Sex and gender differences in control of blood pressure

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Abstract

In recent years, the interest in studying the impact of sex steroids and gender on the regulation of blood pressure and cardiovascular disease has been growing. Women are protected from most cardiovascular events compared with men until after menopause, and postmenopausal women are at increased risk of cardiovascular complications compared with premenopausal women. The pathophysiological mechanisms have not been elucidated, but are not likely to be as simple as the presence or absence of oestrogens, since hormone replacement therapy in elderly women in the Women's Health Initiative or HERS (Heart and Estrogen/progestin Replacement Study) did not provide primary or secondary prevention against cardiovascular events. Men are also thought to be at risk of cardiovascular disease at earlier ages than women, and these mechanisms too are not likely to be as simple as the presence of testosterone, since androgen levels fall in men with cardiovascular and other chronic diseases. In fact, many investigators now believe that it is the reduction in androgen levels that frequently accompanies chronic disease and may exacerbate cardiovascular disease in men. In the present review, the roles of sex steroids and gender in mediating or protecting against hypertension and cardiovascular disease will be discussed.

Key words: androgen, endothelin, menopause, obesity, oestrogen, renin-angiotensin system, sympathetic nervous system

INTRODUCTION

The roles played by sex and gender in controlling blood pressure have not been completely elucidated, and studies have shown that these mechanisms are not as easily understood as the presence or absence of sex steroids or the sex chromosomal complement present. As discussed below, both sex steroids and chromosomes probably have an impact on the mechanisms of blood pressure control at multiple levels.

THE DIFFERENCE BETWEEN ‘SEX’ AND ‘GENDER’

As defined by the Institute of Medicine in its white paper, entitled “Does Sex Matter?” published in 2010 [1], ‘sex’ differences are defined as those caused by sex organs and subsequent sex steroids, oestrogens or androgens, and by sex chromosomes, XX and XY. ‘Gender’ is a sociological term that is “a person’s self representation as male or female, or how that person is responded to by social institutions based on the individual’s gender presentation. Gender is rooted in biology and shaped by environment and experience”. For simplicity, unless stated otherwise, animal studies using males and females are studies into the ‘sex differences’ in a system, since animals do not consciously self-represent as ‘male’ or ‘female’. In humans, unless a single sex trait is studied, e.g. prostate cancer in men or the effect of hormone replacement therapy in postmenopausal women, differences in traits in men and women connote a ‘gender difference’.

SEX STEROIDS VERSUS SEX CHROMOSOMES

There is a significant amount of evidence that sex steroids contribute to cardiovascular disease progression or protection, but more studies are needed to more fully determine their effects. However, in order to evaluate the role played by sex steroids versus...
sex chromosomes on physiological parameters, Arnold et al. [2] have developed the four core genotype mouse models that are either Sry-null or have Sry transgene expressed on an autosome providing mice that have XX females, XY males, XY females and XX males. When the sex steroids are manipulated by castration or ovarioectomy, the contribution of sex steroids to physiology in these animals can be separated from the contribution made by sex chromosomal complement.

To date, to our knowledge, there is no information suggesting that chromosomes alone affect cardiovascular disease including hypertension. In fact, Liu et al. [3] have used the four core genotype mouse models and reported that ACE (angiotensin-converting enzyme) 2 activity in the kidney is modulated by oestradiol and the ovarian milieu, but not by testicular steroids or the Y chromosome. However, Chen et al. [4] have recently exploited the four core genotype models and have shown that adiposity is due to the dosage of X chromosomes independent of the gonadal sex or Y chromosome, due to the lack of X chromosome inactivation of several genes. Mice with two X chromosomes, regardless of their type of gonad, had greater adiposity and food intake during daylight hours. They also had greater weight gain when fed on a high-fat diet with higher lipid and insulin levels. Blood pressure was not measured in these animals, but obesity is known to increase blood pressure [5,6]. Thus the contribution of chromosomes to hypertension or cardiovascular disease independent of gonadal steroids remains to be determined.

SEX STEROIDS AND RECEPTORS

Oestrogens are the most important female sex hormones and are synthesized in the granulosa cells of the ovary in females or Sertoli cells in males. Oestradiol is produced by the conversion of testosterone and androstenedione by the aromatase enzyme. There are three types of oestrogen: oestradiol, which is more prevalent in premenopausal women; oestrone, which is more prevalent during the postmenopausal period; and oestriol, which is increased during pregnancy primarily due to synthesis in the placenta. There are also three ERs (oestrogen receptors), namely ERα, ERβ [7,8] and the endoplasmic-reticulum-membrane-bound GPER-1 (G-protein-coupled for ER-1), a member of the G-protein-coupled receptor superfamily [9]. GPER-1 is located in both intercalated cells and in tubular cells of the kidney [10], and ERβ and ERα are both present in the kidney and the vasculature [8,11,12]. In the heart, ERα expression is similar in males and females, but ERβ expression is higher in males [13]. The age of the animals/humans studied may also be important in ER localization, since Brandenberger et al. [14] found, in the midgestational human fetus, that the prevalence of ERβ was greater in the kidney than ERα regardless of gender. In the brain, although ERα was located in the nuclei of hypothalamic cells in men and premenopausal women, ERα was located in the cytoplasm in postmenopausal women [15]. GPER-1 is located in highest concentrations in the hypothalamic–pituitary axis and does not bind either testosterone or progesterone [10]. There is some evidence that GPER-1 may act as a mineralocorticoid receptor and bind aldosterone, but this hypothesis is controversial [10]. In any case, the potential differential expression and cellular and intracellular localization of the ERs and GPER-1 may explain some of the sex differences in the physiological responses to oestrogens.

Testosterone is the primary male sex hormone and is synthesized in theca cells in ovaries of females and Leydig cells in testis of males. The AR (androgen receptor) has been found in most tissues. In humans, two different forms of AR have been found: A and B. AR-B is predominant in all tissues in which both isoforms were detected. In fact, AR-A is not found in the kidneys of adult humans [16]. In the heart, ARs are found in both males and females [13], but the subtype(s) present is not clear.

Both ERs and ARs are transcription factors that bind to EREs (oestrogen-response elements) or ARES (androgen-response elements) upstream in the promoter regions of genes that regulate the synthesis of proteins whose transcriptional and translational regulation is controlled by the steroids [7,8,16]. In addition to genomic effects, sex steroids also have acute, non-genomic effects, especially in the vasculature, where both oestrogen and androgens cause acute vasodilation [17].

OESTROGENS AND HYPERTENSION

Premenopausal women typically have lower blood pressure than age-matched men [18]. The NHANES (National Health and Nutrition Examination Survey) III and IV studies showed that the prevalence of hypertension was greater in women 60 years of age and older than men, regardless of ethnicity [19]. The SIMONA (Study on Hypertension Prevalence in Menopause in the Italian population) epidemiological study, a large cross-sectional study on 18326 women ranging in age from 46 to 59 years, showed that menopause is associated with a slightly, but significantly, higher blood pressure, even after adjustment for age and BMI (body mass index), as well as other confounding factors. Similar results were found in other studies that demonstrated natural menopause as a risk factor for higher blood pressure, independent of age and BMI [20]. The results of the WHI (Women’s Health Initiative) and HERS (Heart and Estrogen/progestin Replacement Study) I and II are well known now, with HRT (hormone replacement therapy) with CEE (conjugated equine oestrogen) and progestin or CEE alone failing to provide prevention against primary or secondary cardiovascular events [21–23]. Moreover, these studies have shown other deleterious effects of HRT, such as ischaemic stroke, pulmonary emboli and other cardiovascular complications.

The effect of HRT on blood pressure is controversial. There are studies that show HRT increases blood pressure, decreases blood pressure or has no effect [18,24–26]. However, the positive or negative potential effect of oestradiol on hypertension and cardiovascular disease may be age-related, since observational studies in women with premature or early menopause have shown that oestrogen is protective for ischaemic stroke before 50 years of age, but may become a risk factor after 50–60 years of age [27].
Animal models also show age-related effects on blood pressure, some dependent on sex steroids, some not. For example, the Dahl salt-sensitive rat exhibits increases in blood pressure when ovariectomized [28]. In contrast, we have shown that the blood pressure in the SHR (spontaneously hypertensive rat) is independent of oestrogens [29] and, even in the postmenopausal period, treatment with oestradiol reduces blood pressure only transiently (L.L. Yanes and J.F. Reckelhoff, unpublished work).

**ANDROGENS AND HYPERTENSION**

The role of androgens in mediating hypertension and cardiovascular disease is not clear. Animal studies suggest that androgens may promote cardiovascular-related diseases, including hypertension, since certain diseases are more common in men, such as myocardial infarction, at an earlier age when testosterone levels are elevated. However, androgen levels are actually decreased in men with chronic diseases, including hypertension, obesity, heart disease and chronic kidney disease [30–34]. This has led many investigators to propose that the reduction in testosterone with chronic disease may somehow contribute to the disease progression and is not just a consequence of the disease [30].

Men typically have higher blood pressure and develop cardiovascular diseases earlier than women. The sexual dimorphism in blood pressure begins at puberty and persists through adult age [32–34]. The prevalence of hypertension is also higher in men until after menopause in women when the prevalence of hypertension is higher in women than age-matched men [18]. However, as mentioned above, androgen deficiency is associated with cardiovascular disease. For example, the Fourth Tromsø study (1994–1995) in which 1568 randomly selected community dwelling men were analysed for the impact of endogenous testosterone levels on risk for myocardial infarction and all-cause mortality. Men with free testosterone levels in the lowest quartile had a 24% greater risk of all-cause mortality due to ischaemic heart disease [35]. These studies did not distinguish whether the men in the lowest testosterone quartile had some undiagnosed disease process and thus had lower testosterone or if they were healthy and yet had lower testosterone that ultimately contributed to their cardiovascular disease and death. However, in other studies, total testosterone levels in older men have been shown to be inversely associated with systolic blood pressure and an increased risk of death over the subsequent 20 years, independent of multiple risk factors and several pre-existing health conditions [36,37].

**SEX DIFFERENCES IN BLOOD PRESSURE**

We and others have a significant amount of data showing that there are sex differences in blood pressure control in most of the major systems known to be responsible for causing hypertension. For example, we showed many years ago that, although renal denervation reduced the blood pressure in both male and female SHRs, the reduction was similar in both males and females and that the blood pressure remained significantly elevated [38]. These findings show that the hypertension in both male and female SHRs is mediated by the SNS (sympathetic nervous system), but that the SNS is not responsible for the sex difference in blood pressure. In addition, the fact that renal denervation failed to reduce the blood pressure to normotensive levels suggests that there are other mechanisms responsible for the hypertension in male and female SHRs.

Other mechanisms include the RAS (renin–angiotensin system). We found that blockade of the RAS with enalapril, an ACEI (ACE inhibitor), reduces blood pressure in both males and females and removes the sex difference [39]. In addition, we found that treatment with ovariectomized females with testosterone increases blood pressure in a dose-dependent manner [39]. In retrospect, enalapril also did not normalize blood pressure in the SHRs, as defined as blood pressure equal to 100 mmHg, suggesting that other mechanisms contribute to the hypertension. Berecek et al. [40] have shown that ACEIs given intracerebroventricularly reduced blood pressure to 140 mmHg in male SHRs, also showing that other mechanisms than central AngII (angiotensin II) contribute to the hypertension in these rats. As the SNS can stimulate the RAS and vice versa, it would be interesting to see whether simultaneous blockade of both systems would normalize blood pressure in male and female SHRs.

In normotensive animals, sex differences in the pressor response to AngII occur. Xue et al. [41] have reported that chronic AngII treatment increased blood pressure to a higher level in male rats than females. Sartori-Valinotti et al. [42] performed similar studies in rats chronically treated with enalapril and found that females had a greater depressor response to the ACEI and had a subsequently greater pressor response to chronic AngII infusion than did the males. When placed on a high-salt diet, the males responded with a further increase in blood pressure, whereas females did not. This is a species effect, since male mice exhibited a great pressor response to chronic AngII regardless of their treatment with chronic ACEI [43].

There are also sex differences in the role that oxidative stress plays in mediating hypertension. In male SHRs, inhibiting oxidative stress with SOD (superoxide dismutase) mimetics or NADPH oxidase inhibitors reduces blood pressure without having an effect on blood pressure in females [26]. In addition, increasing oxidative stress with molsidomine in males increases their blood pressure [44]. A competent NO system is necessary for antioxidants to reduce blood pressure, as blockade of NOS (nitric oxide synthase) with L-NAME (N^G-nitro-L-arginine methyl ester) prevents antioxidants from reducing the blood pressure in male SHRs [45]. Whether there are gender differences in the depressor response to antioxidants has not been tested, but in general antioxidants have not been successful in clinical trials in reducing blood pressure. Perhaps the antioxidants reduced blood pressure in men but not women. In addition, if the individuals exhibited considerable endothelial dysfunction, perhaps due to chronic long-term hypertension, the antioxidants may not have been able to reduce the blood pressure in the clinical trials.
There are also sex differences in the mechanisms responsible for hypertension in SHRs with aging when the blood pressure in female SHRs increases to levels similar to or higher than in males [18]. In males, the hypertension is mediated by the RAS, as blockade with losartan, an AT1 receptor (angiotensin type 1 receptor) antagonist, normalizes their blood pressure [46]. In aging female SHRs, the hypertension is mediated by endothelin [47], the RAS [46] and 20-HETE (hydroxyeicosatetraenoic acid) [48]. The combination of losartan (AT1 receptor antagonist), Abt 627 (endothelin ETA receptor antagonist) and 1-ABT (ω-hydroxylase inhibitor) reduced blood pressure to 110 mmHg in these old females, but still failed to normalize the blood pressure [49]. There is a contribution by the SNS as well, as α1- and β1,2-adrenergic receptor blockade or renal denervation reduce blood pressure, but alone do not normalize it (R. Lima and J.F. Reckelhoff, unpublished work).

HOW SEX STEROIDS COULD MODULATE BLOOD PRESSURE

Oestradiol is a vasodilator and increases NO by chronically up-regulating eNOS (endothelial NOS) expression [50], but can also acutely increase NO due to its effect to increase intracellular calcium, a cofactor for eNOS activity. Oestradiol is also a modest antioxidant, although the role of oxidative stress in mediating hypertension in females has not been fully elucidated (see above) [51].

Oestrogens can affect the RAS in several ways. Oestrogens down-regulate ACE, contributing to attenuation of the synthesis of AngII, while increasing the synthesis of ACE2, the enzyme mainly responsible for synthesis of the vasodilatory peptide Ang-(1–7) [angiotensin-(1–7)] [52]. Both effects would be antihypertensive. Oestradiol can also down-regulate the AT1 receptor [53], which would also be antihypertensive.

The effect of oestrogens on SNS activity is not clear. Xue et al. [54] reported a sex difference in the pressor response to aldosterone and salt with higher blood pressures developing in male rats compared with females, ovariecctomy exacerbating, but castration having no attenuating effect on the hypertension compared with intact males. Interestingly, the sex difference in pressor response took 10–12 days to occur in these groups, and females were protected with no significant increase in blood pressure in response to aldosterone. Central administration of oestradiol blocked the hypertension in male rats given aldosterone and salt, and this protection was afforded via ERs, where a non-specific ER antagonist blocked the depressor response. ER antagonists given centrally also caused a pressor response to aldosterone in intact females, suggesting that the presence of oestrogens protected the females from aldosterone-induced hypertension. They concluded that oestradiol attenuated the sympathetic outflow in response to aldosterone and thus protected the animals from hypertension.

With aging, Seals and Esler [55] have reported that sympathetic activity increases in most tissues except the kidney. However, the incidence of obesity increases with aging, and this is accompanied with increases in sympathetic activation [56]. Thus studies are needed to fully characterize the effect of sympathetic activation on blood pressure control in pre- and post-menopausal women.

As mentioned above, studies in animals show that androgens promote hypertension and cardiovascular disease. Studies in children by Weise et al. [57] have shown that noradrenaline (norepinephrine) levels increased significantly with puberty and were associated with increases in testosterone in boys, suggesting that puberty (and perhaps testosterone itself) is associated with sympathetic activation.

Other mechanisms by which androgens could promote hypertension include their effects to directly stimulate sodium re-absorption via the proximal tubule of the kidney, a mechanism that is blocked by AT1 receptor antagonists [58]. Testosterone also increases angiotensinogen synthesis in the kidney [59,60], which could increase renin enzyme activity. Androgens have also been shown to increase endothelin levels in transsexuals taking masculinizing levels of testosterone [61].

Although testosterone supplements have become commonplace, there are few safety studies done on long-term androgen treatment. However, in a short-term crossover study, testosterone replacement in obese men decreased diastolic blood pressure and improved other components of the metabolic syndrome [62]. In male Zucker rats that are morbidly obese and have testosterone levels that are reduced by 50% compared with lean controls, testosterone supplements for 10 weeks attenuated body weight, inflammation, dyslipidaemia and insulin resistance, but, unlike in the human studies, increased blood pressure [63], suggesting that additional long-term studies with chronic testosterone in men with obesity are necessary since androgen supplements may be beneficial for improvement of characteristics of the metabolic syndrome, but their blood pressures need to be monitored carefully.

ANDROGEN SUPPLEMENTS IN AGING WOMEN AND CARDIOVASCULAR DISEASES

Androgens improve libido in women and thus are given to post-menopausal women to treat sexual dysfunction [64,65]. However, there are no long-term studies regarding the safety of androgen supplements, cardiovascular disease and hypertension in women. Boxer et al. [66] have reported data from a double-blind randomized placebo-controlled trial of 99 aging frail women showing that short-term therapy with dehydroepiandrosterone was safe with regard to cardiovascular risk factors. Nevertheless, other studies suggest that, in menopause, androgen therapy could increase the risk factors for cardiovascular diseases, such as obesity, diabetes and hypertension. In a rat model of PCOS (polycystic ovary syndrome) produced by DHT (dihydrotestosterone) supplements, DHT caused increased food intake and body weight, and increased adiposity, insulin resistance and elevated blood pressure [67]. In women there is evidence that the SNS is activated and may contribute to the elevated blood pressure in young women with PCOS [68], although the specific role played by androgens in the increase in sympathetic activity and the increase
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Figure 1 Potential mechanisms by which oestrogens and androgens may control blood pressure

in blood pressure is not clear. In addition, the presence of PCOS with elevated androgens is a risk factor for increased cardiovascular disease after menopause [69]. There is also evidence that androgen levels remain elevated after menopause in women who have had PCOS during their reproductive years [70]. Thus the role of androgens in mediating hypertension and cardiovascular disease in aging women requires significantly more investigation.

SUMMARY

We hypothesize that hypertension is multifactorial in both animals and humans, and that both sex chromosomes and sex steroids probably modulate the many factors that control blood pressure. As shown in Figure 1, based on the literature, the most important factors that control blood pressure in males and females are activation of the SNS and activation of the RAS to increase AngII, the combination of which would increase sodium reabsorption by the kidney shifting pressure natriuresis to the left and increasing blood pressure. The SNS can also activate the RAS by increasing renin release, and there is evidence, mainly in rodents, that increases in AngII can activate the sympathetic nervous system. There is evidence that oestrogens [54] can attenuate sympathetic activity [5] and we have data showing that androgens can stimulate the SNS, at least in females (R. Lima and J.K. Reckelhoff, unpublished work). Oestrogens have been shown to attenuate the RAS by reducing AT1 receptor [11,53] and ACE expression [52], leading to reductions in AngII, whereas androgens can stimulate synthesis of angiotensinogen [59,60], leading to increases in AngII. Androgens have also been shown to increase sodium reabsorption in the proximal tubule via both ARs and AT1 receptors [58]. Whether oestrogens affect sodium reabsorption directly is not clear. Because oestrogens stimulate NO production [50], they will attenuate renal vasoconstriction both directly and by attenuating oxidative stress. Acutely androgens cause vasodilation, but whether androgens chronically cause vasoconstriction is not clear. In fact, there is evidence to suggest that the afferent arterioles of male SHRs may be vasodilated compared with females, since males exhibit renal injury at an earlier age than do females [71]. This is supported by data from Wu and Schwartzman [72] showing that 20-HETE in the renal vasculature is up-regulated via the androgen-mediated up-regulation of cytochrome P450 4A ω-hydroxylases. Whether oestrogens can attenuate this response has not been studied. Androgens probably stimulate oxidative stress, since hypertension in male, but not female, SHRs and Dahl salt-sensitive rats is mediated in part by oxidative stress [51]. Androgens have also been shown to increase [15] and oestrogens to attenuate [73] endothelin that would promote or attenuate renal vasoconstriction and sodium reabsorption, respectively.

On the basis of recent studies using the four core chromosomal mice, sex chromosomes may also contribute to hypertension, whether directly or indirectly, as via X chromosomal increases in adiposity [4] that would then activate the SNS. It is also likely that sex steroids modulate the sex chromosomal effects, and that this modulation may be different in the two sexes. For example, while oestrogens limit adiposity in females, androgens via their anabolic effects stimulate adiposity in females [67]. Androgens decrease adiposity in males [63], but whether oestrogens increase adiposity in males is not clear.

Thus more research is needed to determine how sex steroids and sex chromosomes modulate the important physiological
factors that control blood pressure, and makes these exciting times for sex and gender differences studies!

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