Gender, sex hormones, and vascular tone

Julia M. Orshal and Raouf A. Khalil

Research and Development, Department of Veterans Affairs Medical Center, West Roxbury; and Department of Medicine, Harvard Medical School, Boston, Massachusetts 02132

Invited Review

CARDIOVASCULAR DISEASES such as hypertension and coronary artery disease are among the most common and costly diseases in the industrialized world. The incidence of cardiovascular diseases is greater in men aged 30–50 yr compared with women of similar age (38, 43). Among women, the incidence of cardiovascular diseases is greater in postmenopausal compared with premenopausal women. Although reports from Heart and Estrogen-Progestin Replacement Study (HERS), HERS2, and Women’s Health Initiative (WHI) studies do not support beneficial vascular effects of hormone replacement therapy (HRT) particularly in elderly hypertensive women (37, 47, 74, 89, 117, 119), other studies have suggested beneficial effects of HRT in reducing the incidence of coronary artery disease in postmenopausal women and have suggested putative vascular protective effects of female sex hormones (43, 48, 108). Sex hormone receptors have been identified in the cytosol and nuclear compartments of various cell types including the endothelium and vascular smooth muscle (VSM) (Table 1). The interaction of sex hormones with cytosolic/nuclear receptors has long been known to stimulate a host of genomic effects that could affect vascular cell growth and proliferation. Recent evidence suggests that sex hormones may also interact with specific plasmalemmal receptors and induce additional nongenomic vascular effects (38, 43). Several excellent reviews have described the role of gender and female sex hormones in modifying the incidence of cardiovascular disease (38, 43, 48). Previous reviews have focused on possible estrogen-induced beneficial effects such as modification of circulating lipoproteins, inhibition of lipoprotein oxidation (106), attenuation of atherosclerotic lesions, favorable modulation of homocysteine (134), changes in blood coagulation (4), and inhibition of intravascular accumulation of collagen (8) (Table 2). Also, several studies have suggested significant effects of sex hormones on the renal control mechanisms of the blood pressure, particularly the renin-angiotensin system (104, 105). For example, estradiol has been suggested to inhibit renin release and the angiotensin converting enzyme (126), whereas testosterone may increase the blood pressure by activating the renin-angiotensin system (105). Although alterations in vascular tone play a major role in the control of blood pressure and the coronary circulation and thereby the incidence of hypertension and coronary artery disease, little information is available regarding the gender differences and the effects of sex hormones on vascular tone.

The purpose of this review is to provide an insight into the gender differences in vascular tone and the effects of sex hormones on vascular cells, namely the endothelium and smooth muscle. We will first provide an overview on vascular tone and its modification with gender. The vascular sex hormone receptors, agonists, and antagonists will then be de-
scribed. We will follow with a description of the genomic effects of sex hormones on endothelial as well as VSM cell growth and proliferation. The nongenomic effects of sex hormones on the endothelium-dependent mechanisms of vascular relaxation will then be discussed. We will follow with a detailed description of the effects of sex hormones on the signaling mechanisms of VSM contraction. The review will end with a perspective on potential areas for future investigations to better understand the mechanisms underlying the gender differences and the effects of sex hormones on vascular tone and the possible clinical uses of HRT to reduce the incidence of cardiovascular disease.

Table 1. Examples of sex hormone receptor distribution, agonists, and antagonists in the endothelium and vascular smooth muscle

<table>
<thead>
<tr>
<th>Subcellular distribution</th>
<th>Blood vessel distribution</th>
<th>Estrogen</th>
<th>Progesterone</th>
<th>Testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mouse aorta</td>
<td>- Mouse aorta</td>
<td>- Mouse aorta</td>
<td>- Mouse aorta</td>
</tr>
<tr>
<td></td>
<td>Rat aorta</td>
<td>- Rat aorta</td>
<td>- Rat aorta</td>
<td>- Rabbit aorta</td>
</tr>
<tr>
<td></td>
<td>Rabbit uterine artery</td>
<td>- Rabbit uterine artery</td>
<td>- Bovine aortic endothelial cells</td>
<td>- Bovine aortic endothelial cells</td>
</tr>
<tr>
<td></td>
<td>Bovine aortic endothelial cells</td>
<td>- Bovine aortic endothelial cells</td>
<td>- Primate aorta, coronary artery</td>
<td>- Bovine aorta</td>
</tr>
<tr>
<td></td>
<td>Primate coronary, carotid artery</td>
<td>- Primate aorta, coronary artery</td>
<td>- Human aorta, internal carotid, coronary, uterine artery</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Human coronary, uterine artery, umbilical vein endothelial cells</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Subcellular distribution                  | Plasma membrane                                                                           | +              | +                | +                |
|------------------------------------------|Cytosol                                                                                     | +              | +                | +                |
|                                          | Nucleus                                                                                   | +              | +                | +                |
|                                          | Mitochondria, Reticulum, Golgi, Lysosomes                                                  |                |                  |                  |

**Agonists**
- 17β-Estradiol
- Estradiol benzoate, cypionate, valerate
- Ethinyl estradiol
- Diethylstilbestrol
- Idoxifene
- Phytoestrogens: genistein, daidzein, coumestrol, zearalenone, α-zearalanol, naringenin, taxifolin, biochanin A

**Antagonists**
- ICI-182780 (Pulvastrent), ICI-164384
- RR-tetrahydrochrysene (ER-β)
- RU-486 (Mifepristone)
- ZK-98299, ZK-98734
- Flutamide, Hydroxyflutamide
- Casodex

**Agonists/antagonists**
- Tamoxifen, 4-hydroxytamoxifen
- Raloxifene, toremifene
- 6-Carboxymethyl genistein
- Enterodiol, enterolactone

Table 2. Beneficial vascular effects and possible clinical applications of sex hormones

<table>
<thead>
<tr>
<th></th>
<th>Estrogen</th>
<th>Progesterone</th>
<th>Testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelium</td>
<td>- Promotes proliferation/migration</td>
<td>- Promotes release of EDRFs</td>
<td>- Promotes release of EDRFs</td>
</tr>
<tr>
<td></td>
<td>- Promotes release of EDRFs</td>
<td>- Inhibits release of EDCFs</td>
<td>- Inhibits proliferation</td>
</tr>
<tr>
<td></td>
<td>- Inhibits proliferation/migration</td>
<td>- Inhibits proliferation/migration</td>
<td>- Facilitates the vascular inflammatory effects of estrogen</td>
</tr>
<tr>
<td></td>
<td>- VSM relaxation and vasodilation</td>
<td>- VSM relaxation and vasodilation</td>
<td>- Acute vascular relaxation</td>
</tr>
<tr>
<td>Smooth muscle</td>
<td>- Antiatherosclerotic</td>
<td>- Antiatherosclerotic</td>
<td>- Antiatherosclerotic</td>
</tr>
<tr>
<td></td>
<td>- Antioxidant</td>
<td>- Decreases low density lipoproteins, increase high density lipoproteins</td>
<td>- Decreases low density lipoproteins</td>
</tr>
<tr>
<td></td>
<td>- Decreases low density lipoproteins, increase high density lipoproteins</td>
<td>- Decrease lipoprotein oxidation</td>
<td>- Decrease platelet adhesion</td>
</tr>
<tr>
<td></td>
<td>- Inhibition of lipoprotein oxidation</td>
<td>- Decrease plasma homocysteine</td>
<td>- Decrease vascular collagen</td>
</tr>
<tr>
<td></td>
<td>- Decreases plasma homocysteine</td>
<td>- Increases antiplatelet aggregation factors, decrease platelet adhesion</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Increases antiplatelet aggregation factors, decrease platelet adhesion</td>
<td>- Increases antiplatelet aggregation factors, decrease platelet adhesion</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Decreases vascular collagen</td>
<td>- Decreases vascular collagen</td>
<td></td>
</tr>
<tr>
<td>Other vascular effects</td>
<td>- Coronary artery disease</td>
<td>- Coronary artery disease</td>
<td>- Reduction of myocardial ischemia in men with coronary artery disease</td>
</tr>
<tr>
<td></td>
<td>- Postmenopausal hypertension</td>
<td>- Thromboembolic events</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Thromboembolic events</td>
<td>- Thromboembolic events</td>
<td></td>
</tr>
</tbody>
</table>

EDRF, endothelium-derived relaxing factor; EDCF, endothelium-derived contracting factor; VSM, vascular smooth muscle.
GENDER DIFFERENCES IN VASCULAR TONE

Vascular tone is defined as the degree of constriction of a blood vessel relative to its maximal diameter in the dilated state. Under basal conditions, most resistance and capacitance vessels exhibit some degree of smooth muscle contraction that determines the diameter or tone of the vessel. Vascular tone is influenced by both the endothelium and VSM. The vascular tone is also determined by a multitude of vasoconstrictor factors such as norepinephrine, ANG II, vasopressin, and 5-hydroxytryptamine as well as vasodilator factors such as bradykinin and prostacyclin (PGI2). These factors can be divided into extrinsic factors that originate from outside the blood vessel and intrinsic factors that originate from the vessel itself. Extrinsic factors primarily serve the function of regulating arterial pressure by altering systemic vascular resistance, whereas intrinsic mechanisms are concerned with regulation of local blood flow within an organ (30).

Gender differences in vascular tone have been described in a multitude of vascular beds in both human and experimental animals (123). For example, α-adrenergic agonists such as norepinephrine cause less forearm vasoconstriction in women than in men (77). Also, oxidized low-density lipoprotein enhances 5-hydroxytryptamine-induced contraction to a greater extent in coronary arteries from male than female pigs (23). In addition, the contraction to norepinephrine or phenylephrine (Phe) is greater in the aorta of intact male than intact female rats (25, 123). Interestingly, vasopressin-induced contraction in rat aorta exhibits sexual dimorphism, but the contraction in females is almost twice that in males (123). The difference could be related to possible tachyphylactic effects of vasopressin in isolated vessels, which are different from its effects in vivo. This is supported by reports that the pressor response to vasopressin infusion in vivo is greater in male than female rats (123).

We recently found that the vascular contraction is not different between castrated and intact male rats, but significantly enhanced in ovariectomized (OVX) females compared with intact females, suggesting that the gender differences in vascular tone are less likely related to androgens and more likely related to estrogens (25, 69). The data also suggest that the gender differences in vascular tone are due to direct vascular effects of sex hormones, possibly through interaction with specific hormone receptors in the vasculature.

SEX HORMONE RECEPTORS IN BLOOD VESSELS

Receptors for estrogen, progesterone, and testosterone are expressed in varying numbers in both the endothelium and VSM of multiple vascular systems (75, 129). For instance, a significant association between the number of estrogen receptors (ER) and normal endothelial cell function has been reported, and suggested that decreased number of endothelial ER may represent a risk factor for cardiovascular diseases (109). Also, the sex hormone receptors appear to have different subtypes, tissue distribution, and subcellular location and can be modulated by various agonists and antagonists (Table 1).

Two ER subtypes have been identified, ER-α and ER-β (84). Several variants of ER-α, such as ER-αA, ER-αC, ER-αE, and ER-αF (79), as well as ER-β, such as ER-β1, ER-β2, ER-β4, and ER-β5, have been described (14, 115). Some studies suggest that ER-α promotes the protective effects of estrogen in response to vascular injury (98). However, ER-β is more widely distributed in the body than ER-α. Also, ER-β is the receptor form that is predominantly expressed in human VSM, particularly in women (84). Induction of ER-β mRNA expression has also been demonstrated after balloon vascular injury to the aorta of the male rat. Furthermore, experiments on transfected HeLa cells have shown that in response to 17β-estradiol (E2), ER-α is a stronger transactivator than ER-β at low receptor concentrations. However, at higher receptor concentrations, ER-α activity self-squelches, and ER-β becomes the stronger transactivator. These data support a role for ER-β in the direct vascular effects of estrogen and in the regulation of vascular function (57).

ERs have been localized in the nucleus, and continuous shuffling of the receptor between the cytoplasm and the nucleus has been suggested (49). Sex steroids, such as estrogen, diffuse through the plasma membrane and form complexes with specific cytosolic and/or nuclear receptors, which then bind to chromatin and stimulate the transcription of a set of genes with a specific sex steroid-responsive regulatory element (60, 80) (Fig. 1). ER-mediated transcription requires coactivators to exert transcriptional activity. The steroid receptor coactivator-3 (SRC-3) is highly expressed in VSM. SRC-3 interacts with estrogen-bound ERs and coactivates the transcription of target genes in cultured VSM cells, suggesting that SRC-3 facilitates ER-dependent vasoprotective effects during vascular injury (144). However, estrogen can also bind to the plasma membrane of various vascular cells and induce rapid cellular events, suggesting additional nongenomic action triggered by a signal-generating receptor on the cell surface (26, 38). Recent studies also suggest possible interactions of ER with signal-modulating proteins or coactivators in the plasma membrane (103).

Another hormone receptor that has been located in the endothelium and VSM is the progesterone receptor (129, 135). Progesterone receptor-A and -B have been identified (97). Progesterone receptors, particularly the B isoform, appear to have a direct role in the regulation of gene transcription and VSM cell proliferation (99).

Testosterone or androgen receptors have also been identified in endothelial cells and VSM. The expression of androgen receptors in VSM appears to vary depending on the gender and the status of the gonads. The androgen receptor protein, as detected by Western blot in rat aortic VSM, is less in the cells of females than those of males (55). In the smooth muscle of primates, androgen receptor mRNA levels are upregulated by combined estradiol plus testosterone treatment, whereas estradiol treatment alone had little or no effect, suggesting that a collaborative action of estradiol and testosterone enhances androgen receptor expression (142).

GENOMIC EFFECTS OF SEX HORMONES

The interaction of sex hormones with nuclear/cytosolic receptors triggers a host of genomic effects leading to endothelial cell growth. The effects of sex hormones on endothelial cell growth appear to be mediated by activation of mitogen-activated protein kinase (MAPK; Fig. 1). This is supported by reports that E2 induces the phosphorylation of p38 and p42/44 MAPK as well as the migration and proliferation of porcine aortic endothelial cells (42).
Although estradiol activates signaling pathways that stimulate endothelial cell proliferation, the steroid appears to inhibit cell growth and to induce antiproliferative effects in VSM (33). For example, the rate of growth in VSM of female aorta is slower than that in male aorta (3). The inhibitory effects of estrogen on VSM growth may be enhanced by its interaction with steroid receptor coactivators such as SRC-3 (144). Estrogen has also been shown to inhibit MAPK activity in VSM, and this effect is blocked by the estrogen antagonist ICI-182780, suggesting that inhibition of the MAPK pathway via ERs contributes to the inhibitory effects of estrogen on VSM growth (33). Estrogen may also antagonize the growth-promoting effect of ANG II on VSM via the induction and activation of protein phosphatases through genomic as well as nongenomic mechanisms (126). Interestingly, nitric oxide (NO) has been shown to inhibit VSM growth and proliferation. Whether an estrogen-induced increase in NO production plays a role as a possible mediator of estrogen-induced inhibition of VSM growth remains to be clarified. Furthermore, estradiol may stimulate cAMP production and the cAMP-derived adenosine may regulate VSM growth via adenosine receptors, suggesting that the cAMP/adenosine pathway may contribute to the antiproliferative effects of estradiol (34).

Progesterone also inhibits VSM proliferation and migration and may facilitate the inhibitory effects of estrogen. Progesterone appears to mediate its inhibitory effects on VSM growth by reducing MAPK activity (93). Some studies have suggested that androgens accelerate vascular growth by stimulating the proliferation of VSM, whereas other studies show androgen-induced inhibition of growth and proliferation (121, 142). The discrepancy in these reports may be related to the concentration of androgens used. Androgens may control the proliferation of their target cells by first increasing cell proliferation and later by inhibiting the proliferation of the same cells. For example, dihydrotestosterone modulates human umbilical VSM cell proliferation in a dose-dependent manner, with low concentrations (3 nM) stimulating...
[\textsuperscript{3}H]thymidine incorporation, and high concentrations (300 nM) inhibiting [\textsuperscript{3}H]thymidine incorporation (121, 142).

We should note that the genomic effects of sex hormones may alter the expression of a multitude of regulatory and signaling proteins in endothelial cells and VSM. To avoid repetition, these genomic effects will be described with the nongenomic effects of sex hormones on the endothelium and VSM as described below.

**Nongenomic Effects of Sex Hormones**

The interaction of sex hormones with plasmalemmal receptors in the endothelium and VSM may initiate additional nongenomic vascular effects. For example, estrogen may induce acute inhibition of vascular contraction (24, 129). Also, progestins may have direct vascular effects or modify the effects of estrogen on vascular contraction (24). Interestingly, direct vascular effects of testosterone have also been described (142, 145). The acute nongenomic vasodilator effects of sex hormones appear to have both endothelium-dependent as well as endothelium-independent mechanisms involving direct effects on VSM.

**Sex Hormones and the Endothelium**

The vascular endothelium plays an important role in mediating the gender-related and the estrogen-induced vasodilation (72). Physiological levels of E\textsubscript{2} potentiate endothelium-dependent flow-mediated vasodilation in postmenopausal women (45). Also, endothelium-dependent relaxation of isolated aorta is greater in female than in male spontaneously hypertensive rats (SHR; 67, 72). Similar to estrogen, progesterone may promote endothelium-dependent vasodilation in porcine coronary artery (92). Also, some studies have shown that testosterone induces endothelium-dependent vascular relaxation (20, 22). The vascular endothelium is known to release relaxing factors such as NO, prostacyclin (PG\textsubscript{I2}), and endothelium-derived hyperpolarizing factor (EDHF), as well as contracting factors such as endothelin (ET-1) and thromboxane A\textsubscript{2}, and the sex hormones appear to induce vascular relaxation by modifying the synthesis/release/bioactivity of one or more of these factors.

**Sex Hormones and NO**

NO is a powerful vasodilator and relaxant of VSM. NO is produced from the transformation of L-arginine to L-citrulline by the enzyme NO synthase (NOS; Figs. 1 and 2). Three NOS isoforms have been described: neuronal nNOS (NOS I), inducible iNOS (NOS II), and endothelial eNOS (NOS III; 141). iNOS is Ca\textsuperscript{2+}- independent and may be involved in long-term regulation of vascular tone, whereas eNOS is Ca\textsuperscript{2+}-dependent and plays a role in the short-term regulation of vascular tone.

Under basal conditions, eNOS is firmly attached to the inhibitor protein caveolin, a scaffolding transmembrane protein in the plasma membrane caveolae (Fig. 2). Agonist activation of endothelial cells causes an increase in intracellular Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{i}) and an initial activation of eNOS. The initial Ca\textsuperscript{2+}-dependent activation of eNOS involves its dissociation from caveolin and its translocation to intracellular sites (Fig. 2).
close to the nucleus. During maintained endothelial cell stimulation with an agonist, activation of MAPK and/or the protein kinase B/Akt pathway and subsequent phosphorylation of eNOS causes a second translocation of cytosolic eNOS back to the cell membrane where it undergoes myristoylation and palmitoylation, a process required for its full activation (Fig. 2). These rapid receptor-mediated effects on the NO pathway are seen not only for “classic” eNOS agonists, such as ACh and bradykinin, but also for estradiol (86; Figs. 1 and 2).

Total NO production is greater in premenopausal women than in men (39). The cellular origin of the increased NO in women is not entirely clear, but differences in vascular endothelial NO production may underlie the gender differences in vascular tone (39). NO release from the endothelium is increased in arteries of females compared with males (71, 78, 139). This is supported by reports that the inhibitory effect of the NOS inhibitor Nω-ω-arginine methyl ester (L-NMMA) on ACh-induced relaxation is more pronounced in the mesenteric artery of female than male rats (67). Estrogen appears to be responsible for the gender differences in endothelial NO release (28, 41, 78, 85). E2 replacement in OVX female guinea pigs enhances the sensitivity to vasodilators in the coronary microcirculation through increased endothelial NO production (130). Also, prolonged treatment of human coronary endothelial cells with E2 increases the basal, adenosine triphosphate-, and A23187-induced NO release (143).

Estrogen may influence NO production by increasing NOS expression (41, 78). Activation of genomic ERs may cause upregulation of eNOS (71; Fig. 1). It has been shown that ER-α gene transfer into bovine aortic endothelial cells induces eNOS gene expression (128). Also, estrogen increases the level of eNOS mRNA in ovine fetal pulmonary artery endothelial cells (83). Estrogen may also prevent destabilization of eNOS mRNA induced by tumor necrosis factor-α through an ER-mediated mechanism (125).

In addition to the genomic effects of estrogen on eNOS expression, estrogen may regulate NOS activity and thereby NO production and vascular tone by interacting with specific ERs in the endothelial cell plasma membrane and activation of rapid nongenomic signaling pathways (41, 78). Membrane-impermeant forms of estrogen bind to specific ERs at the cell surface and stimulate NO release from human endothelial cells (112). Also, in bovine aortic endothelial cells, E2 causes transient translocation of eNOS from the plasma membrane to intracellular sites close to the nucleus, although during prolonged exposure to E2, most of the eNOS returns to the plasma membrane for its full activation (86).

The acute effect of E2 on eNOS activity and NO release has been suggested to occur via activation of ER-α in COS-7 cells (19) and mouse aorta (27). However, studies have shown that overexpression of ER-β in COS-7 cells enhances rapid eNOS activation by E2 and the ER-β protein association to the plasma membrane caveolae and that these events occur independent of ER-α. These findings indicate that endogenous ER-β also plays a prominent role in the nongenomic effects of E2 on eNOS activity (17).

The acute effects of E2 on eNOS activity and NO release appear to be dependent on [Ca\textsuperscript{2+}]. Gender differences in the regulation of endothelial [Ca\textsuperscript{2+}] have been related to direct or indirect effects of estrogen on the Ca\textsuperscript{2+} handling mechanisms in the vascular endothelium (78, 102, 139). Estrogen activation of cell surface ERs has been suggested to be coupled to increases in [Ca\textsuperscript{2+}] and acute stimulation of NO release from human endothelial cells (124). Studies have also suggested possible gender differences in the eNOS sensitivity to [Ca\textsuperscript{2+}]. Experiments on pressurized coronary arteries of rats have shown that increases in [Ca\textsuperscript{2+}], cause activation of eNOS with a similar slope and half-activation constant for both female and male arteries. However, at [Ca\textsuperscript{2+}]. > 100 nM, eNOS activity is higher in females compared with males (78). Interestingly, E2 may promote the association of heat shock protein 90 with eNOS and thereby reduce the Ca\textsuperscript{2+} requirement for eNOS activation (112). However, E2 may stimulate eNOS activity and increase NO release from human endothelial cells, independent of cytosolic Ca\textsuperscript{2+} mobilization (16), perhaps through eNOS phosphorylation via a mechanism involving MAPK or Akt (56, 112). In studies of ovine endothelial cells, E2 caused acute activation of eNOS as well as rapid activation of MAPK, and inhibition of MAPK kinase prevented the activation of eNOS by E2. These data suggest that the acute vascular effects of estrogen are mediated by ER functioning in a nongenomic manner to activate eNOS via MAPK-dependent mechanisms (19). Also, E2 rapidly induces phosphorylation and activation of eNOS through the phosphatidylinositol-3 (PI3)-kinase-Akt pathway and thereby reduces its [Ca\textsuperscript{2+}], requirement for activation (52; Fig. 2).

The effects of estrogen on the NO pathway may also be related to its antioxidant effects. It has been shown that the increase in arterial pressure in OVX female rats is associated with lower plasma antioxidant levels, reduced thiol groups, and increased plasma lipoperoxides and vascular free radicals, and that estrogen replacement prevents the increase in free radicals and the decrease in plasma levels of nitrites/nitrates (54). Also, E2 inhibits NAPDH oxidase expression and the generation of reactive oxygen species and peroxynitrite (ONO\textsubscript{O}−) in human umbilical vein endothelial cells (136; Fig. 1). ANG II stimulation of endothelial cells has been shown to increase the expression of NAPDH oxidase, which may contribute to oxidative stress, as evidenced by ONOO\textsuperscript{−} formation. E2 appears to inhibit ANG II-induced increases in oxidative-stress effects, possibly through reduced ANG II type 1 receptor expression (46). Also, measurements of superoxide anion (O\textsubscript{2}−) in the isolated aorta have shown greater amounts in male than in female rats (12). Furthermore, estrogen decreases the generation of O\textsubscript{2}− from cultured bovine aortic endothelial cells and thereby enhances NO bioactivity and decreases ONOO\textsuperscript{−} release (5). These data suggest that the decrease in vascular tone and arterial pressure with estrogen administration may be related to preventing oxidative stress and improving endothelial function.

Although gender and estrogen treatment may affect NO production/bioactivity, their influence on factors downstream in the NO signaling pathway is unclear. NO produced from the endothelium is known to activate guanylate cyclase in the smooth muscle leading to increased cGMP and stimulation of cGMP-dependent protein kinase (PKG; 140). PKG may decrease [Ca\textsuperscript{2+}], by stimulating Ca\textsuperscript{2+} extrusion pumps in the plasma membrane and Ca\textsuperscript{2+} uptake pumps in the sarcoplasmic reticulum membrane and/or decrease the sensitivity of the contractile myofilaments to [Ca\textsuperscript{2+}], and thereby promote VSM relaxation (Fig. 1). It has been shown that the relaxation of mesenteric arterial rings by the exogenous NO donor sodium
nitroprusside is greater in female than male SHR (67), suggesting gender differences in smooth muscle reactivity to NO and possible hormonal regulation of PKG. However, in aortic rings from male and female SHR, sodium nitroprusside-induced relaxation is similar, making the possibility of gender differences in smooth muscle reactivity to NO and the hormonal regulation of PKG rather less likely (72).

Carbon monoxide is formed by heme oxygenase-2 in the vascular endothelium and has been found to activate soluble guanylate cyclase and dilate blood vessels independently from NO. Because of the parallels between NO and carbon monoxide, it has been suggested that estrogen might affect carbon monoxide production in vascular endothelium. Studies in human umbilical vein and uterine artery endothelial cells have shown that treatment with E2 causes significant increases in intracellular carbon monoxide production, heme oxygenase-2 protein levels, and cellular cGMP, suggesting a potential role for carbon monoxide as a biological messenger molecule in estrogen-mediated regulation of vascular tone (133).

The effects of progesterone on the vascular endothelium are less clear. Experimental studies on canine coronary arteries have suggested that progesterone may counteract the stimulatory effects of estrogen on endothelium-dependent NO production and vascular relaxation (88). However, studies in postmenopausal women have shown that progesterone does not appreciably attenuate estradiol-induced endothelium-dependent vasodilatation of the brachial artery (44). Other studies have shown that progesterone may stimulate NO production in rat aortic strips (116) and induce endothelium-dependent NO-mediated relaxation in rat resistance mesenteric arteries (18). Also, intravenous infusion of progesterone in pigs has been shown to induce endothelium-dependent coronary vasodilatation via a mechanism involving the release of NO (92). Furthermore, chronic administration of progesterone has been shown to increase the expression of eNOS in the endothelium of ovine uterine artery (111).

In regard to testosterone, acute intracoronary administration of the hormone in canine coronary epicardial and resistance vessels has been shown to induce vasodilatation that is mediated in part by NO (20). Testosterone may also modulate the effects of other agonists on endothelial NO production. Bradykinin, a known activator of endothelium-dependent NO pathway, has been shown to increase intracellular inositol 1,4,5-trisphosphate (IP3) and stimulate rapid release of Ca2+ from the endoplasmic reticulum in cultured endothelial cells of male rats. Treatment of the cells with testosterone blocks bradykinin-induced increases in [Ca2+]i, and thereby NO production in endothelial cells, perhaps through an effect of testosterone on membrane-bound bradykinin receptors or on bradykinin-induced Ca2+ release mechanism (110). Recent studies have shown that treatment of human endothelial cells with androgens such as dehydroepiandrosterone (DHEA) triggers NO synthesis by enhancing the expression and stabilization of eNOS. DHEA appears to activate eNOS through an MAPK-dependent mechanism, but not the PIP3 kinase/Akt pathway (120). Also, administration of DHEA in ovarioctomized female Wistar rats has been shown to restore aortic eNOS levels and eNOS activity (120). Interestingly, androgen antagonists such as flutamide may also affect the NO pathway. It has been shown that flutamide produces direct vasodilation by inducing NO release from the endothelium and subsequent activation of guanylyl cyclase in rat aortic smooth muscle; however, these vascular effects of flutamide do not appear to be mediated via androgen receptors (61). Additionally, administration of flutamide reduces blood pressure in hypertensive transgenic TGR(mREN2)27 rats, which have an overactive renin-angiotensin system. Furthermore, flutamide reduces the blood pressure in TGR(mREN2)27 rats with an additional testicular feminizing mutation (tfm), suggesting that the vascular effects of flutamide may be caused by androgen receptor-independent mechanisms (61).

SEX HORMONES AND PGI2

PGI2 is an endothelium-derived relaxing factor that is produced from the metabolism of arachidonic acid by the enzyme cyclooxygenase (COX; Fig. 1). COX has two isoforms, COX-1 and COX-2. COX inhibitors such as indomethacin inhibit a significant component of endothelium-dependent vascular relaxation, and gender differences in the indomethacin-sensitive component of vascular relaxation have been attributed to differences in the COX products (6). Estrogen may augment the production of COX products such as PGI2 (41). Physiological levels of E2 cause upregulation of COX-1 expression and PGI2 synthesis in ovine fetal pulmonary artery and human umbilical vein endothelial cells (66). Also, E2 causes rapid ER-β-mediated stimulation of PGI2 synthesis in ovine fetal pulmonary artery endothelial cells via a Ca2+-dependent, but MAPK-independent, pathway (118). It has also been suggested that the COX-2 pathway plays a specific role in the rapid E2-induced potentiation of cholinergic vasodilation in postmenopausal women (13). However, other studies have reported that indomethacin does not affect the E2-induced relaxation in endothelium-intact coronary artery, suggesting that the release of vasodilator prostanoids may not be involved in the E2-induced coronary relaxation (62). It has also been suggested that estrogen may modulate cross-talk between the NO synthase and COX pathways of vasodilation and that estrogen-induced increase in the NO component of endothelium-dependent dilatation may be associated with a decrease in the COX component (15).

In regard to other sex hormones, some studies have shown that concomitant administration of progesterone with estrogen prevents the stimulatory effects of estrogen on PGI2 production in cultured human umbilical vein endothelial cells (87). However, other studies have shown that progesterone may exert a direct nongenomic effect on rat aorta, which involves COX activation and increased PGI2 production (116). On the other hand, experiments on the aorta of female rat have shown that treatment with testosterone is associated with a decrease in PGI2 synthesis (137).

SEX HORMONES AND EDHF

The endothelium may release other relaxing factors even during complete inhibition of the NO-cGMP and the PGI2-cAMP pathways. Such factors have been shown to activate Ca2+-activated K+ channels (BKca) and to cause hyperpolarization and relaxation of the smooth muscle and thereby designated EDHF.

The greater endothelium-mediated relaxation in females compared with males may be related to differences in the endothelium-dependent hyperpolarization of VSM (67). It has
been shown that ACh-induced hyperpolarization and relaxation of mesenteric arteries are reduced in OVX female and intact male rats compared with intact female rats and that the differences in the ACh responses in OVX female compared with intact female rats are eliminated in the presence of K⁺ channel blockers such as apamin or charybdotoxin. Also, the hyperpolarizing response to ACh is improved in OVX female rats treated with E₂. These data suggest that estrogen-deficient states attenuate relaxation transduced by EDHF (81, 113).

Testosterone may also promote endothelium-mediated hyperpolarization of VSM. In aortic rings of both Wistar-Kyoto (WKY) and SHR, testosterone induces concentration-dependent relaxation (58). Testosterone-induced relaxation is reduced by denudation of endothelium in SHR, but not WKY. Indomethacin and l-NAME show little influence on testosterone-induced relaxation in both WKY and SHR aortic rings. 4-Aminopyridine, inhibitor of voltage-dependent K⁺ channels, and tetraethylammonium, inhibitor of BKCa, reduce testosterone-induced relaxation in SHR, but not WKY. On the other hand, glibenclamide, inhibitor of ATP-sensitive K⁺ channels, reduces testosterone-induced relaxation in both WKY and SHR aortic rings. These data suggest that in SHR aortic rings, testosterone may release endothelium-derived substances that cause hyperpolarization of the cells by a mechanism that involves voltage-dependent and BKCa channels. However, a significant component of testosterone-induced vasorelaxation in both WKY and SHR appears to be endothelium-independent and may involve ATP-sensitive K⁺ channels in aortic smooth muscle (58).

**SEX HORMONES AND ENDOTHELIUM- DERIVED CONTRACTING FACTORS**

The gender differences in vascular tone may be related to differences in the release of or sensitivity to endothelium-derived contracting factors (EDCF) such as ET-1 and thromboxane A₂. ET-1 release from endothelial cells appears to be reduced in females and may explain the decreased vascular tone and blood pressure in female compared with male SHR reduced in females and may explain the decreased vascular activation of endothelial ET B causes the release of various contracting factors such as thromboxane A₂. ET-1 release from endothelial cells appears to be derived contracting factors (EDCF) such as ET-1 and thromboxane A₂ is more pronounced in male than in female SHR (67).

**SEX HORMONES AND VSM CONTRACTION**

In addition to the nongenomic effects of sex hormones on the endothelium, rapid nongenomic effects on VSM have been described (24, 62, 64, 129; Fig. 3). For example, estrogen causes vasodilation in endothelium-denuded vessels, suggesting that the estrogen-induced inhibition of vascular tone has an endothelium-independent component that involves direct action on VSM (24, 38, 62, 64). Also, estrogen causes relaxation in endothelium-denuded rabbit, porcine, and human coronary arteries precontracted by ET-1, PGF2α, and high KCl depolarizing solution (24, 51, 64; Fig. 4). The vasodilator effects of estrogen do not appear to be mediated by the classic cytosolic-nuclear ER or stimulation of protein synthesis, but rather through a direct effect of estrogen on plasmalemmal receptors in VSM (24, 38).

The acute vasodilator effects of estrogen may be influenced by gender, vessel type, estrous cycle, and previous exposure to estrogen. For example, in rat aorta, E₂ causes greater relaxation in males than females (25). Among female rats, the largest E₂-induced vasodilation is seen in the tail and mesenteric arteries from females at the proestrous stage. However, the magnitude of relaxation in microvessels of estradiol-replaced OVX female rats is smaller than that of nonreplaced OVX rats, suggesting that chronic estradiol replacement may downregulate the acute nongenomic vasorelaxation effects of estrogen in small arteries of OVX rats (68).

The effects of progesterone on vascular reactivity are less clear and range between no effect, inhibition of vasorelaxation, and potent vascular relaxation (24, 63, 129). Progesterone may cause endothelium-independent relaxation of VSM, although it is smaller than that induced by estrogen (24; Fig. 4). Progesterone induces relaxation of primate, porcine, rabbit, and rat coronary arteries (24, 36, 63, 90). The vasodilator effect of progesterone in isolated VSM suggests that its benefits in hormone replacement therapy may be related to its nongenomic relaxant effects on VSM.
Some studies suggest that testosterone enhances vascular contraction either by inhibiting endothelium-dependent relaxation or by directly stimulating VSM contraction (142). For example, treatment of porcine coronary artery with nanomolar concentrations of testosterone impairs bradykinin- and A23187-induced endothelium-dependent vascular relaxation. Also, testosterone enhances thromboxane A2-induced coronary vasoconstriction in guinea pigs. Flutamide, a testosterone receptor antagonist, has been shown to cause direct vasodilatation in rat vessels and flutamide-induced vascular relaxation is smaller in females compared with males, suggesting that testosterone may promote vascular contraction (2). However, other studies have shown that testosterone induces relaxation of rabbit coronary artery and aorta, rat aorta, and canine and porcine coronary artery (24, 145; Fig. 4). A significant portion of the testosterone-induced vascular relaxation appears to be endothelium independent because only small differences could be observed between the relaxation in vessels with and without endothelium. Also, inhibition of endothelium-dependent relaxation pathways such as NOS and COX may not abolish the vasorelaxing effect of testosterone (145), providing evidence that a significant component of testosterone-induced relaxation is endothelium independent and involves direct action on VSM. The testosterone-induced vasorelaxation appears to be a structurally specific effect of the androgen molecule and is enhanced in more polar analogs that have a lower permeability to the VSM cell membrane (31).

We should note that although both the endogenous presence and exogenous application of sex hormones may be associated with reduction in vascular contraction, the mechanisms of hormone-induced relaxation in isolated vascular strips or cells and the possible vasorelaxant effects of the hormone in vivo may not be identical. The acute effects of estrogen on vascular contraction in vitro are often observed at micromolar concen-
ERs in their arteries than males (21). The gender differences in vascular contraction could also be related to effects of sex hormones on the gene expression of the specific receptors of vasoconstrictor agonists such as ANG II (Table 3). Western blot analyses in VSM have revealed that estrogen induces a downregulation and progesterone an upregulation of the angiotensin AT1 receptor protein. Also, E2 decreases the AT1 receptor mRNA half-life, whereas progesterone induces stabilization of AT1 receptor mRNA (96). Other studies have shown that progesterone replacement in OVX monkeys decreases thromboxane A2 receptors in coronary arteries (91). Nevertheless, the gender differences in vascular contraction may also be related to gender differences in the signaling mechanisms of VSM contraction downstream from receptor activation.

**SIGNALING MECHANISMS OF VSM CONTRACTION**

It is widely accepted that VSM contraction is triggered by increases in [Ca2+]i due to initial Ca2+ release from the sarcoplasmic reticulum and maintained Ca2+ entry from the extracellular space (73, 95). Also, activation of protein kinases such as myosin light chain (MLC) kinase, Rho kinase, and MAPK as well as inhibition of MLC phosphatase may contribute to smooth muscle contraction (59, 122; Fig. 3). Additionally, the interaction of an α-adrenergic agonist such as Phe with its receptor is coupled to increased breakdown of plasma membrane phospholipids and increased production of diacylglycerol (DAG), which activates protein kinase C (PKC; 70). PKC is mainly cytosolic under resting conditions and undergoes translocation from the cytosolic to the particulate fraction when it is activated by DAG or phorbol esters. PKC is now known to be a family of several isoforms that have different enzyme properties, substrates, and functions and exhibit different subcellular distributions in the same blood vessel from different species and in different vessels from the same species (69, 70).

**Table 3. Potential causes of the gender differences in vascular tone**

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Premenopausal Female</th>
<th>Postmenopausal Female</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma level</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrogen, pg/ml</td>
<td>15–25</td>
<td>100–700</td>
<td>0–60</td>
</tr>
<tr>
<td>Progesterone, pg/ml</td>
<td>25–100</td>
<td>50–700</td>
<td>200–500</td>
</tr>
<tr>
<td>Testosterone, ng/ml</td>
<td>4–10</td>
<td>0.3–0.7</td>
<td>0.1–0.7</td>
</tr>
<tr>
<td><strong>Vascular hormone receptor</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrogen</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Progesterone</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Testosterone</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Endothelium eNOS</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Ca2+</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td><strong>Smooth muscle</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANG II Receptor</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>TXA2 Receptor</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>[Ca2+]i, (nM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>–100</td>
<td>~60</td>
<td>~100</td>
</tr>
<tr>
<td>Transient</td>
<td>~400</td>
<td>~400</td>
<td>~400</td>
</tr>
<tr>
<td>Maintained</td>
<td>~200</td>
<td>~150</td>
<td>~200</td>
</tr>
<tr>
<td>α-PKC</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

eNOS, endothelial nitric oxide synthase; [Ca2+]i, intracellular Ca2+ concn; PKC, protein kinase C.
SEX HORMONES AND VSM [Ca2+].

Because [Ca2+]i is important for the initiation of smooth muscle contraction, several studies have used isolated vascular strips and smooth muscle cells from intact and gonadectomized male and female experimental animals to investigate the effect of gender and sex hormones on [Ca2+]i, and the Ca2+ mobilization mechanisms of smooth muscle contraction (25, 95, 146). Studies in isolated VSM cells have shown that the resting cell length is longer and the basal [Ca2+]i is smaller in intact female compared with intact male rats, suggesting gender differences in the Ca2+ handling mechanisms (95). The gender differences in resting cell length and [Ca2+]i appear to be related to estrogen because the cell length and [Ca2+]i are greater in OVX females compared with intact females, but not different between OVX females with E2 implants and intact females or between castrated and intact males (95; Fig. 5).

In cells incubated in the presence of external Ca2+, Phe causes an initial peak in [Ca2+]i mainly due to Ca2+ release from the intracellular stores, followed by a smaller but maintained increase in [Ca2+]i, due to Ca2+ entry from the extracellular space (95; Fig. 5). In Ca2+-free solution, Phe causes a transient increase in smooth muscle contraction and [Ca2+]i, that is not different between intact and gonadectomized male and female rats, suggesting that the IP3-mediated Ca2+ release is not involved in the gender differences in cell contraction and [Ca2+]i (95). Also, caffeine, which stimulates the Ca2+-induced Ca2+ release mechanism, causes a small cell contraction and a transient increase in [Ca2+]i, that are similar in magnitude in intact and gonadectomized male and female rats, suggesting that the gender differences in cell contraction and [Ca2+]i, are not related to the Ca2+-induced Ca2+ release mechanism (95).

On the other hand, the maintained Phe-induced [Ca2+]i in VSM cells incubated in the presence of external Ca2+ is greater in intact male than intact female rats, suggesting gender differences in the Ca2+ entry mechanism of VSM contraction. The maintained Phe-induced [Ca2+]i is enhanced in OVX compared with intact females, but not different between OVX females with estrogen implants and intact females, or between castrated and intact males, suggesting that the gender differences are more likely related to estrogen than androgen (95; Fig. 5).

Membrane depolarization by high KCl mainly stimulates Ca2+ entry from the extracellular space. The reports that the KCl-induced smooth muscle contraction, Ca2+ influx, and [Ca2+]i, are greater in intact males than intact females further support gender differences in the Ca2+ entry mechanisms (25, 95). Also, the KCl-induced cell contraction and [Ca2+]i, are enhanced in OVX females compared with intact females, but not different between OVX females with estrogen implants and intact females, lending support to the contention that the gender differences are more likely related to endogenous estrogen. The causes of the gender differences in the Ca2+ entry mechanism are not clear, but may be related to the plasmalemmal density and/or the permeability of the Ca2+ channels depending on the presence or deficiency of endogenous estrogen. This is supported by reports that the expression of the L-type Ca2+ channels in cardiac muscle is substantially increased in ER-deficient mice and that the L-type Ca2+ current is significantly greater in coronary smooth muscle of males compared with females (11, 65).

Inasmuch as VSM contraction and [Ca2+]i, are often enhanced in animal models of hypertension, any gender differences in the Ca2+ mobilization mechanisms of VSM contraction are expected to be more apparent in hypertensive SHR than normotensive WKY rats. Aortic strips of SHR show greater vascular contraction and Ca2+ entry than those of WKY rats (25). Also, VSM cells of SHR show shorter resting cell length, greater basal [Ca2+]i, and greater maintained Phe- and KCl-induced contraction and [Ca2+]i, than those of WKY rats (95; Fig. 5). Additionally, the reduction in vascular contraction, Ca2+ entry, and [Ca2+]i, in intact females or OVX females with estrogen implants compared with intact males or OVX females is greater in SHR than WKY rats, suggesting possible differences in the number of ERs or the number and permeability of the plasma membrane Ca2+ channels (25, 95).

We should note that the gender differences in the mechanisms of Ca2+ mobilization into VSM could be due to a multitude of effects of sex hormones in vivo. However, E2 causes relatively rapid relaxation of isolated vascular strips of rabbit, porcine, and human coronary artery, suggesting that it may be mediated by an effect on Ca2+ mobilization and/or fluxes (24; Fig. 5).
Several studies have shown that estrogen does not inhibit caffeine- or carbachol-induced smooth muscle contraction or \([\text{Ca}^{2+}]_i\) in \([\text{Ca}^{2+}]_i\)-free solution, suggesting that it does not inhibit \([\text{Ca}^{2+}]_i\) release from the intracellular stores (24, 94). However, supraphysiological concentrations of estrogen may inhibit thromboxane \(A_2\)-induced \([\text{Ca}^{2+}]_i\) release in porcine coronary artery (50). On the other hand, estrogen inhibits the high KCl-induced contraction, \([\text{Ca}^{2+}]_i\) influx, and \([\text{Ca}^{2+}]_i\), suggesting that it may act by inhibiting \([\text{Ca}^{2+}]_i\) entry through voltage-gated channels (24, 40, 76, 94; Fig. 6).

Estrogen may inhibit \([\text{Ca}^{2+}]_i\) entry by direct or indirect action on plasmalemmal \([\text{Ca}^{2+}]_i\) channels. Some studies have shown that estrogen blocks \([\text{Ca}^{2+}]_i\) channels in cultured A7r5 and aortic smooth muscle cells (146). Other studies have shown that estrogen activates BK\(_{\text{ca}}\) channels in coronary smooth muscle cells, which could lead to hyperpolarization and decreased \([\text{Ca}^{2+}]_i\) entry through voltage-gated channels (140). However, estrogen-induced vasorelaxation and inhibition of \([\text{Ca}^{2+}]_i\) influx into VSM have been observed even in the absence of activation of K\(^+\) efflux, lending support to direct effects of estrogen on \([\text{Ca}^{2+}]_i\) channels (114).

Estrogen may also decrease \([\text{Ca}^{2+}]_i\) by stimulating \([\text{Ca}^{2+}]_i\) extrusion via plasmalemmal \([\text{Ca}^{2+}]_i\) pump (101). However, this mechanism seems less likely because the rate of decay of caffeine- and carbachol-induced contraction and \([\text{Ca}^{2+}]_i\), transients in smooth muscle incubated in \([\text{Ca}^{2+}]_i\)-free solution, which are often used as a measure of \([\text{Ca}^{2+}]_i\) extrusion, are not affected by estrogen (24, 94).

In contrast to estrogen, the effects of progesterone on \([\text{Ca}^{2+}]_i\) have not been clearly established. However, several studies have shown that acute application of progesterone decreases \([\text{Ca}^{2+}]_i\) influx and \([\text{Ca}^{2+}]_i\), in rabbit and porcine coronary smooth muscle (24, 94; Fig. 6). There have also been inconsistent reports regarding the effects of testosterone on VSM \([\text{Ca}^{2+}]_i\). However, the majority of studies suggests that testosterone has a potent vasorelaxant effect in the rabbit coronary artery and aorta and porcine coronary artery and that testosterone decreases VSM \([\text{Ca}^{2+}]_i\), by inhibiting \([\text{Ca}^{2+}]_i\) entry from the extracellular space (24, 94, 145; Fig. 6). It has been shown that the relaxing effect of testosterone is attenuated by K\(^+\) channel blockers, suggesting that stimulation of K\(^+\) conductance through specific K\(^+\) channels, e.g., voltage-dependent (delayed rectifier) K\(^+\) channel may be involved in the inhibitory effects of testosterone on \([\text{Ca}^{2+}]_i\), (145).

The progesterone- and testosterone-induced inhibition of PGF\(_{2\alpha}\)-induced contraction is greater than the inhibition of the KCl-induced responses (24). These data suggest that progesterone and testosterone not only inhibit \([\text{Ca}^{2+}]_i\) entry through voltage-gated channels, but may also inhibit additional VSM contraction mechanisms activated by PGF\(_{2\alpha}\) such as PKC.

**SEX HORMONES AND PKC**

Recent studies have investigated whether the gender differences in vascular contraction reflect differences in the expression/activity of PKC isoforms in VSM. Phorbol esters, which activate PKC, produce greater contraction in isolated vessels of intact male than intact female rats (69). The greater Phe- and phorbol ester-induced contraction and PKC activity in intact male compared with intact female rats have suggested gender differences in the PKC-mediated pathway of VSM contraction (69), which may be related to differences in the amount of PKC expressed in VSM and/or the sensitivity of the PKC pathway to endogenous sex hormones.

Immunoblot analysis in aortic smooth muscle of intact male WKY rat has shown significant amounts of \(\alpha\)-, \(\delta\)-, and \(\zeta\)-PKC (Fig. 7). In the same preparation, both Phe and phorbol ester cause activation and redistribution of \(\alpha\)- and \(\delta\)-PKC from the cytosolic to the particulate fraction. The amount of \(\alpha\)-, \(\delta\)-, and \(\zeta\)-PKC, and the Phe- and phorbol ester-induced redistribution of \(\alpha\)- and \(\delta\)-PKC are reduced in intact females compared with intact males, suggesting that the gender differences in vascular contraction are related, in part, to underlying changes in the amount and activity of \(\alpha\)-, \(\delta\)-, and \(\zeta\)-PKC (69; Fig. 7).
One important question is related to the subtypes, distribution, and function of sex hormone receptors in vascular cells. In blood vessels of wild-type mice, estrogen attenuates vasoconstriction via an ER-β-mediated increase in iNOS expression. Initial studies in ER knockout mice have shown that deficiency of ER-β renders the aortic wall supersensitive to relaxation by E2, but does not change the vascular wall morphology, suggesting that ER-β may not be involved in vascular structure development. Other studies have shown that ER-β-deficient mice develop hypertension as they age, and their blood vessels show abnormal ion channel functions (84, 147), supporting a role for ER-β in the regulation of vascular function and blood pressure. However, complex tissue-specific effects of sex hormones may be mediated by the expression of heterogeneous forms of their cognate receptors. Variant estrogen, progesterone, and testosterone receptor transcripts are expressed in human vascular cells and may alter the physiological effects of estrogen, progestins, and androgens on the endothelium and VSM.

In addition to the nuclear ERs that mediate the classic transcriptional effects of estrogen, ERs may associate with the cell membrane, and a subpopulation of these membrane-bound ERs may mediate the rapid effects of estrogen. However, little is known regarding the pathways that regulate the distribution of ER between the nuclear and membrane fractions. Phosphorylation of transcription factors plays an important role in regulation of gene expression, and subcellular trafficking of specific transcription factors is regulated by phosphorylation/dephosphorylation. Steroid hormone receptors are phosphoproteins, and mutations in phosphorylation sites may affect the transactivation capacity of these transcription factors (10). Studies in human VSM cells transiently transfected with ER-α have shown translocation of ER-α from the membrane to the nucleus. Nuclear localization of ER-α was blocked by both pharmacological and genetic inhibition of MAPK. Also, constitutive activation of MAPK resulted in nuclear translocation of ER-α. These studies suggest that MAPK-mediated phosphorylation of ER-α induces its nuclear localization (82).

Another question relates to the effect of sex hormones on cell growth and proliferation. Why estrogen enhances the proliferation of endothelial cells but inhibits the proliferation of VSM cells remains an enigma and should represent an important area for future studies.

The rapid vasodilator effects of estrogen have suggested other mechanisms in addition to the classic genomic pathway of steroid action, possibly involving effects on the cellular mechanisms of vascular relaxation and/or contraction. Recent evidence indicates that Ca2+ and K+ channels in VSM cells play an important role in mediating estrogen-induced relaxation of many vascular beds; however, elucidation of the signal transduction mechanisms coupling ER-α and ER-β activation to generation of second messengers and effector mechanisms remains an area of intense study.

Although the gender differences in vascular contraction may be related to effects of sex hormones on vascular [Ca2+], or PKC activity, other protein kinases such as MLC kinase, Rho kinase, and tyrosine kinase as well as MLC phosphatase could regulate smooth muscle contraction. Whether the expression and activity of smooth muscle protein kinases and phosphatases differ with gender and by the presence or deficiency of...
gonadal hormones is unclear and should be examined in future investigations.

There is considerable evidence that both female and male sex hormones affect the mechanisms of vascular contraction; however, the vascular effects of sex hormones may not be uniform. Sex hormones have different sexual effects in both sexes, and it is reasonable to believe that the vascular effects of sex hormones are different in the two sexes. Preliminary studies suggest gender differences in the effects of estrogen on the mechanisms of vascular contraction (25), a research area that should be more thoroughly examined.

Because the vascular effects of estrogen and progestrone may involve modulation of the Ca^{2+} channels, HRT may represent a natural approach to decrease the severity of certain forms of hypertension that are responsive to Ca^{2+} channel blockers. To use or not to use HRT in postmenopausal women with hypertension or coronary artery disease is still controversial. Reports from HERS, HERS2, and WHI studies do not appear to support beneficial vascular effects of HRT, particularly in elderly hypertensive women (37, 47, 74, 89, 117, 119). HRT is accompanied with a reduction in arterial pressure when compared with the role of estradiol in the regulation of vascular protective effects, and nonfeminizing estradiol metabolism may be an important determinant of its cardiovascular effects and in doses that produce hormone levels similar to those in the premenopausal state (32). Furthermore, estradiol metabolism may be an important determinant of its cardiovascular protective effects, and nonfeminizing estradiol metabolites may confer cardiovascular protection in both genders (7).

Finally, compared with the role of estradiol in the regulation of vascular tone, there are sparse data on the effects of androgens and androgen receptors on the vascular control mechanisms. Whether the recently discovered effects of testosterone on the mechanisms of vascular relaxation/contraction justify its potential use in prevention of cardiovascular disease remains to be explored (107).

GRANTS

This work was supported by grants from National Heart, Lung, and Blood Institute (HL-52696, HL-65998, and HL-70659). R. A. Khalil is an Established Investigator of the American Heart Association.

REFERENCES


70. Kanashiro CA and Khalil RA. Invited Review. SEX HORMONES AND VASCULAR TONE R247


