0163-769X/95/$03.00/0
Endocrine Reviews
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Vol. 16, No. 6
Printed in U.S.A.

Estrogen, Progesterone, and Vascular Reactivity: Potential Cellular Mechanisms*

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I. Introduction

FEMALE hormones are broadly recognized as affecting susceptibility to vascular disease, yet comparatively little is known about the cellular mechanisms by which they exert their effects. While clinical and epidemiological data support a protective effect of estrogen on cardiovascular function, a female predominance in other vascular disorders suggests that sex steroids influence the pathogenesis of vascular disease as well. Most of the literature on cellular effects of estrogen and progesterone has emerged from studies in the breast, uterus, and brain. Whether similar mechanisms occur in the vasculature and influence susceptibility to vascular disease is largely unknown.

The purpose of this review is to provide a framework for identifying the mechanisms by which female sex steroids influence vascular function. A diverse literature encompassing clinical observations, whole animal, isolated vessel, and cell culture studies has been incorporated in the hopes of providing a common basis for considering the influences of the female sex steroids on the vascular system. In view of the importance of the vascular endothelium and underlying smooth muscle in vascular control, emphasis is placed on the mechanisms by which estrogen and progesterone may influence these cell types and the interactions between them. Specifically, the effects of the female hormones on the production of vasoactive endothelial products, adrenergic sensitivity, and Ca^{++} homeostasis will be reviewed. Data from nonvascular tissue relevant to potential mechanisms of steroid effects in the vasculature are also presented. It is hoped that such a review will prompt further studies that will provide a resolution of the present paradoxical association between female hormones and protection from some vascular diseases (*e.g.* coronary artery disease) and susceptibility to others (*e.g.* migraines, Raynaud's phenomena, primary pulmonary hypertension).

II. Clinical Correlations

A. Protective effects of female hormones

Several studies point to a role for female sex steroids in favoring the balance of vasodilators to vasoconstrictors. Serum levels of nitrate and nitrite, stable metabolites of the endothelium-derived vasodilator nitric oxide (NO), increase during the follicular phase of the menstrual cycle in conjunction with rising 17β-estradiol levels and decrease during the postovulatory, high progesterone phase (1). Lower en-

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*This review was supported by NIH HL07171, HL14985 and a Maternal Fetal Medicine fellowship. We would like to express our appreciation to Dr. Caleb Awoniyi for his thoughtful comments and advice.

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dothelin (ET-1) levels, an endothelium-derived vasoconstrictor, have been reported in premenopausal women compared to age-matched men, and a reduction in ET-1 levels in male transsexuals after estrogen treatment has also been observed (2). Circulating norepinephrine levels are also reduced in the follicular compared with the luteal phase of the menstrual cycle (3). These are consistent with the possibility that estradiol enhances vasodilator and/or inhibits vasoconstrictor activity whereas progesterone antagonizes estrogen's effects.

1. Cardiovascular disease. The presence of female hormones is associated with a decreased incidence of cardiovascular disease. Premenopausal women lag behind men in the development of coronary artery disease by approximately 10 yr (4), but the difference between men and women narrows after 50 yr of age (5). Additionally, women with systemic hypertension have a lower morbidity and mortality when compared with hypertensive men (6, 7). The observation that hypertensive women matched with men for age, race, and mean arterial pressure have a lower blood pressure response to isometric exercise (8) suggests that alterations in vascular reactivity may be involved. It is well documented that women receiving estrogen replacement therapy have a 40–50% reduction in the risk of coronary artery disease (9–12). While a substantial portion of this effect has been attributed to estrogen-mediated increases in high-density lipoprotein-cholesterol and decreases in low-density lipoprotein-cholesterol (5, 13, 14), the alterations in lipid profile account for only about 50% of the cardiovascular benefit observed in estrogen-treated women, suggesting that other mechanisms may be involved (15).

Estrogen therapy appears beneficial for women suffering from cardiac syndrome X, a recently recognized disorder marked by severe angina in the presence of normal coronary arteries. This condition is associated with ovarian insufficiency (16) and a diminished endothelium-dependent hyperemic response (17). In one of the few studies to examine chronic estrogen administration, 2 months of 17β-estradiol treatment restored the hyperemic response to control values and markedly improved cardiac symptoms (16).

2. Pregnancy. Pregnancy is associated with widespread vascular changes that are important for ensuring fetal as well as maternal well-being. There is a fall in blood pressure and systemic vascular resistance, rise in cardiac output, and reduction in the pressor responses to several vasoconstrictors in humans, baboons, sheep, guinea pigs, and rats (18–22). The fall in vascular resistance is due to vasodilation in both the uterine and nonuterine circulations (23) and is accompanied by alterations in levels of vasoactive substances as well as changes in the vasoconstrictor and vasodilator responses of isolated vessel preparations. The ratios of several vasodilator relative to vasoconstrictor substances change with pregnancy. In normal human pregnancy, a rise in the circulating levels of prostacyclin relative to thromboxane occurs with advancing gestation (24). In rats, pregnancy is associated with increases in the circulating levels and urinary excretion of nitrate and cGMP, the second messenger involved in prompting NO-induced vascular smooth muscle relaxation (Fig. 1) (25). In a study on the effect of intravenous

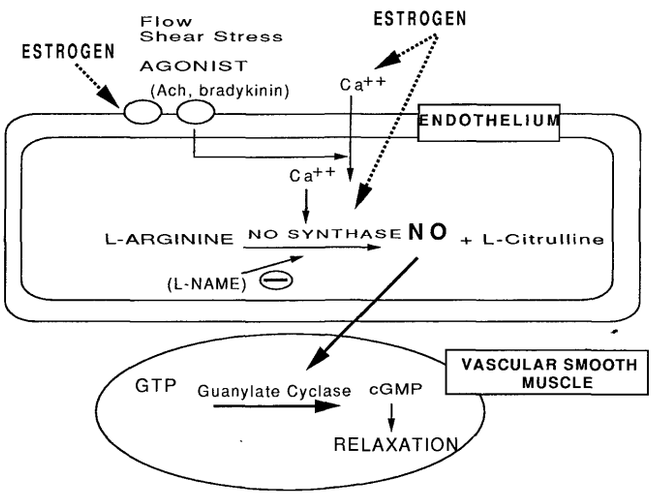

FIG. 1. Biosynthesis of NO. Shown here are potential mechanisms by which estrogen may modulate endothelium-dependent vasodilation through 1) effects on number and/or sensitivity of cholinergic receptors, 2) modification of Ca^{++} influx, and 3) induction of NO synthase. Flow, shear stress, or receptor activation by acetylcholine or bradykinin results in influx of Ca^{++}. Increased intracellular Ca^{++} stimulates the constitutive NO synthase. The NO formed from L-arginine diffuses to smooth muscle cells, stimulating guanylate cyclase and resulting in increased synthesis of cGMP from GTP. Nitro-L-arginine methyl ester (L-NAME) is a competitive inhibitor of NO synthase and causes vasoconstriction *in vitro*.

infusion of glyceryl dinitrate (GTN), an NO donor *in vivo*, and prostacyclin on uterine artery blood flow during human pregnancy, GTN administered during the first trimester decreased uterine vascular resistance while prostacyclin had no effect (26). The GTN-associated increase in uterine artery blood flow occurred without significant alterations in heart rate or systemic blood pressure and mimicked the normal physiological increase observed during the second trimester, suggesting a potential role for NO in uterine artery vasodilation. The effect of pregnancy on the vasoconstrictor, ET-1, is unclear; increases, decreases, as well as no differences between pregnant and nonpregnant women have been reported (2, 27, 28).

B. Speculations about involvement of female hormones in vascular disease

Migraine headaches, primary pulmonary hypertension, preeclampsia, and Raynaud's phenomenon are a diverse group of vascular disorders. A range of etiological mechanisms have been implicated in their pathogenesis, including alterations in central nervous system neurons and pain-modulating pathways, immunological function, and vascular remodeling (29–31). However, a number of features are common to these diseases. They all demonstrate a marked female preponderance (31–34), or, in the case of preeclampsia, occur exclusively in women. There is an overlap in their occurrence such that the presence of one disease increases the risk of developing another of these conditions (35, 36). Women with severe migraines are at an increased risk of developing preeclampsia (37). Migraine or Raynaud's phenomenon predisposes women to variant angina, a condition characterized by

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transient coronary arterial vasospasm (38). The frequency of Raynaud's is greater among women with primary pulmonary hypertension than in the general population in most (31, 39–43) but not all studies (44).

Alterations in vasoactive substances and Ca^{++} homeostasis are present in many if not all of these disorders. Increased thromboxane relative to prostacyclin has been described in patients with preeclampsia, primary pulmonary hypertension, and Raynaud's phenomenon (45–47). In preeclampsia, a disease of pregnancy characterized by systemic hypertension, edema, proteinuria, and coagulation abnormalities (48), the increased ratio of vasodilator to vasoconstrictor prostaglandins of normal pregnancy is reversed in proportion to the severity of disease (49, 50). Further, a decrease in the circulating levels of the NO metabolite, nitrite, has also been noted in preeclamptic patients (51). Pregnant rats treated chronically with the NO inhibitor, nitro-L-arginine methyl ester, developed hypertension and proteinuria, and delivered growth-retarded pups (52). Increased levels of ET-1 and reduced expression of NO synthase have been reported in the lungs of patients with primary pulmonary hypertension (53, 54). A role for alterations in Ca^{++} homeostasis is suggested by the therapeutic benefits of Ca^{++} channel blockers in Raynaud's phenomenon, primary pulmonary hypertension, and migraines (29, 55, 56). Ca^{++} supplementation has also been associated with a decreased incidence of preeclampsia (57). When compared with normotensive controls, platelets from preeclamptic women have increased intracellular Ca^{++} concentration (58, 59). Further, the observation of a greater contractile response to the Ca^{++} channel agonist BayK8644 in inferior epigastric arteries from preeclamptic women suggests that voltage-gated Ca^{++} channel activity may be enhanced in comparison to normal women (60).

Despite the clear female preponderance of these disorders, the mechanisms by which female sex steroids influence their occurrence remains speculative. Studies reviewed below and in *Section II.C* suggest that such mechanisms may involve alterations in sex hormone levels and/or ratios and hormonal stimulation of vasoconstrictor pathways.

Surprisingly little is known about the role of sex steroids, their ratios, or the levels of their metabolites in the development of preeclampsia. Preeclampsia is characterized by an increase in systemic vascular resistance, vascular reactivity, and alterations in pelvic blood flow distribution that precede the onset of hypertension, suggesting a failure of the normal vascular adjustments to pregnancy (61–64). In addition to alterations in endothelial cell vasoactive substances, evidence of sympathetic activation has also been reported in preeclamptic compared with normal pregnant women (65, 66). Older studies suggesting an association between preeclampsia and low serum 17β-estradiol levels were limited by the small number of subjects, the absence of rigorous diagnostic criteria for preeclampsia, and the likelihood that the low estradiol levels were due to other obstetrical conditions (67, 68). We have recently described a decrease in serum estradiol and increase in serum progesterone before the onset of hypertension in preeclamptic women residing at high altitude (3100 m), where the incidence of preeclampsia is markedly increased in comparison with low altitude (1600 m) (69).

Migraine headaches tend to cluster just before or during menstruation in a majority of affected women and exclusively with the menses in a substantial proportion (29, 32–34). In addition to the alterations in estradiol and progesterone levels, both of which have been shown to be higher in women with migraines than in asymptomatic controls (32, 70, 71), fluctuations in hormone levels or, in particular, estrogen withdrawal may be important (72, 73). In a small descriptive study, headaches began immediately before menses, and their onset was delayed by estrogen treatment (73). Additionally, in two controlled trials, percutaneous estradiol gel reduced the frequency of migraine headaches (74, 75). Further, the vast majority (86%) of menstrual migraines improve with pregnancy, a time when steroid hormone levels rise and their fluctuations are eliminated (74, 75). Thus, while estrogen withdrawal appears to be associated with the development of menstrual migraines, whether these effects are mediated through alterations in vascular function is unknown.

A link between hormonal fluctuations and symptoms of Raynaud's phenomenon is suggested by the observation that whereas normal women decrease their digital blood flow response to cold during the follicular compared with the luteal phase of the menstrual cycle, women with Raynaud's show no such variation (76). Further studies are needed to characterize the nature of these hormonal alterations or how they may be related to the altered vasoreactivity seen in Raynaud's phenomenon.

Estrogen and progesterone may also influence vasoconstriction depending on the vascular bed and the site of action (see *Section II.C*). Such effects may contribute to the gender-related differences in certain vascular diseases. Studies in isolated rat lungs, for instance, indicate that estradiol enhances vasoconstriction to the thromboxane mimetic U46619 which may, in turn, explain the increased vasoconstrictor response noted in the female compared with male rat lung (77). This observation may be relevant for understanding the association between primary pulmonary hypertension and female hormones. It is well known that primary pulmonary hypertension occurs twice as often in women as men (31). Further, it is associated with significant mortality during pregnancy (78, 79). In 23 women that presented with primary pulmonary hypertension before becoming pregnant, half died while pregnant, with a majority of the deaths occurring during delivery (79). Most authors attribute maternal demise to the collapse of a compromised maternal circulation further stressed by the hemodynamic changes of labor and delivery, but pregnancy-associated increases in hormones and/or their decline at the time of delivery may also be important.

C. Summary

Female hormones are linked both to protection and susceptibility to vascular disease. There are several possibilities by which this apparent contradiction might be resolved. One possibility concerns the levels of female hormones. While fluctuations in hormone levels appeared to be associated with the onset of menstrual migraines and primary pulmonary hypertension in some studies, there is not sufficient information to judge whether there are differences in circulating levels of estrogen and progesterone, their fluctuations,

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or their ratios in women with vasospastic conditions compared with well matched controls. A second possibility concerns the separate *vs.* combined effects of the hormones involved. It is well recognized that estrogen and progesterone can act in ways that are antagonistic to each other. For example, in isolated dog coronary artery rings, progesterone attenuates estrogen-induced stimulation of the endothelium-dependent responses (80). These antagonistic effects may, in turn, be receptor-mediated. In reproductive tissues such as the uterus and breast, estrogen has been shown to up-regulate progesterone receptors while progesterone down-regulates its own receptor (81–86). It has also been demonstrated that in the chick oviduct and rodent uterus, progesterone prevents the continuation of an estrogen effect (87–89), possibly by interfering with the replenishment of cytosolic estrogen receptors (90–92). A third possibility concerns the site of hormonal action. Since the same action, namely an increase in intracellular Ca^{++} promotes vasodilation or vasoconstriction depending on whether it occurs in the endothelial or vascular smooth muscle cell, respectively, one possibility for this apparent paradox is that female hormones have endothelial *vs.* vascular smooth muscle cell sites of action in different disease states.

III. Potential Mechanisms of Hormone-Mediated Effects

A. Receptor-mediated actions

Estrogen and progesterone are lipophilic molecules whose receptor-mediated modes of action have been well studied in the breast, uterus, and brain (86, 93–95). Expression of estrogen and progesterone receptors in the vasculature has been controversial, perhaps reflecting intervessel and/or species variability as well as limitations of the different techniques employed.

Using radioactive ligand binding, estrogen and progesterone receptors have been demonstrated in cytosolic fractions of the canine aorta and inferior vena cava (96). Immunocytochemistry has been used to demonstrate the presence of estrogen receptors in guinea pig uterine artery vascular smooth muscle and endothelial cells (97). While estrogen and progesterone receptors have been found in rabbit and human uterine artery vascular smooth cells (98), these studies were not able to demonstrate receptors in rabbit or guinea pig aortas or rabbit renal, femoral, hepatic, or pulmonary arteries using the same technique.

More recently, studies have investigated both the presence and functional status of steroid receptors in vascular tissue. Using Northern analysis and a monoclonal antiestrogen receptor antibody that recognizes the DNA-binding domain of the estrogen receptor, Orimo *et al.* (99) demonstrated estrogen receptor mRNA and protein, respectively, in cultured rat aortic vascular smooth muscle cells. The authors also showed that a concentration of 17β-estradiol at the high end of the physiological range (10 nM) up-regulated the expression of estrogen-dependent c-*fos* mRNA, suggesting estrogen stimulation of gene transcription in vascular smooth muscle. Using the ribonuclease protection assay and immunofluorescence techniques, others have demonstrated low levels of estrogen receptor mRNA and protein in human vascular smooth muscle cells derived from the mammary artery and saphenous vein (100). The low level of estrogen receptor found in these cells may be related to the use of cell cultures. The concentration of estrogen receptor decreases markedly over time in cultured rabbit uterine myocytes (101). Functional integrity of the receptor was demonstrated by transfection of vascular smooth muscle cells with a reporter plasmid containing an estrogen response element driving the expression of the luciferase gene (ERE-Luc) or the control plasmid lacking the estrogen response element (Tk-Luc) to 17β-estradiol. Vascular smooth muscle cells containing the luciferase gene, but not the control cells, had a dose-dependent increase in activity of the ERE-Luc plasmid after exposure to 17β-estradiol (100).

Some studies suggest that receptor levels within the vasculature are altered by changes in the hormonal milieu. Spiral arteries of the human uterus demonstrate intense staining of both estrogen and progesterone receptors during the first trimester of pregnancy (102). While immunocytochemistry revealed no estrogen receptor in guinea pig heart, aorta, and carotid specimens, receptors were demonstrated in the uterine artery (97). In addition, a midpregnancy fall in estrogen receptor levels compared with nonpregnant or term animals was demonstrated, which may have been due to a rise in serum progesterone relative to estradiol levels (97). The recent observation that estrogen receptors are diminished in coronary arteries of premenopausal women with atherosclerosis compared with normal premenopausal controls suggests that receptor levels may also be altered in disease states (103).

B. Effects of estrogen and progesterone on vascular function

1. Cardiovascular disease. The protective effect of female hormones in coronary artery disease may be due to the effects of estradiol. Several studies have reported that estradiol administration increases endothelium-dependent vasodilation in response to acetylcholine stimulation in the coronary circulation (Fig. 1). Using quantitative coronary angiography in monkeys fed a high cholesterol diet, Williams *et al.* (104) demonstrated that infusion of the endothelium-dependent vasodilator acetylcholine constricted the coronary arteries of ovariectomized animals but dilated those of an estrogen-treated group. Although the estrogen-treated group had reduced plaque formation, the altered vasoreactivity did not correlate with the degree of plaque present. These findings have been supported by studies in postmenopausal women with evidence of endothelial cell dysfunction as indicated by a vasoconstrictor response to acetylcholine. Administration of physiological levels of 17β-estradiol and the synthetic steroid, ethinyl estradiol, by intraarterial or intracoronary infusion, potentiated vasodilation to acetylcholine in large and microvascular coronary arteries as well as in the forearm circulation (105–107). Estradiol also increased the vasodilator response to sodium nitroprusside, an endothelium-independent vasodilator, in the forearm but not the coronary circulation (105), suggesting that estradiol influences vascular smooth muscle sensitivity in some vascular beds. At supraphysiological doses, estradiol or progesterone caused relax-

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ation of precontracted, endothelium-denuded rabbit isolated coronary artery rings, also supporting the involvement of endothelial-independent actions (108, 109). The extent to which baseline circulatory parameters are affected is unclear; the synthetic steroid, ethinyl estradiol, but not 17β-estradiol, increased baseline coronary blood flow and decreased resistance (106, 107).

2. Pregnancy. A vasodilatory effect of estradiol in the sheep uterine circulation has long been recognized. Acute or chronic administration of 17β-estradiol to oophorectomized ewes reduces systemic and uterine vascular resistance, markedly increases uterine blood flow, and decreases the pressor response to angiotensin II (110–113). However, the vasodilatory effects of estradiol administration are not consistent across species or study preparations. Whereas acute intravenous administration of 17β-estradiol decreased the systemic pressor response to angiotensin II in oophorectomized rats (114), 2–3 week treatment with subcutaneous pellets or intraperitoneal injection did not have similar effects (115–117). Even within a species, the effects of chronic estradiol administration differ in the uterine and nonuterine circulations. Systemic vasodilation and refractoriness to angiotensin II in sheep were maintained throughout (and in the case of angiotensin II, even beyond the infusion period), but uterine values returned to baseline after 7 days. Given the prolonged nature of the hormone stimulation of pregnancy and the consistency among species in pregnancy's vasodilatory effects, these discrepancies suggest that estradiol alone cannot account for pregnancy-associated vasodilation. The possible involvement of progesterone is supported by studies in which progesterone alone or in combination with estrogen decreased pressor response to angiotensin II in whole animals or isolated tail arteries of rats (115, 118). However, other studies in the rat using more physiological levels of hormone showed no effect (114, 116, 117).

Ca^{++} channels may play a role in the physiological changes observed during pregnancy. Voltage-gated Ca^{++} channels are activated by depolarization of the plasma membrane when extracellular $[K^+]$ is increased (Fig. 6). In isolated porcine uterine arteries, pregnancy and estradiol-treatment decreased the contractile response to potassium chloride. That this decrease was associated with a reduction in Ca^{++} uptake into uterine vascular smooth cells (119–121) suggests that pregnancy and estradiol may reduce intracellular Ca^{++} via an effect on voltage-gated Ca^{++} channels.

C. Steroid-mediated effects on endothelial and vascular smooth muscle cell function

Included among the mechanisms that modulate vascular tone and reactivity are alterations in endothelial vasodilator or vasoconstrictor activity and vascular smooth muscle cell function. Reviewed below are several lines of evidence suggesting that estrogen and progesterone influence the production or activity of the endothelial-derived substances, NO, ET-1, and eicosanoids. There are a number of other endothelial-derived factors that represent potential sites of influence that are beyond the scope of this review, including endothelial-derived hyperpolarizing factor, angiotensin II, and platelet-activating factor (Table 1). In addition, since this review is primarily concerned with vascular reactivity, factors important in angiogenesis such as vascular endothelial growth factor (VEGF) (122) are not discussed.

1. NO. Endothelial-derived NO mediates vascular smooth muscle relaxation by stimulating soluble guanylate cyclase (Fig. 1). NO is produced by the endothelium under basal conditions as well as upon stimulation by agonists such as acetylcholine and bradykinin that act via endothelial surface membrane receptors (Fig. 1 and Table 1), some of which are linked to G protein activation (123, 124). Vascular smooth muscle, neural, and other cell types can, under certain circumstances, also produce NO (125). Studies from a broad range of experimental preparations suggest that physiological alterations in female hormones modulate endothelial cell function through effects on basal as well as stimulated NO production and/or activity.

Female gender has been associated with increased basal NO release. Aortic rings isolated from female compared with male rabbits and precontracted with phenylephrine showed greater relaxation to the inhibitor of NO inactivation, superoxide dismutase, and greater constriction after inhibition of NO synthesis by nitro-methyl-L-arginine (126). Oophorectomy abolished this gender difference, suggesting that physiological levels of female hormones stimulate basal NO activity. The absence of gender differences in relaxation to acetylcholine indicated that stimulated NO activity was not affected.

Pregnancy has been associated with increased stimulated release of NO. Guinea pig uterine artery and rat aortic rings demonstrate increased relaxation to acetylcholine during pregnancy (127–129). These effects of pregnancy are similar to those observed with acute and chronic estradiol treatment, suggesting that the pregnancy-associated changes are

TABLE 1. Endothelial targets for actions of estrogen and progesterone

I. Endothelial Derived Products	
Vasodilators	Vasoconstrictors
PGE_2	Angiotensin II
PGI_2	Endothelin 1
Nitric oxide	Arachadonic acid
Histamine	PGH_2
PGH_2	Thromboxane A_2
Platelet activating factor	Histamine
Superoxide anion	Superoxide anion
Leukotrienes	Platelet activating factor
EDHF	Leukotrienes

II. Endothelial Membrane Receptors
Acetylcholine
Bradykinin
ATP/ADP
Serotonin
Thrombin
α_2-Adrenergic
Endothelin B (ET_B)
Histamine
Thromboxane A_2
Angiotensin II
Vasopressin
Substance P
Vascular endothelial growth factor (VEGF)

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mediated, at least in part, by estradiol. In oophorectomized ewes, 80% of the rise in uterine blood flow with acute estradiol infusion was blocked by the NO synthesis inhibitor, nitro-L-arginine methyl ester (130). The possibility that estradiol increased NO synthesis was supported by the observation that the inhibition of the uterine blood flow response could be reversed with excess administration of L-arginine (Fig. 1). Chronic treatment with 17β-estradiol also increased relaxation to acetylcholine in isolated rabbit femoral artery rings and aortas from spontaneously hypertensive rats (131, 132). Not all studies support that estradiol-induced vasodilation is mediated through NO; estradiol relaxed precontracted rabbit isolated coronary artery rings, and this relaxation was reversed by NO inhibition in some but not other studies (133, 134).

Estradiol may stimulate endothelium-dependent relaxation through both receptor-mediated and non-receptor-mediated mechanisms. Chronic treatment with 17β-estradiol increased relaxation to acetylcholine in isolated rabbit femoral artery rings and aortas from spontaneously hypertensive rats (131, 132). Increased relaxation to acetylcholine but not to non-receptor-mediated substances such as the Ca^{++} ionophore A23187 has been observed in rabbit femoral artery rings, suggesting that chronic estrogen treatment may increase the number or sensitivity of muscarinic receptors (132). However, increased relaxation to A23187 in rabbit isolated aortic rings indicated that non-receptor-mediated mechanisms of NO release may also be present in some species or vessels (135).

The stimulatory effects of pregnancy and estradiol treatment on NO activity may take place by increasing endothelial constitutive NO synthase (NOS III) production or activity. Pregnancy has been associated with a 4-fold increase in Ca^{++} dependent NOS III activity in the guinea pig uterine artery as measured by conversion of radiolabeled L-arginine to citrulline (136). Using semiquantitative polymerase chain reaction, increased relaxation to acetylcholine in the pregnant rat aorta correlated with a 2-fold increase in NOS III mRNA in pregnant compared with nonpregnant rings (129). Five-day intraperitoneal injection of 17β-estradiol to gonadally intact female guinea pigs also increased NOS III activity in heart, kidney, esophagus, and skeletal muscle, while progesterone had no effect (136). Additionally, 17β-estradiol increased NO production and protein levels of NOS III in cultured human aortic endothelial cells and up-regulated NOS III gene expression in the aorta of gonadectomized rats (129, 137). Of interest, the effect of estradiol on NOS III was only 50% of that seen with pregnancy, suggesting that other factors during pregnancy also influence expression of this gene. Finally, the presence of two left-half palindromic sites of an estrogen receptor-binding element on the human endothelial NOS gene supports a potential receptor-mediated effect of estrogen on gene expression (138).

Thus, data in several species suggest that estradiol modulates endothelium-dependent relaxation in a number of vascular beds through increased NO activity which, in turn, may be due to up-regulation of NOS III gene expression. However, the stimulation observed during pregnancy is not fully reproduced by estradiol treatment and the effects of estradiol treatment are not as generalized among vascular beds as those observed during pregnancy. Thus, other mechanisms may also be involved, such as increased sensitivity or affinity of endothelial receptors or effects on intermediary steps involved in receptor-mediated NO release, such as modulation of G-protein activation.

2. ET-1. In subnanomolar concentrations, ET-1 is a potent endothelial-derived vasoconstrictor, but at lower concentrations, it can also induce transient vasodilation (139). Two classes of ET-1 receptors, ET-1 A (ET_A) and ET-1 B (ET_B), both of which appear to be G-protein linked, have been identified in vascular beds. Activation of ET_A in vascular smooth muscle cells causes constriction which, in turn, is mediated by Ca^{++} channel activation and activation of phospholipase C (Figs. 2 and 3). ET_B activation on endothelial cells may also

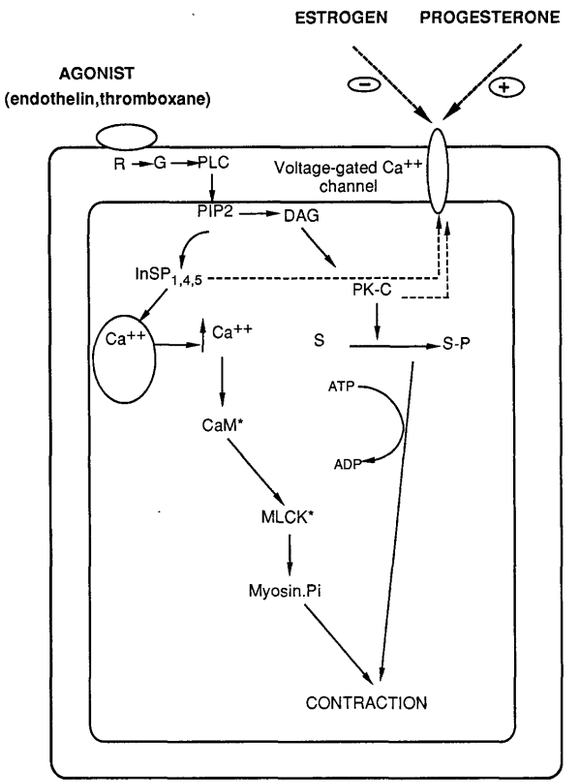

FIG. 2. Simplified schema of receptor-mediated mechanism of smooth muscle contraction. Estrogen may directly inhibit voltage-gated Ca^{++} channels in vascular smooth muscle cells, thereby decreasing Ca^{++} influx. Progesterone may increase Ca^{++} entry through activation of these channels. Female sex steroids may also influence the sensitivity of contractile proteins such as myosin light chain kinase (MLCK*) and calmodulin (CaM*) to Ca^{++}. Binding of agonist to a receptor linked to a G protein (G) results in activation of phospholipase C (PLC). PLC hydrolyzes a membrane phospholipid phosphotidyl-inositol-4,5-biphosphate (PIP_2) into two second messengers: diacylglycerol (DAG) and inositol-1,4,5-triphosphate ($InSP_{1,4,5}$). $InSP_{1,4,5}$ diffuses across the cytoplasm triggering the release of intracellular Ca^{++} from internal stores. Increased cytoplasmic Ca^{++} promotes activation of the Ca^{++} binding protein calmodulin (CaM*), which in turn activates myosin light chain kinase (MLCK*). Muscle contraction is initiated with myosin phosphorylation. DAG activates protein kinase C (PKC), which in turn phosphorylates a number of proteins resulting in smooth muscle contraction. Agonists may also activate Ca^{++} channels through effects of second messengers such as $InSP_{1,4,5}$ and PKC.

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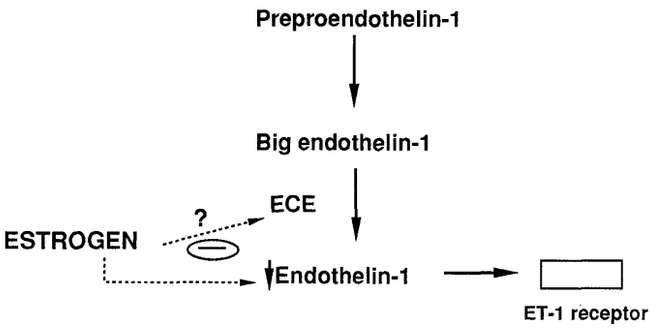

FIG. 3. Biosynthesis of ET-1. ET-1 is synthesized from prepro-ET-1. The mechanism(s) by which estrogen decreases ET-1 levels is unknown but may involve effects on gene transcription of important enzymes such as endothelin-concerting enzyme (ECE).

stimulate NO and prostacyclin release. In cultured bovine and porcine endothelial cells, ET-1 release can be stimulated by a number of agonists including thrombin, arginine vasopressin, angiotensin II, and A23187 (140, 141). In the porcine isolated aorta, increased NO resulting from the inhibition of the NO scavenger, superoxide dismutase, or the nonhydrolyzable analog of cGMP, 8-bromo-cGMP, reduced thrombin-stimulated ET-1 production (142). Thus, a dynamic balance exists, involving feedback relations between NO and ET-1 such that ET-1 stimulates NO which, in turn, reduces ET-1 production. ET-1 may also stimulate prostacyclin release. In cultured human umbilical vein endothelial cells, high doses of ET-1 (10^{-9} M) stimulated prostacyclin production as measured by increased 6-keto-prostaglandin $F_{1\alpha}$ formation (143).

Evidence for steroid-mediated effects on ET-1 is limited. Doses of 17β-estradiol 1000-fold greater than the normal physiological range (3–30 uM) decreased the contractile response to ET-1 in isolated rabbit coronary artery rings (134). However, the decrease was similar in rings from male and nonpregnant female animals, and there were no gender differences in the magnitude of the ET-1-induced contraction. Further, nitro-L-arginine methyl ester did not alter the response to 17β-estradiol, suggesting that estradiol acted via an endothelium-independent mechanism. Consistent with this possibility, similar doses of 17β-estradiol (10–30 uM) decreased the contractile response to ET-1 in endothelium-denuded coronary arteries from male and female animals. The authors hypothesized that estradiol affected vascular smooth muscle cell Ca^{++} channels since estradiol reduced the contractile response to BAY K8644, a specific agonist of voltage-dependent Ca^{++} channels (134). While 17β-estradiol (10^{-8} M) potentiated the ET-1 increase in prostacyclin production in human umbilical vein endothelial cells, the mechanism remains unknown (143).

The effects of pregnancy and physiological alterations in estradiol or progesterone on ET-1 have not been fully explored. Among the possibilities requiring further investigation are direct effects of pregnancy and female hormones on vascular smooth muscle cell response to ET-1 stimulation and indirect effects on NO activity which, in turn, exert a feedback inhibition on ET-1 production.

3. Eicosanoids. Endothelial cells metabolize arachadonic acid to produce eicosanoids through cyclooxygenase, peroxidase, and lipooxygenase enzymatic pathways (Fig. 4). In general, vasodilation is mediated by prostacyclin and prostaglandin E_2 while thromboxane A_2, prostaglandin $F_{2\alpha}$, and some leukotrienes are vasoconstrictors (144). Vascular smooth muscle cell relaxation results from formation of cAMP and activation of cAMP-dependent protein kinase (Fig. 5). Thromboxane A_2 is thought to antagonize the effects of prostacyclin through inositol-1,4,5-triphosphate ($InSP_{1,4\ 5}$)-mediated release of intracellular Ca^{++} (Fig. 2).

Conflicting evidence exists regarding the effects of physiological alterations in estrogen or progesterone on eicosanoid production in the vasculature. Two- to three-week treatment with intraperitoneal injections of estradiol increased prostacyclin release in isolated aortic rings of male and intact female rats as measured by elevated levels of the stable metabolite 6-keto-prostaglandin $F_{1\alpha}$ (145) (whether blood estradiol levels obtained were within the physiological range is unknown). Physiological concentrations of 17β-estradiol have been shown to increase prostacyclin production and biosynthetic activity in cultured rat and rabbit aortic smooth muscle cells (145, 146). Estradiol also appears to stimulate the production of contractile prostanoids, as demonstrated by the observation that indomethacin reduced the estradiol-induced increase in contractile response to norepinephrine in isolated rabbit aortic rings (135). However, cyclooxygenase inhibition did not reverse the vasodilator effects of pregnancy in whole animals (21, 22), or in isolated

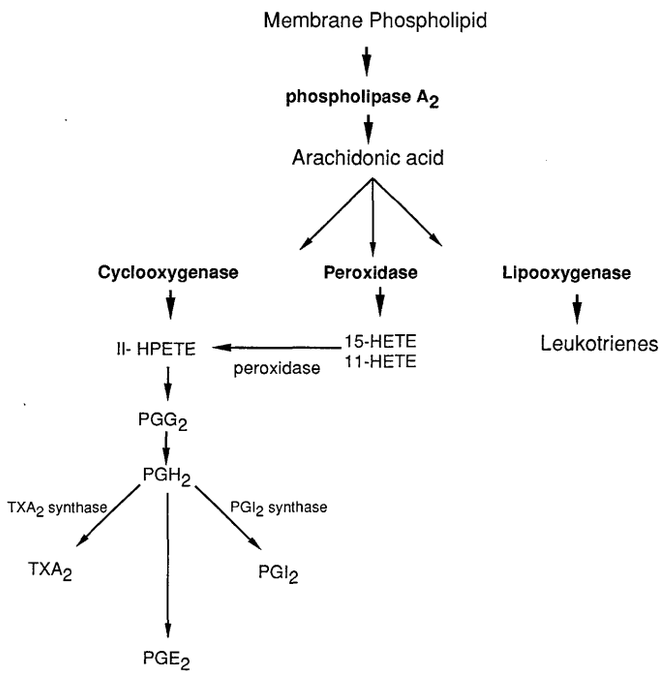

FIG. 4. Endothelial cells produce eicosanoids from arachadonic acid through the cyclooxygenase, lipooxygenase, and peroxidase pathways. Primary end products include thromboxane A_2 (TXA_2), prostaglandin E_2 (PGE_2), and prostacyclin (PGI_2) from the cyclooxygenase pathway and leukotrienes from the lipooxygenase pathway. Hydroxyperoxyeicosatetraenoic acid (II-HPETE) and hydroxyeicosatetranoic acid (HETE) are intermediate products in the cyclooxygenase and peroxidase pathways. Precise mechanisms by which estradiol increases PGI_2 production remain unclear.

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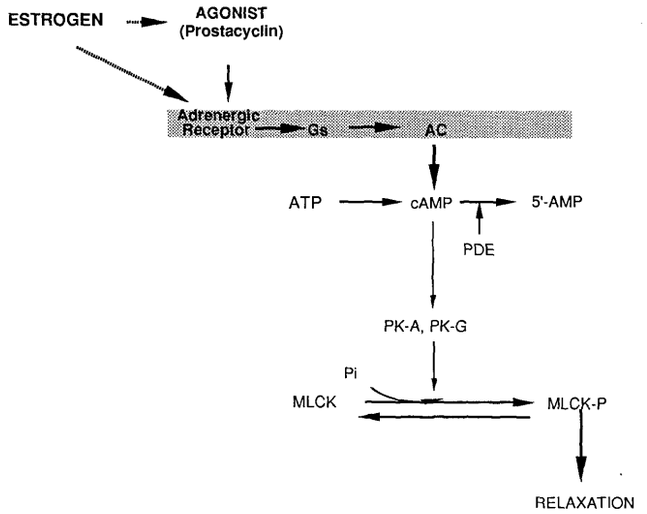

FIG. 5. Simplified schema of cAMP pathway of smooth muscle relaxation. Estrogen has been shown to modify expression of β-receptors in a number of tissues and may stimulate prostacyclin production in endothelial cells. Key proteins in the pathway of vascular smooth muscle cell relaxation include hormone receptors (Rec), a stimulatory protein (Gs), catalytic adenylyl cyclase (AC), and phosphodiesterase (PDE) that hydrolyzes cAMP. cAMP stimulates cAMP-dependent protein kinases (PK-A, PK-G) that are involved in the phosphorylation of myosin light chain kinase (MLCK) triggering relaxation.

rabbit femoral or coronary artery rings (108, 132), suggesting that estradiol-mediated vasodilation is not dependent on cyclooxygenase products (Fig. 4). Since most studies look at single blockers and do not examine interactions between the cyclooxygenase and lipooxygenase pathways, the possibility that estradiol or pregnancy acts on more than one eicosanoid pathway to affect the balance of vasodilator to vasoconstrictor eicosanoids has not been investigated. The effects of female hormones on endothelial cells may also differ from those seen in vascular smooth muscle cells; supraphysiological doses of estradiol did not alter prostacyclin or thromboxane B2 production in cultured human umbilical vein endothelial cells (143).

4. *Adrenergic responsiveness.* Most (21, 128, 147) but not all (148) studies suggest that pregnancy decreases contractile response to adrenergic stimulation in a broad range of species and study preparations. However, the effects of estrogen and progesterone on adrenergic responsiveness vary among species and vascular beds. Femoral artery rings from oophorectomized female rabbits treated chronically with physiological concentrations of 17β-estradiol decreased their contractile response to norepinephrine (149) while mesenteric arteries from female and male rats administered higher dosages of estrogen demonstrated increased adrenergic sensitivity (150). Few studies have examined the effect of chronic progesterone treatment on adrenergic responses. In the perfused tail artery of female rats, 3-day treatment with supraphysiological levels of progesterone decreased the pressor response to norepinephrine while estradiol treatment had no effect (118). The results from these studies may reflect not only interspecies and vessel differences but also effects of varying dosages of hormones as well as the use of gonadally intact *vs.* castrated preparations. Additionally, the hormones may be exerting their effects at alternate sites; endothelial α_2-receptor stimulation causes NO release and vasodilation, while vascular smooth muscle α_1- and α_2-receptor activation produces vasoconstriction (151, 152).

Ovarian steroids may influence adrenergic responsiveness via alterations in adrenergic receptor affinity. Both estrogen and progesterone appear to increase α_1-receptor affinity. The mesenteric arteries of estrogen-treated male and female rats showed an increased contractile response to adrenergic stimulation that was associated with increased α_1-adrenergic receptor affinity (150). In the gilt uterine artery, decreased uterine blood flow during the luteal (high progesterone) phase was associated with increased α_1-receptor binding affinity (153).

An additional potential mechanism of steroid-mediated effects involves modification of catecholamine levels via alterations in uptake, storage, and efflux from nerve endings (147, 154). Sensitivity to norepinephrine was increased in pregnant compared with nonpregnant rat mesenteric arteries in the presence of cocaine, an inhibitor of neuronal reuptake, suggesting that pregnancy may be associated with increased neuronal reuptake and/or deactivation of norepinephrine (147). Studies in canine saphenous veins suggest that estradiol may also stimulate norepinephrine release from nerve endings (154). Less is known concerning the effects of female hormones on β- receptors. While β_1 and β_2 have been identified in cultured bovine pulmonary artery endothelial cells (155), and ovarian hormones have been shown to modify the number and/or affinity of β-adrenergic receptors in the myometrium, brain, lung, and heart (156, 157), little is known about their effects in the vasculature.

Thus, while pregnancy and ovarian steroids appear to modulate adrenergic responses in a number of vascular beds, their specific effects on endothelial *vs.* vascular smooth muscle cells await study.

5. *Ca^{++}homeostasis.* Ca^{++} homeostasis is central to both endothelial and vascular smooth muscle cell function; endothelial release of NO and prostacyclin is Ca^{++} dependent (158), and increased intracellular Ca^{++} prompts vascular smooth muscle contraction (159). The regulation of cytosolic Ca^{++} in both cell types is a function of extracellular Ca^{++} influx, intracellular Ca^{++} release or reuptake, and intracellular Ca^{++} efflux (Fig. 6). Ion channels play a key role in both cell types. In the vascular smooth muscle, the predominant current is carried by voltage-dependent Ca^{++} channels that are activated by depolarization of the plasmalemma (160). Voltage-gated channels have not been identified in large vessel endothelium although they appear to exist in microvascular endothelial cells (161). Ca^{++} entry also occurs through leak channels, stretch-activated channels, and receptor-operated channels (158) (Fig. 6).

Little is known about the effects of estrogen and progesterone on ion channels in vascular endothelial and smooth muscle cells. Studies in other cell types suggest that estrogen and progesterone influence the expression and activity of ion channels through genomic and nongenomic mechanisms. In myometrial smooth muscle cells, estrogen and progesterone act on ion channels through the classic mechanism of gene

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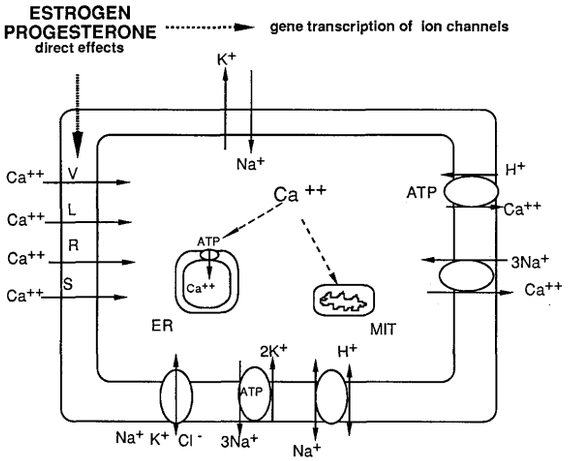

FIG. 6. Mechanism of calcium homeostasis. Ovarian steroids may inhibit voltage-gated Ca^{++} channels at the cellular membrane or influence synthesis of Ca^{++}, K^+, and Na^+ channels via gene transcription. Ca^{++} entry may occur through leak (L), stretch-activated (S), voltage-dependent (V), or receptor-activated (R) channels. Ca^{++} may be extruded from cell by Ca^{++}-H^+ ATPase and Na^+-Ca^{++} exchange. Elevated intracellular Ca^{++} may be sequestered into the mitochondrion (MIT) or the endoplasmic reticulum (ER) via an ATP-Ca^{++} pump. Other factors that influence Ca^{++} homeostasis by regulating membrane potential include K^+ and voltage-gated Na^+ channels as well as the Na^+-K^+- Cl^- antiporter, the Na^+-H^+ exchanger, and the Na^+-K^+ pump. K^+ channels identified in vascular smooth muscle cells include receptor-operated, voltage-dependent, and independent channels.

transcription. Three-day progesterone treatment increases the number and/or density of Ca^{++} channels and increases Ca^{++} current in cultured myometrial cells (162). Activation of the progesterone receptor is likely involved, since progesterone injection did not increase Ca^{++} current in the presence of the progesterone antagonist RU486. Chronic estrogen treatment has also been shown to increase the number of nitrendipine binding sites in myometrial smooth muscle cell membranes (nitrendipine is a dihydropyridine Ca^{++} channel blocker) (163), suggesting an estrogen-induced increase in the number of voltage-gated Ca^{++} channels.

Although estrogen and progesterone receptors have been demonstrated in a number of vascular beds, it is unclear whether the effects of these hormones are exerted through genomic or nongenomic mechanisms and, if genomic, the specific gene products involved. The immediate effects of estrogen and progesterone on Ca^{++} homeostasis in vascular as well as nonvascular tissue support the existence of nongenomic influences. Acute administration of progesterone and estradiol alone caused relaxation in endothelium-denuded rabbit coronary artery rings (108, 109) preconstricted with Bay K8644, a voltage-gated Ca^{++} channel agonist. Incubation with 17β-estradiol shifted the Ca^{++}-dependent concentration curve to the right in a high K^+ depolarization medium (108). These observations suggest that the female hormones may alter vascular smooth muscle Ca^{++} influx through effects on voltage-gated Ca^{++} channels (Fig. 6). In support of this possibility, using the whole cell patch-clamp technique, 17β-estradiol (10 μM) was shown to have an inhibitory effect on voltage-dependent Ca^{++} channels in the A7r5 vascular smooth muscle cell culture line (164).

In nonvascular tissue, progesterone inhibited Ca^{++}-induced contraction in uterine smooth muscle cells within 1–3 min of Ca^{++} addition, and the inhibitory effect was immediately reversed when the hormone was washed out (165). In guinea pig myocytes, 17β-estradiol decreased intracellular Ca^{++} within 1 min of administration and inhibited Ca^{++} current in a dose-dependent fashion (166), suggesting an immediate, negative inotropic effect in these cells. These acute effects of ovarian hormones may occur at the cell membrane. Human sperm cells have been shown to have progesterone receptors in the cell membrane (167). In these cells, acute progesterone administration stimulates rapid Ca^{++} influx (168, 169). The progesterone receptor has been hypothesized to interact with Ca^{++} channels directly or via a cell membrane G protein. The membrane receptor appeared to be different from the classic intranuclear receptor because the progesterone receptor blockers RU38486 and ZK98,299 were ineffective in inhibiting the progesterone-induced Ca^{++} influx. K^+ and Na^+ channels also play a role in altering intracellular Ca^{++} levels in nonvascular tissues. In isolated rat myometrial smooth muscle cells, hormonal status of the animal influenced the expression of K^+ channels (170). Rat myometrial cells have also been shown to express putative K^+ channel mRNA after chronic estrogen treatment of the animal and to undergo an increase in fast Na^+ channels density during gestation (171, 172). Whether similar changes occur in vascular smooth muscle or endothelial cells is unknown.

Results from the above studies should be interpreted with caution since concentrations of hormone used are either not reported or in some studies involve supraphysiological levels ranging from 10 μM to 30 μM of estradiol and 0.3 to 30 μM of progesterone. While effects of these steroid concentrations may well be nonspecific, the hormone levels to which the cell is actually exposed may exceed reported blood levels (169). Clearly further studies are needed on genomic and nongenomic effects of estradiol or progesterone on Ca^{++} homeostasis in vascular smooth muscle and endothelial cells.

IV. Summary, Conclusions, and Directions for Future Research

Gender differences have long been described in the occurrence of several vascular disorders. Female hormones exert protective effects against some disease (*e.g.* coronary artery disease), and restoration of hormone levels improves symptoms in some disorders such as menstrual migraines and cardiac syndrome X. In other circumstances, alterations in female hormones may be linked to the onset of symptoms.

The protective effects of female sex steroids may be related to their vasodilatory properties. Studies suggest that estradiol enhances vasodilation by stimulating endothelial NO and prostacyclin activity and by attenuating vascular smooth muscle cell responses to vasoconstrictors such as ET-1. Estradiol may also have an inhibitory effect on voltage-gated Ca^{++} channels.

The influence of female hormones on the onset of symptoms or disease remains speculative and may include alterations in hormone levels and/or ratios. In certain vascular

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beds, however, estrogen and progesterone may also stimulate vasoconstrictor pathways by increasing α_1-adrenergic activity and/or catecholamine release.

The cellular mechanisms underlying these hormonal effects remain to be determined. Studies primarily conducted in nonvascular cell types support the likelihood that estrogen and progesterone can influence Ca^{++} homeostasis through genomic as well as nongenomic mechanisms of action. Research is needed to better define the effects of estrogen and progesterone in different vascular beds, the cellular mechanisms through which these effects are mediated, whether receptor-mediated induction of enzymes and proteins and/or immediate effects at the cell membrane are involved, and how the normal mechanisms are altered under conditions of vascular disease.

In addition, studies are needed to determine the clinical relevance of many of these observations. For instance, does chronic estrogen replacement therapy enhance endothelial cell vasodilator/vasoconstrictor function? The effects of progesterone also require study since, thus far, the primary focus has been on the effects of estrogen alone. The cardiovascular effects of long-term use of progesterone-only contraceptives, such as Norplant, remain to be determined. Finally, studies on the effects of both hormones combined are important as rarely, if ever, is the level of only one altered *in vivo*.

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