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***Abstract*—**The greater incidence of hypertension in men and postmenopausal women compared with premenopausal women has suggested gender differences in vascular function. Vascular effects of the female sex hormones estrogen and progesterone and the male hormone testosterone have been described. Sex steroid receptors have been identified in vascular endothelium and smooth muscle. Interaction of sex hormones with cytosolic/nuclear receptors initiates long-term genomic effects that stimulate endothelial cell growth but inhibit smooth muscle proliferation. Activation of sex hormone receptors on the plasma membrane triggers nongenomic effects that stimulate endothelium-dependent vascular relaxation via NO–cGMP, prostacyclin–cAMP, and hyperpolarization pathways. Sex hormones also cause endothelium-independent inhibition of vascular smooth muscle contraction, $[Ca^{2+}]_i$, and protein kinase C. These vasorelaxant/vasodilator effects suggested vascular benefits of hormone replacement therapy (HRT) during natural and surgically induced deficiencies of gonadal hormones. Although some clinical trials showed minimal benefits of HRT in postmenopausal hypertension, the lack of effect should not be generalized because it could be related to the type/dose of sex hormone, subjects' age, and other cardiovascular conditions. The prospect of HRT relies on continued investigation of the molecular mechanisms underlying the vascular effects of sex hormones and identification of compounds that specifically target the vascular sex hormone receptors. Naturally occurring hormones and phytoestrogens may be more beneficial HRT than synthesized compounds. Also, the type/dose, time of initiation, and duration of HRT should be customized depending on the subject's age and preexisting cardiovascular condition, and thereby enhance the outlook of sex hormones as potential modulators of vascular function in hypertension. **(*Hypertension*. 2005; 46:249-254.)**

Key Words: estrogen ■ endothelium ■ nitric oxide ■ muscle, smooth, vascular ■ calcium

Hypertension is more common in men 30 to 45 years of age than in women of similar age, suggesting gender differences in the physiological control mechanisms of blood pressure. Hypertension is also more prevalent in postmenopausal than premenopausal women, suggesting vascular protective effects of female sex hormones.[1,2] Experimental and initial clinical data have suggested that hormone replacement therapy (HRT) may reduce cardiovascular disease in postmenopausal women.[3–6] On the other hand, reports from Heart and Estrogen-Progestin Replacement Study (HERS), HERS2, and Women's Health Initiative (WHI) clinical trials did not support vascular benefits of HRT in postmenopausal women.[1,7,8] However, the lack of beneficial effects of HRT could be explained by the small number of subjects studied and that subjects were mainly elderly women.[1] Thus, despite an initial setback, investigations have continued to examine the effects of sex hormones on blood pressure. Significant effects of sex hormones on the neuronal and renal control mechanisms of blood pressure have been proposed.[9–13] For example, estradiol inhibits renin release, whereas testosterone activates the renin-angiotensin system.[10,13,14] Also, previous reviews have provided detailed information on the effects of sex hormones on the vascular control mechanisms of blood pressure.[1,2,15] This brief report highlights the gender differences in vascular function and the genomic effects of sex hormones on endothelial cell and vascular smooth muscle (VSM) growth. The nongenomic effects of sex hormones on endothelium-dependent vascular relaxation and on VSM contraction are then described. The report finalizes with a perspective on potential areas for research to better understand the effects of sex hormones on vascular function and blood pressure and the potential use of HRT in hypertension.

Gender Differences in Vascular Reactivity

Gender differences in vascular function have been described.[2,15–17] Vascular contraction is greater in blood vessels of intact male than intact female rats, not different between castrated and intact males, but greater in ovariectomized (OVX) than intact females.[18,19] Also, estrogen replacement in OVX female rats restores the vascular contraction to its level in intact females,[3] suggesting that the gender differences in

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vascular contraction may involve direct effects of estrogen on specific hormone receptors in the vasculature.

Sex Hormone Receptors in Blood Vessels

Estrogen, progesterone, and testosterone receptors are expressed in the endothelium and VSM.[2,15,20,21] Two estrogen receptor (ER) subtypes (ER-α and ER-β) and several ER variants have been described.[20,22] Estrogen diffuses through the plasma membrane and forms complexes with cytosolic/nuclear receptors, which then bind to chromatin, stimulate gene transcription, and induce genomic effects. Estrogen also binds to signal-generating receptors on the plasma membrane of vascular cells and induces rapid nongenomic events.[2]

Genomic Effects of Sex Hormones

The interaction of sex hormones with cytosolic/nuclear receptors triggers a host of genomic effects leading to endothelial cell growth. For example, 17β-estradiol (E2) induces the phosphorylation and activation of mitogen-activated protein kinase (MAPK) and proliferation of endothelial cells. In contrast, E2 inhibits MAPK activity and cell growth and proliferation in VSM.[23,24] Estrogen also antagonizes the growth-promoting effect of angiotensin II (Ang II) on VSM via the induction/activation of protein phosphatases.[25] Additionally, estrogen stimulates cAMP production, and cAMP-derived adenosine may regulate VSM growth and thereby contribute to the antiproliferative effects of estrogen.[26]

Progesterone inhibits VSM proliferation and facilitates the inhibitory effects of estrogen.[2] Testosterone modulates VSM cell proliferation in a dose-dependent manner, with low concentrations stimulating and high concentrations inhibiting [^{3}H]thymidine incorporation.[27]

Nongenomic Effects of Sex Hormones

The interaction of sex hormones with plasmalemmal receptors in the endothelium and VSM may initiate additional nongenomic effects. For example, estrogen causes acute inhibition of vascular contraction.[2,15,28] Progestins may have direct effects or modify the effects of estrogen on vascular contraction. Although androgens could play a role in the development of some forms of hypertension,[10,29] testosterone induces direct vasodilation in several vascular preparations.[2,21,28] The nongenomic vascular effects of sex hormones appear to have endothelium-dependent mechanisms as well as endothelium-independent mechanisms involving direct effects on VSM.

Sex Hormones and the Endothelium

A significant portion of the gender-related and estrogen-induced vasodilation involves the endothelium.[30] E2 potentiates endothelium-dependent flow-mediated vasodilation in postmenopausal women.[31] Also, endothelium-dependent vascular relaxation is greater in female than male spontaneously hypertensive rats (SHR).[30] Additionally, selective ER-α agonists improve endothelial dysfunction in blood vessels of OVX SHR.[32] Similar to estrogen, progesterone and testosterone may induce endothelium-dependent vascular relaxation.[2,21] The vascular endothelium releases relaxing factors such as NO, prostacyclin (PGI_2), and endothelium-derived hyperpolarizing factor (EDHF), as well as contracting factors such as endothelin-1 (ET-1) and thromboxane A_2. Sex hormones could induce vascular relaxation by modifying the synthesis/release/bioactivity of these factors.

Sex Hormones and NO

There is considerable evidence that sex hormones modify the synthesis/bioactivity of NO. Total NO production is greater in premenopausal women than in men.[33] Also, endothelial NO release is greater in blood vessels of female than male rats.[2] Estrogen may influence NO production by activating ER-mediated genomic pathways and upregulation of endothelial NO synthase (NOS). For example, ER-α gene transfer into endothelial cells induces endothelial NOS (eNOS) gene expression. Also, estrogen increases eNOS mRNA in endothelial cells. On the other hand, cross-sectional data suggest an association between eNOS gene polymorphisms and hypertension, and the eNOS gene may influence the long-term burden and trend of blood pressure since childhood in females and may contribute to their predisposition to hypertension.[34] Estrogen may also regulate NOS activity by interacting with ERs in endothelial cell plasma membrane and activation of rapid nongenomic signaling pathways. For instance, membrane-impermeant estrogen binds to ERs at the cell surface and stimulates NO release from human endothelial cells. Also, in endothelial cells, E2 causes transient translocation of eNOS from the plasma membrane to intracellular sites close to the nucleus, whereas during prolonged exposure to E2, eNOS returns to the plasma membrane for its full activation.[2]

The acute effect of E2 on eNOS activity and NO release may be dependent on $[Ca^{2+}]_i$. Gender differences in endothelial cell $[Ca^{2+}]_i$ have been related to direct or indirect effects of estrogen on the Ca^{2+}-handling mechanisms. For example, estrogen-induced activation of cell surface ERs is coupled to increased $[Ca^{2+}]_i$ and NO release in human endothelial cells. Also, E2 promotes the association of heat shock protein 90 with eNOS, and thereby reduces the Ca^{2+} requirement for its activation. E2 also induces the phosphorylation/activation of eNOS by increasing the activity of MAPK or the phosphatidylinositol 3-kinase–Akt pathway.[2]

Estrogen has antioxidant properties that could affect NO bioactivity. In OVX female rats, increased blood pressure is associated with lower plasma antioxidant levels, reduced thiol groups, and increased plasma lipoperoxides and vascular free radicals, and E2 replacement prevents these effects. Also, the amount of superoxide anion is greater in isolated vessels of male rats than in females. Furthermore, E2 inhibits nicotinamide adenine dinucleotide phosphate oxidase expression and the generation of superoxide anion and peroxynitrite, and thereby enhances NO bioactivity.[2]

Although progesterone may counteract the stimulatory effects of estrogen on NO production and vascular relaxation in canine coronary artery, it stimulates NO production and endothelium-dependent NO-mediated relaxation in rat aorta and porcine coronary artery and increases eNOS expression in ovine uterine artery.[2] With regard to testosterone, acute intracoronary administration of the hormone in canine coronary vessels induces NO-mediated vasodilation. Also, in

human endothelial cells, dehydroepiandrosterone stimulates NO production by enhancing the expression and stabilization of eNOS.[21]

Sex Hormones and PGI_2

PGI_2 is produced from the metabolism of arachidonic acid by cyclooxygenase (COX). COX inhibitors such as indomethacin inhibit a significant portion of endothelium-dependent vascular relaxation, and gender differences in indomethacin-sensitive vascular relaxation have been attributed to differences in COX products.[35] Also, E2 causes upregulation of COX-1 expression and PGI_2 synthesis in endothelial cells.[2]

Progesterone may also cause direct nongenomic COX activation and increased vascular PGI_2 production, whereas testosterone decreases PGI_2 synthesis in blood vessels of female rats.[2]

Sex Hormones and EDHF

Gender differences in endothelium-dependent vascular relaxation may involve differences in EDHF. Acetylcholine (ACh)-induced hyperpolarization and relaxation of mesenteric arteries are less in intact male and OVX female than intact female rats, and the differences in ACh responses are eliminated by K^+ channel blockers. Also, the hyperpolarizing response to ACh is improved in E2-replaced OVX female rats, confirming that estrogen-deficient states attenuate vascular relaxation by EDHF.[2]

Testosterone may promote endothelium-mediated hyperpolarization of VSM. In SHR blood vessels, testosterone appears to release EDHF, which causes VSM hyperpolarization by a mechanism involving voltage-dependent BK_{Ca} channels. However, a portion of testosterone-induced vasorelaxation is endothelium independent and may involve ATP-sensitive K^+ channels in VSM.[36]

Sex Hormones and Endothelium-Derived Contracting Factors

The gender differences in vascular reactivity may involve endothelium-derived contracting factors such as ET-1 and thromboxane A_2. ET-1 release from endothelial cells is less in female than male SHR, and the gender differences in ET-1 production may be related to estrogen.[2]

ET-1 activates endothelial ET_{B1} receptor and causes the release of relaxing factors that promote vascular relaxation. On the other hand, the interaction of ET-1 with ET_A and ET_{B2} receptors causes VSM contraction. Gender differences in vascular responses to ET-1 have been shown in deoxycorticosetrone acetate (DOCA)-salt hypertensive rats, with the arteries of males producing more contraction than those of females.[37] In mesenteric arteries of DOCA rats, the ET_B agonist IRL-1620 induces mild vasoconstriction in intact females but marked vasoconstriction in OVX females. E2 replacement decreases IRL-1620–induced vasoconstriction in OVX females. Ovariectomy is also associated with increased ET-1 and ET_B receptor mRNA in mesenteric arteries, and E2 replacement reverses these changes. These data suggest that ovarian hormones attenuate ET-1/ET_B receptor expression and their vascular responses in DOCA-salt hypertension.[37] Studies have also shown that prolonged treatment of endothelial cells with E2 inhibits basal and stimulated ET-1 production in response to serum, tumor necrosis factor-α, transforming growth factor-β1 and Ang II.[38]

Similar to estrogen, progesterone inhibits serum- and Ang II–induced ET-1 production in endothelial cells, whereas androgens appear to stimulate ET-1 production.[21]

Gender differences in COX-derived constricting factors have also been observed, and thromboxane A_2–induced vasoconstriction is greater in male than female SHR.[2]

Sex Hormones and VSM Contraction

Estrogen, progesterone, and testosterone cause relaxation in endothelium-denuded blood vessels.[28] The acute effects of estrogen on vascular contraction in vitro are observed at micromolar concentrations, which exceed the physiological nanomolar concentrations in vivo. Although genomic effects of estrogen may underlie the reduced cell contraction in VSM of intact females, they may not account for the inhibitory effects of micromolar concentrations of E2 on vascular contraction. The acute vasorelaxant effects of estrogen may represent additional nongenomic effects on the mechanisms of VSM contraction.

The vasorelaxant effects of estrogen surpass those of progesterone or testosterone. Thus, the greater plasma estrogen levels in females may explain the reduced vascular contraction in females compared with males. However, the gender differences in vascular contraction may be related to the relative abundance of sex hormone receptors. For instance, females have more ERs in their arteries than males.[39] Sex hormones could also cause changes in the expression of vascular Ang II receptors. Western blot analyses in VSM suggest that estrogen induces a downregulation and progesterone an upregulation of the Ang II type 1 (AT_1) receptor protein. Also, E2 decreases AT_1 receptor mRNA half-life, whereas progesterone promotes stabilization of AT_1 receptor mRNA.[2] The gender differences in vascular contraction could also be attributable to differences in the signaling mechanisms of VSM contraction downstream from receptor activation.

Signaling Mechanisms of VSM Contraction

VSM contraction is triggered by increases in $[Ca^{2+}]_i$ attributable to Ca^{2+} release from the sarcoplasmic reticulum and Ca^{2+} entry from the extracellular space.[40] Activation of myosin light chain (MLC) kinase, Rho kinase, and MAPK, as well as inhibition of MLC phosphatase, also contributes to VSM contraction. Also, the agonist–receptor interaction is coupled to increased production of diacylglycerol, which activates protein kinase C (PKC). PKC is a family of several isoforms that have different substrates, functions, and subcellular distributions.[19]

Sex Hormones and VSM $[Ca^{2+}]_i$

Studies in isolated VSM cells have shown that the resting cell length is longer and basal $[Ca^{2+}]_i$ is smaller in female than male rats, suggesting gender differences in the Ca^{2+}-handling mechanisms in VSM.[40] In VSM cells incubated in the presence of external Ca^{2+}, phenylephrine (Phe) causes an initial peak in $[Ca^{2+}]_i$, mainly attributable to Ca^{2+} release

from the intracellular stores, and a maintained $[Ca^{2+}]_i$ attributable to Ca^{2+} entry from the extracellular space. In Ca^{2+}-free solution, Phe or caffeine causes transient cell contraction and $[Ca^{2+}]_i$ that are not different between intact and gonadectomized male and female rats, suggesting that the gender differences in VSM contraction do not involve the Ca^{2+} release mechanism from the intracellular stores.[40]

The maintained Phe-induced $[Ca^{2+}]_i$ in VSM cells is greater in intact male than female rats, suggesting gender differences in the Ca^{2+} entry mechanism of VSM contraction. The maintained Phe-induced $[Ca^{2+}]_i$ is greater in OVX than intact females but not different between E2-replaced OVX and intact females or between castrated and intact males, suggesting that the gender differences are likely related to estrogen.[40] The cause of the gender differences in Ca^{2+} entry may be related to the plasmalemmal density or permeability of VSM Ca^{2+} channels.

The gender differences in the mechanisms of Ca^{2+} mobilization in VSM could be attributable to a multitude of effects of sex hormones in vivo. However, E2 causes rapid relaxation of isolated blood vessels (possibly through an effect on Ca^{2+} mobilization or fluxes).[28] Estrogen does not inhibit caffeine- or carbachol-induced VSM contraction or $[Ca^{2+}]_i$ in Ca^{2+}-free solution, suggesting that it does not inhibit Ca^{2+} release from the intracellular stores. On the other hand, estrogen inhibits maintained agonist- and KCl-induced contraction, Ca^{2+} influx, and $[Ca^{2+}]_i$, suggesting inhibition of Ca^{2+} entry through voltage-gated channels.[28,40,41]

Estrogen activates BK_{Ca} channels in coronary VSM, leading to hyperpolarization and decreased Ca^{2+} entry through voltage-gated channels. However, estrogen-induced vasorelaxation and inhibition of Ca^{2+} influx in other types of VSM occurs even in the absence of increased K^+ efflux, suggesting direct effects on Ca^{2+} channels.[2] Estrogen may also decrease $[Ca^{2+}]_i$ by stimulating Ca^{2+} extrusion via plasmalemmal Ca^{2+} pump; however, this mechanism seems less likely because the rate of decay of caffeine- and carbachol-induced contraction and $[Ca^{2+}]_i$ transients in VSM incubated in Ca^{2+}-free solution, which is often used as a measure of Ca^{2+} extrusion, is not affected by estrogen.[28,41]

The effects of progesterone on VSM $[Ca^{2+}]_i$ are not clearly established, but acute application of progesterone decreases Ca^{2+} influx and $[Ca^{2+}]_i$ in rabbit and porcine coronary VSM.[28,41] Most studies suggest that testosterone is a potent vasorelaxant that decreases VSM $[Ca^{2+}]_i$ by inhibiting Ca^{2+} entry from the extracellular space.[21,28,41] The vasorelaxant effect of testosterone is attenuated by K^+ channel blockers, suggesting that stimulation of K^+ conductance is involved in the inhibitory effects of testosterone on VSM $[Ca^{2+}]_i$.[21]

Sex Hormones and PKC

The gender differences in vascular contraction may reflect differences in the expression/activity of PKC isoforms in VSM. Like Phe, phorbol esters, which activate PKC, produce greater contraction in isolated vessels of intact male than female rats, suggesting gender differences in the PKC-mediated pathway of VSM contraction.[19]

Immunoblot analysis in VSM of intact male rats has shown significant amounts of α-, δ-, and ζ-PKC, and Phe and phorbol esters cause activation and redistribution of α- and δ-PKC from the cytosolic to the particulate fraction. The amount of α-, δ-, and ζ-PKC, and the Phe- and phorbol ester–induced redistribution of α- and δ-PKC are less in intact female than male rats, suggesting that the gender differences in VSM contraction are related, in part, to underlying changes in the amount/activity of α-, δ-, and ζ-PKC.[19]

The Phe- and phorbol ester–induced VSM contraction and PKC activity are not different between castrated and intact male rats but greater in OVX than intact females, suggesting that the differences are related to estrogens. This is supported by reports that E2 implants in OVX female rats are associated with reduction in vascular contraction and PKC activity.[19]

A genomic action of estrogen on PKC expression in VSM might well underlie the reduction in vascular contraction and PKC activity in female rats compared with males. However, additional nongenomic effects of sex hormones on the PKC molecule or its lipid cofactors or other protein kinases upstream from PKC cannot be excluded. For example, progesterone inhibits phorbol ester–induced contraction and PKC translocation in VSM, an effect possibly mediated by increasing cAMP levels in VSM.[2]

Perspectives

Gender differences in the regulation of vascular function may partially explain the greater incidence of hypertension in men and postmenopausal women than in premenopausal women. Numerous studies have shown genomic and nongenomic effects of sex hormones on the endothelium and VSM, but many questions remain unanswered.

The sex hormone receptor subtypes, distribution, and function in vascular cells need to be examined further. Variants of sex steroid receptors are expressed in vascular cells and may alter the physiological effects of sex hormones. Also, the subcellular distribution of sex hormone receptors could determine the effects of sex steroids. Additionally, sex steroid receptors are phosphoproteins, and mutations in phosphorylation sites may affect their transactivation capacity. For example, human VSM cells transiently transfected with ER-α show translocation of ER-α from the surface membrane to the nucleus. Nuclear translocation of ER-α occurs as a result of constitutive activation of MAPK and is blocked by inhibition of MAPK, suggesting that MAPK-mediated phosphorylation of ER-α induces its nuclear localization.[2] Differences in sex hormone receptor distribution/signaling pathways may also explain why estrogen enhances endothelial cell growth but inhibits VSM proliferation.

The rapid vasodilator effects of sex hormones have suggested additional effects on the cellular mechanisms of vascular relaxation/contraction. Although the gender differences in vascular contraction may be related to the effects of sex hormones on VSM $[Ca^{2+}]_i$ or PKC, other signaling pathways such as MLC kinase and phosphatase and Rho kinase and tyrosine kinase could regulate VSM contraction. Whether the expression and activity of VSM protein kinases and phosphatases differ with gender and gonadal hormones should be examined further.

Female and male sex hormones affect the mechanisms of vascular contraction. However, sex steroids have different sexual effects, and their vascular effects may be different in the 2 sexes. Previous studies suggest gender differences in the effects of estrogen on vascular contraction.[18] Also, ethnic background could influence the effects of sex hormones on blood pressure, and determinants of salt sensitivity may vary in black and white normotensive and hypertensive women.[42] The vascular effects of sex hormones could also vary with aging.[16,17,43] For example, ovariectomy augments hypertension in aging female Dahl salt-sensitive rats,[44] and age-related reduction in ER-mediated mechanisms of vascular relaxation has been observed in blood vessels of female SHR.[45]

Because the vascular effects of estrogen and progesterone involve modulation of the Ca^{2+} channels, HRT may represent a more natural approach for treatment of certain forms of hypertension that respond to Ca^{2+} channel blockers. To use or not to use HRT in postmenopausal hypertension is still controversial. Although some experimental and clinical data suggest that HRT may reduce cardiovascular complications in postmenopausal women,[3,5,6,46] reports from HERS, HERS2, and WHI clinical trials do not support vascular benefits of HRT, particularly in elderly hypertensive women.[1,7,8] However, the lack of vascular benefits of HRT in these studies could be related to the timing of HRT and the subjects' age or preexisting cardiovascular condition. The prospect of HRT would require continued investigation of the mechanisms underlying the vascular effects of sex hormones and the identification of compounds that specifically target the vascular sex hormone receptors. Selective ER-α agonists have been shown to improve endothelial dysfunction in estrogen-deficient rats.[32] Also, postmenopausal HRT may be more efficient in reducing blood pressure when natural hormones are used in a manner that avoids first-pass liver effects and in doses that produce hormone levels similar to those in premenopausal women. Estradiol metabolism may also determine its cardiovascular effects, and nonfeminizing estradiol metabolites may confer cardiovascular protection in both genders. Furthermore, phytoestrogens may provide a more natural dietary source of estrogen replacement than synthesized compounds. Other factors, such as the use of medications for treatment of preexisting conditions or following a specific dietary regime, may modify the effects of sex hormones.[47,48] Thus, the type/dose, time of initiation, and duration of HRT should be customized depending on the subject's age and preexisting cardiovascular condition, and thereby enhance the outlook of sex hormones as potential modulators of vascular function in hypertension.

Finally, although androgens could be involved in some forms of hypertension, perhaps by upregulating the renal renin-angiotensin system,[10,29] there is sparse data on the effects of androgens on the vascular control mechanisms of blood pressure. The recently discovered effects of testosterone on the mechanisms of vascular relaxation/contraction may warrant further examination of its role in cardiovascular disease and hypertension.

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