Renal Denervation Abolishes Hypertension in Low-Birth-Weight Offspring From Pregnant Rats With Reduced Uterine Perfusion

Barbara T. Alexander, Andrew E. Hendon, Geoffrey Ferril, Terry M. Dwyer

Abstract—Low birth weight is a risk factor for the subsequent development of hypertension in humans. We previously reported that reduced uterine perfusion in the pregnant rat results in growth-restricted offspring predisposed to the development of hypertension. The purpose of this study was to determine whether the sympathetic nervous system plays a role in mediating hypertension in this model of low birth weight. Weight at birth was significantly decreased in male growth-restricted offspring (5.9±0.1 grams) as compared with male control offspring (6.5±0.2 grams; P<0.05). At 10 weeks of age, growth-restricted offspring and control offspring were randomly assigned to either an intact group (sham-denervated) or a group subjected to bilateral renal denervation. For sham-denervated offspring, mean arterial pressure was significantly elevated in growth-restricted offspring (145±4 mm Hg; n=7) as compared with control offspring (134±3 mm Hg; P<0.05; n=9) at 12 weeks of age. Bilateral renal denervation resulted in a marked reduction in arterial pressure in growth-restricted offspring (125±3 mm Hg; P<0.01; difference of 20 mm Hg versus sham growth-restricted; n=8) but no significant decrease in control offspring (127±3 mm Hg; difference of 7 mm Hg versus sham control; n=9). Adequacy of renal denervation was verified by >90% reduction in renal norepinephrine content. Therefore, these findings indicate the renal nerves play an important role in mediating hypertension in adult growth-restricted offspring. (Hypertension. 2005;45[part 2]:754-758.)

Key Words: hypertension, pregnancy ■ rats ■ renal nerves ■ denervation ■ sympathetic nervous system

Hypertension is a multifactorial disorder thought to result from both genetic and environmental factor interactions. Recent epidemiological studies1-2 suggest that a predisposition for development of hypertension may be programmed by factors initiated in utero,3 an observation supported by many animal studies.4-7 Fetal malnutrition limits fetal growth and results in small-for-gestational-age newborns.8,9 Findings from both epidemiological and animal studies suggest that fetal malnutrition may also program or permanently alter fetal structure and physiology, resulting in an increased risk for development of hypertension and cardiovascular disease.3,10-12 However, the mechanisms mediating low birth weight (LBW) and hypertension remain to be elucidated.

In humans, few studies have examined the relationship between the sympathetic nervous system (SNS) and LBW, and controversy exists with regards to whether LBW individuals are associated with increases13-15 or decreases in sympathetic activity.16 Evidence for alterations in the SNS is noted in some animal models of intrauterine growth restriction (IUGR), because plasma levels of circulating catecholamines are increased.17-21 This observation has been noted in growth-restricted offspring from pregnant rats fed a protein-restricted diet during gestation,17 in a naturally occurring model of IUGR using piglets,18,19 and in a model of IUGR induced by placental insufficiency in the rat20 and in sheep.21 Additionally, hypoxia during fetal development leads to increased sympathetic innervation.22,23 However, no further assessment of the SNS in association with IUGR has been examined.

Nutrient and oxygen supply limitations are the components of the intrauterine environment that limit fetal growth and result in small-for-gestational-age newborns. Because IUGR within the Western world is more likely the result of reduced uterine perfusion,24 we have developed a model of fetal programming induced by placental insufficiency caused by hypoxia and fetal undernutrition.25 Using this model, we previously reported that reduced uterine perfusion initiated at day 14 of gestation in the rat results in growth-restricted offspring that are hypertensive as early as 4 weeks of age.25 Furthermore, marked elevations in mean arterial pressure (MAP) remain evident in male growth-restricted offspring at 12 weeks of age. Because increased renal sympathetic nerve activity appears to be a causal mechanism in primary hypertension,26 we determined the role of the renal nerves in...
mediating the hypertension observed in adult male growth-restricted offspring, a product of placental insufficiency.

Methods
All experimental procedures were in accordance with National Institutes of Health guidelines with approval by the Animal Care and Use Committee at the University of Mississippi Medical Center. Rats were housed in a temperature-controlled room (23°C) with a 12:12-hour light/dark cycle with food and water available ad libitum. Timed pregnant Sprague Dawley rats were purchased from Harlan Inc (Indianapolis, Ind). At day 14 of gestation, rats destined for reduced uterine perfusion were clipped as described. All dams were allowed to deliver at term, with birth weight recorded within 12 hours. At this time, a control litter was size-matched per growth-restricted litter, with a minimum litter size of 8 pups. Animals were weighed twice weekly. Pups were weaned at 3 weeks of age. Bilateral renal denervation (RDNX) or sham denervation was performed as described at 10 weeks of age. Male offspring from 8 control pregnant and 6 reduced uterine perfusion pregnant litters were randomly assigned into 4 groups: control sham (n=9), control bilateral RDNX (n=9), growth-restricted sham (n=7), and growth-restricted bilateral RDNX (n=8). MAP was determined simultaneously in conscious chronically instrumented control offspring and growth-restricted offspring at 12 weeks of age.

Reduced Uterine Perfusion in the Pregnant Rat
Reduced placental perfusion was used for induction of IUGR. As previously described, silver clips were slipped around the lower abdominal aorta and on both branches of the ovarian arteries at day 14 of gestation.25 As described previously, IUGR was not present in sham-operated control pregnant rats.25

Acute Arterial Pressure Measurements in Conscious Rats
Rats were surgically instrumented with a carotid arterial catheter, and MAP was measured 2 days after surgery as previously described.25

Isolation of Total Cellular Proteins and Western Blot Analyses
Total cellular proteins were quantitated by Western blot analysis as previously described.27 Anti-tyrosine hydroxylase polyclonal antibody (Chemicon, Temecula, Calif) was the primary antibody for quantitation of tyrosine hydroxylase, anti-actin (Amersham, Arlington Heights, Ill) was the internal control, and horseradish peroxidase-conjugated anti-host–specific IgG (Amersham, Arlington Heights, Ill) antibodies were secondary antibodies. Detection was by chemiluminescence (ECL Plus kit; Amersham), quantitation by densitometry (BioRad, Richmond, Va), control (n=9), and growth restriction (n=8).

Bilateral Renal Denervation
Rats were anesthetized with isoflurane as described previously.25 After a midline abdominal incision, renal denervation was initiated by stripping nervous and connective tissues from the renal arteries and veins, followed by coating of these vessels with 10% phenol in alcohol. Sham-operated rats received a similar midline abdominal incision, but the renal nerves were left intact.

Measure of Renal Norepinephrine Content
Completeness of renal denervation was determined by measurement of renal norepinephrine content from kidneys, quick-frozen, and stored at −80°C. Kidneys were homogenized in chilled 0.1 N perchloric acid. Norepinephrine was extracted using alumina and measured by reverse-phase high-performance liquid chromatography using an analytical column (ESA HR-80) optimized for electrochemical detection (ESA Coulochem III).

Statistical Analyses
GB-STAT version 6.5 was used for all statistical analysis. All data are expressed as mean±SEM. Comparisons of control offspring with growth-restricted offspring were analyzed using factorial ANOVA followed by Scheffe test. A value of P<0.05 was considered statistically significant.

Results

Birth and Body Weights
Weight at birth was significantly reduced in offspring from reduced uterine perfusion dams (5.9±0.1 grams) as compared with offspring from control pregnant dams (6.5±0.2 grams; P<0.05). This observation was evident in animals selected for sham denervation (5.7±0.1 versus 6.6±0.01 grams; P<0.05; growth-restricted versus control, respectively) and animals selected for bilateral renal denervation (5.9±0.2 versus 6.7±0.02 grams; growth-restricted versus control, respectively). Before initiation of either sham denervation or bilateral renal denervation, average body weight remained significantly decreased in growth-restricted offspring as compared with control offspring (Table). Weight gain from 10 to 12 weeks of age was similar for all growth-restricted offspring, an average of 120 grams, both sham-denervated and bilateral renal denervated; weight gain was similar for all control offspring, ~80 grams, both sham-denervated and bilateral renal-denervated (Table). After measure of MAP at 12 weeks of age, body weight was similar in the sham-denervated group on comparison of growth-restricted offspring to control offspring (Table); however, body weight was reduced in the bilateral renal-denervated group on comparison of growth-restricted offspring to control offspring (Table). Thus, although average weight gain was similar for all growth-restricted offspring, both sham-denervated and bilateral renal-denervated, postnatal catch-up growth was evident by 12 weeks of age in the sham-denervated group. Average kidney weight did not differ on comparison of the 4 groups, and only animals with no visible

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<tr>
<td>Control</td>
<td>260±6</td>
<td>263±5*</td>
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<td>IUGR</td>
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Bilateral renal denervation (RDNX) was initiated at 10 weeks of age.

*P<0.05 vs. control sham, †P<0.05 vs. control bilateral renal denervated.
sign of renal damage caused by the bilateral renal denervation protocol were used for study (Table).

**Effect of Renal Denervation on MAP**

MAP was significantly elevated in growth-restricted sham offspring as compared with control sham offspring at 12 weeks of age (145±4 versus 134±3 mm Hg; P<0.05; growth-restricted sham versus control sham, respectively) (Figure 1). Although MAP tended to be lower in control offspring subjected to bilateral renal denervation (127±3 mm Hg, control RDNX) (Figure 1), this difference was not statistically significant. However, hypertension observed in growth-restricted offspring at 12 weeks of age was markedly attenuated by renal denervation (125±3 mm Hg, growth-restricted RDNX; P<0.05; versus growth-restricted sham) (Figure 1).

**Renal Protein Expression of Tyrosine Hydroxylase**

Renal protein expression of tyrosine hydroxylase (ratio of tyrosine hydroxylase to actin) was not significantly increased in growth-restricted (n=8) offspring as compared with control (n=9) offspring (9.9±2 versus 7.6±1 densitometric units, growth-restricted versus control, respectively) (Figure 2).

**Verification of Renal Denervation**

Renal norepinephrine was significantly decreased after renal denervation in both control (188±17 versus 9±2 ng/g; P<0.01; control sham versus control RDNX, respectively) and growth-restricted (198±12 versus 18±8 ng/g; P<0.01; growth-restricted sham versus growth-restricted RDNX, respectively) offspring.

**Discussion**

Fetal malnutrition and hypoxia, caused by either an insufficient nutrient and oxygen delivery via reduced uteroplacental perfusion or improper transport of nutrients and oxygen across the maternal placental barrier limits fetal growth and results in an increased risk for development of hypertension and cardiovascular disease. We recently reported that placental insufficiency initiated at day 14 of the 22-day gestational period in the pregnant rat results in growth-restricted offspring predisposed to the development of hypertension. Although this model of LBW induced by reduced uterine perfusion results in IUGR in both male and female offspring, only male growth-restricted offspring remain hypertensive into young adulthood, or at 12 weeks of age. Therefore, only male offspring were selected for this study. The present findings indicate that the renal nerves are essential for the maintenance of hypertension in adult male LBW offspring.

We previously reported that the marked increases in arterial pressure observed in growth restricted offspring were not associated with significant decreases in glomerular filtration rate or glomerular filtration rate normalized to kidney weight at either 8 or 12 weeks of age. Because hypertension in our growth-restricted offspring was not associated with reductions in renal hemodynamics, this suggested that increases in tubular sodium reabsorption may play an important role in mediating the hypertension observed in these growth-restricted offspring. Many known regulatory mechanisms control sodium balance and any alterations in natriuretic factors, such as nitric oxide, or antinatriuretic factors, such as the renal sympathetic nervous system and/or angiotensin II, can result in abnormal pressure natriuresis and hypertension. Thus, although this previous study provided support for an inverse relationship between birth weight and hypertension, the mechanisms linking this inverse relationship between LBW and hypertension have yet to be fully elucidated. Therefore, the purpose of this study was to examine one such regulatory system that may be involved in mediating the hypertension observed in our model of IUGR, the SNS.

In animal models of IUGR induced by an adverse fetal environment, increased levels of circulating catecholamines have been observed. To test the hypothesis that the renal nerves play a role in mediating the hypertension observed in adult growth-restricted offspring, we performed bilateral renal denervation at 10 weeks of age, or 2 weeks before measurement of MAP. MAP, determined in conscious chronically instrumented animals at 12 weeks of age, was significantly decreased in renal-denervated growth-restricted offspring as compared with the sham-denervated growth-restricted offspring (P<0.01; Δ 20 mm Hg). The effect of renal denervation appeared to be specific to growth-restricted
offspring, because bilateral renal denervation did not signif-
imcantly decrease MAP in renal-denervated control offspring as compared with sham-denervated control offspring (Δ 7 mm Hg). Therefore, these results indicate that the renal nerves may play an important role in mediating the hyper-
tension observed in adult growth-restricted offspring because bilateral renal denervation completely abolished the hypertension.

Tyrosine hydroxylase, the rate-limiting enzyme in the biosynthesis of catecholamines, or norepinephrine, dopamine and epinephrine, has been used as an indirect measure of catecholamine production. No significant increase in renal protein expression of tyrosine hydroxylase was observed on comparison of growth-restricted offspring to control offspring (Figure 2). However, in some instances elevations in tyrosine hydroxylase have correlated with increased levels of norepinephrine and hypertension, but in other instances decreased levels of tyrosine hydroxylase activity have been associated with higher blood pressure. Thus, although renal tyrosine hydroxylase protein expression was not significantly elevated at 12 weeks of age in growth-restricted offspring, blockade of the renal nerves attenuated the hypertension observed in growth-restricted offspring.

Fetal programming of the renin-angiotensin system (RAS) is suggested in a model of IUGR induced by maternal protein restriction administered during gestation. Specifically, Woods et al noted suppression of the RAS, as determined by renal renin mRNA levels, was evident at birth in growth-restricted offspring from protein-restricted dams. Furthermore, these growth-restricted offspring were hypertensive and exhibited a significant reduction in nephron number. Vehaskari et al have also examined components of the RAS in offspring from protein-restricted dams and found that plasma renin activity, although reduced in young rats, was elevated in adult rats after establishment of the hypertension. Thus, results from studies using a low-protein model of IUGR suggest that permanent alterations in both structure and physiology do occur in response to fetal undernutrition. Furthermore, a pathway for fetal programming in this animal model is suggested involving suppression of the RAS during fetal development leading to structural alterations in the kidney with hypertension, followed by activation of the RAS in the adult animal. Although fetal undernutrition results in suppression of the RAS at birth in low-protein offspring, the stimulus for activation of the RAS in adult low-protein offspring remains unknown. Our findings suggest that one possible mechanism may be increased renal sympathetic nerve activity.

Alternately, because angiotensin II stimulates the SNS, it is possible that sympathetic activation in this model of LBW is secondary to the generation of angiotensin II. Establishing the temporal interactions between the RAS and the SNS will provide insight into this issue and is a goal of future studies.

In this study, we used bilateral renal denervation to examine the role of the SNS in mediating hypertension in adult LBW offspring. Our results suggest that hypertension in LBW offspring produced in response to reduced uterine perfusion is caused by increased renal sympathetic nerve activity. However, because bilateral renal denervation results in destruction of both afferent and effenter renal nerves, we cannot exclude a role for the renal afferent nerves in mediating the hypertensive response observed in the adult LBW offspring. The afferent renal nerves may also be involved in the cause of hypertension in this model of LBW. Afferent renal innervation occurs early in fetal life, and the renal nerves are suggested to play an important role in the proper development of the kidney. Thus, in this model of reduced uterine perfusion, hypoxia may serve as a stimulus for increased renal afferent nerve activity, resulting in alterations during kidney development, leading to both structural and physiological changes linked to the increased risk for development of hypertension in the adult animal.

Perspectives
The present study examined the role of the renal nerves in mediating the sustained hypertension observed in adult growth-restricted offspring. In this model of IUGR-induced hypertension, marked elevations in MAP are apparent as early as 4 weeks of age. Whether increased renal sympathetic nerve activity is an initiating event in the evolution of the hypertension or a secondary response in this model merits further investigation.

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References
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