Renal Denervation

Maternal Gestational Hypertension-Induced Sensitization of Angiotensin II Hypertension Is Reversed by Renal Denervation or Angiotensin-Converting Enzyme Inhibition in Rat Offspring

Baojian Xue, Haifeng Yin, Fang Guo, Terry G. Beltz, Robert L. Thunhorst, Alan Kim Johnson

Abstract—Numerous findings demonstrate that there is a strong association between maternal health during pregnancy and cardiovascular disease in adult offspring. The purpose of the present study was to test whether maternal gestational hypertension modulates brain renin–angiotensin–aldosterone system (RAAS) and proinflammatory cytokines that sensitize angiotensin II–elicited hypertensive response in adult offspring. In addition, the role of renal nerves and the RAAS in the sensitization process was investigated. Reverse transcription polymerase chain reaction analyses of structures of the lamina terminalis and paraventricular nucleus indicated upregulation of mRNA expression of several RAAS components and proinflammatory cytokines in 10-week-old male offspring of hypertensive dams. Most of these increases were significantly inhibited by either renal denervation performed at 8 weeks of age or treatment with an angiotensin-converting enzyme inhibitor, captopril, in drinking water starting at weaning. When tested beginning at 10 weeks of age, a pressor dose of angiotensin II resulted in enhanced upregulation of mRNA expression of RAAS components and proinflammatory cytokines in the lamina terminalis and paraventricular nucleus and an augmented pressor response in male offspring of hypertensive dams. The augmented blood pressure change and most of the increases in gene expression in the offspring were abolished by either renal denervation or captopril. The results suggest that maternal hypertension during pregnancy enhances pressor responses to angiotensin II through overactivity of renal nerves and the RAAS in male offspring and that upregulation of the brain RAAS and proinflammatory cytokines in these offspring may contribute to maternal gestational hypertension-induced sensitization of the hypertensive response to angiotensin II. (Hypertension. 2017;69:669-677. DOI: 10.1161/HYPERTENSIONAHA.116.08597.) • Online Data Supplement

Key Words: brain □ denervation □ hypertension, □ inflammation □ prenatal programming □ renin-angiotensin system

Preeclampsia and gestational hypertension are common conditions affecting 5% to 7% of all pregnancies. Multiple case-control and cohort studies have demonstrated that offspring of hypertensive pregnancies have higher blood pressure (BP) in childhood and adolescence and are at increased risk of developing adult hypertension. Studies investigating the mechanisms that contribute to the development of hypertension in the offspring of mothers with preeclampsia and gestational hypertension found that male adolescents had increased aldosterone levels, a trend for increased circulating renin activity and increased sympathetic activity before and during isometric exercise. These results suggest the renin–angiotensin–aldosterone system (RAAS) and sympathetic nerve activity (SNA) are involved in the development of higher BP in the offspring of mothers with high BP during pregnancy.

Animal experiments have shown that uteroplacental insufficiency, protein restriction, chronic secondary hypertension, or glucocorticoid treatment during pregnancy leads to hypertension in the offspring. Consistent with the RAAS and SNA playing a role in mediating the fetal programming of hypertension, several studies have demonstrated that both captopril, an angiotensin-converting enzyme-1 (ACE1) inhibitor, and losartan, an angiotensin II (ANG II) receptor blocker, administered in the drinking water reduced increased BP in the offspring of dams fed low-protein diet and of spontaneously hypertensive rats, whereas renal denervation (RD) abolished hypertension in the offspring from diabetic rats or from pregnant rats with reduced uterine perfusion. Moreover, Mizuno et al demonstrated that the RAAS contributes to the enhancement of the renal...
sympathetic and pressor responses to physical stress in male offspring exposed to maternal protein restriction. Recent studies also indicate that activation of inflammatory pathways is present early in the kidney of offspring from diabetic dams and that early life nuclear factor-kB dyshomeostasis in conduit arteries induced by prenatal inflammatory exposure plays a role in the development of hypertension through triggering RAAS overactivity. Most of these previous studies have focused on the roles of the peripheral RAAS and SNA in the development of hypertension in prenatally programmed hypertensive rats. Whether the RAAS, proinflammatory cytokines, or SNA in the central nervous system (CNS) is affected during the prenatal period to predispose the offspring to higher BP is not clear.

Recently, a link between the peripheral RAAS activated in hypertension and the CNS as a source of neurohumoral drive has been identified. In this context, structures associated with the lamina terminalis (LT) and hypothalamic paraventricular nucleus (PVN) have emerged as sites that sense and process information related to humoral signals generated peripherally in response to challenges leading to augmented sympathetic drive and hypertension. An earlier study by Pladys et al demonstrated that intracerebroventricular injection of an ACE inhibitor or an ANG II receptor type 1 (AT1R) antagonist significantly reduced BP of fetal protein-restricted offspring. Expression of the AT1R in the subfornical organ and the vascular organ of the LT was increased in these offspring. These data implicate the brain RAAS in hypertension associated with antenatal nutrient deprivation.

It has been shown that the risk to the offspring is graded and greatest in those whose mothers had more severe hypertensive signs, such as early-onset hypertension or preeclampsia. This phenomenon was further confirmed in a recent study by Staley et al who compared the BP of offspring from mothers with hypertension present at the start of pregnancy with those from mothers with pregnancy-induced hypertension or preeclampsia. These investigators found that a higher BP in early pregnancy was associated with higher BP during childhood in the offspring.

In previous studies in adult animals, we used an induction–delay–expression experimental design to study sensitization of the hypertensive response. In these studies, during induction rats were exposed over a relatively short period of time to various types of challenges that did not produce by themselves any sustained effect on BP. Then, after a period of delay, a sensitized hypertensive response to a slow-pressor dose of ANG II was observed in these rats when challenged during a period of expression. The present study addressed the question of the permanence of sensitization of the hypertensive response and associated CNS changes in expression of RAAS components and proinflammatory cytokines. We did this by inducing sensitization of the offspring during the prenatal period by subjecting pregnant dams to ANG II–elicited hypertension. Expression of the sensitized hypertensive response was studied when the offspring were adults, whereas changes in the mRNA expression of components of the RAAS and of proinflammatory cytokines were primarily studied in the offspring at the time of weaning and as adults. We also determined whether RD or blockade of the RAAS reversed the sensitization and whether these effects were related to changes in the RAAS and proinflammatory cytokines in forebrain cardiovascular regulatory nuclei associated with the LT and PVN.

### Methods

Twenty-eight female and 28 male rats (Sprague–Dawley, 10-week-old, Harlan) were used for breeding. The dams were chronically instrumented with telemetry probes (TA11PA-C40, DSI) through the femoral artery for continuous monitoring of mean arterial pressure (MAP) and heart rate (HR). After baseline MAP and HR recordings were made, female dams were given vehicle (saline) or ANG II (0.01, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, 25.6 mg/kg/min, model 2002, 2 weeks; Alzet) and to evaluate the effects of either RD or ACE inhibition on sensitization. The RD experiments included 4 groups: (1 and 2) offspring of NT dams and HT dams were used to determine whether maternal hypertension during pregnancy sensitized the hypertensive response to slow-pressor doses of ANG II (0.01, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, 25.6 mg/kg/min, model 2002; 2 weeks; Alzet) and to evaluate the effects of either RD or ACE inhibition on sensitization. The RD experiments included 4 groups: (1) offspring of NT dam with sham surgery plus ANG II (NT dam offspring/sham+ANG II; n=6); (2) offspring of HT dam with sham surgery plus ANG II (HT dam offspring/sham+ANG II; n=6); and (3) offspring of NT dam with sham surgery plus ANG II (NT dam offspring/sham+ANG II; n=6). The ACE inhibition experiments were performed on 2 groups: (1) offspring of NT dam offspring/sham+ANG II (NT dam offspring/sham+ANG II; n=6). The ACE inhibition experiments were performed on 2 groups: (1) offspring of NT dam offspring/sham+ANG II (NT dam offspring/sham+ANG II; n=6); (2) offspring of HT dam with sham surgery plus ANG II (HT dam offspring/sham+ANG II; n=6). The ACE inhibition experiments were performed on 2 groups: (1) offspring of NT dam offspring/sham+ANG II (NT dam offspring/sham+ANG II; n=6) and (2) offspring of HT dam with sham surgery plus ANG II (HT dam offspring/sham+ANG II; n=6). The ACE inhibition experiments were performed on 2 groups: (1) offspring of NT dam offspring/sham+ANG II (NT dam offspring/sham+ANG II; n=6) and (2) offspring of HT dam with sham surgery plus ANG II (HT dam offspring/sham+ANG II; n=6). The ACE inhibition experiments were performed on 2 groups: (1) offspring of NT dam offspring/sham+ANG II (NT dam offspring/sham+ANG II; n=6) and (2) offspring of HT dam with sham surgery plus ANG II (HT dam offspring/sham+ANG II; n=6). The ACE inhibition experiments were performed on 2 groups: (1) offspring of NT dam offspring/sham+ANG II (NT dam offspring/sham+ANG II; n=6) and (2) offspring of HT dam with sham surgery plus ANG II (HT dam offspring/sham+ANG II; n=6). The ACE inhibition experiments were performed on 2 groups: (1) offspring of NT dam offspring/sham+ANG II (NT dam offspring/sham+ANG II; n=6) and (2) offspring of HT dam with sham surgery plus ANG II (HT dam offspring/sham+ANG II; n=6). The ACE inhibition experiments were performed on 2 groups: (1) offspring of NT dam offspring/sham+ANG II (NT dam offspring/sham+ANG II; n=6) and (2) offspring of HT dam with sham surgery plus ANG II (HT dam offspring/sham+ANG II; n=6).

### Data Analysis

MAP and HR are presented as mean daily values. Differences for MAP and HR were calculated for each animal based on the mean of the 5-day baseline subtracted from the mean of the final 5 days of ANG II treatment. Two-way ANOVA for the experimental groups was then conducted on daily MAP, HR, or the means of calculated differences. After establishing a significant ANOVA, post hoc analyses were performed with Tukey multiple comparison tests between pairs of mean changes. One-way ANOVAs and post hoc Tukey analyses were used to analyze the differences in....

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*Note: The text continues with further details, experimental procedures, and data analysis specific to the study described.*
norepinephrine content, mRNA, or protein expression of the RAAS components and proinflammatory cytokines in the LT and PVN. All data are expressed as means±SE. Statistical significance was set at \( P<0.05 \).

**Additional Methods**

For additional methods, please see the online-only Data Supplement.

**Results**

**Effects of Hypertension During Gestation on the Dams and Neonates**

There were no differences in baseline BP and HR between NT and HT dams. During pregnancy, BP was significantly increased in dams receiving ANG II infusions when compared with female treated with saline, but there were no differences in HR (Figure 1A and 1B). ANG II–induced hypertension did not impair reproduction, and all females conceived. Fourteen hypertensive dams produced a total of 147 pups, including 71 males and 76 females, whereas 14 normotensive dams produced a total of 166 pups, including 77 males and 89 females. Although there was a tendency for the normotensive dams to have more pups than the hypertensive dams, there were no significant differences in litter sizes (10.7±1.4 pups versus 11.8±0.6 pups; \( P>0.05 \)) or birthweights of the pups (5.98±0.44 g/pup versus 5.96±0.36 g/pup; \( P>0.05 \)) from HT versus NT dams. However, there was a declining trend in daily MAP of the final 5 days of pregnancy of baseline recording to recovery from delivery (n=6/group; +ANG II, beginning of ANG II infusion, −ANG II, stop of ANG II infusion; #\( P<0.05 \) vs saline-treated dams).

**mRNA and Protein Expression of RAAS Components and Proinflammatory Cytokines in the Brains of Young and Adult Offspring Without ANG II Treatment**

In brain tissues collected from pups at 3 weeks of age, offspring from HT dams exhibited increased mRNA expression of RAAS components (ACE1 in the LT and AT1R in the PVN) and the proinflammatory cytokines (ie, tumor necrosis factor-\( \alpha \) [TNF-\( \alpha \)], interleukin-6 [IL-6], interleukin-1\( \beta \) [IL-1\( \beta \)] in both the LT and the PVN) when compared with offspring of NT dams (\( P<0.05 \); Figure 2A and 2B). In contrast, in brain tissues collected at 10 weeks of age, the offspring of HT dams showed upregulation of mRNA expression of the RAAS components (renin, angiotensinogen [AGT], AT1R, ACE1, and mineralocorticoid receptor [MR]) only in the LT and of the proinflammatory cytokines (ie, TNF-\( \alpha \), IL-6, and IL-1\( \beta \)) only in the PVN (\( P<0.05 \); Figure 2C and 2D), which were significantly inhibited by either RD or captopril treatment. Western blotting analysis confirmed the upregulated effects of maternal hypertension during pregnancy on genomic expression in the adult offspring by determining the protein expression for one of RAAS components (ACE1) or for one of the components of TNF-\( \alpha \) proinflammatory pathway (TNF-\( \alpha \) receptor I). The increased protein expression of ACE1 in the LT and TNF-\( \alpha \) receptor I in the PVN was evident (\( P<0.05 \); Figure 2E and 2F).

**Effects of Maternal Hypertension on the Hypertensive Response of Adult Offspring and Effects of RD on Sensitization of ANG II–Induced Hypertension**

At 10 weeks of age (baseline), there were no significant differences in MAP (108.6±1.6 versus 110.2±1.9 mm Hg) and HR (345.7±8.9 versus 342.8±10.5 beats/min) between RD sham offspring of NT and HT dams. However, during infusion of the slow-pressor dose of ANG II, the male offspring of HT dams showed a significantly enhanced hypertensive response (43.7±3.1 mm Hg) compared with the male offspring of NT dams (23.6±5.4 mm Hg; \( P<0.05 \); Figure 3A and 3B).

RD significantly reduced baseline MAP in both offspring of NT (108.6±1.6 to 103.2±1.2 mm Hg; \( P<0.05 \); Figure 3A) and HT dams (110.2±1.9 to 103.7±0.6 mm Hg; \( P<0.05 \); Figure 3A), but did not alter baseline HR. Bilateral RD significantly reduced the ANG II–elicited pressor response, including daily MAP, and the means of difference score for MAP, in the offspring of the HT dams (28.7±4.7 mm Hg; \( P<0.05 \); Figure 3A and 3B). In RD offspring of NT dams, a declining trend in daily MAP of the final 5 days of ANG II treatment was noticed when compared with sham offspring of NT dams. However, RD did not result in a significant reduction in the ANG II–induced pressor response because of a lower baseline BP produced by RD (the means of difference score for MAP, 17.2±5.5 mm Hg; \( P>0.05 \); Figure 3A and 3B).

**Effect of RD on ANG II–Induced mRNA Expression of RAS Components and Proinflammatory Cytokines in the Brain**

In LT tissues, the slow-pressor ANG II infusion resulted in a significant increase in mRNA expression of ACE1 and proinflammatory cytokines (ie, TNF-\( \alpha \), IL-6, and IL-1\( \beta \)) in the offspring of NT dams when compared with the saline group (\( P<0.05 \); Figure 4A). The mRNA expression of renin, AGT, AT1R, and MR was not higher after ANG II (\( P>0.05 \)). Compared with the NT dam offspring treated with ANG II,
the offspring of HT dams exhibited enhanced expression of AT1R, MR, ACE1, TNF-α, and IL-6 (*P<0.05) but not IL-1β (P>0.05) after ANG II treatment (Figure 4A). RD significantly attenuated the increased mRNA expression of ACE1, MR, TNF-α, and IL-6 (P<0.05), but AT1R and IL-1β expression remained high in both offspring of NT and HT dams after slow-pressor ANG II administration (P>0.05). Even mRNA expression of AT1R was upregulated in RD offspring of NT dams when compared with sham RD offspring of NT dams (P<0.05; Figure 4A).

In PVN tissues, ANG II infusion elicited a significant increase in the mRNA expression of renin, AGT, ACE1, and IL-6 (P<0.05) but not MR and TNF-α when compared with saline group. The mRNA expression of MR and TNF-α was not higher after ANG II (P>0.05). The offspring of HT dams showed enhanced mRNA expression of renin, ACE1, MR, TNF-α, IL-1β, and IL-6 after ANG II treatment (P<0.05; Figure 4B). RD significantly attenuated increased gene expression produced by the slow-pressor ANG II infusion (P<0.05; Figure 4B) in offspring of both NT and HT dams.

Effect of Captopril Treatment on Prenatal Gestational Hypertension-Induced Sensitization of ANG II Hypertension

Captopril administered in the drinking water after weaning significantly reduced baseline MAP in both offspring of NT (108.6±1.4 to 95.8±1.7 mmHg; P<0.05; Figure 5A) and HT dams (107.8±1.1 to 96.5±3.4 mmHg; P<0.05; Figure 5A) but had no effect on basal HR. Furthermore, the captopril...
treatment significantly attenuated the enhanced hypertensive response produced by the slow-pressor dose of ANG II compared with animals without captopril treatment in the offspring of HT dams (40.3±4.8 versus 25.6±5.8 mm Hg; *P<0.05; Figure 5A and 5B). In captopril-treated offspring from NT dams, a declining trend in daily MAP of the final 5 days of ANG II treatment was evident when compared with the non–captopril-treated offspring of NT dams. However, the alteration in the ANG II–induced pressor response produced by captopril treatment was not significant (the means of difference score for MAP, 18.7±1.7 versus 20.1±3.6 mm Hg; P>0.05; Figure 5A and 5B).

Effect of Captopril Treatment on ANG II–Induced mRNA Expression of RAS Components and Proinflammatory Cytokines in the Brain

In LT tissues, captopril treatment significantly attenuated ANG II–induced increases in mRNA expression of TNF-α and IL-1β (*P<0.05; Figure 6A) but not ACE1 and IL-6 (P>0.05) in offspring of NT dams. mRNA expression of AT1R was also upregulated in the captopril-treated offspring of NT dams. In contrast, the enhanced mRNA expression of ACE1, MR, TNF-α, IL-1β, and IL-6 after ANG II were significantly reduced by captopril treatment in the offspring of HT dams (P<0.05; Figure 6A).

In PVN tissues, the slow-pressor ANG II infusion elicited enhanced increases in the mRNA expression of renin, ACE1, MR, TNF-α, IL-6, and IL-1β in the offspring of HT dams when compared with those in the offspring of NT dams (P<0.05; Figure 6B). Captopril treatment reversed most of the increased expression in both offspring of NT and HT dams.

Surprisingly, in the offspring of NT dams, captopril treatment did not attenuate the increased mRNA expression of AGT in the PVN, but upregulated mRNA expression of MR...
in the PVN and mRNA expression of AT1R in both the LT and the PVN after delivery of the slow-pressor dose of ANG II. In contrast, the increased mRNA expression of AGT and MR in the PVN and AT1R in the LT was not altered, and mRNA expression of AT1R in the PVN was upregulated in the offspring of HT dams (Figure 6A and 6B).

**Discussion**

The major findings of the present study are the following: (1) The offspring of HT dams exhibited normal BP at 10 weeks of age but had a significantly enhanced hypertensive response to a slow-pressor dose of ANG II; (2) before challenging adult offspring with a slow-pressor dose of ANG II, the offspring of HT dams (Figure 6A and 6B) of adult offspring with or without captopril (Cap) after saline or angiotensin (ANG) II administration (n=6/group; P<0.05 vs NT dam-offspring+ANG II; #P<0.05, HT dam-offspring+ANG II vs NT dam-offspring+ANG II; † or ‡P<0.05 Cap-treated NT- or HT-dam offspring+ANG II vs non–Cap-treated NT or HT-dam offspring+ANG II, respectively). ACE1 indicates angiotensin-converting enzyme-1; AGT, angiotensinogen; AT1R, angiotensin II receptor; and TNF-α, tumor necrosis factor-α.

The predisposition of the offspring of HT dams to display an enhanced hypertensive response seems to be similar to sensitization of this response produced by previous challenges that we have found in adult animals.25–28 In these previous studies, we used an induction–delay–expression experimental design to investigate whether peripheral or central pretreatment with nonpressor doses of ANG II, aldosterone, leptin, or TNF-α, or feeding of high dietary fat sensitized the hypertensive response to a slow-pressor dose of ANG II. We found that these mild physiological or dietary challenges upregulated mRNA expression of several components of the RAAS and proinflammatory cytokines in the brain, whereas central inhibition of AT1R, MR, and inflammation reversed the changes in gene expression and prevented the sensitization of ANG II–elicited hypertension. The results suggest that the sensitization of hypertension depends on the functional integrity of the brain RAAS and proinflammatory cytokines.25–28 In the present study, induction during the prenatal period was sufficient to sensitize the hypertensive response observed during expression after an intervening 10-week postnatal delay. Also, at 10 weeks of age, before administering a pressor challenge, the adult offspring showed evidence of long-term neurochemical changes in brain regions implicated in the control of BP. The present experiments demonstrate that reprogramming of the mechanisms controlling BP during a sensitive prenatal period produces long-lasting phenotypic changes in the CNS and increased responsiveness to a hypertensinogenic challenge.

Increased basal BP has been shown in childhood and adolescence of preeclamptic or hypertensive mothers2–7 or in the offspring of rat dams with diabetes mellitus,13 protein restriction,15 and uteroplacental insufficiency16 during pregnancy. Alterations of the RAAS and proinflammatory cytokines in peripheral tissues and plasma have been implicated as important mediators in the fetal programming of increased BP.30 Washburn et al10 found increased plasma aldosterone levels in male adolescents born to mothers with preeclampsia. In animal experiments, the offspring from dams with protein restriction,11,12 diabetes mellitus,19 or inflammation20 showed significant increases in intrarenal RAAS components, such as angiotensinogen and ANG II, renal and arterial proinflammatory cytokines, and pulmonary and plasma ACE activity, which were associated with higher BP in these offspring. However, not all studies involving treatments increasing the BP of pregnant dams have reported increased basal BP in the offspring. For example, there was no increase in basal BP in adult male offspring from mothers with aldosterone-induced11 or 2-kidney, 1-wrapped hypertension12 during pregnancy. The absence of changes in resting BP in these models is similar to the finding of a normal basal BP seen in the present study.

It has been shown that hypertension is associated with exaggerated sympathetic activity which is because of an imbalance between inhibitory and excitatory mechanisms within specific areas in the CNS, including the LT and PVN, 2 forebrain regions involved in regulation of BP and sympathetic activity.22 On the basis of previous evidence, it is reasonable to hypothesize that elevated activity of RAAS components and proinflammatory cytokines in these forebrain nuclei play a pivotal role in the process of sensitization of SNA and the hypertensive response.25–28 In the present study, we found an upregulation of mRNA expression
of several RAAS components and proinflammatory cytokines in both the LT and PVN at 3 weeks of age, whereas at 10 weeks of age, upregulation of RAAS components was evident only in the LT and upregulation of proinflammatory cytokines was seen only in the PVN. It is unclear why the pattern of expression of message and protein of these RAAS components and proinflammatory cytokines changes over the ontogenetic period studied, but considering the dynamic developmental changes that occur in progressing from early childhood through puberty into adulthood, such effects might be expected. The important point to recognize is that exposure to hypertension during pregnancy results in a distinct brain phenotype, which might predispose offspring to develop high BP in response to challenges or pressor stimuli that come into play later in life. As expected, we did find that the gestational hypertension induced by a high dose of ANG II sensitized the ANG II–induced hypertension in male offspring, resulting in an augmented increase in BP.

Increased renal SNA and overactivity of RAAS and proinflammatory cytokines have been implicated as causal mechanisms in prenatal programming of hypertension. RD or chronic blockade of the RAAS and inflammation has been demonstrated to reduce elevated BP through restoration of renal and arterial function in offspring of dams after different types of insult to the mothers during pregnancy.11,12,14–16,20 Mizuno et al17,18 demonstrated that the RAAS plays a significant role not only in the generation of increased basal BP but also in the development of the enhanced renal sympathetic and pressor responses to physical stress in prenatally programmed adult hypertensive rats. On the basis of such observations, we investigated whether disrupting the integrity of the renal nerves or of the RAAS would alter the maintenance of sensitization of ANG II–induced hypertension in offspring of HT dams. The results of these experiments showed that either bilateral RD or captopril treatment in the offspring of HT dams reduced the sensitized hypertensive response to a level seen in the offspring of NT dams.

The protective effects of both RD and blockade of the RAAS were accompanied by attenuation of the enhanced expression of LT and PVN RAAS components and proinflammatory cytokines induced by either prenatal gestational hypertension or ANG II treatment. The results indicate that RD and ACE1 inhibition seem to have similar effects and that the renal nerves and the RAAS affect neural activity of cardiovascular nuclei that have implicated the sensitization of ANG II–induced hypertension.22 The central changes observed in our study are consistent with previous work showing that maternal dietary protein restriction induced an increase in brain AT1R expression in the offspring and central blockade of ACE or AT1R abolished increased BP.23 In addition, the present findings are reinforced by a recent study showing that RD ameliorated heart failure through downregulation of the brain RAAS and markers of inflammation in rats.34

In contrast to the consistency of RD and ACE1 inhibitor abrogating the sensitizing effects on BP, the alterations of mRNA expression found in the LT and PVN after 2 weeks of infusion of a slow-pressor dose of ANG II did not provide a consistent pattern of change. For example, at the end of expression, the AT1R was significantly increased in the LT in the RD offspring from NT dams and in the PVN of the RD offspring from HT dams. Also, there was the increased expression of IL-1β in the LT that was not reduced by RD in both types of offspring (Figure 4). Likewise, captopril treatment did not attenuate the increased mRNA expression of several RAAS components, and some of these RAAS components were actually upregulated (Figure 6). At present, there is no clear explanation for these changes except to speculate that the increased level of message and proteins in key brain nuclei may reflect compensatory mechanisms necessary for maintaining viable BP after RD or captopril treatment.

There are some potential limitations to our study. (1) Age may serve as an important factor determining the impact of the development of adverse cardiovascular function in offspring of dams after prenatal programming.15,16,12 We only examined basal BP and HR at 10 to 12 weeks of age in the offspring of HT dams and saw no difference from those of offspring of NT dams. Studying BP in older offspring (eg, 6–12 months of age) may show differences in basal BP and warrants further investigation. (2) The elevated maternal RAAS activity has been demonstrated to influence development of renal sympathetic nerves and contribute to programming of adult hypertension.19 Besides this, alterations in gene expression and the delayed evolution of hypertension seems to be linked to the epigenetic changes induced during pregnancy.10 Epigenetic mechanisms involved in altering brain RAAS and proinflammatory cytokines of offspring in the ANG II maternal hypertensive programming model also need to be investigated in the future. (3) We have considered the effects of maternal ANG II–induced hypertension per se, increased circulating ANG II, ANG II–induced cytokines, or combination of these factors to produce sensitization of the hypertensive response because of factors operating while the pup is in utero.30 However, because the pups were raised to the time of weaning by their natural mothers, it cannot be ruled out that maintained changes in the mothers’ physiology or behavior may also have played a role in the process of sensitization. Future studies using cross-fostering of pups need to be conducted to determine whether there are potential effects of mothering on sensitization of the hypertensive response.

**Perspectives**

The present study demonstrated the influence of prenatal hypertensive programming on BP in adult offspring. Such programming was associated with altered gene expression of the RAAS and proinflammatory cytokines in the CNS that may, at least in part, explain the increased sensitization of ANG II–induced hypertension. The protective effects of RD and RAAS blockade on the sensitization of ANG II–induced hypertension in these offspring suggest that maternal hypertension-induced sensitization of male offspring can be reversed by interventions delivered between the time of weaning and testing for the expression of the hypertensive response. This new model of sensitization opens up many possibilities to investigate interventions that will reverse presumed epigenetic and other
molecular changes that mediate nervous system plasticity and the long-term maintenance of sensitization.

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Disclosures

None.

References


Novelty and Significance

What Is New?
• These studies demonstrate that maternal hypertension during pregnancy sensitized the hypertensive response to angiotensin II in adult male offspring through upregulation of message for components of the brain renin-angiotensin-aldosterone system (RAAS) and proinflammatory cytokines. Either earlier renal denervation or prior RAAS inhibition prevents the prenatal gestational hypertension-elicited sensitization of angiotensin II hypertension.

What Is Relevant?
• The demonstration that maternal hypertension during pregnancy facilitated mRNA expression of the central RAAS components and of proinflammatory cytokines in the offspring indicates that the neurohumoral systems are likely to play an important role in the pathogenesis of prenatal programming of hypertension. Also of importance is the finding that either renal denervation or postnatal administered angiotensin-converting enzyme inhibitor reversed the effects of fetal programming of the sensitized hypertensive response.

Summary
The study indicates that prenatal gestational hypertension results in reprogramming of mechanisms involved in the control of blood pressure and that these changes are maintained for a long period time in the offspring. Overactivity of renal sympathetic nerve and RAAS, as well as altered mRNA expression of RAAS and proinflammatory cytokines, within components of a forebrain cardiovascular control network is associated with the sensitizing process.
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Methods

Animals

Twenty-eight female and twenty-eight male rats (Sprague-Dawley, 10 wk old, Harlan) were used for breeding. Parents and offspring were housed in a temperature- and humidity-controlled facility, and maintained on a 12:12-h light-dark cycle (6:00 AM to 6:00 PM). They were provided with rat chow (7013 NIH-31 modified rat diet, 0.25% NaCl) ad libitum. Under Ketamine-xylazine mixture (100 mg/kg & 10 mg/kg), The dams were chronically instrumented with telemetry probes (TA11PA-C40; DSI) through the femoral artery for continuous monitoring of mean arterial pressure (MAP) and heart rate (HR). After baseline MAP and HR recordings were made, the female dams were anesthetized by inhalation of isoflurane to allow the implantation of osmotic pumps for infusion of vehicle (saline) or ANG II (sc, 250 ng/kg/min, model 2004 to provide 4-week of delivery, Alzet) throughout mating and pregnancy. Osmotic pumps were depleted by 1 or 2 days after giving birth. The offspring were weighed and counted at birth and male offspring were used in all experiments. Each experimental group was composed of individual subjects that were randomly selected from different litters.

At three weeks, male offspring from normotensive (NT) or hypertensive (HT) dams were deeply anesthetized with isoflurane and then decapitated. The brains were collected for analyses of mRNA expression of the RAAS components and PICs (n=5 per group). Likewise, 10 week old offspring from NT or HT dams and offspring from HT dams with renal denervation (RD) performed at 8 weeks of age or application of ACE inhibitor in the drinking water from weaning until beginning baseline recording were also used to determine the changes in RAAS components and proinflammatory cytokines in brain (n=5 per group). The structures lying along the lamina terminalis [LT, i.e., the subfornical organ (SFO), median preoptic nucleus (MnPO), organum vasculosum of the lamina terminalis (OVLT)] and the paraventricular nucleus (PVN) were used for these analyses.

In separate functional experiments, at 10 weeks of age, male offspring of both NT dams and HT dams were used to determine whether maternal hypertension during pregnancy sensitized the hypertensive response of male offspring and to evaluate the effects of either RD or ACE inhibition on sensitization. Upon completion of the studies, the offspring were anesthetized with isoflurane and brains were harvested. In the RD study, the both kidneys were also collected. All tissues were immediately frozen in liquid nitrogen and stored at ~80 °C. The kidneys were assayed for tissue norepinephrine (NE) content; and the brains were analyzed for mRNA expression of the RAAS components and PICs in the LT and PVN.

All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and approved by the University of Iowa Animal Care and Use Committee.

RD and telemetry probe or osmotic pump implantations

At 8 weeks of age, sham or bilateral RD was performed via a midline approach in male offspring from NT or HT dams. Under a surgical microscope, the renal artery and vein were isolated, and all visible renal nerves from the length of the artery and vein were then carefully removed. The hilus of the kidney was also cleared of fat and any visible nerve fibers followed by painting the
renal artery and vein with 10% phenol in an alcohol solution to destroy the remaining nerves. After a 7-day recovery period, rats were instrumented with telemetry probes (TA11PA-C40, DSI, St. Paul, MN) through the femoral artery for monitoring of BP and HR and then infused with a slow pressor dose of ANG II for 2 weeks (120 ng/kg/min, model 2002, Alzet). This experiment included four groups: 1) offspring of NT dam with sham surgery plus ANG II (NT dam-offspring/sham+ANG II, n=6); 2) offspring of HT dam with sham surgery plus ANG II (HT dam-offspring/sham+ANG II, n=6); 3) offspring of NT dam with RD plus ANG II (NT dam-offspring/RD+ANG II, n=6); 4) offspring of HT dam with RD plus ANG II (HT dam-offspring/RD+ANG II, n=6).

**Application of ACE1 inhibitor and implantation of telemetry probes and osmotic pumps**

At weaning, pups were separated into four groups: 1 and 2) offspring of NT or HT dams, tap water alone (vehicle) was given until baseline recording and then ANG II infusion at 10 week old (NT dam-offspring/vehicle+ANG II, HT dam-offspring/vehicle+ANG II, n=6 per group); 3 and 4) offspring of NT or HT dams, an ACE1 inhibitor, captopril (Cap, 0.5 mg/ml drinking water), was given in drinking water continuously until baseline recording began and then ANG II infusion at 10 weeks of age (NT dam-offspring/Cap+ANG II, HT dam-offspring/Cap+ANG II, n=6 per group).

At week 9, these offspring were instrumented with telemetry probes. After one week for recovery, the treatment with captopril was stopped and 5 days of baseline recording was conducted. After this, sc infusions with a slow-pressor dose of ANG II (120 ng/kg/min, model 2002, Alzet) were carried out.

**Real-time RT-PCR analysis**

The total RNA was extracted using RNaseasy® Mini Kit (Qiagen, Valencia, CA, USA) and reverse transcribed into cDNA. mRNA levels for RAAS components [renin, angiotensinogen (AGT), ACE1 AT1-R, mineralocorticoid receptor (MR)], proinflammatory cytokines (TNF-α, IL-1β and IL-6) and GAPDH were analyzed with SYBR Green real-time PCR. The sequences for the primers are summarized in Table S1. Real-time RT-PCR was performed with the ABI prism 7300 Sequence Detection System (Applied Biosystems, Carlsbad, CA). The values were corrected by GAPDH and the final concentration of mRNA was calculated using the formula
\[ x=2^{\Delta \Delta Ct} \]
where x=fold difference relative to control.

**Western blot analysis**

The LT or PVN tissues were homogenized in lysis buffer and the protein concentration in the supernatant was measured with the BCA protein assay Kit (Pierce, Rockford, IL, USA). Equivalent amounts of protein were separated on 12% SDS-polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Millipore Corporation, Bedford, MA, USA). The membranes were blocked with 5% nonfat dry milk and then incubated using primary antibody at 4°C overnight. The primary antibodies used in this study were anti-ACE1 (sc-20791, Santa Cruz Biotechnology Inc), anti-TNF-α receptor I (ab19139, Abcam) and anti- β-actin (Cat. 4970, Cell Signaling Inc). After three washing, the membranes were incubated with horseradish peroxidase-conjugated second antibody (sc-2004, Santa Cruz Biotechnology Inc) for 1 h at room temperature. The signal was visualized using an enhanced chemiluminescence (ECL) detection
system (Amersham) and densities of the immunobands were quantitated using NIH ImageJ software (Bethesda, MD, USA). All data were corrected by β-actin.

**RD confirmation**

Samples of renal tissue were homogenized and processed to perform an ELISA (GenWay Biotech, San Diego, CA) for NE. To normalize the NE content to protein concentration, a protein assay kit (Pierce, Rockford, IL) was used according to the manufacturer’s directions. NE levels of bilateral kidney tissues were compared between the sham groups and RD groups.

**Results**

**Kidney Norepinephrine Content**

Kidneys were collected at the end of infusions to determine the amounts of NE to verify the success of RDs. RD abolished the contents of the NE in the denervated kidneys (Fig. S2).

**Changes in heart rate (HR) after ANG II infusion in male offspring with renal denervation or ACE inhibition**

ANG II administration induced comparable decreases in HR in both sham and RD offspring from NT and HT dams (Fig. S3A and Fig. S3B). Likewise, ANG II infusion also resulted in comparable decreases in HR in both vehicle and captopril-treated offspring from NT and HT dams (Fig. S4A and Fig. S4B).
Table S1. Primer Sequences for Real Time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Product size (bp)</th>
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<tbody>
<tr>
<td>GAPDH</td>
<td>TGACTCTACCCACGGCAAGTCAA</td>
<td>ACGACATACTCAGCACCAGCATCA</td>
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<tr>
<td>Renin</td>
<td>CTGCCACCTTGTGTTGTGAG</td>
<td>ACCTGGCTACAGTTCAACACG</td>
<td>154</td>
</tr>
<tr>
<td>AGT</td>
<td>TCCCTCGCTCTCTGGACTTA</td>
<td>AAGTGAACGTAGTGTTGAAA</td>
<td>209</td>
</tr>
<tr>
<td>ACE1</td>
<td>GTGGTTGTGGAACGATAACGC</td>
<td>CTTCTTTATGATCCGCTTGA</td>
<td>187</td>
</tr>
<tr>
<td>AT1R</td>
<td>CTCAAGCCTGTCTACGAAAATGAG</td>
<td>GTGAACTGTGCTTTTGGTCGT</td>
<td>188</td>
</tr>
<tr>
<td>MR</td>
<td>GCCCGCAAATCTCAACAACCTCA</td>
<td>TTAGGGAAGGAAACGTCGTGAGCA</td>
<td>235</td>
</tr>
<tr>
<td>IL-1β</td>
<td>AGCAACGAACAAAATCTTCT</td>
<td>GAAAGACCGTCCTTCC</td>
<td>209</td>
</tr>
<tr>
<td>IL-6</td>
<td>GCCTATTGAAATCTCCTCTG</td>
<td>GGAAGTTGGGGTACCGAGGA</td>
<td>160</td>
</tr>
<tr>
<td>TNF-α</td>
<td>GCCGATTGTGCCAATTC</td>
<td>AAGTAGACCTGACCCGGACTC</td>
<td>209</td>
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</tbody>
</table>

AGT, angiotensinogen; ACE1, angiotensin converting enzyme 1; AT1R, angiotensin II type 1 receptor; MR, mineralocorticoid receptor; IL-1β, interleukin-1β; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α.

Table S2. Averaged Ct values of renin-angiotensin-aldosterone system components and proinflammatory cytokines in the lamina terminalis (LT) and the paraventricular nucleus (PVN) in 10-week old offspring of normotensive (NT) and hypertensive (HT) dams.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Offspring of NT dams</th>
<th>Offspring of HT dams</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LT (Ct)</td>
<td>PVN (Ct)</td>
</tr>
<tr>
<td>Renin</td>
<td>29.48±0.29</td>
<td>30.61±0.43</td>
</tr>
<tr>
<td>AGT</td>
<td>21.68±0.23</td>
<td>22.11±0.46</td>
</tr>
<tr>
<td>AT1R</td>
<td>28.29±0.31</td>
<td>27.88±0.46</td>
</tr>
<tr>
<td>ACE1</td>
<td>24.99±0.37</td>
<td>27.41±0.43</td>
</tr>
<tr>
<td>MR</td>
<td>25.53±0.53</td>
<td>26.32±0.38</td>
</tr>
<tr>
<td>IL-1β</td>
<td>32.49±0.27</td>
<td>33.50±0.31</td>
</tr>
<tr>
<td>IL-6</td>
<td>31.90±0.21</td>
<td>33.37±0.42</td>
</tr>
<tr>
<td>TNF-α</td>
<td>32.82±0.29</td>
<td>33.93±0.44</td>
</tr>
<tr>
<td>GAPDH</td>
<td>17.64±0.08</td>
<td>17.63±0.13</td>
</tr>
</tbody>
</table>

AGT, angiotensinogen; AT1R, angiotensin II type 1 receptor; ACE1, angiotensin converting enzyme 1; MR, mineralocorticoid receptor; IL-1β, interleukin-1β; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α.
Figure S1. Representative time line of the study designs for reproduction of normotensive and hypertensive dams (Fig. S1A) and for evaluating the effects of either renal denervation (Fig. S1B) or ACE1 inhibitor, captopril (Cap) (Fig. S1C) on angiotensin (ANG) II-induced hypertension in adult male offspring from normotensive and hypertensive dams.
Figure S2. Effect of sham surgery (Sham) and renal denervation (RD) on norepinephrine (NE) content in left and right kidneys (Sham n=12; RD n=12; * p<0.05 vs. Sham).
Figure S3. Bradycardiac effects induced by angiotensin (ANG) II in adult offspring with sham or renal denervation (RD) from normotensive (NT) and hypertensive (HT) dams (Fig. S3A). Figure S3B shows the change in heart rate (HR) after ANG II infusion in all groups (n=6/group).
Figure S4. Bradycardiac effects induced by angiotensin (ANG) II in adult offspring with or without ACEI inhibitor (vehicle), captopril (Cap), treatment from normotensive (NT) and hypertensive (HT) dams (Fig. S4A). Figure S4B shows the changes in and heart rate (HR) after ANG II infusion in all groups (n=6/group).