REVIEW

Hypertension after the Menopause: What Can We Learn from Experimental Studies?

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Summary

Hypertension is the most prevalent cardiovascular disease of the adult population and is closely associated with serious cardiovascular events. The burden of hypertension with respect to vascular and other organ damage is greater in women. These sex differences are not fully understood. The unique feature in women is their transition to menopause accompanied by profound hormonal changes that affect the vasculature that are also associated with changes of blood pressure. Results from studies of hormone replacement therapy and its effects on the cardiovascular system are controversial, and the timing of treatment after menopause seems to be important. Therefore, revealing potential sex- and sex hormone-dependent pathophysiological mechanisms of hypertension in experimental studies could provide valuable information for better treatment of hypertension and vascular impairment, especially in postmenopausal women. The experimental rat models subjected to ovariectomy mimicking menopause could be useful tools for studying the mechanisms of blood pressure regulation after menopause and during subsequent therapy.

Key words

Hypertension • Sex differences • Menopause • Ovariectomy • Timing of treatment • Experimental models

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Introduction

Hypertension is the most prevalent cardiovascular disease affecting 30–50 % of the adult population. It is closely associated with other major cardiovascular diseases such as stroke, coronary artery disease, heart and kidney failure and arrhythmias [1]. Moreover, the systematic analysis for the Global Burden of Disease Study 2017 identified hypertension as the leading risk factor responsible for the largest number of all-cause death [2]. The burden of hypertension is greater in women than in men because women are more prone to develop organ damage caused by elevated blood pressure. For example, women developing left ventricular hypertrophy due to hypertension are losing protection against cardiovascular events [3]. The mechanisms underlying these sex differences are not fully understood. Moreover, the interpretation of sex differences became even more complicated due to unique features in women, such as menarche, pregnancy and, mainly, the transition to menopause, which are accompanied by robust and rapid changes of sex hormones with less predictable impact on vascular system and blood pressure (BP). Therefore, the aim of this review is to highlight potential sex- and sex hormone-dependent pathophysiological mechanisms of hypertension in experimental studies focused on rat models, which could provide valuable information for a better strategy of hypertension treatment in women.

Sex differences in experimental hypertension

Sex differences in BP were described in several rat models in which male rats have higher BP. This has been reported in spontaneously hypertensive rats (SHR)

with genetic hypertension [4, for review see 5], in transgenic rats with murine renin gene with angiotensin II-dependent hypertension [6,7], in Dahl salt-sensitive (DS) rats with salt hypertension [8, for review see 9], in rats with deoxycortisterone acetate (DOCA)-salt hypertension [10] or NO-deficient hypertension induced by chronic L-NAME administration [11], and also in a model of prediabetes, i.e. hereditary hypertriglyceridemic (hHTG) rats with moderate hypertension but abnormal lipid and carbohydrate metabolism [12,13, for review see 14].

Spontaneously hypertensive rats – genetic hypertension

SHR are the most widely used model of experimental hypertension. Female SHR are characterized by lower BP compared to males [4, 15-18]. Males show an increased rate of vascular smooth muscle cells (VSMC) proliferation, as well as greater growth response and enhanced cytosolic calcium response to angiotensin II as compared to VSMC from female SHR [19,20]. Numerous sex differences in presynaptic and postsynaptic adrenergic mechanisms [21-23], the renin-angiotensin system (RAS) [24-26], nitric oxide bioavailability and oxidative stress [27-29] and immune system [30-32] have been described in this model. The potential role of SHR in the investigation of sex differences was recently summarized by Elmarakby and Sulivan [5], who stated that the sexual dimorphism in BP is based directly or indirectly upon different regulation of the sympathetic nervous system (SNS), the RAS, oxidative stress, NO bioavailability and immune cells.

mRen2 transgenic rats – angiotensin II-dependent hypertension

Another model in which sexual dimorphism has been described is the (mRen2-27 transgenic rat (TGR). This is a monogenic form of hypertension established through the insertion of murine Ren-2 gene into the genome of normotensive Sprague-Dawley rats [33]. Lee *et al.* [34] demonstrated a sexual dimorphism in BP, which was higher in males than in females, and this was true in both homozygous and heterozygous animals. Both males and females had the highest BP at the age of 4 months, thereafter BP gradually decreased in both sexes, but from the age of 5 months this decrease was significant only in females. The cause of this BP decrease is still not fully understood, although the RAS has been suggested as a major player. Another piece of evidence was provided by Vaněčková *et al.* [35], who not only confirmed a sexual dimorphism in BP levels between male and female TGR, but also observed that hypertension and cardiac hypertrophy were attenuated by castration only in males but not in females. In addition, castration partially decreased BP and had a profound antiproteinuric effect in males, whereas ovariectomy had no effect on BP in female TGR. Since there were no differences in plasma and tissue angiotensin II levels at the end of the experiments, the role of the RAS in the sex-dependent BP regulation seems to be negligible in aging TGR. Rauchová *et al.* [36,37] demonstrated similar elevations of BP in 3-month-old male and female heterozygous TGR, but this was not true for animals aged 6 and 9 months, in which BP spontaneously decreased in females with age. This sexual BP dimorphism (at 6 and 9 months) was not associated with differences in oxidative stress or abnormal cholesterol metabolism [36,37]. Sex differences in salt sensitivity were analyzed in TGR rats by Husková *et al.* [38], who showed that the salt-sensitive component of hypertension is more pronounced in females than in males and that females develop hypertension more rapidly.

Salt-dependent hypertension - Dahl rats

Other examples of sex dimorphism are salt-dependent forms of experimental hypertension in which BP rises more rapidly and BP increase is usually more pronounced in male than female rats. This is true for DOCA-salt hypertension [10,39] as well as for salt hypertension in Dahl salt-sensitive (DS) rats [8,40], although these sex differences in BP were not always very pronounced [41,42]. Salt hypertension in DS rats is caused by the enhanced sympathetic vasoconstriction not only in males [9] but also in females [43,44]. DS females fed a high-salt diet (HS) had smaller total and renal vascular resistance than males [40]. Small resistance arteries from DS females fed a HS diet showed smaller vasoconstriction and greater vasodilation compared to those from DS-HS males [45]. This is in line with smaller vascular norepinephrine release and higher levels of vasodilatory prostaglandins [40]. In addition, a more severe salt hypertension in DS males was associated with more pronounced glomerulosclerosis [46].

Salt intake and sex as factors modifying blood pressure

Human perspective

One potential mechanism for the different

development of hypertension in men and women is association between salt sensitivity and BP changes. This topic was recently reviewed by Barris *et al.* [47] with a conclusion that women of all ethnicities and ages are more salt-sensitive than men, yet the exact mechanisms are not well understood. Nevertheless, it is known that menopause further enhances salt sensitivity regarding BP increase and/or female sex chromosomes and sex hormones may be important in moderating the relationship between salt intake and hypertension. To better understand all these mechanisms, this topic should be addressed in experimental studies.

Experimental perspective

A possible pathophysiological explanation for the sex dimorphism in salt sensitivity for BP comes from experimental models in rodents (rats and mice) and points to an inappropriately enhanced activation of the aldosterone-mineralocorticoid receptor axis in endothelial cells, which promotes impaired vascular function only in females. The increased response of adrenal tissue to angiotensin II, associated with higher mineralocorticoid receptor expression and activation of epithelial sodium channels in endothelial cells of females than males, may be of critical importance [47]. In addition, the effect of estrogen on the central nervous system plays a protective role in the development of aldosterone/NaCl-induced hypertension, which may result from reduced sympathetic outflow [48]. Endothelial mineralocorticoid receptors are sex-specifically upregulated in the vasculature of females. This sex difference, which is driven by endothelial progesterone receptor activation and increased activity of endothelial mineralocorticoid receptors, is an important mediator of endothelial dysfunction and potentially hypertension, in female models of experimental obesity [49]. Nevertheless, it should be kept in mind that the salt sensitivity depends not only on sex, but also strain, age of rats and other factors (Table 1).

Menopause/ovariectomy and timing of treatment on blood pressure and vascular physiology

In addition to sex dimorphism, the changes of reproductive status in females are of primary importance in understanding the mechanisms underlying changes in BP and associated vascular changes. Regarding experimental approach, it was suggested that the rise in BP associated with the cessation of ovarian function induced by ovariectomy in rodents, particularly rat models of hypertension might mimick menopausal hypertension in women.

Human perspective

Menopause is clinically diagnosed when a woman has not menstruated for 12 months, which usually occurs around the age of 45-55 years [50,51]. In general, postmenopausal women have a higher prevalence of hypertension than age-matched men [52,53]. This is not the case in premenopausal women in which the prevalence of hypertension is lower than in age-matched men. However, the mechanisms and sufficient evidence supporting causative association between menopause and hypertension are not clear. One study that followed women prospectively for 5 years found that peri- and postmenopausal women had a rise in systolic BP compared to premenopausal women and men. Importantly, postmenopausal women had higher systolic BP at baseline and these women experienced significant increase of systolic SBP throughout the 5-year follow-up [54]. Although the above study used body mass index-matched women, other studies have shown that the development of hypertension with menopause can be mostly explained by body mass index and age [55]. Similarly, in our cross-sectional population study in middle aged women around the age of menopause, the rise in BP after the menopause appeared to be due to increased body mass index rather than ovarian failure *per se* [56].

However, these controversial findings do not mean that sex hormones and the changes seen in the menopausal period cannot affect BP and other cardiovascular factors; these effects have been detected even on the level of cell membranes [57]. It has been also repeatedly demonstrated that a decrease in estradiol and an increase in testosterone after the menopause alter the estrogen/androgen ratio and this has been proposed as one of the mechanisms responsible for BP increase in this period [58]. It has been hypothesized that a continuation of androgen production in postmenopausal women may result in increased arterial stiffness and vascular inflammation, leading to endothelial dysfunction and resultant hypertension [59]. However, this association between menopause and subsequent hormonal changes with endothelial dysfunction leading to hypertension is far from definitive. In addition, menopause itself appears to activate a cluster of genes that lead to hypertension [60]. The decline in sex hormones, particularly estrogens,

is also associated with vascular endothelial dysfunction [61] very probably leading to increased BP after the menopause. These hormonal changes also result in upregulation of the RAS, leading to increased vasoconstriction [62] and increased salt sensitivity [63].

Another piece of evidence comes from studies in which menopausal transition was considered as the critical period for the progression of atherosclerosis [64]. One of the main limitations in obtaining a reliable picture is very fast and mostly unpredictable "rollercoaster" fluctuations of sex hormones during this period. This makes it extremely difficult to interpret reliably the actual impact of hormonal status on the progression of atherosclerosis and vascular impairment, including the development of hypertension. One of the proposed mechanisms could be epigenetic factors, including miRNA and methylation processes of DNA [65], which are also under the control of sex hormones [66]. In addition to clinical and epidemiological data, the experimental studies could help to clarify these mechanisms regarding the treatment in the "window of opportunity" based on the "timing hypothesis" which stressed the importance of early treatment starting soon after menopause [67,68]. For example, when women used hormone replacement therapy (HRT) soon after menopause (for up to 10 years), the overall mortality decreased by 30 % and the incidence of coronary heart disease by 48 % (relative risk) [69]. The sooner after the onset of menopause the HRT was administered, the greater was the benefit [70-72]. Unfortunately, there are no data regarding the timing of treatment by hypolipidemic or antihypertensive drugs after the menopause.

Regarding the effect of HRT on BP the evidence is rather ambiguous. An observational cohort study of 43,405 previously normotensive postmenopausal women in Australia even found that HRT was associated with significantly higher odds of elevated BP. These odds increased with the duration of HRT use [73]. A French observational study showed slight but significant increase in hypertension risk in postmenopausal women using combined oral estrogen and progesterone therapy [74]. In contrast, the Kronos Early Estrogen Prevention Study (KEEPS) showed that HRT use, regardless of formulation (oral conjugated estrogen or weekly transdermal estradiol, each with intermittent progesterone administration) did not affect BP in normotensive postmenopausal women [75]. With respect to BP changes, HRT may also improve fat mass and distribution, dyslipidemia, and insulin resistence after menopause. As shown in the meta-analysis performed by Salpeter *et al.* [76], HRT had a beneficial effect in patients with metabolic syndrome. In this meta-analysis of 107 trials that enrolled 33,315 subjects, HRT increased lean body mass and reduced abdominal fat, HOMA-IR, and the onset of new diabetes in previously non-diabetic postmenopausal women. However, the effect of HRT on BP was negligible. Unfortunately, the effect of the timing of such preventive measures besides hormone HRT in women transitioning to menopause has not been studied and reliable data are not available for this population.

In summary, premenopausal women have a lower incidence of hypertension and other cardiovascular events than men of the same age, but the reduced sex differences after menopause suggest that 17β-estradiol (E2) is a protective agent. The cardioprotective effects of E2 are mediated by nuclear estrogen receptors (ERα and ERβ) and a G protein-coupled estrogen receptor but the exact mechanisms involved in the association of sex differences and E2 are not fully identified, which should encourage not only clinical trials but also experimental studies in this field [77].

Experimental perspective

Salt-dependent forms of hypertension in ovariectomized rats

Postmenopausal hypertension in the rat is usually induced by ovariectomy (OVX), which enhances both DOCA-salt hypertension [10,78] and salt hypertension in Dahl rats [8,79,80]. Increased BP and higher degree of glomerulosclerosis were found in ovariectomized salt-loaded F2(DSxDR) hybrids compared to intact F_2 hybrids [81,82]. Under the conditions of high salt intake, OVX of DS females was accompanied by distinct renal alterations such as increased density of α_2-adrenergic receptor [83] or angiotensin AT_1 receptors in the kidney [80]. There was a blunted pressure natriuresis in ovariectomized DS rats [84]. Chronic administration of estrogens (17β-estradiol) to ovariectomized DS females fed a HS diet lowered their BP, glomerular filtration rate and adrenal density of AT_1 receptors, while this hormonal therapy increased the expression of estrogen receptors ERα and the density of AT_1 receptors in the kidney [80,85]. Ovariectomized DS females fed a HS diet showed increased oxidative stress because the formation of oxygen free radicals was

enhanced and their scavenging was reduced; these changes were attenuated by estrogen administration [86].

It has been observed in Dahl salt-sensitive rats [8] that males generally developed salt hypertension more rapidly than females. However, final BP was similar in both sexes and, more importantly, castration of males had no effect on BP, whereas OVX caused an increase in BP in the females. In addition, OVX resulted in greater body growth compared to control females, whereas castration of males had the opposite effect. It was speculated that these changes were due to an increase of pituitary growth hormone after OVX or enhanced growth hormone receptor sensitivity in females, while the opposite effect was proposed in males. It was considered unlikely that the BP effect was simply due to the increased dietary salt intake associated with greater growth since growth was independent of salt intake. It was therefore suggested that the rise in BP associated with the cessation of ovarian function might mimic "menopausal" hypertension in women. However, the increase in growth hormone associated with decline in ovarian function after the menopause could be the stimulus for the development of hypertension earlier than would be expected from chronological age. In this case, there is an indirect evidence from human studies focusing on the treatment of central precautious puberty by gonadotropin-releasing hormone agonists (GnRHa), which could also cause hypertension. These compounds have a high affinity for the pituitary LHRH receptor and are resistant to enzymatic degradation. By continuous stimulation, GnRHa inhibit the pulsatile secretion of gonadotropin, resulting in hormonal suppression [87].

Salt-independent postmenopausal hypertension in ovariectomized Dahl rats

A long-term development of postmenopausal hypertension has been described in OVX DS females fed a low-salt (LS) diet in which the elevated BP was reduced by chronic estrogen administration [42,88-90]. This form of hypertension was accompanied not only by glomerulosclerosis and tubulointerstitial fibrosis but also by an increased density of AT_1 receptors in the glomeruli and adrenal cortex. The severity of these changes was attenuated by E2 administration [91]. OVX decreased the expression of iNOS and eNOS in the renal medulla of DS-LS females, while these changes were attenuated by a concomitant estrogen administration. In addition, OVX increased the number of renal CD68 macrophages, which was reduced by estrogen treatment [92].

Ovariectomy and estrogen treatment in SHR

Castration of male SHR substantially lowered their BP [4,16,25,93-95], whereas OVX had much smaller BP effects in SHR females [4,16,25, 96]. In contrast, Iams and Wexler [94] described the late BP rise in OVX SHR females, which was attenuated by estrogen treatment. Much attention has been paid to the role of testosterone in the higher BP of male SHR or testosterone-treated ovariectomized SHR females [96-98]. It should be mentioned that BP differences between male and female SHR as well as between the castrated SHR males and ovariectomized SHR females could be abolished by chronic treatment with the ACE inhibitor enalapril [25,] implying the effect of the RAS. Although ovariectomized SHR females are often used for the studies of postmenopausal BP changes, it is not clear whether OVX performed in the prepuberty (at the age of 3-6 weeks) or in the early adulthood (at the age of 3-4 months) is an optimal model for human postmenopausal hypertension [28,58,100,101].

Prepubertal OVX of young SHR females aged 3 weeks increased their sensitivity to hypertensogenic effects of high salt intake, while dietary phytoestrogens attenuated this BP increase by reducing sympathetic tone [102]. Phytoestrogens are known to have a high affinity to ERβ receptors. Thus, the treatment of young ovariectomized SHR females with ERβ receptor agonist 8β-VE2 lowered their elevated BP by decreasing total peripheral resistance, improved NO-dependent vasodilatation, attenuated cardiac hypertrophy but increased their cardiac output through the augmentation of stroke volume [103]. In contrast, chronic treatment with estrohen receptor-α agonists (16α-LE2 or Cpd1471) did not significantly reduce BP of young ovariectomized SHR females but attenuated cardiac hypertrophy, increased cardiac output and myocardial contractility [104] and improved their endothelial dysfunction [105].

OVX of young adult SHR females aged 3 months substantially increased BP through the augmentation of sympathetic tone, which was associated with the enhanced expression of AT_1 receptors in the brainstem [106]. Enhanced sympathetic tone and/or decreased vagal tone were also detected in ovariectomized SHR, in which only a moderate BP elevation was observed [107,108]. Decreased baroreflex gain and increased variance of systolic arterial pressure were also seen in ovariectomized SHR without a significant BP change [109]. OVX increased BP in SHR females and enhanced the reactivity of their

mesenteric vessels to angiotensin II. This was due to an increase in mRNA expression of AT_1 (but not AT_2) receptors in the kidney, aorta and mesenteric arteries, causing thus a pronounced rise in the AT_1/AT_2 ratio [26].

Ovariectomized SHR females are characterized by attenuated acetylcholine-induced vasodilatation due to impaired release of endogenous NO [110-112], enhanced production of superoxide radicals [108,113] and the presence of vasoconstrictor prostaglandins [114,115]. On the other hand, vascular formation of vasodilator prostaglandins was not altered by OVX in SHR [110]. The increased superoxide generation appears to be mediated by augmented NADPH oxidase activity [113,116], which is partly under the control of angiotensin II acting *via* AT_1 receptors. Therefore, the chronic blockade of the RAS by ACE inhibitors [112] or AT_1 receptor blockers [113,114] reduced oxidative stress and increased NO bioavailability. The aforementioned endothelial dysfunction in ovariectomized SHR females can be reduced by estrogen supplementation [112,113,115,117].

Late OVX (carried out in SHR aged 5-9 months) also caused a substantial endothelial dysfunction due to a decreased NO bioavailability and increased superoxide formation, as well as enhanced expression of vascular AT_1 receptors. These changes were corrected by chronic estrogen treatment or stimulation of estrogen receptors [118-120].

Fortepiani *et al.* [121] proposed to use the "postcycling" 18-month-old SHR females with a BP equal to that of the age-matched SHR males. This model showed lower estradiol and higher testosterone serum levels, elevated plasma renin activity, high urinary F_2-isoprostanes as well as considerable alterations of renal function (decreased glomerular filtration rate, low renal plasma flow, high renal vascular resistance) [121]. This form of postmenopausal hypertension can be attenuated by chronic antioxidant treatment [121], chronic blockade of endothelin ET_A receptors [122,123], chronic inhibition of 20-HETE formation [124], chronic ACE inhibition [125,126] and combined α_1- and β-adrenergic blockade [127].

Ovariectomy and estrogen treatment in mRen2 transgenic rats

OVX in heterozygous mRen2.Lewis rats, a congenic rat strain established from the original (mRen2)-27 transgenic rats [128], dramatically increased BP as well as plasma angiotensin II, ACE activity and plasma renin concentrations, while renal levels of eNOS or ANG(1-7) were decreased [128]. Estrogen replacement or treatment with olmesartan (AT_1 receptor blocker) lowered BP even below the level of sham-operated controls. Estrogen supplementation shifted the vasoconstrictor/vasodilator balance of the RAS towards a higher contribution of the vasodilator ANG(1-7) while reducing vasoconstrictor effect of angiotensin II [129]. Estradiol treatment started 7 weeks after OVX decreased cardiac angiotensin II and ANG(1-7) concentrations, which were increased after OVX [130].

Chappell *et al.* [131] analyzed the interaction of estrogen and high salt intake in mRen2.Lewis females showing that early OVX (performed at 4-5 weeks of life) substantially exacerbated the development of hypertension and that high salt intake increased BP in intact animals. Estrogen depletion increased their BP response to high salt intake. The inability of this strain to downregulate various components of the RAS has been suggested as the mechanism leading to their elevated BP following either OVX or high salt intake [131]. Interestingly, high salt intake applied for four weeks at the age of 60 weeks in mRen2.Lewis rats that underwent OVX at 15 weeks of age did not alter their BP, while proteinuria, glomerulosclerosis and creatinine were lower in ovariectomized females suggesting that OVX performed in older females is protective against salt-induced renal damage [132]. In addition, Groban *et al.* [133] demonstrated an exacerbation of diastolic dysfunction after high-fat diet, which was further deteriorated by OVX. This may explain a higher susceptibility of postmenopausal women to diastolic heart failure. Experiments using GPR30, an estrogen receptor agonist, in ovariectomized mRen2. Lewis rats suggested a possible role of estrogen in diastolic dysfunction. In addition, Lindsey *et al.* [134] demonstrated that the vasorelaxant effects of GPR30 agonist or E2 on resistance mesenteric arteries were due to NO release from endothelium and the activation of guanylate cyclase in vascular smooth muscle. The chronic activation of GPR30 reduced BP in ovariectomized females but not in male mRen2.Lewis rats. Furthermore, GPR30 activation also reduced oxidative stress and proteinuria in ovariectomized females on a high-salt diet, independent of BP reduction [135].

The participation of renal NO in BP increase after OVX was demonstrated in mRen2.Lewis female rats, which showed reduced renal eNOS that correlated negatively with BP [136]. In line with this finding, Jessup

et al. [137] found that chronic nNOS inhibition in rats treated 7 weeks after OVX improved diastolic dysfunction and reduced oxidative stress in the same model. In (mRen2)-27 transgenic females, estrogen augmented endothelial NO release and thus contributed to the modulation of agonist-induced contractile response [138].

Timing of treatment after ovariectomy in experimental studies

Unfortunately, only one paper was focused on the timing of sex hormone therapy after ovariectomy. In this study the 2-month-old ovariectomized Sprague-Dawley rats were implanted with E2 in oil capsules 1, 4, and 8 months after surgery [139]. While most of the vascular changes were reversed in the groups treated one or four months after surgery, no such effect was observed when the treatment was delayed for 8 months after OVX, indicating irreversible changes. After such a long period of hypoestrogenicity, acetylcholine-mediated aortic relaxation was attenuated and insensitive to E2 administration despite sustained endothelial integrity. Whereas no rapid vasorelaxant responses were elicited by a selective estrogen receptor-β agonist, the responses to E2 and a selective estrogen receptor-α agonist waned after OVX at any given time and were restored by E2 treatment after 1 and 4 months, but not 8 months, after OVX. Accordingly, endothelial ERα mRNA and protein expression decreased approximately six-fold after prolonged hypoestrogenicity and was restored by estrogen replacement starting 1 month but not 8 months after OVX. Furthermore, the amount of active phosphorylated endothelial NO synthase increased significantly after E2 replacement after 1 and 4 months but not 8 months after OVX. The present findings document that the functional impairment of the ERα/endothelial NO synthase signaling network after the extended period of hypoestrogenicity was not restored by E2 administration, providing experimental support for early initiation of estrogen replacement with preferential ERα targeting to improve cardiovascular outcomes [139].

Surprisingly we also found only one human study addressing the effect of hypolipidemic therapy after menopause in a small group of postmenopausal women (n=32). This study compared six months of transdermal E2 supplementation plus oral medroxyprogresterone acetate with oral simvastatin treatment. Both approaches reduced basal and exercise test-induced endothelin-1 plasma levels. E2 supplementation gradually increased NO release, whereas simvastatin initially reduced and finally increased NO release. It was concluded that 6 months of oral simvastatin treatment had a beneficial influence on endothelial function similar to that of continuous transdermal E2 supplementation combined with medroxyprogesterone acetate [140].

Conclusions and future directions

To summarize, in contrast to human studies, most animal studies show a favorable effect of estrogens on the protection against hypertension, coronary heart disease, stroke, and heart failure. Female rodents are protected against increased BP, vascular injury, and heart failure compared to males, but this cardioprotection is abolished after OVX and reversed back by estrogen substitution [131,141,142]. Furthermore, the changes in metabolic factors caused by OVX and their reversal by estradiol treatment in an experimental study could provide valuable information to hemodynamic parameters, as already demonstrated in study of hereditary hypertriglyceridemic (hHTG) rats as a model for prediabetes [143]. In our experimental study performed in ovariectomized rats of this strain [144], we found not only favorable changes of hemodynamic factors after estradiol substitution but also an increase of triglyceride content in myocardium reflecting a potentially ambiguous role of HRT. Therefore, in parallel with the study of hypertension the evaluation of metabolic factors is also important. In addition, from this point of view, one of the scientific approaches to fill the gaps in our knowledge of postmenopausal hypertension is to study the effect of the timing of treatment not only with HRT but also with other cardioprotective drugs such as statins and antihypertensives in already established rat models of experimental hypertension.

Intensive research in this area should be also encouraged by the fact that hypertension affects women at all stages of life and is a major contributor to cardiovascular morbidity and mortality. The combination of age, sex hormones, genetic background and unhealthy lifestyle contribute to the development of hypertension, particularly in postmenopausal women. Despite the multiple mechanisms participating in these processes such as SNS, RAS and others (Fig. 1), there are several gaps in our understanding of the sex-specific prevention, detection and management of hypertension at particular periods of woman´s life including menarche, pregnancy

and especially menopause. Similarly, the experimental studies with rodent models, particularly rats, have shown great variability regarding the strain, time of ovariectomy, type and dosage of hormonal or other therapies and especially the timing of therapy after ovariectomy (Table 1). The study of the effect of ovariectomy, focusing also on metabolic factors accompanying development of hypertension, may provide further valuable data useful for humans.

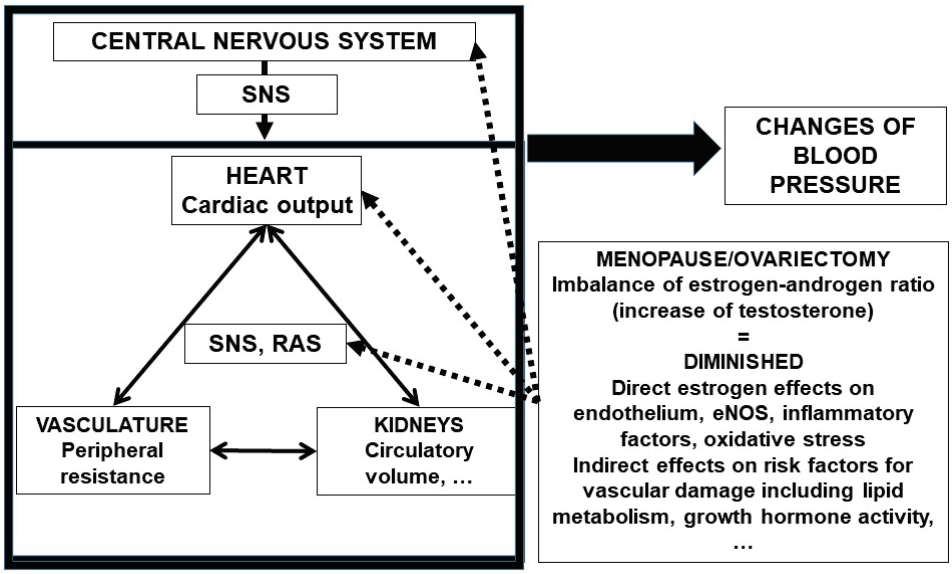

Fig. 1. Interplay between factors associated with hypertension and menopause/ovariectomy. SNS - sympathetic nervous system, RAS - renin-angiotensin system

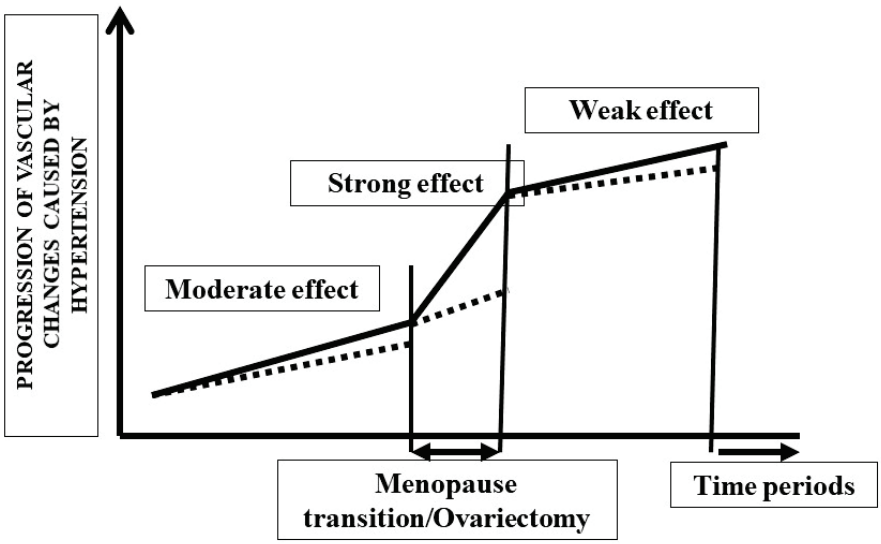

Fig. 2. The effect of intervention could be modified by its timing after menopause/ovariectomy

Rapid changes of estrogen and other sex hormones associated with the menopausal transition may play an important role in the development of risk factors including hypertension and their cardiovascular consequences at the population level. Therefore, it is of great importance to study the effect of the timing of interventions in women transitioning to menopause. In this respect, the identification of particular mechanisms of this approach at the experimental level could provide definite proof of this concept and lead to changes in clinical practice. If the hypothesis of the menopausal transition as a very sensitive method for intervention in hypertension (Fig. 2) is correct, it can be immediately exploited in everyday clinical practice.

Table 1. Management of hypertension and associated vascular impairment with regard to the timing of treatment after ovariectomy (OVX) in rats.

Strain of rats (reference)	Age of ovariectomy	Intervention type/dosages	Onset and duration of intervention	Results
Dahl salt-sensitive rats Harrison-Bernard *et al.* [80]	4-5 weeks	17β-estradiol (1.7 mg) subcutaneous pellet or candesartan (10 mg/kg/day)	At the time of OVX 13 weeks	Development of hypertension in OVX rats was prevented by estrogen replacement or AT_1 receptor blockade. Chronic estrogen administration lowered BP, glomerular filtration and density of AT_1 receptors in the adrenals, while this hormonal therapy increased the expression of estrogen ER-α receptors and the density of AT_1 receptors in the kidney.
Dahl salt-sensitive SS/Jr rats (Rapp strain) Sartori-Valinotti *et al.* [145]	6 weeks	Rosiglitazone (5 mg/kg/day)	At the time of OVX 2 weeks	PPARγ activation lowered BP and salt sensitivity due to increased NO release and reduction of renal resident macrophages and inflammation.
Dahl salt-sensitive SS/Jr rats (Rapp strain) Zheng *et al.* [85]	Approximately 2 months of age (200-250 g)	17β-estradiol benzoate (10 μg/day)	At the time of OVX 2 weeks	Ovariectomy increased BP in rats kept on high-salt diet. The ovariectomy-induced BP increase was prevented by 17β-estradiol treatment, suggesting that the loss of E2 causes this BP increase.
Dahl salt-sensitive SS/Jr rats (Rapp strain) Zhang *et al.* [86]	8 weeks	17β-estradiol (0.5 mg) subcutaneous pellet	At the time of OVX 4 weeks	Ovariectomy led to amplification of oxidative stress in rats fed a high-salt diet through an increase in reactive oxygen species (ROS)-generating system and a decrease in ROS-eliminating system, as shown by the increase in superoxide production and urinary H_2O_2 excretion. Supplementation of estrogens counteracted these changes.
Dahl salt-sensitive SS/Jr rats (Rapp strain) Hinojosa-Laborde *et al.* [90]	8 weeks	17β-estradiol (5 mg) subcutaneous pellet implants replaced every 12 weeks	At the time of OVX 10 months	Rats fed a low-salt diet have developed hypertension with age that was accelerated by OVX and attenuated by estrogen replacement. Concurrently, AT1Rs were upregulated by age and OVX, which was prevented by estrogen replacement. Increased activity of the renin-angiotensin system might contribute to the development of hypertension, and estrogen protects against this process.
Dahl salt-sensitive SS/Jr rats (Rapp strain) Maric *et al.* [91]	12 weeks	17β-estradiol (5 mg) subcutaneous pellet implants replaced every 12 weeks	At the time of OVX 1 or 9 months	E2 is renoprotective in the ageing rat by attenuating glomerulosclerosis and tubulointerstitial fibrosis.
Dahl/Iwai salt-sensitive rats Sasaki *et al.* [89]	12 weeks	17β-estradiol (0.5 mg) subcutaneous pellet	At the time of OVX 8 weeks	Estrogen replacement suppressed hypertension development and attenuated platelet aggregation.

Dahl salt-sensitive SS/Jr rats (Rapp strain) Maric *et al.* [92]	12 weeks	17β-estradiol (5 mg) subcutaneous pellet implants replaced every 12 weeks	At the time of OVX 1 or 9 months	E2 loss with ageing may contribute to the development of age-related renal disease through downregulation of iNOS and eNOS and increased renal inflammation. Furthermore, E2 supplementation may be protective in ageing kidney by attenuating these changes.
SHR Reckelhoff *et al.* [96]	3 weeks	Testosterone (5 mg s.c.) Replaced every 3 weeks	Nine weeks after OVX 6 weeks	Male sex hormones contribute to the exacerbation of hypertension by reducing pressure-natriuresis
SHR Fang *et al.* [102]	3 weeks	dietary phytoestrogens	At the time of OVX 9 weeks	Dietary phytoestrogens protect ovariectomized females from NaCl-sensitive hypertension through attenuated sympathetic tone.
SHR Iams and Wexler [94]	4 weeks	Estradiol (0.5 mg/2 weeks) Injections repeated every 2 weeks	At the time of OVX 30 weeks	Estradiol lowered blood pressure in both intact or gonadectomized animals. Besides lowering of BP, estradiol also increased pituitary and adrenal weights, caused hyperlipidemia, and increased circulating levels of corticosterone and 11-deoxycorticosterone.
SHR Jazbutyte *et al.* [103]	6 weeks	17β-estradiol (2 μg/kg/day) or ER-β receptor agonist 8β-VE2 (30 μg/kg/day)	At the time of OVX 12 weeks	Ligand-dependent activation of ER β receptors lowered BP more than 17β-estradiol or ERα agonist.
SHR Pelzer *et al.* [104]	6 weeks	17β-estradiol (2 μg/kg/day) or ER-α receptor agonist 16 α-LE2 (30 μg/kg/day	At the time of OVX 12 weeks	Activation of ERα receptors favorably affects cardiac hypertrophy, myocardial contractility and gene expression in OVX rats.
SHR Loh and Salleh [146]	8 weeks	Testosterone propionate (10 mg s.c.)	Two weeks after OVX 6 weeks	Chronic testosterone treatment of ovariectomized rats increased their blood pressure although there was decrease not only in plasma aldosterone and Na^+ but also in ENaC levels in kidneys.
SHR Reckelhoff *et al.* [25]	8 weeks	Testosterone (10 mg s.c.) Replaced every 3 weeks ACE inhibitor enalapril	One week after OVX 8-10 weeks	ACE inhibitors reduced BP to similar levels in all gender groups. Untreated males and OVX females given testosterone had significantly higher levels of urinary protein excretion than the other groups. ACE inhibitors had no effect on proteinuria in any group. Thus, the development of hypertension regardless of sex steroids is mediated by the renin-angiotensin system.
SHR Huang *et al.* [110]	9 weeks	17β-estradiol (50 μg/kg s.c., every 48 hours)	One week after OVX 3 weeks	Estrogen preserved NO-mediated portion of flow/shear stress-induced dilation resulting in lower wall shear stress in females
SHR Bonacasa *et al.* [147]	10 weeks	17β-estradiol (1.5 mg pellet) Tempol (90 mg/kg/day)	At the time of OVX 8 weeks	Tempol prevented BP rise after OVX but a combination of E2 with tempol had detrimental effects on myocardial arteriolar remodeling.
SHR Garcia *et al.* [148]	10 weeks	17β-estradiol (1.5 mg pellet) Captopril (5 mg/kg/day)	At the time of OVX 8 weeks	Estrogens enhanced the improvement of vascular remodeling induced by chronic administration of ACE inhibitor.

SHR Gimenez *et al.* [149]	10 weeks	17β-estradiol (1.5 mg pellet) Captopril (5 mg/kg/day)	At the time of OVX 8 weeks	Both chronic treatments with estrogen and captopril attenuated hypertension development and improved micro-vascular density (additive effects).
SHR Gimenez *et al.* [112]	10 weeks	17β-estradiol (1.5 mg pellet)	At the time of OVX 8 weeks	Treatment with 17β-estradiol prevented the blunted acute BP response to captopril in ovariectomized rats. Kinins and nitric oxide may be involved in the mechanisms of 17β-estradiol potentiation of the hemodynamic effects of captopril.
SHR Widder *et al.* [105]	12 weeks	17β-estradiol (2 μg/kg/day) or selective ERα agonist Cpd1471 (30 μg/kg/day)	At the time of OVX 4 weeks	After ovariectomy, endothelium-dependent NO-mediated vasorelaxation and eNOS expression are attenuated. The novel selective ERα agonist Cpd1471 prevented these pathophy-siological changes to a similar extent as 17β-estradiol. Thus, the selective activation of ERα receptors mediates positive vascular effects.
SHR Dantas *et al.* [113]	12 weeks	Subcutaneous pellets containing estradiol (0.05 mg) or estradiol (0.05 mg) with progesterone (50 mg)	Thirty days after OVX 4-6 weeks	Estrogens reduced superoxide overproduction induced by OVX. The antioxidant effect of estrogen, which can contribute to a less pronounced endothelial dysfunction, may be dependent on a direct modulatory action of estrogen on NADPH activity
SHR Ceravolo *et al.* [116]	12 weeks	Conjugated equine estrogen (CEE) given by gavages at the dose of 0.625 mg/day	Thirty days after OVX 2 weeks	Benefit of CEE therapy through a mechanism that involves reduction of oxidative stress, improving endothelial function
SHR Silva-Antonialli *et al.* [26]	12 weeks	17β-estradiol (50 μg/ s.c.)	Thirty days after OVX 2 weeks	OVX increased expression of AT_1 receptors, while it lowered expression of AT_2 receptors. Estrogen treatment reversed these changes.
SHR Riveiro *et al.* [114]	13 weeks	Irbesartan (50 mg/kg/day)	No hormonal substitution ACEi at the time of OVX 30 weeks	Irbesartan enhanced basal nitric oxide availability and ameliorated vascular relaxation by decreased production of cyclooxygenase-dependent contracting factors in smooth muscle cells, regardless of estrogen status
SHR Dantas *et al.* [115]	13 weeks	17β estradiol (50 μg) subcutaneous pellet	Fifteen days after OVX 2 weeks	Estrogen deprivation leads to activation of prostaglandin endoperoxide synthase, which is reversed by estrogens.
SHR Wassmann *et al.* [118]	16 weeks	17β-estradiol (1.7 mg) subcutaneous pellets Irbesartan (50 mg/kg/day)	At the time of OVX 5 weeks 2 weeks after OVX 5 weeks	Ovariectomy increased vascular free radical production and enhanced angiotensin II-induced vasoconstriction, resulting in endothelial dysfunction. Estrogen replacement therapy and AT_1 receptor antagonism prevented these pathological changes. Thus, estrogen deficiency induced AT_1 receptor overexpression and oxidative stress that play important role in menopause
SHR Jazbutyte *et al.* [150]	12 weeks or 24 months	17β-estradiol (2 μg/kg/day)	At the time of OVX 6 weeks	Aging attenuated antihypertrophic effects of estradiol with major alterations of ERα and estradiol metabolism. This explains low efficiency of estrogen substitution in senescent SHR.

SHR Vera *et al.* [120]	6 months	Phytoestrogen genistein (10 mg/kg/day p.o.) or 17β-estradiol (2 mg/kg/week s.c.)	Three weeks after OVX 5 weeks	Genistein prevented all cardiovascular changes induced by estrogen depletion to a similar extent as estradiol but had no uterotrophic effect.
SHROB (obese) Bitto *et al.* [151]	7 months	Genistein (54 mg/day)	At the time of OVX 4 weeks	Genistein ameliorated endothelial dysfunction and insulin resistance, increased HDL cholesterol and enhanced liver expression of PPARα and PPARγ
SHR Males, females Leitzbach *et al.* [119]	9 months	Estrogen receptor modulator raloxifene (10 mg/kg/day)	At the time of OVX 12 weeks	Long-term treatment of rats with raloxifene has beneficial effects on the cardiovascular system in old male and female OVX *via* an increased NO bioavailability.
SHR Fortepiani *et al.* [121]	OVX at 8 months or Spontaneous menopause	Intact or OVX rats were studied at the age of 18 months	No hormonal substitution	Postmenopausal rats, but not OVX rats, may be a suitable model for the study of postmenopausal hypertension. Oxidative stress plays a role in the increased BP.
SHR Lima *et al.* [123]	Spontaneous menopause studied at the age of 18 months	Blockade of three systems contributing to hypertension: renin-angiotensin (enalapril), eicosanoids (1-amino-benzotriazole), and endothelin (ET_A receptor antagonist).	No OVX or hormonal substitution Short-term blockade of each system for one week	ANG II, eicosanoids, and endothelin contribute together and independently to BP control in old female rats. However, other systems might also contribute to the postmenopausal hypertension.
SHR Yanes *et al.* [124]	Spontaneous menopause studied at the age of 18 months	Chronic inhibition of 20-Hydroxyeicosatetraenoic acid (20-HETE) formation by 1-aminobenzotriazole (50 mg/kg/day)	No OVX or hormonal substitution Short-term blockade for one week	20-hydroxyeicosatetraenoic acids (20-HETE), produced by cytochrome *P*-450 ω-hydroxylase, contribute to postmenopausal hypertension in SHR.
SHR Maranon *et al.* [127]	Spontaneous menopause studied at the age of 18 months	Adrenergic blockade (terazosin and propranolol , each 10 mg/kg/day) Renal denervation after uninephrectomy Melanocortin 3/4 receptor antagonism	No OVX or hormonal substitution Short-term studies for one week	Hypertension in old female SHR is partly due to the activation of sympathetic nervous system. The renal nerves also contribute to this hypertension. The sympathetic activation in old females is independent of melanocortin 3/4 receptor.
Sprague-Dawley rats Pinna*et al.* [139]	At about 2 months of age (200-225 g)	17β-estradiol (235 μg/kg/5 days),	1, 4, and 8 months after OVX 5 days	Functional impairment of the ERα/endothelial NO synthase signaling network after an extended (8 months) period of hypoestrogenicity was not restored by E2 administration, providing experimental support to early initiation of estrogen replacement with preferential ERα a targeting to improve cardiovascular outcomes.
Heterozygous mRen2.Lewis rats Jessup *et al.* [137]	4 weeks	nNOS inhibitor (L-VNIO) N5-(1-imino-3-butenyl)-L-ornithine (0.5 mg/kg /day)	No hormonal substitution	Chronic nNOS inhibition lowered BP and improved diastolic dysfunction and reduced oxidative stress in OVX mRen2.Lewis female rats.

Heterozygous mRen2.Lewis rats Chappell *et al.* [128]	5 weeks	17β-estradiol (1.0 mg subcutaneous pellet replaced after 3 weeks) Additional groups of 12-week-old OVX mRen2.Lewis rats were treated with AT_1 receptor antagonist olmesartan for 4 weeks	At the time of OVX 6 weeks	OVX increased BP which was lowered by estrogens or olmesartan to a similar extent. Ovarial hormones considerably lowered blood pressure potentially by limiting activation of the renin-angiotensin system.
Heterozygous mRen2.Lewis rats Yamaleyeva *et al.* [132]	15 weeks	High-salt diet feeding of OVX rats at the age of 60 weeks	No hormonal substitution	No BP increase in this strain 15 or 45 weeks after OVX. However, OVX in older female mRen2.Lewis rats conveys protection against salt-dependent increase in renal injury.
Heterozygous mRen2.Lewis rats Lindsey *et al.* [135]	No OVX	Membrane-bound estrogen receptor GPR30 agonist G-1 (0.4 mg/kg/day) for the last 2 weeks of high-salt diet 4% NaCl diet (5 to 15 weeks of age)	Eight weeks after the onset of high-salt diet 2 weeks	GPR30-mediated beneficial effects in salt-sensitive mRen2.Lewis rats were independent of changes in BP. The failure of G-1 to influence BP may reflect a salt-induced impairment in GPR30-mediated vasorelaxation. The renoprotective action of GPR30 may involve attenuation of tubular oxidative stress and activation of megalin-mediated protein reabsorption.

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