Placental insufficiency results in temporal alterations in the renin angiotensin system in male hypertensive growth restricted offspring

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Submitted 13 October 2006; accepted in final form 25 May 2007

Grigore D, Ojeda NB, Robertson EB, Dawson AS, Huffman CA, Bourassa EA, Speth RC, Brosnihan KB, Alexander BT. Placental insufficiency results in temporal alterations in the renin angiotensin system in male hypertensive growth restricted offspring. Am J Physiol Regul Integr Comp Physiol 293: R804–R811, 2007. First published May 30, 2007; doi:10.1152/ajpregu.00725.2006.—Reduced uterine perfusion initiated in late gestation in the rat results in intrauterine growth restriction (IUGR) and development of hypertension by 4 wk of age. We hypothesize that the renin angiotensin system (RAS), a regulatory system important in the long-term control of blood pressure, may be programmed by placental insufficiency and may contribute to the etiology of IUGR hypertension. We previously reported that RAS blockade abolished hypertension in adult IUGR offspring; however, the mechanisms responsible for the early phase of hypertension are unresolved. Therefore, the purpose of this study was to examine RAS involvement in early programmed hypertension and to determine whether temporal changes in RAS expression are observed in IUGR offspring. Renal renin and angiotensinogen mRNA expression were significantly decreased at birth (80 and 60%, respectively); plasma and renal RAS activity did not differ in conjunction with hypertension (mean increase of 14 mmHg) in young IUGR offspring; however, hypertension (mean increase of 22 mmHg) in adult IUGR offspring was associated with marked increases in renin-angiotensin-converting enzyme (ACE) activity (122%) and renal renin and angiotensinogen mRNA (7-fold and 7.4-fold, respectively), but no change in renal ANG II or angiotensin type 1 receptor. ACE inhibition (enalapril, 10 mg·kg−1·day−1, administered from 2 to 4 wk of age) abolished hypertension in IUGR at 4 wk of age (decrease of 15 mmHg, respectively) with no significant depressor effect in control offspring. Therefore, temporal alterations in renal RAS are observed in IUGR offspring and may play a key role in the etiology of IUGR hypertension.

intrauterine growth restriction; kidney; brain; angiotensin; rat

Epidemiological and experimental studies provide strong evidence to suggest cardiovascular disease and hypertension are programmed by exposure to adverse conditions in utero (1, 6, 11, 12, 13, 17, 30, 51, 55). The inverse relationship observed in epidemiological studies between birth weight and blood pressure suggests that factors involved in prenatal development, which affect fetal growth, are responsible for the in utero programming of arterial blood pressure (6, 7, 25, 31). Mechanisms involved in the prenatal programming of hypertension are unclear. However, animal studies suggest a role for the kidneys (1, 2, 30, 51, 55, 56) in the pathogenesis of prenatal programmed hypertension with inclusion of the renin angiotensin system (RAS) (30, 44, 51, 55).

Numerous investigators have examined the role of the RAS in models of prenatal programming induced by protein restriction during gestation in the rat (33, 34, 40, 43, 45, 48, 51, 52, 55) and prenatal exposure to glucocorticoids (41, 43). Suppression of the RAS observed at birth (51, 55) may lead to permanent structural changes associated with the pathogenesis of hypertension in offspring from protein-restricted dams (55). Upregulation of the renin angiotensin type 1 receptor (AT1R) following late gestational protein restriction is observed as early as 4 wk of age by some investigators (30, 33, 51), but not others (35), and characterization of the renal RAS, including AT1R, has not yet been determined in the adult hypertensive offspring. Marked increases in plasma renin activity (PRA) following late gestational protein restriction are also observed as early as 4 wk of age by some investigators (30) but not by other investigators who observe suppression of PRA at 4 wk of age with a gradual increase leading to inappropriate activation at 6 mo of age (30). Thus, temporal alterations in the RAS are reported by investigators using similar models of prenatal programming induced by gestational protein restriction (30, 33, 48, 51, 52, 55). More importantly, the critical role of the RAS in the etiology of hypertension programmed by protocols of in utero protein restriction is indicated by RAS blockade studies (24, 48). Furthermore, alterations in the RAS may also be responsible for marked increases in blood pressure programmed by prenatal exposure to glucocorticoids, indicating that fetal insults lead to similar pathways of programmed hypertension (41, 43).

Our laboratory uses a model of prenatal programmed hypertension initiated by reduced uterine perfusion that may better reflect the pathophysiological induction of intrauterine growth restriction (IUGR) in humans in the Western world. IUGR induced by placental insufficiency is associated with development of hypertension in male growth-restricted offspring (1, 40). We previously reported that angiotensin-converting enzyme (ACE) inhibition abolishes hypertension in adult male growth-restricted offspring (40), suggesting that the RAS plays a critical role in the maintenance of established hypertension in this model of IUGR. Thus, the purpose of this study was to determine whether temporal alterations in the RAS are present...
in male growth-restricted offspring from reduced uterine perfusion dams, and to determine the quantitative importance of the RAS in the early phase of programmed hypertension in male growth-restricted offspring.

MATERIALS AND METHODS

All experimental procedures were done in accordance with National Institutes of Health guidelines with approval by the Animal Committee at the University of Mississippi Medical Center. Rats were housed in a temperature-controlled room (23°C) with a 12:12-h light-dark cycle with food and water available ad libitum. At day 14 of gestation, female timed pregnant Sprague-Dawley rats (Harlan, Indianapolis, IN) destined for reduced uterine perfusion were clipped as described below. All dams were allowed to deliver at term with one control litter size-matched to a reduced uterine perfusion litter. Minimum litter size was 8 pups. Birth weight was recorded within 12 h and offspring from all litters were weaned at 3 wk of age. Only male offspring were used for all studies since only male IUGR offspring develop and remain hypertensive into adulthood. For ACE inhibition studies, young male offspring from 12 control and 11 reduced uterine perfusion litters were administered either enalapril, 10 mg·kg⁻¹·day⁻¹ by gavage, using a dose shown previously by our laboratory or others (47) to result in an effective blockade of the ANG II response, as determined by a decrease in arterial pressure and increase in PRA, or vehicle (water and corn syrup, 50:50). Administration of enalapril or vehicle was initiated at 2 wk of age and continued for 2 wk of until 4 wk of age. Mean arterial pressure was measured at 4 wk of age in one group of animals and at 5 wk of age in a second group. For measurement of RAS components at birth, tissues were pooled from offspring of a single litter for collection of adequate tissue for analysis. Six litters of control and 8 litters of reduced uterine perfusion rats were utilized. For studies involving quantification of RAS components in older animals, kidneys were not pooled, as kidneys were sufficient in size for analysis, and therefore, each measurement represents one kidney per animal. For these determinations, tissues and serum were collected from offspring born to a total of 12 control rats and 13 reduced uterine perfusion rats. Kidneys and plasma were collected from animals following decapitation.

Reduced uterine perfusion in the pregnant rat. As previously described (1), reduced uterine perfusion was used to induce IUGR. Briefly, all rats undergoing surgical procedures were anesthetized with 2% isoflurane (W. A. Butler) delivered by Vaporizer (Ohio Medical Products, Madison, WI). At day 14 of gestation, a midline abdominal incision was done and the lower abdominal aorta was isolated and a silver clip (0.203 mm ID) was placed around the aorta above the iliac bifurcation. Because compensation of blood flow to the placenta occurs through an adaptive increase in ovarian blood flow, flow to both the right and left ovarian arteries that supply the uterus was reduced using silver clips (0.100 mm ID). Pregnant rats used for the control group were not exposed to surgical procedures. On the basis of previous observations, no differences have been observed between offspring from pregnant rats undergoing a sham operation and offspring from pregnant rats not exposed to surgical procedures. (B. T. Alexander, unpublished data, 2003).

Acute arterial pressure measurements in conscious rats. Mean arterial pressure (MAP) was measured acutely, as previously described (1). Briefly, rats were anesthetized with isoflurane, as described above and surgically instrumented with a carotid arterial catheter [polyethylene (PE)-50 tubing]. After 3 days of recovery, rats acclimated to restraint were placed in modified restraining cages with arterial pressure monitored with a pressure transducer connected to a data acquisition kit (DATAQ Instruments, Akron, OH) and computer for continuous recording. All animals were acclimated to restraint for 2 h before a 1-h equilibration period followed by two 20-min pressure determinations. All pressure measurements were made during mid-morning hours.

Measure of plasma renin activity: Renin activity in plasma was measured using RIA using a modification of Haber and associates (22) with ANG I standards, tracer, and antibody from the National Institute for Standards and Technology (Gaithersburg, MD), Perkin Elmer Life Sciences (Waltham, MA), and Arnel, respectively.

ACE activity assay. Isolated kidney cortices were homogenized in an assay buffer consisting of (in mmol/l) 50 HEPES, pH 7.4, 150 NaCl, 0.5% Triton X-100, 10 μM ZnCl₂, and 1.0 PMSF, and then clarified by centrifugation at 10,000 g for 15 min. ACE activity against a synthetic substrate (p-hydroxybenzoyl-glycyl-L-hisidyl-L-leucine) was determined using the colorimetric based ACEcolor kit (Fujirebio). For the assay, tissue samples were standardized to 1 μg protein/μl. For serum ACE activity, 50 μl of serum was used. Optical density was read at 505 nm with a spectrophotometer. Results were calculated as mIU/mg of protein. All data were reported as means ± SE.

Isolation of total cellular RNA and real-time PCR. Total RNA was utilized for quantification of the mRNA message by real-time PCR. Kidneys were removed, quick frozen in liquid nitrogen, and stored at −80°C. Each kidney was first ground using a liquid nitrogen chilled mortar and pestle, and total RNA was isolated using a guanidine thiocyanate, acid phenol:chloroform procedure (ToTALLY RNA kit, Ambion, Austin, TX). Total RNA concentration and purity were determined spectrophotometrically using A260 and A260/280 ratio, respectively. Total RNA integrity was checked using 1% agarose gel electrophoresis. All RNA isolates were DNA free by treatment with DNase (DNA-free kit, Ambion, Austin, TX). Two micrograms of total RNA were reverse transcribed using a modified Maloney murine leukemia virus-derived RT and a unique blend of oligo (dT) and random hexamer primers (iScript cDNA Synthesis Kit, Bio-Rad, Hercules, CA). One microliter of the resulting cDNA was amplified by real-time PCR using SYBR Green (iQ SYBR Green Supermix, Bio-Rad) as fluorophore in an iCycler real-time thermal cycler (Bio-Rad). Specific pairs of primers were used for each gene amplification. PCR conditions were optimized for each pair of primers. DNA sequencing of the fragment amplified confirmed specificity of the PCR products: renin (37) forward: GCTACATGGGAAT- GGGAATGGA and reverse: ACCACATCTTTGCTGAAAC; angiotensinogen (37) forward: ACGACGACGACCCCTATT and reverse: AGAACTCAGGGGAC; AT1R (34) forward: AATTACCGTGACCAAAGTCTTGGA and reverse: AGAA-CATGGGAAGGGGCAACCA; and AT₂R primers (34) forward: GCCG-CAGTTGTTTTCTTTGATAGC and reverse: GTTCCCTCTTGTGTGTGTTGTAT.

ACE mRNA expression was assessed using RT² PCR Primer Set for Rat ACE (SuperArray Bioscience). Results were calculated using the 2⁻¹⁹ΔCt method and expressed in folds increase/decrease of the gene of interest in IUGR vs. control rats. All reactions were performed in triplicate, and β-actin was used as an internal control (RT-PCR primer and control set; Invitrogen, Carlsbad, CA). Level of mRNA expression was calculated using the mathematical formulas for delta/ΔCT and expressed as fold change vs. control. Gene expression was calculated using the mathematical formulas of the ΔCt method and expressed as fold change of the gene of interest relative to control. All reactions were performed in triplicate, and β-actin was used as an internal control (RT-PCR primer and control set; Invitrogen, Carlsbad, CA).

Radioimmunoassay for ANG II. Briefly, frozen kidneys were quickly weighed and quick frozen in liquid nitrogen. For analysis, they were homogenized and quick extracted using Sep-Pak columns. Solvent was evaporated, and recovery of radiolabeled angiotensin added to the samples was determined during the extraction. Recovery of radiolabeled peptide was corrected for recovery. ANG II was measured using the Alpco Diagnostics kit (Windham, NH).

Radioiodination autoradiography for ANG II receptors and ACE. Quantitative autoradiography was used to determine renal AT₁R, AT₂R, and ACE binding from whole kidneys as previously described (5, 28, 29). Briefly, kidneys were snap-frozen in liquid nitrogen, cut sagittally into sequential 20-μm-thick frozen sections within 1 wk of harvest, thaw-mounted onto gelatin-coated slides, air-dried, and stored at −20°C. Within 1 wk of sectioning, the sections were thawed, and the autoradiography procedure was carried out. For AT₁ and AT₂
receptor autoradiography, sections were preincubated for 30 min at room temperature in isotonic buffer (50 mM NaPO₄, 150 mM NaCl, 5 mM EDTA, 0.1 mM bacitracin, pH 7.1–7.2). Consecutive sections were incubated for 2 h in isotonic buffer containing 0.4 nM ¹²⁵I-SI ANG II plus a saturation concentration (3 µM) of ANG II to block both AT₁ and AT₂ receptors for nonspecific binding; 0.4 nM ¹²⁵I-SI ANG II plus 10 nM PD123177 (an AT2R-selective compound) for measurement of AT₁R; and 0.4 nM ¹²⁵I-SI ANG II plus 10 mM losartan (an AT₁-selective antagonist) for measurement of AT₂R, as previously described (35). For ACE autoradiography, sections were preincubated in 50 mM Tris, 100 mM NaCl buffer (pH 7.4 at 22°C) for 30 min at room temperature, and then incubated with ¹²⁵I-MK-422 plus or minus 1 µM MK-422 for 1 h at room temperature for determination of ACE as previously described (4, 24). Following radioligand incubation, sections were rinsed, dried, and exposed to Kodak Biomax MR1 X-ray film, at −20°C for ~36 h. A standard slide with calibrated concentrations ranging from 1 to 600 nCi/mg ¹²⁵I (Microscales, GE Healthcare Biosciences, Piscataway, NJ) was included in each cassette. The film was developed using an automated film processor.

**Image analysis.** Images were captured by use of an Advanced Imaging System image analysis system (Imaging Research) via an analog video camera. The density of ANG II receptors was referenced to the ¹²⁵I standards processed with each film.

**Statistics.** All data are presented as means ± SE. Statistical differences were evaluated using repeated-measures one-way ANOVA, followed by a Student’s *t*-test. The criterion for statistical significance was set at *P* < 0.05.

**RESULTS**

**Birth and body weights.** As reported previously (1), weight at birth was significantly reduced in growth-restricted offspring compared with control offspring (5.7 ± 0.1 vs. 6.4 ± 0.3 g, *P* < 0.01, IUGR vs. control, respectively). However, body weight was not significantly decreased at either 4 (80 ± 3 g) or 5 wk of age in growth-restricted offspring (126 ± 4 g) compared with control (80 ± 3 and 119 ± 4 g, respectively). However, treatment with enalapril from 2 to 4 wk of age led to a significant reduction in body weight in growth-restricted offspring relative to their untreated counterparts at 4 wk of age (66 ± 3 g, *P* < 0.05) that persisted at 5 wk of age (103 ± 5 g, *P* < 0.05); enalapril had no significant effect on body weight in control offspring at either 4 (76 ± 2 g) or 5 wk of age (121 ± 2 g). Body weight remained similar at 16 wk of age in growth-restricted offspring (413 ± 50 g) compared with control offspring (411 ± 5 g).

**Effect of RAS blockade on MAP.** To determine the importance of the RAS in the early phase of hypertension in this model of IUGR, enalapril was administered from 2 to 4 wk of age. The increase in MAP at both 4 and 5 wk of age in growth-restricted offspring was abolished by ACE inhibitor treatment. ACE inhibition did not significantly alter blood pressure in control offspring (Fig. 1).

**Measurement of MAP in adult growth-restricted offspring.** For characterization of RAS components in adult growth-restricted offspring, tissues were collected at 16 wk of age, a time at which MAP was significantly elevated in growth-restricted offspring (145 ± 2 mmHg, *P* < 0.05) compared with control (123 ± 2 mmHg). These values, as determined in conscious chronically instrumented animals, are comparable to values of MAP obtained by telemetry (measurement at 16 wk of age following full recovery from probe implantation at 10 wk of age) (40).

Intrarenal expression of RAS components. Expression of renin mRNA in the kidney was significantly decreased by 80% (day 1) in growth-restricted offspring relative to control offspring (Fig. 2A). At 6 wk of age, no difference in expression of renin in the renal cortex was observed in growth-restricted vs. control offspring (Fig. 2B). However, cortical renin mRNA expression was significantly increased by 7-fold at 16 wk of age in growth-restricted offspring (Fig. 2C) compared with control offspring. Renal angiotensinogen mRNA expression followed a similar trend. Renal angiotensinogen mRNA expression, which was significantly decreased at birth (day 1) (Fig. 2D), did not differ significantly at 6 wk of age (Fig. 2E) but was significantly increased at 16 wk of age (Fig. 2F). Renal cortical ACE mRNA expression (Fig. 3, top left) or renal ACE density (Fig. 3, middle left) determined by radioligand autoradiography did not differ at 16 wk of age; however, a significant increase in renal ACE activity (Fig. 3, bottom left) was observed in growth-restricted offspring at 16 wk of age relative to control offspring. Renal AT₁ receptor mRNA (Fig. 3, top right) or renal AT₁ receptor density (Fig. 3, middle right) were not altered in growth-restricted offspring compared with control offspring at 16 wk of age, and despite a significant increase in renal ACE activity, renal ANG II levels were also not increased at 16 wk of age (Fig. 3F). AT₁R binding was greater in the medulla relative to the cortex in both control and growth-restricted offspring (Fig. 4), an observation previously reported by others (20, 57). AT₁ receptor binding, as determined by radioligand autoradiography, also did not differ at 4 wk of age. Binding to the angiotensin type 2 (AT₂) receptor was not detectable in growth-restricted or control
offspring at either 4 or 16 wk of age; \(\text{AT}_2\) receptor mRNA expression was also not detectable.

**Plasma renin activity.** PRA did not differ between control and growth-restricted offspring at either 4 (5.7 ± 2.6 vs. 6.5 ± 2.2 nmol ANG I-1⁻¹·h⁻¹) or 5 wk of age (5.2 ± 1.6 vs. 8.1 ± 0.6 nmol ANG I-1⁻¹·h⁻¹), respectively. ACE inhibition from 2 to 4 wk of age resulted in a significant increase in PRA at 4 wk of age in control and growth-restricted treated offspring (114 ± 42.2 and 95.6 ± 37.4 nmol ANG I-1⁻¹·h⁻¹). At 5 wk of age, PRA returned to baseline values in control (6.3 ± 1.7 nmol ANG I-1⁻¹·h⁻¹) or below in growth-restricted offspring (3.2 ± 1.7 nmol ANG I-1⁻¹·h⁻¹, \(P < 0.05\) vs. untreated growth-restricted).

**Serum ACE activity.** Serum ACE activity was not significantly increased at 16 wk of age in growth-restricted offspring (13.5 ± 1.2 vs. 15.2 ± 1.1 IU ACE/l, control vs. growth-restricted, respectively).

![Fig. 2. Temporal changes in renal renin and angiotensinogen in IUGR offspring. Real-time PCR was used to assess renal renin mRNA expression in newborn (day 1) (A), 6 wk of age (B), and 16 wk of age (C) or renal angiotensinogen mRNA expression of in newborn (day 1) (D), 6 wk of age (E), and 16 wk of age (F). Quantitation of renal RAS components was normalized relative to renal \(\beta\)-actin mRNA expression levels. For newborn samples, mRNA expression was quantitated from whole kidney homogenates representing a pool of tissues collected from a single litter; \(n = 8\) IUGR litters and 6 control litters. Individual cortical sections were analyzed from 8 IUGR and 8 control offspring at 6 wk of age; 7 IUGR and 8 control offspring at 16 wk of age. Standard error is calculated from at least three determinations from at least three independent experiments. \(* P < 0.05\) vs. control. All data are expressed as means ± SE.](http://ajpregu.physiology.org/)

![Fig. 3. Renal angiotensin-converting enzyme (ACE), \(\text{AT}_1\) receptor, and ANG II in IUGR offspring at 16 wk of age. Real-time PCR was used to assess renal ACE (top left) and \(\text{AT}_1\) receptor (top right) mRNA expression. Quantitation of renal RAS components was normalized relative to renal mRNA \(\beta\)-actin mRNA expression levels. Individual cortical sections were analyzed from 7 IUGR and 8 control offspring at 16 wk of age. Standard error was calculated from at least 3 determinations from at least three independent experiments. Quantitative binding of renal ACE (middle left) and \(\text{AT}_1\) receptor (middle right) was determined in 8 IUGR and 8 control offspring. Renal ACE activity (bottom left) and renal ANG II (bottom right) was determined in 6 IUGR and 5 control offspring. All data are expressed as means ± SE.](http://ajpregu.physiology.org/)
DISCUSSION

Fetuses subjected to stressful influences resulting in IUGR are predisposed to the development of cardiovascular disease and hypertension in later life (1, 30, 51, 55). In the rat, reduced uterine perfusion initiated in late gestation results in growth-restricted offspring that develop marked elevations in MAP (1, 40). Significant elevations in MAP observed at 12 wk of age in growth-restricted offspring are not associated with marked changes in glomerular filtration rate or renal plasma flow (1). Therefore, regulatory systems for control of sodium balance and arterial pressure may be altered by placental insufficiency in utero. We previously reported that RAS blockade by use of the ACE inhibitor enalapril abolishes hypertension in adult growth-restricted offspring from reduced uterine perfusion dams (40). This suggests an important role for RAS involvement in the established phase of IUGR-induced hypertension.

In the present study, we determined the quantitative importance of the RAS in the early phase of IUGR-induced hypertension. In addition, we also determined whether temporal alterations in the RAS were associated with the etiology of hypertension in this model of IUGR produced in response to reduced uterine perfusion.

An important role for RAS involvement in the development of hypertension in models of prenatal programming induced by low protein is suggested by RAS blockade studies. Early blockade of the RAS by the ACE inhibitor enalapril (33), or by AT1R blockade (48) prevents development of hypertension in models of programming induced by gestational protein restriction. Although elevated levels of intrarenal ANG II (30, 45) or systemic (32) RAS may not be present at this time, investigators report offspring of protein-restricted dams present increased sensitivity to ANG II (35, 46) that may (46) or may not (35) be associated with increased intrarenal expression of AT1 receptors. In our studies, hypertension is present in growth-restricted offspring from reduced uterine perfusion dams between 4 to 6 wk of age (1). To examine the quantitative importance of the RAS in the early phase of hypertension in this model of IUGR, we administered the ACE inhibitor, enalapril, for 2 wk starting at 2 wk of age. Nephrogenesis is complete by 2 wk of age, so the initiation of an ACE inhibitor at this age in the rat will not interfere with proper renal development (21). Although intrarenal expression of AT1 receptors was not increased at 4 wk of age in growth-restricted offspring, early ACE inhibition abolished hypertension. In addition, the effect of ACE inhibition on arterial pressure was sustained since MAP remained normalized in treated growth-restricted offspring relative to control, treated and untreated, at 5 wk of age, or 1 wk past treatment with enalapril. Thus, the RAS contributes to early hypertension in this model of IUGR induced by placental insufficiency, and increased sensitivity to ANG II may contribute to the etiology of hypertension in growth-restricted offspring.

We next examined whether temporal alterations in the RAS were observed in this model of prenatal programmed hypertension induced by placental insufficiency. RAS components are highly expressed in the developing kidney and play a role in...
in mediating proper nephrogenesis (20, 57). AT₁R blockade during the nephrogenic period in the rat leads to a reduction in nephron number and development of hypertension (57), suggesting that the intrarenal RAS contributes to proper nephrogenesis. Models of programming induced by gestational protein restriction consistently exhibit marked reductions in nephron number and kidney size associated with significant increases in arterial pressure in adulthood (30, 51, 55). In one model of gestational protein restriction used by Woods et al. (57), suppression of the RAS is present at birth. Thus, in utero exposure to low protein may lead to alterations in the RAS, resulting in permanent structural changes and subsequent development of hypertension in adult offspring (55). Similar to observations noted by Woods with the low-protein model, we observed a significant decrease in renal mRNA expression of renin and angiotensinogen in newborn growth-restricted offspring. This reduction in renin and angiotensinogen mRNA expression at birth in growth-restricted offspring may also be associated with reductions in nephron number. Kidney weight is reduced in growth-restricted offspring in response to reduced uterine perfusion (1), and reductions in nephron number have been reported by other investigators using similar models of reduced uteroplacental flow in the rat (36, 42) and other species (8, 10).

The RAS is an important regulator of arterial pressure and body fluid balance through the systemic actions of ANG II (23). Inappropriate activation of the peripheral RAS, noted by a marked increase in PRA, is observed in conjunction with established hypertension in a model of gestational protein restriction utilized by Vehaskari and colleagues (53, 54). Vehaskari also notes that RAS blockade abolishes established hypertension in adult offspring of gestational protein-restricted dams (53). Furthermore, alterations in the RAS may also contribute to marked increases in blood pressure induced by prenatal exposure to glucocorticoids (41, 43). Therefore, a role for RAS involvement is indicated in animal models of hypertension produced in response to an adverse insult during fetal development. However, in growth-restricted offspring from reduced uterine perfusion dams, established marked elevations in MAP were not associated with changes in the peripheral RAS, as we previously reported by measure of PRA and plasma renin substrate (40), and in this study, by serum ACE activity. Thus, inappropriate activation of the peripheral RAS is not associated with hypertension in this model of IUGR.

However, as suggested by studies in transgenic animals, intrarenal ANG II can contribute to development of hypertension despite the absence of an increase in the peripheral RAS (14). Therefore, the intrarenal RAS, not the peripheral RAS, may contribute to the etiology of hypertension in growth-restricted offspring from reduced uterine perfusion dams.

All components of the RAS are present in the kidney (19). Because RAS blockade abolishes hypertension in adult growth-restricted offspring, suggesting RAS involvement (40), we characterized the renal RAS in growth-restricted offspring to determine whether alterations were present in this model of IUGR-induced hypertension. Renal renin and angiotensinogen mRNA expression were no longer suppressed at 6 wk of age as previously observed at birth and were significantly elevated at 16 wk of age in the cortex of adult growth-restricted offspring relative to control offspring. A marked increase in renal ACE activity was also observed at 16 wk of age in adult growth-restricted offspring. However, ACE and AT₁R mRNA expression did not differ upon comparison of adult growth-restricted offspring to control. Quantitative autoradiography also showed no difference between ACE and AT₁R expression in the kidneys of adult growth-restricted offspring relative to control. Although renal ACE activity was increased in adult growth-restricted offspring at 16 wk of age in conjunction with increased levels of renin substrate, ANG II formation was not increased.

Angiotensinogen of hepatic origin can serve as the substrate for generation of intrarenal ANG II; however, angiotensinogen is also present within the proximal tubule (27) and can be stimulated by testosterone (39). Regulation of renal angiotensinogen by testosterone can occur in a dose-dependent manner (16), suggesting that elevated testosterone in adult male growth-restricted offspring may serve as the stimulus for enhanced renal angiotensinogen observed in this model of IUGR hypertension. Enhanced renal angiotensinogen levels can lead to activation of the RAS and hypertension (26), and we have demonstrated the importance of the RAS in mediating hypertension in adult male growth-restricted offspring (40). However, despite enhanced intrarenal angiotensinogen, increased intrarenal ANG II was not observed in conjunction with established hypertension in adult male IUGR. Discrepancies in intrarenal angiotensinogen and ANG II are also observed in the Dahl salt-sensitive (28) and Zucker diabetic rat (50), yet the importance of ANG II in these experimental models is demonstrated by the renoprotective effects mediated via AT₁ receptor blockade (33, 39, 50). Therefore, the intrarenal RAS, independent of the peripheral RAS, may play a role by contributing to impaired sodium reabsorption and hypertension in adult growth-restricted offspring from reduced uterine perfusion dams.

ANG II blockade studies support a role for RAS involvement in both the development and maintenance of established hypertension in this model of IUGR induced by placental insufficiency. Moreover, temporal alterations in the renal RAS are evident in this model of IUGR. However, it appears that the factors that initiate hypertension in this model of IUGR may differ from the factors that maintain established hypertension. Fetal responses to reduced uterine perfusion indicate suppression of intrarenal ANG II at birth, no alteration in the intrarenal RAS in young animals, and marked alterations in the intrarenal RAS in the adult hypertensive animal; in addition, no temporal changes are observed in the peripheral RAS. Because sex differences are observed in this model of IUGR, only male growth-restricted offspring remain hypertensive after puberty, and differential expression of the RAS by sex hormones may contribute to established hypertension in adult male growth-restricted offspring.

Animal studies provide evidence suggesting that insults during gestation result in structural and physiological alterations leading to hypertension associated with permanent changes in the regulatory systems involved in the long-term control of arterial pressure. Alterations in intrarenal RAS contribute to the etiology of hypertension in offspring from reduced uterine perfusion dams, an observation similar to that observed by other investigators using models of gestational protein restriction and prenatal glucocorticoid exposure. The
pathogenesis of hypertension is multifactorial, and although insight provided by different animal models highlights the complexities involved in the developmental origins of adult disease, the presence of common mechanistic pathways is suggested.

GRANTS
This work was supported by National Institutes of Health Grant HL-074927.

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R810 RAS AND HYPERTENSION IN IUGR OFFSPRING

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