Year: 2002

Sex hormones and hypertension

Dubey, R

DOI: https://doi.org/10.1016/s0008-6363(01)00527-2

Originally published at:
Dubey, R (2002). Sex hormones and hypertension. Cardiovascular Research, 53(3):688-708.
DOI: https://doi.org/10.1016/s0008-6363(01)00527-2

ELSEVIER

Cardiovascular Research 53 (2002) 688–708

Cardiovascular Research

www.elsevier.com/locate/cardiores

Review

Sex hormones and hypertension

Raghvendra K. Dubey[a,b,*], Suzanne Oparil[e], Bruno Imthurn[b], Edwin K. Jackson[a,c,d]

[a]Center for Clinical Pharmacology, University of Pittsburgh Medical Center, Pittsburgh, PA 15213, USA
[b]University Hospital Zurich, Department of Obstetrics and Gynecology, Clinic for Endocrinology, Frauenklinikstrasse 10, 8091 Zurich, Switzerland
[c]Department of Medicine, University of Pittsburgh Medical Center, Pittsburgh, PA 15213, USA
[d]Department of Pharmacology, University of Pittsburgh Medical Center, Pittsburgh, PA 15213, USA
[e]The Vascular Biology and Hypertension Program, University of Alabama, Birmingham, AL 35294, USA

Received 8 June 2001; accepted 30 October 2001

Abstract

Gender has an important influence on blood pressure, with premenopausal women having a lower arterial blood pressure than age-matched men. Compared with premenopausal women, postmenopausal women have higher blood pressures, suggesting that ovarian hormones may modulate blood pressure. However, whether sex hormones are responsible for the observed gender-associated differences in arterial blood pressure and whether ovarian hormones account for differences in blood pressure in premenopausal versus postmenopausal women remains unclear. In this review, we provide a discussion of the potential blood pressure regulating effects of female and male sex hormones, as well as the cellular, biochemical and molecular mechanisms by which sex hormones may modify the effects of hypertension on the cardiovascular system.

Keywords: Blood pressure; Endothelial function; Gender; Growth factors; Hypertension; Smooth muscle

---

1. Introduction

Sexual dimorphism in arterial blood pressure appears in adolescence and persists throughout adulthood [1,2]. Average systolic and diastolic blood pressures in men less than 60 years-of-age are higher than in age-matched women by 6–7 and 3–5 mmHg, respectively [3–5]. After that time, blood pressure (particularly systolic blood pressure) increases in women so that hypertension becomes more prevalent [4] or at least as prevalent in women as men. Inasmuch as gender-associated differences in hypertension prevalence either disappear or cross over after women enter menopause, ovarian hormones may be responsible in part for lower blood pressure in premenopausal women and for the increase in blood pressure in postmenopausal women. Similar to humans, sex-associated differences in blood pressure also exist in animals. For example, compared with females, male spontaneously hypertensive rats (SHR; [6–9]), Dahl salt-sensitive rats [10,11], deoxycorticosterone acetate-salt hypertensive rats [12], and New Zealand genetically hypertensive rats [13] have higher blood pressures. In these animal models of hypertension, blood pressure is reduced in males by castration [6–9,14,15], but is not increased in females by ovariectomy [16,17]. Thus, sex-associated differences in blood pressure also may be due to changes in testicular hormones. In the sections that follow, we examine the evidence for and against the involvement of female and male sex hormones in the pathogenesis of hypertension.

2. Estrogens and hypertension

2.1. Effects of estrogens on blood pressure

Cross sectional [18–20], but not longitudinal [21–23], studies show a significant increase in systolic and diastolic blood pressure following the onset of menopause. Staessen et al. [18] reported a four-fold increase in the incidence of

---

*Corresponding author. Clinic for Endocrinology, Department of Obstetrics and Gynecology, University Hospital Zurich, Frauenklinikstrasse 10, CH-8051 Zurich, Switzerland. Tel.: +41-1-2555-383; fax: +41-1-2554-439.

E-mail address: rag@fhk.usz.ch (R.K. Dubey).

Time to primary review 26 days.

0008-6363/02/$ – see front matter
PII: S0008-6363(01)00527-2

hypertension in postmenopausal women (40% in postmenopausal women vs. 10% in premenopausal women). In a subsequent prospective evaluation of blood pressure (conventional and ambulatory) in women who were premenopausal, perimenopausal or postmenopausal, the authors reported that postmenopausal women had a higher systolic blood pressure (4–5 mmHg) compared with premenopausal or perimenopausal women [20]. Also, the rise in systolic blood pressure per decade was 5 mmHg greater in perimenopausal and postmenopausal women compared with premenopausal women [20]. Because menopause is associated with decreased synthesis of estradiol, it is likely that changes in blood pressure induced by menopause may be due in part to reductions in estradiol production.

Support for the conclusion that estradiol has a blood pressure lowering effect in women is provided by the observation that during the menstrual cycle, blood pressure is lower during the luteal phase (when estradiol levels peak) than during the follicular phase [24–26]. Observations made during pregnancy provide additional circumstantial evidence for a blood pressure lowering effect of estradiol. Estradiol levels increase 50–180-fold during pregnancy [27], and these increases are associated with substantial reductions in blood pressure [28]. The timing of maximal decreases in blood pressure and maximal increases in estradiol levels does not coincide completely, however. In the first, second and third trimesters of pregnancy, estradiol levels increase by 8-, 15- and 186-fold [27], respectively. In contrast, ambulatory blood pressure is lowest in the first (systolic 103±7 mmHg) and second (systolic 101±9 mmHg) trimesters and rises in the third trimester (systolic 111±9 mmHg) of pregnancy [28]. This suggests that factors in addition to estradiol modulate blood pressure in pregnancy [29] and/or that other hormones or local modulators generated during pregnancy abrogate the blood pressure lowering effects of estradiol during the third trimester of pregnancy.

If endogenous estradiol does lower blood pressure, administration of estrogenic preparations to women might also be expected to reduce blood pressure. However, data on the effects of estrogenic preparations on blood pressure are inconsistent, and include reports of blood pressure lowering [30–45], blood pressure elevating [46–51], and blood pressure neutral effects [37,52–54]. Evidence for blood pressure elevating effects of estrogen preparations comes primarily from studies conducted between 1970 and the early 1980s that used conjugated estrogens or contraceptive estrogens (different from natural estradiol) and conventional rather than ambulatory blood pressure measurements. For example, in several studies, Premarin™ (a mixture of conjugated estrogens isolated from urine of pregnant mares) increased blood pressure [46,50,51], and various synthetic contraceptive estrogens increased the risk of hypertension [55–60]. Of five women who developed hypertension after using Premarin (1.25 mg/day) for 3 months to 5 years, four became normotensive from 1 to 7 months after they cessation of therapy [46]. Similarly, of 27 women who developed hypertension while taking Premarin (1.25 mg/day), 13 became normotensive within 3.6 months after Premarin was discontinued, and of five women who restarted Premarin, blood pressure was again elevated within 6 weeks to 6 months [50]. In a prospective study of 160 postmenopausal women taking either Premarin ($n=73$) or piperazine estrone sulphate (Ogen; $n=87$), development of hypertension was observed only in women taking Premarin [51]. It should be noted that in the above studies, conjugated estrogens (Premarin) were administered orally. Since conjugated estrogens administered via patches have been shown to have largely neutral or marginally blood pressure lowering effects, the route of administration may have a decisive role in defining the effects of conjugated estrogens on blood pressure.

With regard to contraceptive estrogens, in 22 patients who developed hypertension on oral contraceptive pills, the blood pressure was normalized after oral contraceptives were discontinued (blood pressures before, during and after administration of oral contraceptives were 125/76, 183/110, and 130/82 mmHg, respectively [46]). Similar reversals in oral contraceptive-induced hypertension were observed in eight out of 14 women within 3.6 months of discontinuing the contraceptive estrogen [50], and seven of the eight became hypertensive again 3–6 months after resuming therapy. In a prospective cohort study of 68 297 female nurses, compared with women who had never used oral contraceptives, the age adjusted relative risk of hypertension was 1.5 (95% CI=1.5 to 1.8) for current use and 1.1 (95% CI=0.9 to 1.2) for past use [59]. After adjusting for age, body mass index, hormones, cigarette smoking, family history of hypertension, parity, physical activity, alcohol intake, and ethinicity, current users of oral contraceptives had an increased risk of development of hypertension [relative risk (RR)=1.8; 95% CI=1.5–2.3]. The risk of hypertension associated with oral contraceptives increased with age, duration of use, body mass and progestin potency [56]. It is important to note that the estrogens used in contraceptive pills (e.g., ethinyl estradiol) are different than the natural estrogen estradiol; moreover, the doses for contraceptive estrogens and estrogens used for hormone replacement therapy vary considerably. This underscores the principle that the blood pressure effects of estrogenic preparations cannot be equated just because the preparations belong to the same pharmacological class.

More recent studies indicate that postmenopausal estrogen replacement therapy either does not affect or reduces blood pressure. For example, tibolone, a synthetic estrogen with both androgenic and gestagenic properties, had no effect on blood pressure [61]. In the Postmenopausal Estrogen/Progestin Interventions Trial (PEPI), no change in blood pressure was observed in 875 normotensive postmenopausal women receiving conjugated equine estrogens in combination with a variety of progestins [52]. In smaller placebo-controlled, randomized crossover studies, administration of conjugated estrogens to normotensive postmenopausal women had blood pressure lowering or neutral effects (Table 1).

Administration of estradiol (oral or transdermal patches) to normotensive postmenopausal women either reduced or had no effect on blood pressure (Table 1). For example, in a placebo-controlled, double-blind, crossover study with 24-h ambulatory blood pressure measurements, transdermal estradiol (50 μg/day; achieves physiological levels of estradiol) treatment for 2 months was reported to decrease nocturnal blood pressure (systolic, diastolic and mean), but not daytime (office) blood pressure [40]. Several other studies utilizing 24-h ambulatory blood pressure measurement reported blood pressure lowering effects of estradiol (Table 1). In a non-randomized, non-placebo-controlled study, nocturnal blood pressure was decreased in normotensive women treated chronically with estradiol (transdermal) [41]. Twenty four-hour ambulatory blood pressure was also lowered in postmenopausal women who were treated with estradiol sequentially with the progestin dydrogesterone (5 or 10 mg) for 1 year [32].

Blood pressure lowering effects of estradiol were also observed by Seely et al. [42] who evaluated in healthy postmenopausal women the effects of transdermal estradiol (0.1 mg patches administered twice per week to attain physiologic estradiol levels) administered in combination with intravaginal progesterone (300 mg). Significant decreases in nighttime systolic, diastolic, and mean blood pressure were observed in the group treated with estradiol and estradiol/progesterone. Similar to the findings of Cagnacci et al. [40] and the data from the PEPI trial [52], there were trends toward lower *daytime or office* systolic and diastolic blood pressures in patients on estradiol or estradiol/progestin compared to placebo; however, the changes were not significant. Since women, in particular may be vulnerable to white coat hypertension [62], it is possible that nocturnal blood pressure may be more sensitive to estradiol and progesterone. Moreover, this may be a potential reason for the lack of decrease in office/daytime blood pressure observed in the PEPI trial [52]. This also reaffirms the importance and superiority of 24-h ambulatory blood pressure measurements for ascertaining blood pressure changes accurately and without missing some subtle but potentially important effects of estrogen replacement therapy on blood pressure. Indeed, decreases in nighttime ambulatory blood pressure in response to hormone replacement therapy have been observed in other clinical studies [32,41].

Results from several small clinical trials suggest that estrogen replacement therapy also lowers blood pressure in hypertensive postmenopausal women (Table 1). In a randomized, double-blind crossover trial carried out in 30 postmenopausal women with mild hypertension who were receiving estradiol (transdermal; delivery rate 100 mg/day), the 24-h ambulatory blood pressure was lowered significantly [31]. A significant decrease in blood pressure was also observed in postmenopausal women with mild to moderate hypertension who were receiving transdermal estradiol [33]. In a prospective study conducted in 34 postmenopausal women with treated hypertension, administration of estradiol plus the progestin norgestrel for 19 weeks lowered 24-h ambulatory blood pressure [36]. In 13 postmenopausal women with ongoing treatment for hypertension, acute administration of transdermal estradiol reduced 24-h ambulatory day time systolic blood pressure and nocturnal systolic and diastolic blood pressure [43]. In a follow up study of 75 hypertensive postmenopausal women on estradiol replacement therapy for 36 months, no significant changes in blood pressure were observed [53], a negative result that may have been due to the lack of ambulatory blood pressure measurements.

Estradiol also lowers blood pressure in several animal models of hypertensive, including SHR [63], stroke prone SHR (SHRSP; [64]), rats with deoxycortisterone acetate-salt induced hypertension [65], Dahl salt-sensitive rats [66] and rats with pulmonary hypertension [67,68]. In contrast, ovariectomy does not affect the development of hypertension in SHRs [6], suggesting that the effects of estradiol on blood pressure in rats are pharmacological rather than physiological. Unlike the natural estrogen estradiol, the synthetic estrogen ethinyl estradiol increases blood pressure [55], suggesting that the effects of natural and synthetic estrogens differ markedly and may influence distinct mechanisms involved in the regulation of blood pressure. For example, even though contraceptive estrogens are administered at a lower dose (30–200 μg) than estrogens (0.625–2 mg) used for hormone replacement therapy, contraceptive estrogens increase blood pressure [55].

In summary, although the literature on the effects of estrogens on blood pressure is confusing and inconsistent, several general trends can be gleaned from existing reports. Whether blood pressure is decreased, increased or unchanged in response to estrogen treatment depends primarily on three factors: (1) the type of estrogenic preparation; (2) the dose of estrogens; and (3) how blood pressure is monitored. Contraceptive estrogenic preparations tend to increase blood pressure; conjugated equine estrogens appear to have little effect on blood pressure, and estradiol tends to lower blood pressure. The blood pressure lowering effects of estradiol are more readily observed if 24-h ambulatory blood pressure monitoring is employed. The effects of estradiol on the cardiovascular system and kidneys are discussed below from the perspective of how these effects may contribute to the blood pressure lowering actions of estradiol.

2.2. Effects of estradiol on vascular tone

Functional estrogen receptors (ERs) of the α and β

Table 1
Effects of estrogens on blood pressure

Study	Subjects Normotensive (N)/hypertensive (H)	Treatment	Outcome	Ref.
1	62 PMW (42 no HRT; 20 HRT); N	CEE	24 h ambulatory BP was decreased	[30]
2	30 PMW (randomized, double blind crossover; mild H)	Transdermal 17β-E	24 h BP was decreased	[31]
3	29 PMW (15 placebo; 14 treated; N)	Oral 17β-E daily plus dydrogesterone every 3–4 weeks	Follow-up after 1 year of treatment showed a significant decrease in BP	[33]
4	16 PMW (mild to moderate H)	Transdermal 17β-estradiol	Ambulatory 24 h BP was decreased	[34]
5	12 surgically PMW (N)	Oral CEE Oral CE plus MPA	Auscultatory BP unchanged after 1 week Auscultatory BP lowered after 1 week	[35]
6	73 PMW (38 oral; 35 transdermal; N)	Oral 17β-E+norethindrone Transdermal 17β-E+norethindrone	24 h ambulatory BP decreased after 2 and 6 months 24 h ambulatory BP decreased after 2, but not 6 months	[36]
7	34 PMW (prospective study, 34 PMW with treated H)	Cyclic estradiol+norgestrel (19 weeks)	24 h ambulatory BP decreased after 19 weeks	[37]
8	60 PMW (20 placebo, 20 CEE, 20 17β-E; CAD) treatment for 1 year; transdermal	Oral CEE±MPA (10 days) Transdermal 17β-E±MPA (10 days)	Night time ambulatory BP decreased No significant change in BP	[38]
9	16 PMW (placebo-controlled, randomized crossover study; N)	17β-E plus cyclic NETA	24 h ambulatory BP decreased and this effect was more pronounced in presence of NETA	[39]
10	17 PMW (3 month, placebo-controlled, randomized crossover study; N)	Oral CEE Oral CEE plus MPA	24 h ambulatory BP decreased 24 h ambulatory BP decreased	[40]
11	18 PMW (placebo-controlled, randomized crossover study, N)	Transdermal 17β-E for 2 months	24 h nocturnal, but not day time, BP decreased	[41]
12	15 PMW (placebo-controlled, randomized crossover study, N)	Transdermal 17β-E (8 weeks)±P (vaginal; 2 weeks)	24 ambulatory BP (nocturnal&daytime) decreased by 17β-E alone and the effect was enhanced by P	[43]
13	107 PMW+ERT; 223 PMW-HRT; population based sample; H and N	Oral CEE, 17β-E and other Es oral or transdermal	No change in BP	[55]
14	PEPI trial-875 PMW; N	CEE±various progestins	No change in BP	[53]
15	13 PMW (with ongoing treatment for hypertension, placebo-controlled double blind, crossover study; H)	Transdermal 17β-E	24 h ambulatory daytime diastolic BP decreased at 24 h and 24 h ambulatory nocturnal systolic and diastolic BP decreased at 24 h	[44]
16	90 PMW women (oophorectomized, 30–59 yr, non-randomized, prospective study; N)	Transdermal 17β-E ($n=40$) Oral ERT ($n=50$)	24 h ambulatory nocturnal systolic and daytime as well as night time BP reduced at 3 and 6 months No change in BP in subjects receiving oral ERT	[42]
17	20 PMW, double blind, cross over study (N)	Oral CEE plus oral cyclical MPA	Ambulatory day time diastolic and mean BP reduced and MPA lowered BP dose-dependently in presence of conjugated estrogens	[176]

PMW, Postmenopausal women; ERT, estrogen replacement therapy; HRT, hormone replacement therapy; CEE, conjugated equine estrogens; 17β-E, 17β-estradiol; MPA, medroxyprogesterone; NETA, norethisterone acetate.

subtypes are expressed in vascular endothelial [60,70] and smooth muscle cells [60,70], and it is well established that estradiol can cause vasodilation by both ER-dependent and ER-independent mechanisms. Acute administration of estradiol in vitro and in vivo induces rapid dilation of coronary arteries of cholesterol-fed ovariectomized animals [69–73]. Exogenous estradiol also dilates coronary and brachial arteries in postmenopausal women and men [74–77]. Long-term treatment with estradiol abrogates the vasoconstrictor effects of U46619 (thromboxane mimetic), phenylepinephrine, 5-HT, calcium, potassium and acetylcholine on vascular tissues such as aortic rings and coronary arteries [69,78–81]. Compared with premenopausal women, vasodilator effects of estradiol are decreased in postmenopausal women and are normalized by estrogen replacement therapy [69]. The vasodilator effect of estradiol replacement therapy is diminished by co-administration of synthetic progestins such as medroxyprogesterone and cyproterone acetate [82,83].

Both the acute and long-term vasodilator effects of estradiol are mediated in part via generation of endothelium-derived NO and are attenuated by NO synthesis inhibitors [84,85]. Estradiol induces an increase in intracellular free calcium concentration in endothelial cells [69,86], which could contribute to the increase in endothelial-derived NO. Since inhibition of NO synthesis promotes arterial hypertension [87], it is conceivable that estradiol protects against hypertension by increasing NO synthesis. Long-term administration of estradiol increases acetylcholine-mediated coronary vasodilation in non-human primates [71,72], male-to-female transsexuals [88], and postmenopausal women [89], particularly those with angina and normal coronary arteries [90]. The ability of estradiol to increase endothelium-dependent vasodilation in the forearm of hypertensive postmenopausal women is shared by conjugated equine estrogens [91]. Progestins inhibit estradiol-induced synthesis of endothelium-derived NO [92,93], and this may contribute to the diminished vasodilator effects of estrogen observed in postmenopausal women receiving estradiol plus progestins.

Other mechanisms also contribute to estradiol-induced vasodilation. Estradiol causes coronary vasodilation by opening calcium-activated K^+ channels and relaxes endothelium-denuded porcine coronary arteries by opening large conductance calcium-activated and voltage-activated K+ channels [86,94]. Estradiol inhibits voltage-dependent L-type calcium currents in vascular smooth muscle cells and has potent stimulatory effects on large-conductance, calcium- and voltage-activated K^+ channels in coronary artery vascular smooth muscle cells [95]. Since the vasodilator effects of estradiol in intact arteries are abrogated by blockers of calcium-activated, large conductance K^+ channels and inhibitors of cyclic GMP-dependent protein kinase, estradiol may exert its vasodilator effects by opening calcium-activated, large conductance K^+ channels via NO and cGMP-dependent pathways [94–98].

Additionally, estradiol activates adenylyl cyclase activity and increases the synthesis of cyclic AMP, a vasodilator second messenger [99]. Moreover, estradiol stimulates the production of adenosine in vascular smooth muscle cells via the cyclic AMP-adenosine pathway [99]. Estradiol also increases the synthesis of the vasodilator prostacyclin by inducing the expression of prostacyclin synthase and cyclooxygenase [100,101]. Finally, estradiol reduces the synthesis of potent vasoconstrictors such as angiotensin II (Ang II), endothelin-1 and catecholamines [102–104].

In summary, estradiol is a vasodilator that decreases vascular resistance by multiple mechanisms. Increased production of NO plays a prominent role, and increased synthesis of other endogenous vasodilators, decreased synthesis of endogenous vasoconstrictors and activation of K^+ channels also contribute to the vasodilatory actions of estradiol.

2.3. *Effects of estradiol on vascular growth*

The elevated total peripheral resistance characteristic of hypertension is due in part to accelerated growth of vascular smooth muscle cells [105]. Vascular remodeling in hypertension involves interactions among multiple cell types, such as endothelial cells, smooth muscle cells, adventitial fibroblasts, monocytes, macrophages and leukocytes, and among multiple growth inducers, including local growth factors, circulating growth factors and mechanical forces [106]. The processes that lead to increased vascular resistance involve endothelial cell damage/dysfunction, increased generation of chemotactic and mitogenic factors at injury sites, migration of smooth muscle cells into the intima, proliferation of the migrated cells, hypertrophy of smooth muscle cells (increase in cell size) and deposition of extracellular matrix proteins [106]. In addition to smooth muscle cells, migration of adventitial fibroblasts into the neointima and fibroblast proliferation also play a major role in the vascular remodeling process [107]. The sequence of events in vascular remodeling may vary depending on the type of vascular challenge (mechanical, immunologic, lipid-induced), but the abnormal growth of smooth muscle cells is the final process that leads to increased vascular resistance. In vivo studies conducted in several species using various models (balloon injury-induced neointima formation, allograft-induced dysplasia, cholesterol/lipid-induced atherosclerosis and vascular narrowing-induced neointima formation) provide convincing evidence that estradiol prevents the vascular remodeling processes [69,70].

Estradiol engages key cellular/molecular components of the vascular remodeling process. Specifically, estradiol: (1) protects against endothelial damage caused by mechanical injury, oxidized-low-density lipoprotein (LDL), homocysteine, free radicals and immunological factors; (2) blocks vascular inflammation and decreases the expression of adhesion molecules (intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and endothelial leukocyte adhesion molecule-1) at sites of vascular damage; (3) inhibits neointima formation by attenuating/inhibiting the recruitment of circulating macrophages, lymphocytes and thrombocytes to the site of injury, thus reducing the secretion of cytokines, eicosanoids and growth factors; (4) inhibits the vascular remodeling processes in atherosclerosis by stopping the migration of macrophages into the subendothelial spaces and preventing them from accumulating LDL and becoming foam cells; (5) prevents foam cell-induced inflammatory processes such as platelet adhesion and the release of multiple platelet-derived growth factors and cytokines; and (6) inhibits the mitogenic effects of multiple factors generated at the site of endothelial injury/dysfunction and which trigger a cascade of biochemical events that stimulate hyperplastic and/or hypertrophic growth of vascular smooth muscle cells [69,70,108].

In summary, there is no doubt that estradiol profoundly inhibits the vascular response to injury. This important effect of estradiol is mediated by numerous mechanisms, and this is an active area of contemporary research. The vasculoprotective effects of estradiol may attenuate the development of hypertension and may protect the vasculature from the injurious effects of high blood pressure.

2.4. Effects of estradiol on circulating factors

Estradiol modulates the synthesis of circulating factors known to influence vascular tone and structure. For example, estradiol increases bradykinin levels and may lower blood pressure by increasing bradykinin synthesis [124]. Estradiol down-regulates the expression of angiotensin converting enzyme (ACE) in serum as well as in the vasculature [103,109–111] and decreases renin release and Ang II formation [103]. These effects are in some respects paradoxical since estradiol stimulates angiotensinogen expression in the liver [50]. Ang II is a potent mitogen for vascular smooth muscle cells [112,113] and induces vascular remodeling processes associated with hypertension. Therefore, the inhibitory effects of estradiol on the renin–angiotensin system may lead to reduced vascular growth. Estradiol also down-regulates the expression of Ang II type 1 receptors in smooth muscle cells [114]. Since these receptors mediate the mitogenic effects of Ang II, estradiol may abrogate the effects of Ang II in part via this mechanism. Our own studies show that estradiol inhibits Ang II-induced growth of human smooth muscle cells in vitro [115], thus supporting this concept. Additionally, estradiol induces the synthesis of Ang 1–7, a vasodilator and smooth muscle cell growth inhibitor [116].

Homocysteine contributes to vascular disease by inducing endothelial cell damage, inhibiting endothelial cell growth and inducing smooth muscle cell growth [117,118]. Clinical studies provide evidence that estradiol reduces circulating levels of homocysteine in postmenopausal women [119]. In women-to-men transsexuals, homocysteine levels increase with androgen treatment, whereas in men-to-women transsexuals, homocysteine levels decrease with estrogen substitution [120]. Thus, estrogen may protect the vasculature in part by lowering homocysteine levels.

Endothelin-1 is a vasoconstrictor and mitogenic peptide that is thought to play a role in the pathogenesis of various forms of vascular disease. The synthesis and biological activity of endothelin-1 are regulated by estradiol. Estradiol inhibits serum- and Ang II-stimulated synthesis of endothelin-1 [121] in endothelial cells via ER-receptor dependent mechanisms [122]. Estradiol also blocks the mitogenic effects of endothelin-1 on smooth muscle cells and inhibits endothelin-1 induced MAP kinase activation [123]. Compared with premenopausal women, plasma endothelin-1 levels are increased in postmenopausal women not taking estradiol, and are reduced following estradiol replacement therapy [104,119]. These findings suggest that estradiol may have anti-mitogenic effects on the vasculature in part by reducing endothelin-1 levels.

Estradiol influences the synthesis of a number of factors associated with coagulation and fibrinolysis. For example, estradiol decreases plasma concentrations of clottable fibrinogen, soluble thrombomodulin, plasminogen activator inhibitor 1, antithrombin III and protein S [125–127]. Moreover, most studies report that estradiol decreases levels of von Willebrand factor [128]. Importantly, the effects of estradiol on coagulation and fibrinolytic factors are not the same as those of the oral contraceptive ethinyl estradiol [129]. Even though ethinyl estradiol is administered at a much lower dose than is used for estrogen replacement therapy, it increases coagulation factors such as factor VII, VIII, VIII:Ag, VIII:C, IX and X. Ethinyl estradiol also increases beta-thromboglobulin, plasminogen, fibrinogen antigen and euglobulin lysis activity [130–132], and decreases antithrombin levels and the sensitivity to activated protein C, a process that could increase the risk of thrombosis. Moreover, ethinyl estradiol has a greater ability to induce hepatic synthesis of angiotensinogen than does estradiol, and ethinyl estradiol tends to cause sodium and fluid retention [132]. Unlike the natural estrogens, ethinyl estradiol increases triglycerides and total cholesterol and is associated with impaired glucose tolerance [132].

The clinical significance of the aforementioned effects of estradiol and oral contraceptives on circulating factors is underscored by the well established fact that oral contraceptives induce disorders of coagulation and increase blood pressure, whereas estradiol when used for hormone replacement therapy tends to lower blood pressure and have neutral effects on the coagulation system. In this context, following the early reports in 1960s that blood pressure is elevated in women on oral contraceptives [55], multiple clinical studies have provided evidence that contraceptive estrogens increase blood pressure [55–60].

In a study of 83 women using oral contraceptives for 3 years, increases in both systolic and diastolic blood pressure (9.2 and 5.0 mmHg, respectively) were observed. In a subgroup of these subjects, the mean increases in systolic and diastolic blood pressure were 14.2 and 8.5 mmHg, respectively [133]. Moreover, the blood pressure returned to pretreatment levels within 3 months of stopping the contraceptives [133]. In a longitudinal study of 13 358 women on oral contraceptives, a significant rise in blood pressure was observed, and this increase could be reversed by discontinuing oral contraceptives [134]. The Nurses Health study found that current users of contraceptive estrogens had significantly increased (RR=1.8; 95% CI=1.5–2.3) risk of hypertension compared with never users [59].

The adverse effects of contraceptive estrogens in the past can be attributed to the high doses (≥150 μg) of contraceptive estrogens (ethinyl estradiol) used. Indeed, with the current regimen of 30 μg, the blood pressure elevating effects of contraceptive estrogens have been considerably reduced [132]. Briggs and Briggs [135] reported no rise in blood pressure in women taking 30 μg contraceptive estrogens for 3 years, while there was an increase in women taking 50 μg contraceptive estrogens. Lack of blood pressure elevating effects of 30 μg contraceptive estrogens was also observed in a three year study of 1000 women [136]. Although the lower doses may be safer, contraceptive estrogens still can elevate blood pressure and oral contraceptive-induced hypertension is still the most frequent form of secondary hypertension in younger women [137].

Apart from the blood pressure elevating effects of contraceptive estrogens, clinical studies provide evidence that oral contraceptives can disturb glucose metabolism, and induce glucose intolerance, procoagulant effects, hypercholesterolemia and unfavorable effects on the plasma lipoprotein [high-density lipoprotein (HDL) and LDL] profile [132]. High, but not low, doses of oral contraceptive estrogens were associated with increased risk of ischaemic stroke and venous thromboembolism [132]. Taken together, this once again illustrates the concept that all estrogenic compounds are not created equal.

In summary, the direct vasodilator and vasoprotective effects of estradiol are likely reinforced by the ability of estradiol to modify several important humoral systems and circulating factors, including the renin–angiotensin system, homocysteine, endothelin-1, the coagulation cascade and the fibrinolytic system. As discussed above, in contrast to estradiol, the oral contraceptives, such as ethinyl estradiol, induce unfavorable effects on coagulation, fibrinolysis, as well as on hepatic angiotensinogen synthesis, sodium and fluid balance, triglycerides and total cholesterol levels, and glucose tolerance.

2.5. Effects of estradiol on the heart

Hypertension is importantly associated with a remodeling process that leads to cardiac hypertrophy and abnormal growth and function of cardiac fibroblasts and myocytes. Cardiac fibroblasts contribute to pathological changes in the hypertensive heart by proliferating, depositing extracellular proteins and replacing myocytes with fibrotic scar tissue. Estradiol and progesterone inhibit mitogen-induced proliferation of cardiac fibroblasts and extracellular matrix (collagen) synthesis by cardiac fibroblasts [138], suggesting that these sex hormones may attenuate the structural changes in the heart that are usually associated with hypertension. The direct effects of estradiol on the heart may be amplified by estradiol-induced changes in circulating and local factors such as Ang II, endothelin, NO, prostacyclin, adenosine and bradykinin.

Many studies provide evidence that estradiol ameliorates ischemia/reperfusion-induced myocardial injury and ventricular arrhythmias [139–143]. Estradiol may induce these protective effects by: (1) upregulating NO synthesis and opening calcium-activated potassium channels [139]; (2) inhibiting TNF-α production and limiting the deleterious ICAM-1 mediated binding of leukocytes to injured myocardium [140]; (3) regulating endothelin-1 synthesis and expression of endothelin type-B receptors [141]; and (4) activating mitochondrial ATP-dependent potassium channels in myocardium [142]. Importantly, the protective effects of estradiol on exercise-induced myocardial ischemia in postmenopausal women are enhanced by the naturally occurring progestin, progesterone, but not by the synthetic progestin, medroxyprogesterone [143].

Female sex hormones have other protective effects on cardiac myocytes. For instance, apoptosis causes loss of cardiac myocytes in heart failure [144], and estradiol prevents programmed cell death in cardiac myocytes [144]. In addition, estradiol and progesterone, but not testosterone, upregulate the expression of heat shock factor-1, and overexpression of this factor attenuates cardiac damage [145]. Other protective mechanisms induced by estradiol in cardiac myocytes include induction of NO synthesis (inducible NOS and endothelial NOS; [146]), reduction in L-type calcium channel current and density [147] and inhibition of K^+ currents [148].

The above findings indicate that estradiol has favorable effects on both cardiac myocytes and fibroblasts. The cardioprotective effects of estradiol may attenuate hypertension-induced cardiac damage, as well as ischemia/reperfusion injury of the heart.

2.6. Effects of estradiol on the kidney

The kidneys play a major role in the regulation of blood pressure, and renal abnormalities are involved in the development and maintenance of hypertension. As pointed out repeatedly by Guyton et al. [149], the common defect in hypertension is a shift to the right in the pressure-natriuresis relationship [150]. This contention is strongly supported by the experimental evidence that transplantation of prehypertensive kidneys from SHR to Wistar–

Kyoto rats and from Dahl salt-sensitive to salt-resistant rats produces hypertension [150]. Moreover, blood pressure is normalized in hypertensive humans transplanted with kidneys from normotensive donors [151].

Gender-associated differences in renal hemodynamics [single nephron glomerular filtration rate (GFR), glomerular plasma flow, preglomerular resistance, glomerular pressure] and renal responses to Ang II are well established. In single nephrons, GFR and glomerular plasma flow are higher and preglomerular resistance is lower in male compared with female rats despite similar numbers of glomeruli per kidney [16,17]. A reduction in preglomerular resistance is associated with increased glomerular injury, particularly if there is a concomitant increase in systemic BP.

Premenopausal women are protected against the progression of renal disease [16,152,153], and evidence from epidemiological studies, clinical trials and experimental studies with animal models of renal injury suggests that ovarian hormones are responsible for this renoprotection. A meta-analysis has shown that men are predisposed to the rapid progression of multiple non-diabetic chronic renal diseases, including idiopathic membranous nephropathy and autosomal dominant polycystic kidney disease [153]. Further, aged male rats of various strains exhibit decreased GFR and develop glomerular injury, including glomerulosclerosis, at an earlier age than females rats [16,152]. Moreover, estradiol treatment reduces glomerulosclerosis, cellular infiltration, and expression of adhesion and extracellular matrix molecules and prevents tubular damage in animal models with chronic renal allograft rejection or unilateral nephrectomy [154,155]. Since healthy glomeruli are critical for normal renal hemodynamics and blood pressure, the above findings suggest that estradiol helps maintain a normal blood pressure by protecting the kidney.

Estradiol down-regulates the synthesis of multiple factors known to induce glomerulosclerosis and elevate blood pressure. Of particular relevance to the kidney, estradiol decreases: (1) the local synthesis of Ang II within the kidney [109]; (2) the production of endothelin-1 [121], a strong mitogen for glomerular mesangial cells [106]; (3) plasma levels of homocysteine [119], another endogenous factor that causes glomerular damage and induces glomerulosclerosis; (4) IGF-1, a potent mitogen for mesangial cells [106] and IGF-1 receptors [156]; (5) PAI-1, a mesangial cell mitogen that is decreased by almost 50% by estradiol [126]; (6) free radicals [106,157]; (7) oxidized-LDL [158]; (8) cholesterol [158]; and (9) lipid peroxides [159].

Increased generation and deposition of extracellular matrix proteins is an initial step in the development of glomerular obsolescence and progressive loss of renal function. Estradiol suppresses collagen (types I and IV) synthesis in mesangial cells [70,92], suggesting that it may limit the development of glomerulosclerosis by reducing matrix accumulation after glomerular injury. In particular, estradiol inhibits collagen synthesis induced by Ang II and TGFβ, growth factors implicated in the pathophysiology of progressive renal injury in various experimental models of kidney disease [160–162]. Moreover, estradiol inhibits serum-induced collagen synthesis in mesangial cells by down-regulating TGFβ expression [161]. Since endothelin-1 and Ang II induce proliferation of mesangial cells via TGFâ [163], it is likely that estradiol attenuates the deleterious effects of endothelin-1 and Ang II on the kidney by modulating TGFβ. Thus, there is strong evidence that estradiol protects the kidney in part by abrogating the mitogenic effects of multiple growth factors that participate in the pathophysiology of glomerulosclerosis.

In addition to increased production and deposition of extracellular matrix proteins, increased proliferation of mesangial cells play a key role in glomerulosclerosis. Estradiol inhibits mitogen-induced proliferation of human glomerular mesangial cells [92], suggesting that it may protect against glomerular remodeling by inhibiting mesangial cell growth.

Other mechanisms that may contribute to the renoprotective effects of estradiol include: (1) up-regulation of anti-aggregatory pathways by induction of ecto-ADPase in glomeruli and the vessel wall [164]; (2) up-regulation of renal oxytocin receptor gene expression responsible for regulating renal fluid dynamics [165]; (3) prevention of urinary stone formation with inhibition of crystal deposition and calcium content in renal tissue; (4) decrease in urinary excretion of oxalate; (5) down-regulation of the expression of osteopontin mRNA in renal tissue [166]; (6) up-regulation of the synthesis of anti-mitogenic prostaglandins [100]; (7) attenuation of IgA-induced nephropathy [167]; (8) up-regulation of adenosine synthesis [99]; and (9) increases in NO synthesis by glomerular endothelial cells [92].

Injury to glomerular endothelial cells (ECs) also contributes to glomerulopathies [168], and growth of glomerular ECs participates in capillary repair in glomerulonephritis. Since estradiol induces growth of aortic ECs, it may also facilitate the glomerular repair process by inducing growth of glomerular ECs. It is important to note that estradiol induces VEGF synthesis [169], and VEGF is known to repair glomerular EC injury [170]. This suggests that estradiol may protect the glomeruli by inducing VEGF synthesis in glomerular ECs. Estradiol prevents TNFα and LPS-induced apoptosis of vascular ECs [171]. Thus it is likely that estradiol also protects against the deleterious effects of TNFα and LPS on glomerular ECs.

In summary, estradiol engages multiple mechanisms to protect the kidney from injury. Thus, estradiol-induced renoprotection may importantly contribute to the ability of estradiol to maintain the normotensive state.

2.7. Effects of estradiol on the sympathetic nervous system

In 12 perimenopausal women randomized to receive estradiol valerate ($n=7$) or placebo ($n=5$), Sudhir et al.

[172] demonstrated that estrogen supplementation decreases norepinephrine-induced vasoconstriction and total body norepinephrine spillover. Vogpatanasin et al. [173] recently conducted a randomized, crossover, placebo-controlled study in 12 normotensive postmenopausal women who were treated for 8 weeks with either transdermal estradiol, oral conjugated estrogens or placebo. Measured parameters included 24-h ambulatory blood pressure and sympathetic nerve discharge. Transdermal estradiol, but not conjugated estrogens, decreased sympathetic nerve discharge and blood pressure. Hunt et al. [174] examined the effects of conjugated estrogens administered for 6 months to 11 healthy, postmenopausal women on baroreflex function. In this study, conjugated estrogens did not affect blood pressure or cardiac-vagal baroreflex gain, but significantly increased sympathetic baroreflex gain. The effects of estrogen on sympathetic baroreflex reflexes are also supported by two other studies in postmenopausal women [175,176]. Moreover, in menopausal women, acute administration of estradiol and progesterone attenuated mental stress-induced cardiovascular responses and increases in plasma catecholamines [177], and muscle sympathetic nerve activity measured by microneurography is reduced in women compared with age-matched men [178].

There is also evidence that estradiol inhibits the sympathetic nervous system in animal models of hypertension. In this regard, Fang et al. recently demonstrated that in SHR endogenous estradiol, as well as phytoestrogens (genistein, an ERβ ligand), attenuate NaCl-induced hypertension by inhibiting NaCl-induced activation of the sympathetic nervous system [179]. These findings are consistent with the sexually dimorphic response to dietary NaCl supplementation observed in SHR and WKY rats [180,181].

In summary, it appears that estradiol, but not conjugated estrogens, decreases basal sympathetic tone, and that estrogenic preparations may augment sympathetic baroreflex gain. These effects would reinforce the antihypertensive actions of estradiol.

3. Progestins and hypertension

3.1. Effects of progestins on blood pressure

Data from both human and animal studies indicate that progesterone, the natural progestin, has either neutral or depressor effects on blood pressure. For example, decreases in blood pressure with the progression of pregnancy are positively correlated with increases in progesterone [182]. Moreover, oral administration of natural progesterone significantly lowered blood pressure in six men and four postmenopausal women with mild to moderate hypertension who were not receiving antihypertensive drugs [183]. In contrast, administration of natural progesterone (200 mg, orally) to seven postclimacteric women failed to influence blood pressure [184]. In animal studies, acute administration of progesterone did not alter mean arterial blood pressure in rats [185].

Most studies conducted with synthetic progestins for contraception or hormone replacement therapy have shown a blood pressure elevating effect [132]. Contraceptive progestins have androgenic activity, whereas natural progesterone are non-androgenic. The blood pressure elevating effects of contraceptive progestins may therefore depend on the androgenicity of the individual progestin [186].

In theory, synthetic progestins increase blood pressure by stimulating sodium retention [187]. However, data from small well controlled clinical studies (Table 1) provide evidence that sequential administration of the non-androgenic progestogen medroxyprogesterone acetate (MPA) or the androgenic progestogen norethisterone acetate (NETA) either does not alter or enhances the blood pressure lowering effects of estrogens. In a randomized double-blind crossover study of 53 postmenopausal women treated for 4 weeks with conjugated equine estrogen (0.625 mg daily) and sequentially with either 2.5, 5 and 10 mg MPA during the last 14 days [186], there was a dose-dependent decrease in daytime ambulatory diastolic and mean arterial blood pressure with the MPA treatment [186]. Similar to MPA, the progestin NETA was shown to significantly enhance the blood pressure lowering effects of estradiol in a placebo-controlled randomized crossover study in 16 normotensive postmenopausal women [38]. In summary, based on the human data it is evident that at least some progestins given in the cyclic regimens of hormone replacement therapy have blood pressure neutral or lowering effects.

3.2. Effects of progestins on vascular tone

Similar to estradiol, progesterone induces endothelium-dependent vascular relaxation. In coronary arteries from dogs pretreated with estradiol, endothelium-dependent relaxation is enhanced by progesterone [188]. These findings suggest that natural progesterone has beneficial or neutral, but not adverse, effects on blood pressure. On the other hand, synthetic progestins seem to antagonize the beneficial effects of estradiol on the vasculature. In vitro studies provide evidence that estradiol-induced NO synthesis is inhibited by synthetic progestins [92,93], and in women the vasodilator effects of estradiol are diminished by co-administration of synthetic progestins such as MPA and cyproterone acetate [93]. In atherosclerotic monkeys, estradiol induces coronary artery dilation and increases blood flow reserve, and co-administration of MPA results in a 50% reduction in the dilator response to estradiol [189]. Moreover, estradiol-induced NO synthesis in postmenopausal women is significantly reduced by MPA, as well as by other synthetic progestins such as norethisterone acetate and cyproterone acetate [83,84]. Moreover, MPA

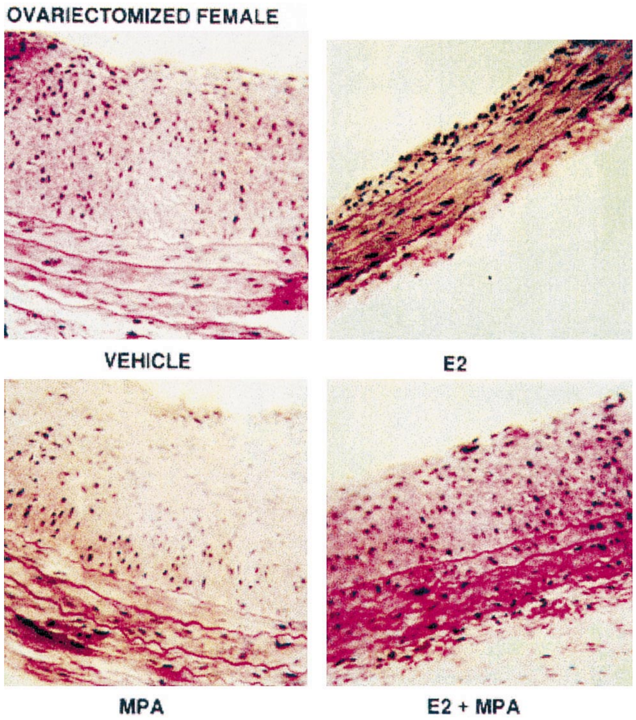

Fig. 1. Representative photomicrographs of right common carotid arteries from ovarectomized female Sprague–Dawley rats 14 days after balloon injury. The rats were treated with vehicle, estradiol (E2), medroxyprogesterone (MPA) or E2+MPA. The inhibitory effects of estradiol on neointima formation were abrogated in the presence of the synthetic progestin, MPA (with permission from the American Heart Association).

can abrogate the antivasoocclusive effects of estradiol following vascular injury ([190]; Fig. 1).

In summary, natural progesterone augments and some synthetic progestins abrogate the beneficial effects of estradiol on vascular tone and the vascular injury response. Nonetheless, synthetic progestins may augment the antihypertensive effects of estradiol. The aforementioned studies teach that like estrogens, not all progestins have the same pharmacological effects.

3.3. Effects of progestins on vascular growth

Similar to estradiol, progesterone inhibits mitogen induced growth and proliferation of cardiac fibroblasts [138], vascular smooth muscle cells [191] and glomerular mesangial cells [106], which contribute to the vascular and glomerular remodeling associated with hypertension, atherosclerosis and glomerulosclerosis. Little is know regarding the effects of synthetic progestins on vascular growth.

4. Androgens and hypertension

4.1. Effects of androgens on blood pressure

Relatively little is known regarding the influence of androgens on blood pressure and cardiovascular disease. There are reports of lower circulating testosterone [192–194] and androstenedione [194] levels in hypertensive men [192–194], and circulating testosterone levels in men with coronary artery disease [195] or myocardial infarction [196] are either unchanged or decreased. These studies suggest that decreased, rather than increased, androgen levels are associated with hypertension, myocardial infarction and coronary artery disease. An important caveat is that the lower testosterone levels observed in the aforementioned studies may merely reflect increased stress. Testosterone levels decrease in response to stress induced by recent myocardial infarction, surgery, head trauma, burns, hypoxia, sleep deprivation and psychological stressors [196]. Thus, the lower levels of testosterone in men with hypertension and cardiovascular disease may not indicate that testosterone reduces blood pressure and protects the cardiovascular system. In support of this conclusion is the low prevalence of coronary disease among men with hypotestosteronemia plus hyperestrogenemia [195]. Also, women suffering from chronic anovulation and exhibiting hypertestosteronemia have an increased risk of coronary artery disease and myocardial infarction [196]. Moreover, men with testosterone deficiency following orchiectomy have a slightly lower mortality from heart disease [196], suggesting that lower testosterone may protect against cardiovascular disease.

In summary, clinical studies are needed to directly define the effects of testosterone on the cardiovascular system in human subjects. Studies should be conducted in men with Klinefelter disease who have testosterone deficiency and require testosterone supplementation. Results from these studies could provide data on the dose-dependent effects of testosterone on cardiovascular function.

Studies in animals strongly suggest that testosterone is a pro-hypertensive hormone. Blood pressure is higher in male SHR, Dahl salt-sensitive rats, deoxycorticosterone acetate-salt hypertensive rats and New Zealand genetically hypertensive rats compared to females [6–13]. The association between testosterone and high blood pressure in these animals is supported by the observation that castration at a young age (3–5 weeks) attenuates the development of hypertension in SHR and Dahl salt-sensitive rats [6–9,14,15]. Furthermore, treatment of ovariectomized normotensive females or castrated males with testosterone increases blood pressure to levels similar to those in intact males [7,9], and testosterone increases blood pressure in ovariectomized female SHRs [7,9]. Moreover, irreversible increases in arterial blood pressure are observed in normotensive, uninephrectomized female rats treated chronically with testosterone [197,198].

Use of anabolic steroids that are synthetic derivatives of testosterone is temporally associated with hypertension, ventricular remodeling, myocardial ischemia and sudden cardiac death [199]. Moreover, in a double blind placebo-controlled study in 21 men, treatment with testosterone enanthate (3.5 mg/kg body weight) for 12 weeks significantly increased systolic blood pressure by 10 mmHg [200]. In contrast, administration of testosterone to 20 male subjects (mean age 70.6±6.2 years) to achieve physiologic and superphysiologic serum testosterone concentrations did not increase blood pressure [201], and no changes in blood pressure were observed in men receiving testosterone undecanoate (120 mg/day for 5 days; [202]). The different outcomes in the above studies may have been due to the age group of the subjects tested (young vs. old) or the duration of treatment (acute vs. long-term). Importantly, the blood pressure elevating effects were observed in subjects who were undergoing regular weight training. In contrast, the subjects with no increase in blood pressure did not exercise. Therefore, it is possible that the effects of testosterone on blood pressure are modulated by exercise.

A number of mechanisms by which testosterone can elevate blood pressure and damage blood vessels have been elucidated. Testosterone increases circulating levels of homocysteine. Homocysteine induces endothelial damage, thus leading to the development of atherosclerosis, and may adversely influence renal function by damaging glomerular endothelial cells [120]. In contrast to estradiol, testosterone increases endothelin-1 levels in subjects undergoing a sex change, providing a humoral mechanism by which it may elevate blood pressure [120]. Finally, in male SHR, elevated levels of catecholamines are associated with high blood pressure [203], and castration reduces catecholamine levels to those observed in normotensive controls [204], suggesting that testosterone can also directly induce catecholamine synthesis [204]. This effect may be explained by the observation that testosterone stimulates tyrosine hydroxylase, the rate limiting enzyme for catecholamine synthesis [203,204]. Thus, testosterone may elevate blood pressure and contribute to the pathogenesis of cardiovascular disease by altering a number of humoral factors, including homocysteine, endothelin-1 and catecholamines.

4.2. Effects of androgens on vascular tone

Androgen receptors are expressed in vascular cells [108], and androgens may have a functional role in modulating vascular tone. Testosterone relaxes precontracted rabbit coronary arteries and aortas in vitro, with or without endothelium [108,205]. Short-term intracoronary infusions of testosterone dilate male and female canine coronary arteries in vivo and increase coronary blood flow. These vasodilator effects of testosterone are largely mediated via ATP-sensitive K^+ channels [206]. Via similar mechanisms, short-term intracoronary administration of physiological concentrations of testosterone induces coronary artery dilatation and increases coronary blood flow in men with established coronary artery disease [207]. Androgen receptor blockers do not alter these rapid onset acute vasorelaxing effects of testosterone. Therefore, the acute vascular effects of testosterone are most likely androgen-receptor independent and non-genomic.

In porcine coronary arteries, testosterone, progesterone and estradiol induce concentration-dependent relaxation of both prostaglandin $F_{2\alpha}$-induced and KCl-induced contraction [208,209]. In contrast to estradiol, testosterone and progesterone have greater relaxing effects on vessels precontracted with prostaglandin $F_{2\alpha}$. Estradiol inhibits Ca^{2+} entry, and progesterone and testosterone cause coronary relaxation by inhibiting other contractile mechanisms [208,209]. Estradiol induces NO synthesis in endothelial cells, and this effect is accompanied by translocation of membrane-associated eNOS [210]. In contrast to estradiol, neither progesterone nor testosterone modulates NO synthesis [210]. Testosterone improves post-exercise ST-segment depression in patients with angina [196,211,212], and short-term administration of testosterone induces beneficial effects on exercise-induced myocardial ischemia in men with coronary artery disease, an effect that may be due to the direct coronary-relaxing actions of testosterone [213].

In contrast to the direct vasodilator and vasorelaxing effects discussed above, testosterone may block the effects of other vasodilator agents. In this regard, coronary vascular perfusion with testosterone blocks adenosine-mediated vasodilation via a rapid non-genomic mechanism [214]. Treatment of cultured cells with testosterone, but not estradiol, significantly increases thromboxane A_2 receptor density. This effect is inhibited by the testosterone receptor blocker hydroxyflutamide, suggesting the involvement of androgen receptors. The testosterone precursor androstenedione also increases thromboxane A_2 receptor density [215,216]. Several lines of evidence support the functional significance of this androgen-induced increase in thromboxane A_2 receptor density. The aortas of male rats show increased (compared to females) contractile responses to thromboxane A_2 [215]. Further, testosterone enhances coronary artery constriction induced by a thromboxane A_2 mimetic in vitro and in vivo [217]. Thromboxane A_2 is implicated as a risk factor for cardiovascular diseases, and it is possible that the contrasting effects of estradiol and testosterone on thromboxane A_2 signaling may, in part, be responsible for some of their differential effects on the cardiovascular system.

Testosterone has additional pro-hypertensive effects. In SHR, testosterone enhances the activity of tyrosine hydroxylase, the rate limiting enzyme in norepinephrine synthesis, and the resultant increase in norepinephrine levels may contribute to the development of hypertension in male animals with this form of genetically-determined hypertension [204]. Moreover, testosterone up-regulates the release of the potent vasoconstrictor neuropeptide Y [218]. Testosterone also enhances, whereas estradiol attenuates, the contractile effects of endothelin-1 in porcine coronary artery rings [219].

These findings indicate that testosterone can activate mechanisms that cause both vasoconstriction and vasorelaxation. The balance between the vasodilating and vasoconstricting effects of testosterone determines its net effect on vascular tone and, perhaps, blood pressure. Thus, the inconsistent effects of testosterone on blood pressure discussed above may be due to its variable effects on vascular tone, in part dependent on the pretreatment condition of the vasculature.

4.3. Effects of androgens on vascular growth and atherogenesis

There is evidence that testosterone can protect against the response to vascular injury and the development of atherosclerosis [205,220,221]. In cholesterol fed rabbits [221], testosterone is anti-atherosclerotic by a mechanism independent of changes in plasma lipids. Testosterone also protects against post-injury-induced plaque development [205]. Despite its anti-atherosclerotic effects in rabbits, testosterone does not inhibit smooth muscle cell proliferation [220]. Testosterone is also ineffective in inhibiting mitogen-induced smooth muscle cell migration [222] and proliferation in vitro [157,223]. These findings suggest that the vasoprotective effects of testosterone are not mediated by inhibition of vascular smooth muscle cell growth.

In contrast, a number of other observations suggest that testosterone is pro-atherosclerotic. Testosterone: (1) exacerbates diet-induced atherosclerosis and has adverse effects on atherosclerosis-related arterial remodeling in female monkeys [224]; (2) induces vascular plaque formation in chicks [225]; (3) increases monocyte adhesion to vascular endothelial cells and increases the expression of vascular cell adhesion molecule-1 in humans [226]; (4) lowers HDL and raises LDL [227]; (5) up-regulates the release of neuropeptide Y [219] which can induce pro-atherosclerotic actions and local vasoconstriction; (6) has a pro-mitogenic effect on vascular smooth muscle cells [228]; and (7) increases the synthesis of Ang II, a vasoconstrictor and mitogen known to induce hypertension and atherosclerosis [6]. Thus, whether testosterone inhibits or accelerates atherosclerosis appears to be model dependent.

4.4. Effects of androgens on cardiac mechanisms

Testosterone and its metabolite dihydrotestosterone (DHT) induce cardiac hypertrophy in part by activating androgen receptors that are expressed in cardiac myocytes [229]. In baroreceptor-denervated rats, left ventricular hypertrophy is gender-dependent; estradiol inhibits, whereas testosterone stimulates, cardiac hypertrophy [230]. Moreover, in vitro studies provide evidence that androgens induce hypertrophic growth in cultured myocytes [229], suggesting that the growth promoting effect is direct. The hypertrophic effects of DHT, but not testosterone, are associated with increased synthesis of atrial natriuretic peptide [229], suggesting that these androgens may induce hypertrophy via different mechanisms.

4.5. Effects of androgens on renal mechanisms

Reckelhoff and Granger have described several mechanisms by which androgens can induce renal injury and precipitate hypertension [231]. Studies in rat models provide evidence that, compared with females, ageing males exhibit decreased glomerular filtration rate and develop glomerular injury, glomerulosclerosis, proteinuria and increased blood pressure [16,152,231]. Castration at an early age attenuates renal injury and prevents the development of hypertension; whereas, administration of exogenous testosterone to castrated animals increases blood pressure to levels similar to those found in intact males [7,152,231]. These findings suggest testosterone can adversely influence renal function and blood pressure, eventually leading to hypertension.

The kidneys express androgen receptors [231,232], and studies have examined whether the effects of testosterone on blood pressure are androgen-receptor mediated. Administration of flutamide, an androgen receptor antagonist, to intact male SHRs lowers blood pressure to levels found in female or castrated male SHRs [232], suggesting that the effects of androgens are receptor mediated. Furthermore, sodium-induced increases in blood pressure in Wistar–Kyoto and SHR are suppressed by androgen-receptor blockade [233], confirming the androgen-receptor dependence of salt sensitive hypertension in this animal model.

Disruption of the CYP4A14 gene (arachidonic acid ω-1-hydroxylase) in mice results in hypertension that is more severe in males and is androgen sensitive, i.e., increases in blood pressure are reduced upon castration and restored upon testosterone supplementation [234]. Renal vascular resistance is dramatically increased in these mice, suggesting that lack of functional CYP4A14 in the kidney may have several interrelated metabolic and regulatory effects whose functional manifestations are increased renal vascular resistance, impaired renal hemodynamics and hypertension. It has been suggested that the final mediator for the blood pressure enhancing effects of androgens in CYP4A14 knockout mice is 20-HETE [234], which is known to alter renal hemodynamics and function.

Another key mechanism by which androgens tilt the cardiovascular system toward a pro-hypertensive state is by shifting the pressure-natriuresis relationship to the right [149,150,152]. At comparable renal perfusion pressures, intact SHR males and ovariectomized females receiving chronic testosterone treatment excrete significantly less sodium and water than intact females, ovariectomized females or castrated males [7,231]. These effects may be mediated by the renin–angiotensin system because testosterone increases plasma renin activity (PRA) [7,152,231], castration of male rats decreases PRA, and administration of testosterone to ovariectomzied female rats increases PRA [7,152,231]. The effect of testosterone on PRA is dose-dependent [7,152,231]; suggesting that the blood pressure regulating effects of testosterone may be concentration-dependent [7,152,231]. Angiotensinogen mRNA levels are also higher in male rats than in females, and castration reduces these levels [235,236]. Thus, animal studies provide convincing evidence that testosterone diminishes the ability of the kidneys to excrete salt, and thereby predisposes to hypertension. Whether this occurs in humans is unknown.

5. Gender independent factors and role of hormone metabolism

Both estradiol and testosterone are present in both sexes, albeit in different concentrations and ratios [196]. Endogenous androgens (dehydroepiandrosterone, androstenedione, and testosterone) are readily converted to estradiol by the sequential actions of 17β-hydroxysteroid dehydrogenase (17β-HSD) and aromatase. Thus, some of the beneficial effects of testosterone observed in males may be due to its conversion to estradiol and estradiol metabolites. This hypothesis is supported by the recent finding that the inhibitory effects of dehydroepiandrosterone, a precursor of androstenedione, on atherosclerosis are blocked by the aromatase inhibitor fadrozole [237]. This finding suggests that the sequential conversion of androgens to estradiol is responsible for their anti-atherosclerotic actions, but whether estradiol is the ultimate mediator remains unclear.

Findings from our laboratory provide evidence that sequential metabolism of estradiol to catecholestradiols and ultimately methoxyestradiols is responsible for the anti-mitogenic effects of estradiol on vascular smooth muscle cells [238], cardiac fibroblasts [239] and glomerular mesangial cells [240]. Importantly, these effects of estradiol on cell growth appear to be ER-independent [92,238]. Increased proliferation of these cell types leads to hypertension, vascular disease, left ventricular hypertrophy and glomerulosclerosis. Thus, some of the cardiovascular and renal protective effects of both testosterone and estradiol may be mediated via their conversion to methoxyestradiols, which have anti-mitogenic effects on multiple cell types (Fig. 2). The importance of estradiol metabolites in vasoprotection is further supported by our finding that in male obese ZSF1 rats that exhibit the metabolic syndrome (i.e., hypertension, obesity, diabetes and hyperlipidemia) and have left ventricular, renal and vascular dysfunction, treatment with 2-hydroxyestradiol decreases body weight, improves vascular endothelial function, decreases nephropathy, exerts antidiabetic actions and lowers blood pressure and blood cholesterol [241].

The hypothesis that estradiol metabolites are responsible for the anti-mitogenic effects of estradiol is supported by several recent findings. Estradiol prevents neointima formation in mice lacking functional ER-α and ER-β, suggesting that the protective effects of estradiol on the cardiovascular system may be ER independent. Estradiol

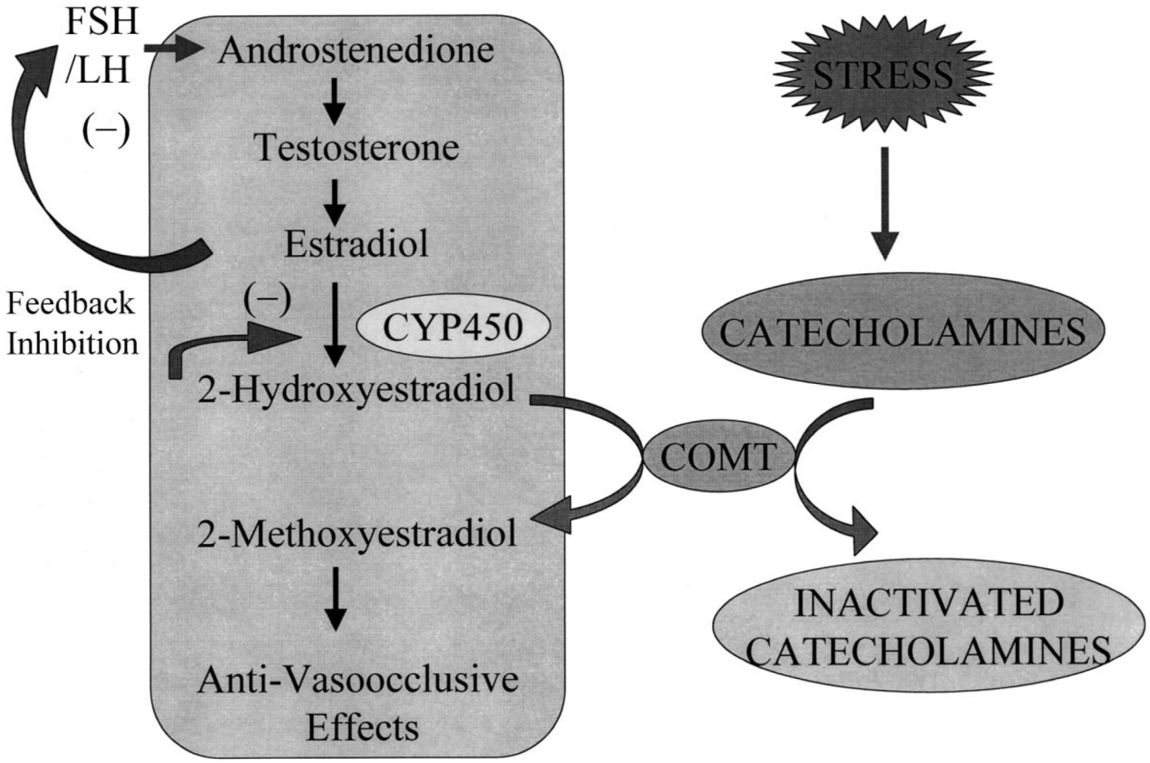

Fig. 2. A schematic representation delineating the possible mechansims via which pathological stimuli such as stress can influence endogenous levels of sex hormones. Catechol-*O*-methyltransferase, COMT; cytochrome P450, CYP450; follicle stimulating hormone, FSH; luteinizing hormone, LH; inhibition (−).

prevents neointima formation in gonadectomized, but not intact male rats, even though male rats express ER-α and ER-β. In female rats the inhibitory effects of estradiol are abrogated by medroxyprogesterone, a synthetic progestin [190]. Since both androgens and medroxyprogesterone are potent inhibitors of the enzyme responsible for the formation of 2-hydroxyestradiol ([242], Dubey et al. unpublished data), these steroids may abrogate the anti-vasoocclusive effects of estradiol by blocking its metabolism to hydroxyestradiols and methoxyestradiols. This hypothesis merits further direct testing.

Lifestyle factors, such as dietary habits and stress, may participate in the development of cardiovascular diseases. Since life style factors can interfere with estradiol metabolism, it is feasible that they may induce their adverse effects in part by preventing methoxyestradiol formation. In this regard, it is important to note that metabolism of 2-hydroxestradiol to 2-methoxyestradiol is mediated by catechol-*O*-methyltransferase (COMT), the enzyme that metabolizes catecholamines to methoxy metabolites. Our recent studies show that catecholamines can abrogate the anti-mitogenic effects of estradiol and 2-hydroxyestradiol on vascular smooth muscle cells, cardiac fibroblasts and glomerular cells, and that these effects are associated with the inhibition of 2-methoxyestradiol formation [243,244].

Although catecholamines may not normally interfere with the metabolism of estradiol to 2-methoxyestradiol, production of 2-methoxyestradiol may be seriously compromised under pathological conditions associated with increased release of catecholamines. As illustrated in Fig. 2, under normal conditions, testosterone is metabolized by aromatase to estradiol; estradiol is metabolized by hydroxylases (CYP1A1, CYP1A2 and CYP1B1) to catecholestradiols (e.g., 2-hydroxyestradiol), and catecholestradiols are metabolized by COMT to methoxyestradiols [245], which induce anti-vasoocclusive effects [238,244]. However, when catecholamine release is increased, catecholamines compete with catecholestradiols and inhibit their metabolism to methoxyestradiols. This results in increased accumulation of catecholestradiols, inhibition of hydroxylases and therefore accumulation of estradiol. Since estradiol inhibits testosterone synthesis by negatively regulating FSH/LH synthesis [246], the net result is a decrease in levels of testosterone. The general principle illustrated by this hypothesis is that the metabolism of estradiol and testosterone may play a critical role in determining the overall effects of sex hormones on the cardiovascular system.

6. Conclusion

Compared to men and postmenopausal women, premenopausal women are relatively protected against hy-

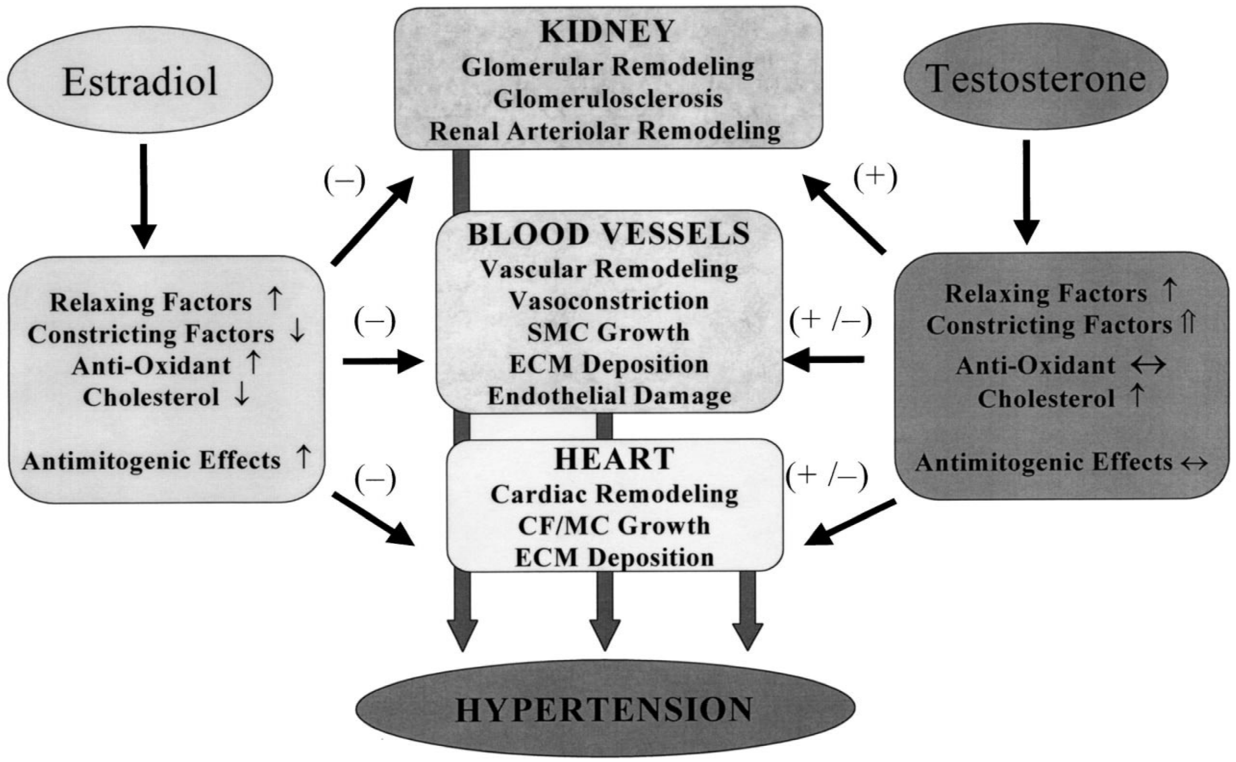

Fig. 3. A schematic representation summarizing and comparing the potential mechansims via which estradiol and testosterone can influence blood pressure and be associated with preventing or inducing hypertension. Smooth muscle cells (SMC); cardiac fibroblast (CF); cardiac myocytes (MC); extracellular matric synthesis (ECM); inhibition, (–); induction (+); increased (↑); decreased (↓); highly increased (⇑); neutral effects (↔).

pertension and its sequelae. Animal studies provide strong evidence that estradiol is an antihypertensive sex hormone, whereas testosterone is pro-hypertensive. As summarized in Fig. 3, the mechanisms by which both estradiol and testosterone affect blood pressure are multifaceted and involve direct effects on vascular, renal and heart cells, as well as indirect effects mediated by humoral factors. It is not yet possible to arrive at any firm conclusions regarding the role of progesterone in hypertension.

Our vocabulary for communicating the effects of sex hormones on the cardiovascular system is misleading. We tend to speak about ‘‘estrogens’’, rather than estradiol, about ‘‘progestins’’, rather than progesterone, and about androgens, rather than ‘‘testosterone.’’ In-point-of-fact, the effects of estradiol, progesterone and testosterone on the cardiovascular system are not necessarily shared by other members of their respective pharmacological classes. This is more than just a semantic nuance. To speak of ‘‘hormone replacement therapy’’ rather than ‘‘estradiol/progesterone replacement therapy’’ implies that conjugated equine estrogens are just as effective as estradiol, and synthetic progestins, as effective as progesterone.

Much additional research is required before we can articulate clear answers to important questions regarding the role of sex hormones in hypertension and cardiovascular disease. In particular, a thorough investigation of the role of hormone metabolism as a determinant of both the beneficial and detrimental effects of sex hormones on the cardiovascular system and blood pressure is required. Many more basic studies need to be performed to determine the mechanisms by which sex hormones affect the blood vessels, heart and kidneys. Further, a long-term prospective clinical trial comparing head-to-head estradiol/progesterone against conjugated equine estrogens/synthetic progestins on blood pressure and cardiovascular disease in a large cohort of hypertensive postmenopausal women is badly needed.

Acknowledgements

This work was supported by grants from the Swiss National Science Foundation (R.K.D. 32-54172.98 and 32-64040.00), and the National Institutes of Health (E.K.J. HL-55314; S.O. HL-64614 and HL-57270), and by an unconditional educational grant from Schering (Schweiz) A.G.

References

[1] Himmelmann A, Svensson A, Hansson L. Influence of sex on blood pressure and left ventricular mass in adolescents: The Hypertension in Pregnancy Offspring Study. J Hum Hypertens 1994;8:485–490.

[2] Yong LC, Kuller LH, Rutan G, Bunker C. Longitudinal study of blood pressure changes and determinants from adolescence to middle age. The Dormont High School Follow-Up Study, 1957–1963 to 1989–1990. Am J Epidemiol 1993;138:973–983.

[3] Burt Vl, Whelton P, Rocella EJ et al. Prevalence of hypertension in the US adult population: results of the Third National Health and Nutrition Examination Survey, 1988–1991. Hypertension 1995;25:305–313.

[4] Stamler J, Stamler R, Riedlinger WF, Algera G, Roberts RH. Hypertension screening of 1 million Americans. Community Hypertension Evaluation Clinic (CHEC) Program, 1973–1975. J Am Med Assoc 1976;235:2299–2306.

[5] August P, Oparil S. Hypertension in women. J Clin Endocrinol Metab 1999;84:1862–1866.

[6] Reckelhoff JF, Zhang H, Srivastava K. Gender differences in the development of hypertension in SHR: role of the renin–angiotensin system. Hypertension 2000;35:480–483.

[7] Reckelhoff JF, Zhang H, Granger JP. Testosterone exacerbates hypertension and reduces pressure-natriuresis in male spontaneously hypertensive rats. Hypertension 1998;31:435–439.

[8] Masubuchi Y, Kumai T, Uematsu A, Komoriyama K, Hirai M. Gonadectomy-induced reduction in blood pressure in adult spontaneously hypertensive rats. Acta Endocrinol (Copenhagen) 1982;101:154–160.

[9] Chen Y-F, Meng Q-M. Sexual dimorphism of blood pressure in spontaneously hypertensive rats is androgen dependent. Life Sci 1991;48:85–96.

[10] Crofton JT, Ota M, Share L. Role of vasopressin, the renin–angiotensin system, and sex in Dahl salt-sensitive hypertension. J Hypertens 1993;11:1031–1038.

[11] Rowland NE, Fregly MJ. Role of gonadal hormones in hypertension in the Dahl salt-sensitive rat. Clin Exp Hypertens 1992;A14:367–375.

[12] Ouchi Y, Share L, Crofton JT, Iitake K, Brooks DP. Sex difference in the development of deoxycorticosterone-salt hypertension in the rat. Hypertension 1987;9:172–177.

[13] Ashton N, Balment RJ. Sexual dimorphism in renal function and hormonal status of New Zealand genetically hypertensive rats. Acta Endocrinol (Copenhagen) 1991;124:91–97.

[14] Ganten U, Schroder G, Witt M et al. Sexual dimorphism of blood pressure in spontaneously hypertensive rats: effects of anti-androgen treatment. J Hypertens 1989;7:721–726.

[15] Iams SG, Wexler BC. Retardation in the development of spontaneous hypertension in SH rats by gonadectomy. J Lab Clin Med 1977;90:997–1003.

[16] Baylis C. Age-dependent glomerular damage in the rat. Dissociation between glomerular injury and both glomerular hypertension and hypertrophy. Male gender as a primary risk factor. J Clin Invest 1994;94:1823–1829.

[17] Goldstein RS, Tarloff JB, Hook JB. Age-related nephropathy in laboratory rats. FASEB J 1988;2:2241–2251.

[18] Staessen J, Bulpitt CJ, Fagard R, Lijnen P, Amery A. The influence of menopause on blood pressure. J Hum Hypertens 1989;3:427–433.

[19] Weiss NS. Relationship of menopause to serum cholesterol and arterial pressure: The United States Health Examination Survey of Adults. Am J Epidemiol 1972;96:237–241.

[20] Staessen JA, Ginnochio G, Thijs L, Fagard R. Conventional and ambulatory blood pressure and menopause in a prospective population study. J Hum Hypertens 1997;11:507–514.

[21] Hjortland MC, McNamara PM, Kannel WB. Some atherogenic concomitants of menopause: The Framingham Study. Am J Epidemiol 1976;103:304–311.

[22] Matthews KA. Meilahn, Kuller L.H. Menopause and risk factors for coronary heart disease. New Engl J Med 1989;321:641–645.

[23] Van Berensteyn ECH, Van ‘t Hof MA, De Waard H. Contributions of ovarian failure and aging to blood pressure in normotensive perimenopausal women: a mixed longitudinal study. Am J Epidemiol 1989;129:947–955.

[24] Chapman AB, Zamudio S, Woodmansee W et al. Systemic and renal

hemodynamic changes in the luteal phase of the menstrual cycle mimic early pregnancy. Am J Physiol 1997;273:F777–F782.

[25] Dunne FP, Barry DG, Ferriss JB, Grealy G, Murphy D. Changes in blood pressure during the normal menstrual cycle. Clin Sci 1991;81:515–518.

[26] Karpanou EA, Vyssoulis GP, Georgoudi DG, Toutouza MG, Toutouzas PK. Ambulatory blood pressure changes in the menstrual cycle of hypertensive women. Significance of plasma renin activity values. Am J Hypertens 1993;6:654–659.

[27] Kletzky OA, Marrs RP, Howard WF, McCormock W, Mishell Jr. DR. Prolactin synthesis and release during pregnancy and puerperium. Am J Obstet Gynecol 1980;136:545–550.

[28] Siamopoulos KC, Papanikolaou S, Elisaf M et al. Ambulatory blood pressure monitoring in normotensive pregnant women. J Hum Hypertens 1996;10(Suppl. 3):S51–S54.

[29] August P, Lenz T, Ales KL et al. Longitudinal study of the renin angiotensin system in hypertensive women: deviations related to the development of superimposed preeclampsia. Am J Obstet Gynecol 1990;163:1612–1621.

[30] Butkevich A, Abraham C, Phillips RA. Hormone replacement therapy and 24-hour blood pressure profile of postmenopausal women. Am J Hypertens 2000;13:1039–1041.

[31] Mercuro G, Zoncu S, Piano D et al. Estradiol-17β reduces blood pressure and restores the normal amplitude of the circadian blood pressure rhythm in postmenopausal hypertension. Am J Hypertens 1998;11:909–913.

[32] van Ittersum FJ, van Baal WM, Kenemans P et al. Ambulatory – not office – blood pressure decline during hormone relacement therapy in healthy postmenopausal women. Am J Hypertens 1998;11:1147–1152.

[33] Mercuro G, Zoncu S, Pilia I et al. Effects of acute administration of transdermal estrogen on postmenopausal women with systemic hypertension. Am J Cardiol 1997;80:652–655.

[34] Regensteiner JG, Hiatt WR, Byyny RL et al. Short-term effects of estrogen and progestin on blood pressure of normotensive postmenopausal women. J Clin Pharmacol 1991;31:543–548.

[35] Cacciatore B, Paakkari I, Hasselblatt R et al. Randomized comparison between orally and transdermally administered replacement therapy regimens of long-term effects on 24-hour ambulatory blood pressure in postmenupausal women. Am J Obstet Gynecol 2001;184:904–909.

[36] Szekacs B, Najo Z, Acs N et al. Hormone replacement therapy reduces mean 24-hour blood pressure and its variability in postmenopausal women with treated hypertension. Menopause 2000;7:31–35.

[37] Pripp U, Hall G, Csemiczky G et al. A randomized trial on effects of hormone replacement therapy on ambulatory blood pressure and lipoprotein levels in women with coronary artery disease. J Hypertens 1999;17:1379–1386.

[38] Sorensen MB, Rasmussen V, Jensen G, Ottesen B. Temporal changes in clinic and ambulatory blood pressure during cyclic post-menopausal hormone replacement therapy. J Hypertens 2000;18:1387–1391.

[39] Manwaring P, Morfis L, Diamond T, Howes LG. Effects of hormone replacement therapy on ambulatory blood pressure responses in normotensive women. Blood Press 2000;9:22–27.

[40] Cagnacci A, Rovati L, Annalisa Z et al. Physiological doses of estradiol decrease nocturnal blood pressure in normotensive postmenopausal women. Am J Physiol Heart Circ Physiol 1999;276:H1355–H1360.

[41] Akkad AA, Halligan AWF, Abrams K, Al-Azzawi F. Differing responses in blood pressure over 24 hours in normotensive women receiving oral or transdermal estrogen replacement therapy. Obstet Gynecol 1997;89:97–103.

[42] Seely EW, Walsh BW, Gerhard MD, Williams GH. Estradiol with or without progesterone and ambulatory blood pressure in postmenopausal women. Hypertension 1999;33:1190–1194.

[43] Manhem K, Ahlm H, Milsom I, Svensson A. Transdermal oestrogen reduces daytime blood pressure in hypertensive women. J Hum Hypertens 1998;12:323–327.

[44] Lind T, Cameron EC, Hunter WM et al. A prospective, controlled trial of six forms of hormone replacement therapy given to postmenopausal women. Br J Obstet Gynaecol 1979;86(Suppl. 3):1–29.

[45] Elias AN, Meshkinpour H, Valenta LJ. Attenuation of hypertension by conjugated estrogens. Nephron 1992;30:89–92.

[46] Crane MG, Harris JJ, Winsor IIIW. Hypertension, oral contraceptive agents, and conjugated estrogens. Ann Intern Med 1971;74:13.

[47] Notelovitz M. Effect of natural oestrogens on blood pressure and weight in postmenopausal women. S Afr Med J 1975;49:2251.

[48] Utian WH. Effect of postmenopausal estrogen therapy on diastolic blood pressure and body weight. Maturitas 1978;1:3.

[49] Pfeffer RI. Estrogen use, hypertension and stroke in postmenopausal women. J Chron Dis 1977;31:389–398.

[50] Crane MG, Harris JJ. Estrogens and hypertension: effect of discontinuing estrogens on blood pressure, exhangeable sodium, and the renin–aldosterone system. Am J Med Sci 1978;276:33–55.

[51] Wren BG, Routledge DA. Blood pressure changes. Oestrogens in climacteric women. Med J Aust 1981;2:528–531.

[52] PEPI Trial Writing Group. Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women. The postmenopausal estrogen/protestin interventions (PEPI) trial. J Am Med Assoc 1995;3:199–208.

[53] Lip GYH, Beevers M, Churchill D, Beevers DG. Hormone replacement therapy and blood pressure in hypertensive women. J Hum Hypertens 1994;8:491–494.

[54] Schunkert H, Danser AHJ, Hense H-W et al. Effects of estrogen replacement therapy on the renin–angiotensin system in postmenopausal women. Circulation 1997;95:39–45.

[55] Woods JW. Oral contraceptives and hypertension. Hypertension 1988;II(Suppl. 2):11–15.

[56] Ramcharan S, Pellegrin FA, Hoag EJ. The occurrence and course of hypertensive disease in users and nonusers of oral contraceptive drugs. In: Ramcharan S, editor, The Walnut Creek Contraceptive Drug Study: a prospective study of the side effects of oral contraceptives, DHEW publication (NIH) No. 76-563, vol. 2, Washington, DC: US Government Printing Office, 1976, pp. 1–16.

[57] WHO Task Force on Oral Contraceptives. The WHO Multicentre Trial of the Vasopressor Effects of Combined Oral Contraceptives. I. Comparisons with IUD. Contraception 1998;40:129–145.

[58] Royal College of General Practitioners. Oral contraception study. Oral contraceptives and health, New York: Pitman, 1974.

[59] Chasan-Taber L, Wilett WC, Manson JE et al. Prospective study of oral contraceptives and hypertension among women in the United States. Circulation 1996;94:483–489.

[60] Spellacy WN, Birk SA. The effect of intrauterine devices, oral contraceptives, estrogens, and progestogens on blood pressure. Am J Obstet Gynecol 1972;112:912–919.

[61] Lloyd G, McGing E, Cooper A et al. A randomized placebo controlled trial of the effects of tibolone on blood pressure and lipids in hypertensive women. J Hum Hypertens 2000;14:99–104.

[62] Meyers MG, Reeves RA. White coat effect in treated hypertensive patients: sex differences. J Hum Hypertens 1995;9:729–773.

[63] Cambotti LJ, Cole FE, Gerall AA, Frohlich ED, MacPhee AA. Neonatal gonadal hormones and blood pressure in the spontaneosly hypertensive rat. Am J Physiol 1984;247:E258–E264.

[64] Sharkey LC, Holycross BJ, Park S et al. Effect of ovariectomy and estrogen replacement on cardiovascular disease in heart failure SHHF/Mac-facp rats. J Mol Cell Cardiol 1999;31:1527–1537.

[65] Crofton JT, Share L. Gonadal hormones modulate deoxycorticosterone-salt hypertension in male and female rats. Hypertension 1997;29:494–499.

[66] Sasaki T, Ohno Y, Otsuka K et al. Oestrogen attenuates the increases in blood pressure and platelet aggregation in ovarectomized and salt loaded Dahl-salt sensitive rats. J Hypertens 2000;18:911–917.

[67] Farhat MY, Chen MF, Bhatti T et al. Protection by oestradiol against the development of cardiovascular changes associated with monocrotaline pulmonary hypertension in rats. Br J Pharmacol 1993;110:719–723.

[68] Resta TC, Kanagy NL, Walker BR. Estradiol-induced attenuation of pulmonary hypertension is not associated with altered eNOS expression. Am J Physiol Lung Cell Mol Physiol 2001;280:L88–L97.

[69] Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. New Engl J Med 1999;340:1801–1811.

[70] Dubey RK, Jackson EK. Estrogen-induced cardiorenal protection: potential cellular, biochemical, and molecular mechanisms. Am J Physiol Renal Physiol 2001;280:F365–F388.

[71] Williams JK, Honoré EK, Adams MR. Contrasting effects of conjugated estrogens and tamoxifen on dilator responses of atherosclerotic epicardial coronary arteries in nonhuman primates. Circulation 1997;96:1970–1975.

[72] Williams JK, Adams MR, Herrington DM, Clarkson TB. Short term administration of estrogen and vascular responses of atherosclerotic coronary arteries. J Am Coll Cardiol 1992;20:452–457.

[73] Farhat MY, Lavigne MC, Ramwell PW. The vascular protective effects of estrogen. FASEB J 1996;10:615–624.

[74] Collins P, Rosano GMC, Sarrel PM et al. 17β-Estradiol attenuates acetylcholine-induced coronary arterial constriction in women but not men with coronary heart disease. Circulation 1995;92:24–30.

[75] Gilligan DM, Badar DM, Panza JA, Quyyumi AA, Cannon III RO. Acute vascular effects of estrogen in postmenopausal women. Circulation 1994;90:786–791.

[76] Guetta V, Quyyumi AA, Prasad A et al. The role of nitric oxide in coronary vascular effects of estrogen in postmenopausal women. Circulation 1997;96:2795–2801.

[77] Reis SE, Bhoopalam V, Zell KA et al. Conjugated estrogens acutely abolish abnormal cold-induced coronary vasoconstriction in male cardiac allografts. Circulation 1998;97:23–25.

[78] Collins P, Shay J, Jiang C, Moss J. Nitric oxide accounts for dose-dependent estrogen-mediated coronary relaxation after acute estrogen withdrawal. Circulation 1994;90:1964–1968.

[79] Keaney JF, Shwaery GT, Xu A et al. 17β-Estradiol preserves endothelial vasodilator function and limits low-density lipoprotein oxidation in hypercholesterolemic swine. Circulation 1994;89:2251–2259.

[80] Andersen HL, Weis JU, Fjalland N, Korsgaard N. Effect of acute and longterm treatment with 17 beta-estradiol on the vasomotor responses in the rat aorta. Br J Pharmacol 1999;126:159–168.

[81] Vedernikov YP, Liao QP, Jain V et al. Effects of chronic treatment with 17β-estradiol and endothelium-dependent and -independent relaxation in isolated aortic rings from ovarectomized rats. Am J Obstet Gynecol 1997;176:603–608.

[82] Imthurn B, Rosselli M, Jaeger AW, Keller PJ, Dubey RK. Differential effects of hormone-replacement therapy on endogenous nitric oxide (nitrate/nitrite) levels in postmenopausal women substituted with 17β-estradiol valerate and cyproterone acetate or medroxyprogesterone acetate. J Clin Endocrinol Metab 1997;82:388–394.

[83] Rosselli M, Imthurn B, Keller PJ, Jackson EK, Dubey RK. Circulating nitric oxide (nitrite/nitrate) levels in postmenopausal women substituted with 17β-estradiol and norethisterone acetate: a two year follow-up study. Hypertension 1995;25:848–853.

[84] Caulin-Glaser T, Garcia-Cardena G, Sarrel P, Sessa WC, Bender JR. 17β-Estradiol regulation of human endothelial cell basal nitric oxide release, independent of cytosolic Ca^{2+} mobilization. Circ Res 1997;81:885–892.

[85] Chen Z, Yuhanna IS, Galcheva-Gargova Z et al. Estrogen receptor α mediates the nongenomic activation of endothelial nitric oxide synthase by estrogen. J Clin Invest 1999;103:401–406.

[86] Stefano GB, Prevot V, Beauvillain JC et al. Cell surface estrogen receptors mediate calcium-dependent nitric oxide release in human endothelia. Circulation 2000;101:1594–1597.

[87] Baylis C, Mitruka B, Deng A. Chronic blockade of NO synthesis in the rat produces systemic hypertension and glomerular damage. J Clin Invest 1992;90:278–281.

[88] New G, Timmins KL, Auffy SJ et al. Long-term estrogen therapy improves vascular function in male to female transsexuals. J Am Coll Cardiol 1997;29:1437–1444.

[89] Herrington DM, Braden GA, Williams JK, Morgan TM. Endothelial-dependent coronary vasomotor responsiveness in postmenopausal women with and without estrogen replacement therapy. Am J Cardiol 1994;73:951–952.

[90] Roque M, Heras M, Roig E et al. Short-term effects of transdermal estrogen replacement therapy on coronary vascular reactivity in postmenopausal women with angia pectoris and normal results on coronary angiograms. J Am Coll Cardiol 1998;31:139–143.

[91] Higashi Y, Sanada M, Sasaki S et al. Effect of estrogen replacement therapy on endothelial function in peripheral resistance arteries in normotensive and hypertensive postmenopausal women. Hypertension 2001;37:651–657.

[92] Xiao S, Gillespie DG, Baylis C, Jackson EK, Dubey RK. Effects of estradiol and its metabolites on glomerular endothelial nitric oxide synthesis and mesangial cell growth. Hypertension 2001;37(Part 2):645–650.

[93] Rosano GMC, Sarais C, Zoncu S, Mercuro G. The relative effects of progesterone and progestins in hormone replacement therapy. Hum Reprod 2000;15(Suppl. 1):60–73.

[94] White RE, Darkow DJ, Lang JLF. Estrogen relaxes coronary arteries by opening BKCa channels through a cGMP-dependent mechanism. Circ Res 1995;77:936–942.

[95] Nakajima T, Kitazawa T, Hamada E et al. 17β-Estradiol inhibits voltage-dependent L-type Ca^{2+} currents in aortic smooth muscle cells. Eur J Pharmacol 1995;294:625–635.

[96] Rusko J, Li L, van Breemen C. 17β-Estradiol stimulation of endothelial K+ channels. Biochem Biophys Res Commun 1995;214:367–372.

[97] Darkow DJ, Lu L, White RE. Estrogen relaxation of coronary artery smooth muscle is mediated by nitric oxide and cGMP. Am J Physiol 1997;272:2765–2773.

[98] Wellman GC, Bonev AD, Nelson MT, Brayden JE. Gender differences in coronary artery diameter involve estrogen, nitric oxide, and Ca^{2+} dependent K^{+} channels. Circ Res 1996;79:1024–1030.

[99] Dubey RK, Gillespie DG, Mi Z et al. Estradiol inhibits smooth muscle cell growth in part by activating the cAMP-adenosine pathway. Hypertension 2000;35(Part 2):262–266.

[100] Chang WC, Nakao J, Orimo H, Murota SI. Stimulation of prostaglandin cyclooxygenase and prostacyclin synthase activities by estradiol in rat aortic smooth muscle cells. Biochem Biophys Acta 1980;620:472–482.

[101] Mikkola T, Turunen P, Avela K et al. 17β-Estradiol stimulates prostacyclin, but not endothelin-1, production in human vascular endothelial cells. J Clin Endocrinol Metab 1995;80:1832–1836.

[102] Pasqualini C, Leviel V, Guibert B, Faucon-Biguet N, Kerdelhui B. Inhibitory action of acute estradiol treatment on the activity and quantity of tyrosine hydroxylase in the median eminence of ovariectomized rats. J Neuroendocrinol 1991;3:575–580.

[103] Schunkert H, Danser AHJ, Hense H-W et al. Effects of estrogen replacement therapy on the renin–angiotensin system in postmenopausal women. Circulation 1997;95:39–45.

[104] Ylikorkala O, Orpana A, Puolakka J, Pyorala T, Viinikka L. Postmenopausal hormonal replacement decreases plasma levels of endothelin-1. J Clin Endocrinol Metab 1995;80:3384–3387.

[105] Folkow B. Physiological aspects of primary hypertension. Physiol Rev 1987;62:347–504.

[106] Dubey RK, Jackson EK, Rupperecht H, Sterzel RB. Factors controlling growth and matrix production in vascular smooth muscle and glomerular mesangial cells. Curr Opin Nephrol Hypertens 1997;6:88–105.

[107] Li G, Chen YF, Greene GC, Oparil S, Thompson JA. Estrogen inhibits vascular smooth muscle cell-dependent adventitial fibroblast migration in vitro. Circulation 1999;100:1639–1645.

[108] Oparil S, Levine RL, Chen Y-F. Sex hormones and vasculature. In: Sowers JR, editor, Contemporary endocrinology: endocrinology of the vasculature, Totowa, NJ: Humana Press, 1997, pp. 225–237.

[109] Gallagher PE, Li P, Lenhart JR, Chappell MC, Broshihan KB. Estrogen regulation of angiotensin-converting enzyme mRNA. Hypertension 1999;33(Part II):323–328.

[110] Hassager C, Riis BJ, Strom V, Guyene TT, Christiansen C. The long term effects of oral and percutaneous estradiol on plasma on plasma renin substrate and blood pressure. Circulation 1987;76:753–758.

[111] Proudler AJ, Ahmed AI, Crook D et al. Hormone replacement therapy and serum angiotensin-converting enzyme activity in postmenopausal women. Lancet 1995;346:89–90.

[112] Dubey RK. Vasodilator-derived nitric oxide inhibits fetal calf serum and angiotensin II-induced growth of renal arteriolar smooth muscle cells. J Pharmacol Exp Ther 1994;269:402–408.

[113] Dubey RK, Jackson EK, Luscher TF. Nitric oxide inhibits angiotensin II-induced migration of rat aortic smooth muscle cell. Role of cyclic-nucleotides and angiotensin1 receptors. J Clin Invest 1995;96:141–149.

[114] Nickenig G, Baumer AT, Grohe C et al. Estrogen modulates A.T. 1 receptor gene expression in vitro and in vivo. Circulation 1998;97:2197–2201.

[115] Rosselli M, Keller PJ, Kern F, Hahn AWA, Dubey RK. Estradiol inhibits mitogen induced proliferation and migration of human aortic smooth muscle cells: implications for cardiovascular disease in women. Circulation 1994;90:I-87, Abstract.

[116] Brosnihan KB, Li P, Ganten D, Ferrario CM. Estrogen protects transgenic hypertensive rats by shifting the vasoconstrictor–vasodilator balance of RAS. Am J Physiol 1997;273:R1908–R1915.

[117] Tsai JC, Perella MA, Yoshizumi M et al. Promotion of vascular smooth muscle cell growth by homocysteine: a link to atherosclerosis. Proc Natl Acad Sci USA 1994;91:6369–6373.

[118] Wall RT, Harlan JM, Harkar LA, Striker GE. Homocysteine-induced endothelial cell injury in vitro: a model for the study of vascular injury. Thromb Res 1980;18:1113–1121.

[119] van Baal VM, Smolders RG, van der Mooren MJ, Teerlink T, Kenemans P. Hormone replacement therapy and plasma homocysteine levels. Obstet Gynecol 1999;94:485–491.

[120] Gilaty EJ, Hoogeveen EK, Elbers JM et al. Effects of sex steroids on plasma total homocysteine levels: a study in transsexual males and females. J Clin Endocrinol Metab 1998;83:550–553.

[121] Morey AK, Pedram A, Razandi M et al. Oestrogen and progesterone inhibit the stimulated production of endothelin-1. Biochem J 1998;330:1097–1105.

[122] Dubey RK, Jackson EK, Keller PJ, Imthurn B, Rosselli M. Estradiol metabolites inhibit endothelin synthesis by an estrogen receptor-independent mechanism. Hypertension 2001;37(Part 2):640–644.

[123] Morey AK, Pedram A, Razandi M et al. Estrogen and progesterone inhibit vascular smooth muscle proliferation. Endocrinology 1997;138:3330–3339.

[124] Sumino H, Ichikawa S, Kanda T et al. Hormone replacement therapy in postmenopausal women with essential hypertension increases circulating plasma levels of bradykinin. Am J Hypertens 1999;12:1044–1047.

[125] Gebara OCE, Mittleman MA, Sutherland P et al. Association between increased estrogen status and increased fibrinolytic potential in the Framingham Offspring Study. Circulation 1995;91:1952–1958.

[126] Koh KK, Mincemoyer R, Sui MN et al. Effects of hormone-replacement therapy on fibrinolysis in postmenopausal women. New Engl J Med 1997;336:683–690.

[127] Nabulsi AA, Folsom AR, White A et al. (for the Atherosclerosis Risk in Communities Study Investigators) Association of hormone-replacement therapy with various cardiovascular risk factors in postmenopausal women. New Engl J Med 1993;328:1069–1075.

[128] Harrison RL, McKee PA. Estrogen stimulates van Willebrand factor production by cultured endothelial cells. Blood 1984;63:657–664.

[129] Helmerhorst FM, Rosendaal FR, Vandenbroucke JP. Venous thromboembolism and the pill. The WHO technical report on cardiovascular disease and steroid hormone contraception: state-of-the-art. Hum Reprod 1998;13:2981–2983.

[130] Davies T, Fieldhouse G, McNocol GP. The effects of therapy with oestriol succinate therapy with ethinyl oestradiol on the haemostatic mechanism in postmenopausal women. Thromb Haemost 1976;35:403–414.

[131] Kluft C, Lansink M. Effect of oral contraceptives on haemostasis variables. Thromb Haemost 1997;78:315–326.

[132] Rosenthal T, Oparil S. Hypertension in women. J Hum Hypertens 2000;14:691–704.

[133] Weir J. Blood pressure in women taking oral contraceptives. Br Med J 1974;23:533–535.

[134] Fisch R, Frank J. Oral contraceptives and blood pressure. J Am Med Assoc 1977;237:2499–2503.

[135] Briggs M, Briggs M. Oestrogen contact of oral contraceptives. Lancet 1977;2:1233.

[136] Rudel HW et al. Safety and efficacy of a new low dose oral contraceptive: a three year study of 1000 women. J Reprod Med 1978;21:79–88.

[137] Calhoun DA, Oparil S. Gender and blood pressure. Hypertension primer. In: Izzo Jr. JL, Black HR, editors, The essentials of high blood pressure, 2nd ed., Baltimore, MD: Lippincott Williams & Wilkins, November 1998, pp. 229–232.

[138] Dubey RK, Gillespie DG, Jackson EK, Keller PJ. 17β-Estradiol, its metabolites and progesterone inhibit cardiac fibroblast growth. Hypertension 1998;31:522–528.

[139] Node K, Kitakaze M, Kosaka H et al. Amelioration of ischemia- and reperfusion-induced myocardial injury by 17β-estradiol: role of nitric oxide and calcium-activated potassium channels. Circulation 1997;96:1953–1963.

[140] Squadrito F, Altavilla D, Squadrito G et al. 17β-estradiol reduces cardiac leukocyte accumulation in myocardial ischemia reperfusion injury in rat. Eur J Pharmacol 1997;335:185–192.

[141] Smith PJ, Ornatsky O, Stewart DJ et al. Effects of estrogen replacement on infarct size, cardiac remodeling, and the endothelin system after myocardial infarction in ovarectomized rats. Circulation 2000;102:2983–2999.

[142] Lee TM, Su SF, Tsai CC, Lee YT, Tsai CH. Cardioprotective effects of 17β-estradiol produced by activation of mitochondrial ATP-sensitive K(+) channels in canine hearts. J Mol Cell Cardiol 2000;32:1147–1158.

[143] Rosano GM, Webb CM, Chierchia S et al. Natural progesterone, but not medroxyprogesterone acetate, enhances the beneficial effect of estrogen on excercise-induced myocardial ischemia in postmenopausal women. J Am Coll Cardiol 2000;36:2154–2159.

[144] Pelzer T, Schumann M, Neumann M et al. 17β-Estradiol prevents programmed cell death in cardiac myocytes. Biochem Biophys Res Commun 2000;268:192–200.

[145] Knowlton AA, Sun L. Heat-shock factor-1, steroid hormones, and regulation of heat-shock protein expression in the heart. Am J Physiol Heart Circ Physiol 2001;280:H455–H464.

[146] Nuedling S, Kahlert S, Loebbert K et al. 17β-Estradiol stimulates expression of endothelial and inducible NO synthase in rat myocardium, in-vitro and in-vivo. Cardiovasc Res 1999;43:666–674.

[147] Patterson E, Ma L, Szabo B, Robinson CP, Thadani U. Ovariectomy and estrogen-induced alterations in myocardial contractions in female inhibits: role of the L-type calcium channel. J Pharm Exp Ther 1998;284:586–591.

[148] Nakajima T, Iwasawa K, Oonuma H et al. Antiarrythmic effect and its underlying ionic mechanism of 17β-estradiol in cardiac myocytes. Br J Pharmacol 1999;127:429–440.

[149] Guyton AC, Coleman TG, Cowley Jr. AW et al. Arterial pressure regulation: overriding dominance of the kidneys in long-term regulation and in hypertension. Am J Med 1972;52:584–594.

[150] Hall JE, Mizelle HL, Hildebrandt DA, Brands MW. Abnormal pressure-natriuresis: a cause or a consequence of hypertension. Hypertension 1990;15:547–559.

[151] Curtis JJ, Luke HP, Dustan HP et al. Remission of hypertension after renal transplantation. New Engl J Med 1983;309:1009–1015.

[152] Reckelhoff JF. Gender differences in the regulation of blood pressure. Hypertension 2001;37:1199–1208.

[153] Neugarten J, Acharya A, Silbiger SR. Effect of gender on the progression of non-diabetic renal disease: a metanalysis. J Am Soc Nephrol 2000;11:319–329.

[154] Mulroney SE, Woda C, Johnson M, Pesce C. Gender differences in renal growth and function after uni-nephrectomy in adult rats. Kidney Int 1999;56:944–953.

[155] Muller V, Szabo A, Viklicky O et al. Sex hormones and gender related differences: their influence on chronic renal allograft rejection. Kidney Int 1999;55:2011–2020.

[156] Lou H, Ramwell PW, Foegh ML. Estradiol 17β represses insulin-like growth factor I receptor expression in smooth muscle cells from rabbit cardiac recipients. Transplantation 1998;66:419–426.

[157] Cathapermal S, Lavinge MC, Leong-Son M, Alibadi T, Ramwell PW. Stereoisomer-specific inhibition of superoxide anion-induced rat aortic smooth-muscle cell proliferation by 17β-estradiol is estrogen receptor dependent. J Cardiovasc Pharmacol 1998;31:499–505.

[158] Neugarten J, Ghossein C, Silbiger S. Estradiol inhibits mesangial cell mediated oxidation of low-density lipoprotein. J Lab Clin Med 1995;126:385–391.

[159] Dubey RK, Tyurina YY, Tyurin VA et al. Estrogen and tamoxifen metabolites protect smooth muscle cell membrane phospholipids against peroxidation and inhibit cell growth. Circ Res 1999;84:229–239.

[160] Neugarten J, Medve I, Lei J, Silbiger SR. Estradiol suppresses mesangial cell type I collagen synthesis via activation of the MAP kinase cascade. Am J Physiol 1999;277:F875–F881.

[161] Silbiger S, Lei J, Neugarten J. Estradiol suppresses type I collagen synthesis by mesangial cells via activation of AP-1. Kidney Int 1998;55:1268–1276.

[162] Sharma K, Ziyadeh FN. The emerging role of transforming growth factor-β in kidney diseases. Am J Physiol 1994;266:F829–F842.

[163] Kagami S, Border WA, Miller DE, Noble NA. Angiotensin II stimulates extracellular matrix protein synthesis through induction of transforming growth factor-β expression in rat glomerular mesangial cells. J Clin Invest 1994;93:2431–2437.

[164] Faas MM, Bakker WW, Klok PA, Baller JF, Schuiling GA. Modulation of glomerular ECTO-ADPase expression by oestradiol. A histochemical study. Thromb Haemost 1997;77:767–771.

[165] Ostrowski NL, Young III WS, Lolait SJ. Estrogen increases renal oxytocin receptor gene expression. Endocrinology 1995;136:1801–1804.

[166] Iguchi M, Takamura C, Umekawa T, Kurita T, Kohri K. Inhibitory effects of female sex hormones on urinary stone formation in rats. Kidney Int 1999;56:479–485.

[167] Greene DM, Azcona-Olivera JI, Murtha JM, Pestka JJ. Effects of dihydrotestosterone and estradiol on experimental IgA nephroparhy induced by vomitoxin. Fundam Appl Toxicol 1995;26:107–116.

[168] Iruela-Arispe L, Gordon K, Hugo C et al. Participation of glomerular endothelial cells in the capillary repair of glomerulonephritis. Am J Pathol 1995;147:1715–1727.

[169] Suzuma I, Mandai M, Takagi H et al. 17β-Estradiol increases VEGF receptor-2 and promotes DANN synthesis in retinal microvascular endothelial cells. Invest Opthalmol Vis Sci 1999;40:2122–2129.

[170] Ostendorf T, Kunter U, Eitner F et al. VEGF [165] mediates glomerular endothelial repair. J Clin Invest 1999;104:913–923.

[171] Spyridopoulos I, Sullivan AB, Kearney M, Isner JM, Losordo DW. Estrogen-receptor-mediated inhibition of human endothelial cell apoptosis. Estradiol as a survival factor. Circulation 1997;95:1505–1514.

[172] Sudhir K, Esler M, Jennings G et al. Estrogen supplementation decreases norepinephrine-induced vasoconstriction and total body norepinephrine spillover in perimenopausal women. Hypertension 1997;30:1538–1543.

[173] Vongpatanasin W, Tuncel M, Mansour Y. Transdermal estrogen replacement therapy decreases sympathetic activity in post-menopausal women. Circulation 2001;103:2903–2908.

[174] Hunt BE, Taylor JA, Hamner JW, Gagnon M, Lipsitz LA. Estrogen replacement therapy improves baroreflex regulation of vascular sympathetic outflow in postmenopausal women. Circulation 2001;103:2909–2914.

[175] DeMeersam R, Zion AS, Giardina EGV et al. Estrogen replacement, vascular distensitibility, and blood pressure in post-menopausal women. Am J Physiol 1998;274:H1539–H1544.

[176] Huikuri H, Pikkujamsa S, Airksien J et al. Sex-related differences in autonomic modulation of heart rate in middle-aged subjects. Circulation 1996;94:122–125.

[177] Del Rio G, Velardo A, Menozzi R et al. Acute estradiol and progesterone administration reduced cardiovascular and catecholamine responses to mental stress in menopausal women. Neuroendocrinology 1998;67:269–274.

[178] Ng VA, Callister R, Johnson DG, Seals DG. Age and gender influence muscle sympathetic nerve activity at rest in healthy humans. Hypertension 1993;21:498–503.

[179] Fang Z, Carlson SH, Chen YF, Oparil S, Wyss JM. Estrogen depletion induces NaCl-sensitive hypertension in female spontaneously hypertensive rats (SHR). Am J Physiol. 2001 (in press).

[180] Calhoun DA, Zhu S-T, Chen Y-F, Oparil S. Gender and dietary NaCl in spontaneously hypertensive and Wistar–Kyoto rats. Hypertension 1995;26:285–289.

[181] Winternitz SR, Katholi RE, Oparil S. Role of renal sympathetic nerves in the development and maintenance of hypertension in the spontaneously hypertensive rat. J Clin Invest 1980;66:971–978.

[182] Kristiansson P, Wang JX. Reproductive hormones and blood pressure during pregnancy. Hum Reprod 2001;16:13–17.

[183] Rylance PB, Brincat M, Lafferty K et al. Natural progesterone and antihypertensive action. Br Med J 1985;5:13–14.

[184] Tapanainem J, Kauppila A, Metsa-Ketela T, Vapatalo H. Prostanoids and catecholamines after oral administration of natural progesterone. Gynecol Endocrinol 1989;3:135–142.

[185] Barbagallo M, Dominguez LJ, Licata G, Shan J et al. Vascular effects of progesterone. Role of cellular calcium regulation. Hypertension 2001;37:142–147.

[186] Harvey PJ, Molloy D, Upton J, Wing LM. Dose response effect of cyclical medroxyprogesterone on blood pressure in postmenopausal women. J Hum Hypertens 2001;15:313–321.

[187] Oelkers W, Schoneshofer M, Blumel A. Effects of progesterone and four synthetic progestagens on sodium balance and the renin–aldosterone system in man. J Clin Endocrinol Metab 1974;39:882–890.

[188] Miller VM, Vanhoutte PM. Progesterone and modulation of endothelium-dependent responses in canine coronary arteries. Am J Physiol 1991;261:R1022–R1027.

[189] Williams JK, Honroe EK, Washburn SA, Clarkson TB. Effects of hormone replacement therapy on reactivity of atherosclerotic coronary arteries in cynomolgus monkeys. J Am Coll Cardiol 1994;24:1757–1761.

[190] Levine RL, Chen SJ, Durand J, Chen YF, Oparil S. Medroxyprogesterone attenuates estrogen-mediated inhibition of neo-intima formation after balloon injury of the rat carotid artery. Circulation 1996;94:2221–2227.

[191] Dubey RK, Gillespie DG, Zacharia LC, Imthurn B, Jackson EK, Keller PJ. Clinically used estrogens differentially inhibit human

aortic smooth muscle cell growth and MAP kinase activity. Arterioscleros Thromb Vas Biol 2000;20:964–972.

[192] Phillips GB, Jing TY, Resnik LM et al. Sex hormones and hemostatic risk factors for coronary heart disease in men with hypertension. J Hypertens 1993;11:699–702.

[193] Jaffe A, Chen Y, Kisch ES et al. Erectile dysfunction in hypertensive subjects. Assessment of potential determinants. Hypertension 1996;28:859–862.

[194] Hughes GS, Mathur RS, Margollus HS. Sex steroid hormones are altered in essential hypertension. J Hypertens 1989;7:181–187.

[195] Phillips GB, Pinkernell BH, Jing TY. The association of hypotestosteronemia with coronary artery artery disease in men. Arterioscler Thromb Vasc Biol 1994;14:1701–1706.

[196] Kalin MF, Zumhoff B. Sex hormones and coronary artery disease: a review of clinical studies. Steroids 1990;55:330–352.

[197] Oudar O, Elger M, Bankir L et al. Differences in rat kidney morphology between males, females, and testosterone-treated females. Ren Physiol Biochem 1991;14:92–102.

[198] Colby HD, Skelton FR, Brownie AC. Testosterone-induced hypertension in the rat. Endocrinology 1970;86:1093–1101.

[199] Sullivan ML, Martinez CM, Gennis P, Gallanger EJ. The cardiac toxicity of anabolic steroids. Prog Cardiovasc Dis 1998;41:1–15.

[200] Giorgi A, Weatherby RP, Murphy PW. Muscular strength, body composition and health responses to the use of testosterone enanthate: a double blind study. J Sci Med Sport 1999;2:341–355.

[201] White CM, Ferraro-Borgida MJ, Moyna NM et al. The effect of pharmacokinetically guided acute intra-venous testosterone administration on electrocardiography and blood pressure. J Clin Pharmacol 1999;39:1038–1043.

[202] Whitworth JA, Scoggins BA, Andrews J, Williamson PM, Brown MA. Haemodynamic and metabolic effects of short term administration of synthetic sex steroids in humans. Clin Exp Hypertens 1992;14:905–922.

[203] Kumai T, Tanaka M, Watanabe M. Possible involvement of androgen in increased norepinephrine synthesis in blood vessels of spontaneously hypertensive rats. Jpn J Pharmacol 1994;66:439–444.

[204] Kumai T, Tanaka M, Watanabe M, Nakura H, Kobayashi S. Influence of androgens on tyrosine hydroxylase mRNA in adrenal medulla of spontaneously hypertensive rats. Hypertension 1995;26:208–212.

[205] Hanke H, Lenz C, Spindler K-D, Weidemann W. Effects of testosterone on plaque development and androgen receptor expression in the arterial vessel wall. Circulation 2001;103:1380–1385.

[206] Chou TM, Sudhir K, Hutchison SJ et al. Testosterone induces dilation of canine coronary conductance and resistance arteries in vivo. Circulation 1996;94:2614–2619.

[207] Webb CM, McNeill JG, Hayward CS, de Zeigler D, Collins P. Effects of testosterone on coronary vasomoter regulation in men with coronary heart disease. Circulation 1999;100:1690–1696.

[208] Crews JK, Khalil RA. Antagonistic effects of 17β-estradiol, progesterone, and testosterone on Ca^{2+} entry mechanisms of coronary vasoconstriction. Arterioscler Thromb Vasc Biol 1999;19:1034–1040.

[209] Murphy JG, Khalil RA. Decreased [Ca(2+)]i during inhibition of coronary smooth muscle contraction by 17β-estradiol, progesterone, and testosterone. J Pharmacol Exp Ther 1999;291:44–52.

[210] Goetz RM, Thatte HS, Prabhakar P et al. Estradiol induces the calcium-dependent translocation of endothelial nitric oxide synthase. Proc Natl Acad Sci USA 1999;96:2788–2793.

[211] Walker TC. The use of testosterone propionate and estrogenic substances in treatment of essential hypertension, angia pectoris and peripheral vascular diseases. J Clin Endocrinol 1942;2:560–568.

[212] Jaffe MD. Effect of testosterone cypionate on post-excercise ST segment depression. Br Heart J 1977;39:1217–1222.

[213] Rosano GMC, Leonardo F, Pagnotta P et al. Acute anti-ischemic effect of testosterone in men with coronary artery disease. Circulation 1999;99:1666–1670.

[214] Ceballos G, Figueroa L, Rubio I et al. Acute and nongenomic effects of testosterone on isolated and perfused rat heart. J Cardiovasc Pharmacol 1999;33:691–697.

[215] Matsuda K, Ruff A, Morinelli TA, Mathur RS, Haluska PV. Testosterone increases thromboxane A2 receptor density and responsiveness in rat aorta and platelets. Am J Physiol 1994;267:H887–893.

[216] Masuda A, Mathur R, Haluska PV. Testosterone increases thromboxane A2 receptors in cultured rat aortic smooth muscle cells. Circ Res 1991;69:638–643.

[217] Schror K, Morinelli TA, Masuda A et al. Testosterone treatment enhances thromboxane A2 mimetic induced coronary artery vasoconstriction in guinea pigs. Eur J Clin Invest 1994;1:50–52.

[218] Zukowska-Grojec Z. Neuropeptide Y. A novel sympathetic stress hormone and more. Ann NY Acad Sci 1995;771:219–233.

[219] Teoh H, Quann A, Leung SWS, Man RYK. Differential effects of 17β-estradiol and testosterone on the contractile responses of porcine coronary arteries. Br J Pharmacol 2000;129:1301–1308.

[220] Bruck B, Brehme U, Gugel N et al. Gender-specific differences in the effects of testosterone and estrogen on the development of atherosclerosis rabbits. Arterioscler Thromb Vasc Biol 1997;17:2192–2199.

[221] Alexandersen P, Haarbo J, Byrjalsen I, Lawaetz H, Christiansen C. Natural androgens inhibit atherosclerosis. A study in castrated, chlosterol-fed rabbits. Circ Res 1999;84:813–819.

[222] Kolodgie FD, Jacob A, Wilson PS et al. Estradiol attenuatcs directed migration of vascular smooth muscle cells in vitro. Am J Pathol 1996;148:969–976.

[223] Akishita M, Ouchi Y, Miyoshi H et al. Estrogen inhibits cuff-induced intimal thickening of rat femoral artery: effects on migration and proliferation of vascular smooth muscle cells. Atherosclerosis 1997;130:1–10.

[224] Adams MR, Williams JK, Kaplan JR. Effects of androgens on coronary artery atherosclerosis and atherosclerosis-related impairment of vascular responsiveness. Arterioscler Thromb Vasc Biol 1995;17:562–570.

[225] Toda T, Toda Y, Cho BH, Kummerow FA. Ultrastructural changes in the comb and aorta of chicks fed excess testosterone. Atherosclerosis 1984;51:47–57.

[226] McCrohon JA, Jessup W, Handelsman DJ, Celermajei DS. Androgen exposure increases human monocyte adhesion to vascular endothelium and endothelial cell expression of vascular cell adhesion molecule-1. Circulation 1999;99:2317–2322.

[227] Goh HH, Loke DFM, Ratnam SS. The impact of long-term testosterone replacement therapy on lipid and lipoprotein profiles in women. Maturitas 1995;21:65–70.

[228] Fujimoto R, Morimoto JK, Morita E et al. Androgen receptors, 5-alpha-reductase activity and androgen-dependent proliferation of vascular smooth muscle cells. J Steroid Biochem Mol Biol 1994;50(3–4):169–174.

[229] Marsh JD, Lehmann MH, Ritchie RH et al. Androgen receptors mediate hypertrophy in cardiac myocytes. Circulation 1998;98:256–261.

[230] Cabral AM, Vasquez EC, Moyses MR, Antonio A. Sex hormones modulation of ventricular hypertrophy in sinoaortic denervated rats. Hypertension 1988;11:193–197.

[231] Reckelhoff JF, Granger JP. Role of androgens in mediating hypertension and renal injury. Clin Exp Pharmacol Physiol 1999;26:127–131.

[232] Reckelhoff JF, Zhang H, Srivastava K, Granger JP. Gender differences in hypertension in spontaneously hypertensive rats: role of androgens and androgen receptor. Hypertension 1999;34:920–923.

[233] Caplea A, Seachrist D, Dunphy G, Ely D. Sodium-induced rise in blood pressure is suppressed by androgen receptor blockade. Am J Physiol Heart Circ Physiol 2001;280:H1793–H1801.

[234] Holla VR, Adas F, Imig JD et al. Alterations in the regulation of androgen-sensitive Cyp4a monooxygenases cause hypertension. Proc Natl Acad Sci USA 2001;98:5211–5216.

[235] Ellison KE, Ingelfinger JR, Pivor M, Dzau VJ. Androgen regulation of rat renal angiotensinogen messenger mRNA expression. J Clin Invest 1989;83:1941–1945.

[236] Chen Y-F, Naftilan AJ, Oparil S. Androgen-dependent angiotensinogen and renin messenger RNA expression in hypertensive rats. Hypertension 1992;19:456–463.

[237] Hayashi T, Esaki T, Muto E et al. Dehydroepiandrostenedione retards atherosclerosis formation through its conversion to estrogen: the possible role of nitric oxide. Arterioscler Thromb Vasc Biol 2000;20:782–792.

[238] Dubey RK, Gillespie DG, Zacharia LC et al. Methoxyestradiols mediate the antimitogenic effects of estradiol on vascular smooth muscle cells via estrogen receptor-independent mechanisms. Biochem Biophys Res Commun 2000;278:27–33.

[239] Dubey RK, Gillespie DG, Zacharia LC et al. Methoxyestradiols mediate the antimitogenic effects of locally applied estradiol on cardiac fibroblast growth. Hypertension 2002 (in press).

[240] Dubey RK, Gillespie DG, Zacharia LC. Role of Methoxyestradiols in the growth inhibitory effects of estradiol on human glomerular mesangial cells. Hypertension 2002 (in press).

[241] Jackson EK, Dubey RK, Tofovic S. 2-Hydroxyestradiol attenuates the development of obesity, metabolic syndrome, and vascular and renal dysfunction in obese ZSF1 rats. J Pharmacol Exp Ther. (in press).

[242] Mondschein JS, Hammond JM, Weisz J. Characteristics of estrogen 2/4-hydroxylase of porcine ovarian follicles: influence of steroidal and non-steroidal agents on the activity of the enzyme in vitro. J Steroid Biochem 1987;26:121–124.

[243] Zacharia LC, Jackson EK, Gillespie DG, Dubey RK. Increased 2-methoxyestradiol formation in human coronary versus aortic vascular cells. Hypertension 2000;37(Part 2):658–662.

[244] Zacharia LC, Jackson EK, Gillespie DG, Dubey RK. Catecholamines abrogate antimitogenic effects of 2-hydroxyestradion on human aortic vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 2001;21:1745–1750.

[245] Zhu BT, Conney AH. Is 2-methoxyestradiol an endogenous estrogen metabolite that inhibits mammary carcinogenesis. Cancer Res 1998;58:2269–2277.

[246] Bardin CW. Pituitary tesicular axis. In: Yen SSC, Jaffe RB, editors, Reproductive endocrinology, physiology, pathophysiology and clinical management, 2nd ed., Philadelphia, PA: W.B. Saunders, 1986, pp. 177–1999.