of sex steroids, no doubt fueled by the realization that sex steroids play essential roles in tissues such as bone, brain and the vasculature (McCullough et al, 2003; Simpson, 2003; Simpson et al., 2000) and the fact that the average lifespan of the population now extends well beyond the reproductive years. Estrogen is not just a “female” hormone nor is testosterone exclusively a “male” hormone. Age-related decline or alteration in sex hormone levels are thought to impact the health of tissues such as the cerebral vasculature. There are male-female differences in risk of stroke

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Conflict of Interest

There is no conflict of interest.

and cerebrovascular disorders such as migraine. What mechanisms are responsible for these observations? What is the impact of hormone replacement therapy, aromatase inhibitors used in cancer, abuse of anabolic steroids? To obtain answers and apply them to better prevention and treatment of cerebrovascular disease, one must first understand the fundamental mechanisms by which sex steroid hormones affect cerebral blood vessels.

Functional effects of sex steroids: Vascular tone of cerebral arteries

Clearly one of the major functions of the cerebral arteries is to regulate blood flow to the brain. Because of the critical metabolic requirements of this vital organ, vascular reactivity and flow are highly regulated in the large cerebral arteries on the surface of the brain. Mechanisms intrinsic to the vessel wall, such as smooth muscle autoregulation and endothelial production of vasoactive factors, are combined with extensive neuroregulation and sensitivity to circulating factors such as hormones (Edvinsson & Krause, 2002).

The effects of sex steroids on cerebrovascular tone have been studied by monitoring the diameter of middle cerebral artery segments that were removed from rodents and pressurized in vitro. A striking difference is seen between arteries taken from males and females; the female arteries are more dilated at any given pressure (Geary et al., 1998, 2000). This is primarily due to a greater influence of endothelial vasodilators, such as nitric oxide (NO), in the females. It appears that the higher level of estrogen exposure in females accounts for much of the male-female differences in cerebral artery tone. Cerebral arteries from ovariectomized females are more constricted and exhibit less NO-mediated dilation than arteries from intact females (Geary et al. 1998). In vivo replacement of 17 β -estradiol in ovariectomized rodents restores the level of NO-mediated dilation in cerebral arteries to that found with normal females (Skarsgard et al., 1997; Geary et al., 1998; Pelligrino et al., 2000). The contractile results are further supported by biochemical analyses of cerebral blood vessels that show estrogen exposure, both in vivo and in vitro, causes an increase in endothelial NO synthase (eNOS) expression and activity (McNeill et al., 1999; 2002; Stirone et al, 2003a; 2005a).

However, the influence of sex steroids on cerebrovascular reactivity is more complex than just estrogen stimulation of eNOS in female arteries. When the effects of gonadectomy and hormone replacement were studied in cerebral arteries isolated from male rats, it was found that testes removal caused a decrease in cerebrovascular tone and also a decrease in NO-mediated dilation. In vivo treatment of these animals with either testosterone or dihydrotestosterone resulted in increased vascular tone and suppressed endothelial-dependent dilation (Geary et al., 2000; Gonzales et al., 2004; 2005). However the endothelial effect of testosterone did not involve NO (Gonzales et al, 2004); in contrast, estrogen treatment of gonadectomized males increased NO-mediated dilation to the level seen in intact males (Geary et al., 2000). Thus it is apparent that both androgenic and estrogenic influences contribute to the net vascular tone observed in cerebral arteries from normal, intact males.

Estrogen and androgens also affect endothelial production of vasoactive prostanoids. The cyclooxygenase inhibitor indomethacin dilates cerebral arteries from intact males and ovariectomized females (Ospina et al., 2003; Gonzales et al., 2005), however, it constricts arteries from females exposed to estrogen (Ospina et al., 2002). Androgens increase endothelium-dependent thromboxane A₂ (TXA₂) production by increasing levels of TXA₂ synthase (Gonzales et al., 2005). Endothelium-dependent TXA₂ constriction is also seen in arteries from ovariectomized females. However estrogen exposure elevates the levels of cyclooxygenase-1 and prostacyclin (PGI₂) synthase and increases endothelial production of the potent dilator PGI₂ (Ospina et al., 2003). Thus, in cerebral arteries, estrogen shifts the

balance of endothelial prostanoid production towards PGI₂, while androgens shift production to TXA₂. The role of prostaglandins may be greater in smaller-diameter cerebral arteries and in larger arteries with dysfunctional eNOS (Li et al., 2004).

Sex steroids can also affect cerebrovascular tone by altering endothelial-derived hyperpolarizing factors (EDHF), which appear to play a greater role in regulating tone in small arteries and arterioles. Testosterone treatment of gonadectomized male rats leads to a down-regulation of EDHF-mediated dilation in pressurized, small branches of the middle cerebral artery (Gonzales et al., 2004). Interestingly, estrogen treatment also has been reported to decrease agonist-stimulated EDHF-mediated dilation in female rat middle cerebral artery (Golding & Kepler, 2001). However, in small, penetrating brain arterioles where EDHF is thought to be an important dilator, estrogen replacement had no effect; although tone in the arterioles was elevated after ovariectomy (Cipolla et al., 2009). These studies underscore the heterogeneity within a given vascular bed and the complexity of vascular regulation that may be impacted by sex hormones.

Taken together, estrogen and androgens generally have opposing effects on the net vascular tone of cerebral arteries. All the mechanisms discussed above involve alterations in endothelial production of vasoactive factors following chronic in vivo exposure to hormone. Estrogen increases dilation by increasing NO and PGI₂ and decreasing TXA₂. Androgens increase constriction by decreasing EDHF (endothelial-derived hyperpolarizing factor) and increasing TXA₂ (Krause et al., 2006).

Functional effects of sex steroids: Inflammatory responses of cerebral arteries

Vascular inflammation is an important injury response, however chronic inflammation is a major contributor to vascular disease. Inflammation in the brain following stroke negatively impacts stroke outcome. We have found that sex steroids modulate inflammatory responses in cerebral blood vessels.

Inflammation was assessed by measuring induction of cyclooxygenase-2 (COX-2) and inducible NOS (iNOS) in cerebral blood vessels following treatment with IL-1 β or lipopolysaccharide endotoxin (LPS), both in vivo and in vitro. Vascular COX-2 and iNOS induction was lowest in vessels taken from animals exposed to estrogen. (Opsina et al., 2004; Sunday et al., 2006; 2007; Razmara et al., 2005). Interestingly, estrogen has similar effects in gonadectomized female and male rats. Estrogen treatment also suppresses the translocation of the inflammatory transcription factor NF κ B to vascular nuclei which occurs in response to IL-1 β and LPS (Opsina et al., 2004; Sunday et al., 2007; Galea et al., 2002).

In contrast, androgen exposure increases the inflammatory response in cerebral blood vessels. When gonadectomized male rats are treated chronically with either testosterone or vehicle and then challenged with an i.p. injection of LPS, COX-2 and iNOS proteins are induced in cerebral vessels (Razmara et al., 2005). This induction is significantly higher in cerebral vessels isolated from animals exposed to androgen. Moreover, dihydrotestosterone treatment, both in vivo and ex vivo, increases activation of NF κ B in cerebral vessels in the absence of added endotoxin or cytokines (Gonzales et al., 2009). Vascular expression of COX-2 and iNOS is also increased by dihydrotestosterone.

Thus, with regard to cerebrovascular inflammation, it appears that estrogen and androgens have opposing actions. Estrogen suppresses whereas androgens increases activation of NF κ B and expression of proinflammatory enzymes such as COX-2 and iNOS. The levels of inflammatory mediators produced by these enzymes in cerebral vessels, PGE₂ and NO

respectively, are also decreased by estrogen and increased by androgen (Ospina et al., 2004; Gonzales et al., 2009). These mediators are vasoactive and examination of vascular tone in isolated pressurized arteries has confirmed that COX-2-mediated dilation is increased after androgens (Gonzales et al., 2009) and decreased after estrogen (Ospina et al., 2004).

Sex steroid receptors and enzymes in cerebral blood vessels

In many of the studies described above, effects of estrogen and androgen were revealed by manipulating circulating levels of hormones in vivo. Thus the effects may or may not be the result of direct hormone action on cerebral vessels. To test if cerebral vessels are a direct target for hormone action, we determined if hormone receptors are present and functional in cerebrovascular tissue.

Using Western blot and immunofluorescent imaging, we have shown the presence of estrogen receptor alpha (ER α), estrogen receptor beta (ER β) and androgen receptor (AR) in cerebral endothelium (Gonzales et al., 2007; Guo et al., 2010; Razmara et al., 2008; Stirone et al., 2003b; 2005a; see Figure 1). We have also found ER α and AR in cerebral artery smooth muscle (Stirone et al., 2003b; Gonzales et al., 2007; Figure 1). Interesting, both estrogen and androgen receptors are found in male and female cerebral vessels. However, levels of ER α are increased by exposure to estrogen (Stirone et al., 2003b); whereas the level of vascular AR is increased by androgens (Gonzales et al., 2007).

We know the most about ER α in cerebral vessels. There are multiple forms, including a truncated form thought to locate to the plasma membrane (Stirone et al., 2003b). Using confocal microscopy, we have confirmed that ER α colocalizes with membrane caveolae and is also located in the nucleus and in the mitochondria (Stirone et al., 2005a; Stirone et al., 2005b). It is likely that in cerebrovascular tissue, membrane receptors mediate rapid effects of estrogen on cell signaling (Stirone et al., 2005a), nuclear receptors mediate genomic hormonal actions (Stirone et al., 2003a), and mitochondrial receptors are involved in estrogen effects on mitochondrial function (Stirone et al., 2005b).

We have also treated isolated vessels and cultured endothelial cells with hormones and hormone receptor antagonists to demonstrate that sex steroids influence cerebrovascular function via specific vascular receptors (McNeill et al., 2002; Gonzales et al., 2009; Guo et al., 2010; Razmara et al., 2008; Stirone et al., 2005a). The effects of estrogen on vascular tone, endothelial vasodilators, and mitochondrial oxidative stress appear to be the result of ER α activation (Geary et al., 2001; Guo et al., 2010; Stirone et al., 2005a, Razmara et al., 2008). Effects of androgens on cerebrovascular inflammation have been shown to be mediated by androgen receptors (Gonzales et al., 2009).

Cerebral arteries also express enzymes involved in sex steroid metabolism (Figure 1). It is well known that target tissues for androgens, such as skeletal muscle, express 5 α -reductase II. This enzyme converts testosterone to the more potent AR agonist, dihydrotestosterone. Confocal immunofluorescent imaging showed that 5 α -reductase II is present in both the endothelium and smooth muscle of rat cerebral arteries (Gonzales et al., 2007). The presence of this enzyme in cerebrovascular tissue was confirmed by Western blot. In addition, we found that aromatase, the enzyme that synthesizes 17 β -estradiol from testosterone is also present but its localization is restricted to the cerebral endothelium (Gonzales et al., 2007). These findings suggest the possibility that local synthesis of the potent estrogen, 17 β -estradiol, and the potent androgen, dihydrotestosterone, can occur within the wall of cerebral vessels. Thus the local concentrations of these two hormones may differ from circulating levels.

Conclusions

The data presented in this brief review are limited to sex steroid influences on cerebral blood vessels. Although not discussed here, a number of the key observations, such as effects of estrogen on eNOS, have been made in other vascular tissues as well (Liu et al., 2003; Miller & Duckles, 2008). However, the body of work on the cerebral vasculature is unique in its level of comprehensiveness, focus on physiological outcomes, and recognition of the balance between estrogenic and androgenic influences. Based on these data, one can begin to visualize the local complexity of sex steroid regulation of cerebral blood vessels (Figure 2).

Endothelium and vascular smooth muscle appeared to be regulated by circulating hormones as well as locally synthesized sex steroids. Hormone concentrations within the vascular wall may be greater than what is appreciated by sampling blood levels. In addition, the local conversion of testosterone to either a more potent androgen or estrogen makes it difficult to predict the overall outcome of hormone exposure. Based on the opposing actions of androgens and estrogens on vascular tone and inflammation, it is possible that aromatase acts as a feedback mechanism to limit androgen effects by conversion to the vasoprotective estrogen. The consequences for the cerebral circulation of use of non-metabolizable anabolic steroids and aromatase inhibitors are not known. Moreover, the effects of both estrogen and androgens are observed in male and female cerebral vessels, so that sex differences in cerebrovascular function and pathophysiology likely reflect differences of degree along a continuum rather than fundamental male-female differences. It appears that both estrogen and androgens have important roles in regulating and protecting cerebral blood vessels.

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Figure 1.

Presence of sex steroid metabolizing enzymes and receptors in the endothelial and smooth muscle layers of rat middle cerebral arteries as revealed by confocal immunofluorescence (either red or green). DAPI fluorescence (blue) indicates cell nuclei which are oriented either in the direction of blood flow (endothelium) or perpendicular to flow (smooth muscle). Data adapted, in part, from Gonzales et al., 2007 and Stirone et al., 2005a. Expression of proteins is found in arteries from both male and female animals.

Figure 2.

Local action and metabolism of sex steroids in cerebral blood vessels. It appears that cerebrovascular ARs are stimulated by circulating androgens as well as by local production of the potent metabolite DHT from testosterone. Cerebrovascular ERs are stimulated by circulating estrogens and also by the local production of 17 β -estradiol from testosterone via endothelial aromatase. The latter reaction shifts the balance from androgenic to estrogenic effects. Abbreviations: A: aromatase; AR: androgen receptor; COX: cyclooxygenase; DHT: dihydrotestosterone; E: estrogen; EDHF: endothelial-derived hyperpolarizing factor; eNOS: endothelial nitric oxide synthase; ER: estrogen receptor; iNOS: inducible nitric oxide synthase; PGI₂S: prostacyclin synthase; R: 5 α -reductase II; T: testosterone; TXA₂S: thromboxane synthase