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

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Abstract

Reduced uterine perfusion initiated in late gestation in the rat results in intrauterine growth restriction (IUGR) and development of hypertension by 4 weeks 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 percent, respectively); plasma and renal RAS 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 renal ACE activity (122%) and renal renin and angiotensinogen mRNA (7-fold and 7.4-fold, respectively), but no change in renal angiotensin II or angiotensin type 1 receptor. ACE inhibition (enalapril, 10 mg/kg/day, administered from 2 to 4 weeks of age) abolished hypertension in IUGR at 4 weeks 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.

Keywords

intrauterine growth restriction; hypertension; kidney; brain; renin; angiotensin; rat

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DISCLOSURES

The authors have no disclosures.

INTRODUCTION

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 pre-natal programming of hypertension are unclear. However, animal studies suggest a role for the kidneys (1,2,30,51,55,54,56) in the pathogenesis of pre-natal 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 pre-natal exposure to glucocorticoids (XX). 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). Up-regulation of the renal angiotensin type 1 receptor (AT₁R) following late gestational protein restriction is observed as early as 4 weeks 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 weeks of age by some investigators (30), but not by other investigators who observe suppression of PRA at 4 weeks of age with a gradual increase leading to inappropriate activation at 6 months of age (30). Thus, temporal alterations in the RAS are reported by investigators utilizing similar models of pre-natal 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 pre-natal exposure to glucocorticoids indicating fetal insults lead to similar pathways of programmed hypertension (XX).

Our laboratory utilizes a model of prenatal programmed hypertension initiated by reduced uterine perfusion that may better reflect the patho-physiological 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 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 Institute 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 hour light/dark cycle with food and water available *ad libitum*. At day 14 of gestation, female timed pregnant Sprague Dawley rats (Harlan Inc., 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 hours and offspring from all litters were weaned at 3

weeks of age. Only male offspring were utilized 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/day by gavage, utilizing 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 weeks of age and continued for 2 weeks until 4 weeks of age. Mean arterial pressure was measured at 4 weeks of age in one group of animals, and at 5 weeks 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 dams were utilized. For studies involving quantitation 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 utilized to induce IUGR. Briefly, all rats undergoing surgical procedures were anesthetized with 2% isoflurane (W.A. Butler Co.) 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. Since 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. Based on 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. (Alexander BT, 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 (PE 50 tubing). Following 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, Inc., Akron, OH) and computer for continuous recording. All animals were acclimated to restraint for two hours prior to a one-hour equilibration period followed by 2-twenty minute pressure determinations. All pressure measurements were made during mid morning hours.

Measure of plasma renin activity

Renin activity in plasma was measured by radioimmunoassay (RIA) using a modification of Haber and associates (22) with AI standards, tracer, and antibody from the National Bureau of Standards, Perkin Elmer Life Sciences, 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 μ M ZnCl₂, and 1.0 PMSF, and then clarified by centrifugation at 10,000g for 15 minutes. ACE activity against a synthetic substrate (p-hydroxybenzoyl-glycyl-L-hisidyl-L-leucine) was determined using the colorimetric based ACEcolor kit (Fujirebio Inc). For the assay, tissue samples were standardized to 1 μ g 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 mean \pm SE.

Isolation of total cellular RNA and Real-time PCR

Total RNA was utilized for quantitation 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 thiocyanide, 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). 2 µg of total RNA were reverse transcribed using a modified MMLV-derived reverse transcriptase and a unique blend of oligo (dT) and random hexamer primers (iScriptTM cDNA Synthesis Kit, BIO-RAD). 1 µl of the resulting cDNA was amplified by real-time PCR using SYBR Green (iQTM SYBR Green Supermix, BIO-RAD) as fluorophore in an iCycler real-time thermal cycler (BIO-RAD). Specific pairs of primers was 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: GCTACATGGAGAATGGGACTGAA Reverse: ACCACATCTTGCTGAGGAAAC
Angiotensinogen (37)	Forward: AGCACGGACAGCACCTATT Reverse: AGAACTCATGGAGCCAGTCA
AT ₂ R primers (34):	Forward: AATTACCCGTGACCAAGTCTTGA Reverse: AGAACATGGAAGGGAAGCCA
AT ₁ R primers (34):	Forward: GGCCAGTGTTCCTTTTGAATTAGCAC Reverse: GTTCCCCTGTTTGGTGTAT

ACE mRNA expression was assessed using RT² PCR Primer Set for Rat ACE (SuperArray Bioscience Corporation). Results were calculated using the $2^{-\Delta\Delta\text{CT}}$ method and expressed in folds increase/decrease of the gene of interest in IUGR vs. control rats. All reactions were performed in triplicates and β -actin was used as an internal control (RT-PCR Primer and Control Set, Invitrogen). Level of mRNA expression was calculated using the mathematical formulas for delta/delta CT recommended by Applied Biosystems (Applied Biosystems User Bulletin, No. 2, 1997).

Radioimmunoassay for angiotensin II

Briefly, frozen kidneys were quickly weighed and quick frozen in liquid nitrogen. For analysis they were homogenized, and 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).

Radioligand autoradiography for ANG II receptors and ACE

Quantitative autoradiography was utilized 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 week of harvest, thaw-mounted onto gelatin-coated slides, air-dried, and stored at -20°C . Within one week of sectioning, the sections were thawed and the autoradiography procedure was carried out. For AT₁ and AT₂ receptor autoradiography, sections were pre-incubated for 30 minutes

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 hours 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 mM 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 pre-incubated 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-351A plus or minus 1 μM MK-422 for 1 hour 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 hours. 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 AIS image analysis system (Imaging Research Inc) 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 ± Std error. Statistical differences were evaluated using repeated-measures one-way ANOVA, followed by 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 as compared to control offspring (5.7±0.1 vs. 6.4±0.3 grams, *P* < 0.01, IUGR vs. control, respectively). However, body weight was not significantly decreased at either 4 (80 ± 3 grams) or 5 weeks of age in growth-restricted offspring (126±4 grams) as compared to control (80±3 and 119±4 grams, respectively). However, treatment with enalapril from 2 to 4 weeks of age led to a significant reduction in body weight in growth-restricted offspring relative to their untreated counterparts at 4 weeks of age (66±3 grams, *P* < 0.05) that persisted at 5 weeks of age (103±5 gram, *P* < 0.05); enalapril had no significant effect on body weight in control offspring at either 4 (76±2 grams) or 5 weeks of age (121±2 grams). Body weight remained similar at 16 weeks of age in growth-restricted offspring (413±50 grams) compared to control offspring (411±5 grams).

Effect of renin angiotensin system blockade on mean arterial pressure

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 weeks of age. The increase in MAP at both 4 and 5 weeks of age in growth-restricted offspring was abolished by ACE inhibitor treatment. ACE inhibition did not significantly alter blood pressure in control offspring (Figure 1).

Measurement of mean arterial pressure in adult growth-restricted offspring

For characterization of RAS components in adult growth-restricted offspring, tissues were collected at 16 weeks of age, a time at which MAP was significantly elevated in growth-restricted offspring (145±2 mmHg, *P* < 0.05) as compared to 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 weeks of age following full recovery from probe implantation at 10 weeks of age) (40).

Intra-renal expression of renin angiotensin system components

Expression of renin mRNA in the kidney was significantly decreased by 80% (day 1) in growth-restricted offspring relative to control offspring (Figure 2a). At 6 weeks of age no difference in expression of renin in the renal cortex was observed in growth-restricted versus control offspring (Figure 2b). However, cortical renin mRNA expression was significantly increased by 7-fold at 16 weeks of age in growth-restricted offspring (Figure 2c) as compared to control offspring. Renal angiotensinogen mRNA expression followed a similar trend. Renal angiotensinogen mRNA expression was significantly decreased at birth (day 1) (Figure 2d), did not differ at 6 weeks of age (Figure 2e), but was significantly increased at 16 weeks of age (Figure 2f). Renal cortical ACE mRNA expression (Figure 3a) or renal ACE density (Figure 3b) determined by radioligand autoradiography did not differ at 16 weeks of age; however, a significant increase in renal ACE activity (Figure 3c) was observed in growth-restricted offspring at 16 weeks of age relative to control offspring. Renal AT₁ receptor mRNA (Figure 3d) or renal AT₁ receptor density (Figure 3e) were not altered in growth-restricted offspring as compared to control offspring at 16 weeks of age, and despite a significant increase in renal ACE activity, renal angiotensin II (ANG II) levels were also not increased at 16 weeks of age (Figure 3f). AT₁R binding was greater in the medulla relative to the cortex in both control and growth-restricted offspring (Figure 4); an observation previously reported by others (20, 57). AT₁ receptor binding as determined by radioligand autoradiography also did not differ at 4 weeks of age. Binding to the angiotensin type 2 (AT₂) receptor was not detectable in growth-restricted or control offspring at either 4 or 16 weeks of age; AT₂ receptor mRNA expression was also not detectable.

Plasma renin activity

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 nmoles AI/L/Hr) or 5 weeks of age (5.2 ± 1.6 vs. 8.1 ± 0.6 nmoles AI/L/Hr), respectively. ACE inhibition from 2 to 4 weeks of age resulted in a significant increase in PRA at 4 weeks of age in control and growth-restricted treated offspring (114 ± 42.2 and 95.6 ± 37.4 nmoles AI/L/Hr). At 5 weeks of age PRA returned to baseline values in control (6.3 ± 1.7 nmoles AI/L/Hr) or below in growth-restricted offspring (3.2 ± 1.7 nmoles AI/L/Hr, $P < 0.05$ vs. untreated growth-restricted).

Serum ACE activity

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

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 weeks 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 pre-natal 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 intra-renal expression of AT₁ receptors. In our studies hypertension is present in growth-restricted offspring from reduced uterine perfusion dams between 4 to 6 weeks 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 two weeks starting at 2 weeks of age. Nephrogenesis is complete by 2 weeks of age so the initiation of an ACE inhibitor at this age in the rat will not interfere with proper renal development (21). Although intra-renal expression of AT₁ receptors was not increased at 4 weeks 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 weeks of age, or one week 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 pre-natal programmed hypertension induced by placental insufficiency. RAS components are highly expressed in the developing kidney and play a role 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 intra-renal 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 utilized by Woods et al., suppression of the RAS is present at birth (55). 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 (32). Vehaskari also notes that RAS blockade abolishes established hypertension in adult offspring of gestational protein restricted dams (32). Furthermore, alterations in the RAS may also contribute to marked increases in blood pressure induced by pre-natal exposure to glucocorticoids (XX). 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 intra-renal 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). Since RAS blockade abolishes hypertension in adult growth-restricted offspring suggesting RAS involvement (40), we characterized the renal RAS in growth-restricted offspring to determine if alterations were present in this model of IUGR-induced hypertension. Renal renin and angiotensinogen mRNA expression were no longer suppressed at 6 weeks of age as previously observed at birth, and were significantly elevated at 16 weeks 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 weeks 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 weeks 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 intra-renal ANG II; however, angiotensinogen is also present within the proximal tubule (27) and can be stimulated by testosterone (39). We previously reported that plasma testosterone levels are elevated two-fold in adult male growth restricted offspring (40). Regulation of renal angiotensinogen by testosterone can occur in a dose dependent fashion (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 intra-renal angiotensinogen, increased intra-renal ANG II was not observed in conjunction with established hypertension in adult male IUGR. Discrepancies in intra-renal 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 intra-renal RAS at birth, no alteration in the intra-renal RAS in young animals, and marked alterations in the intra-renal RAS in the adult hypertensive animal; in addition, no temporal changes are observed in the peripheral RAS. Since sex differences are observed in this model of IUGR, only male growth-restricted offspring remain hypertensive after puberty, 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 intra-renal 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 pre-natal 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.

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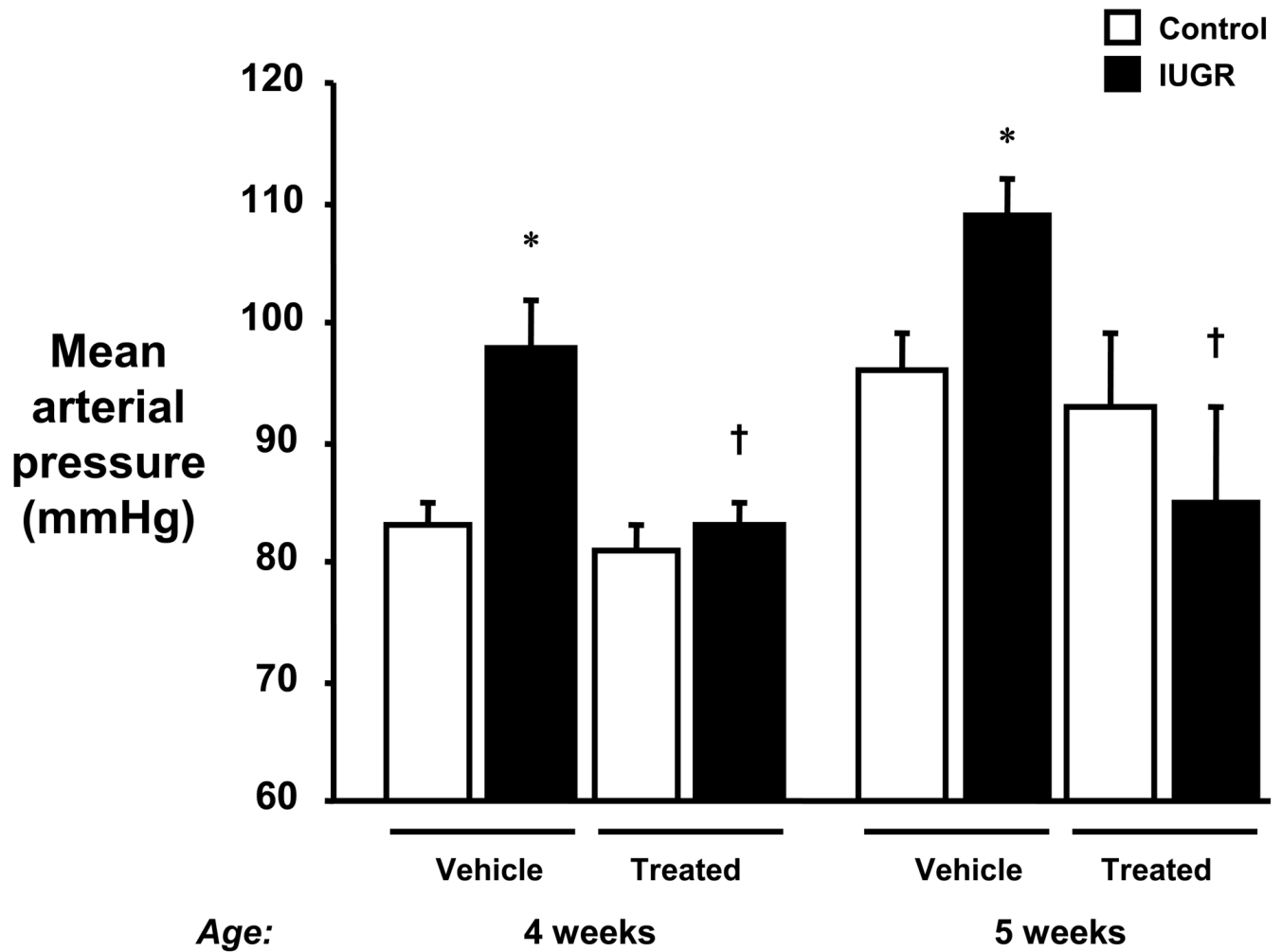


Figure 1.

Inhibition of the renin angiotensin system in intrauterine growth restricted (IUGR) offspring. Mean arterial pressure was measured in conscious chronically instrumented offspring at 4 and 5 weeks of age following treatment with either vehicle or enalapril (treated; 10mg/kg/day by gavage from 2 to 4 weeks of age). 4 week group: control vehicle (n=11), IUGR vehicle (n=7), control treated (n=10), and IUGR treated (n=9). 5 week group: control vehicle (n=8), IUGR vehicle (n=5), control treated (n=7), and IUGR treated (n=6). * $P < 0.05$ vs. untreated control. † $P < 0.05$ vs. untreated IUGR. All data are expressed as mean \pm SEM.

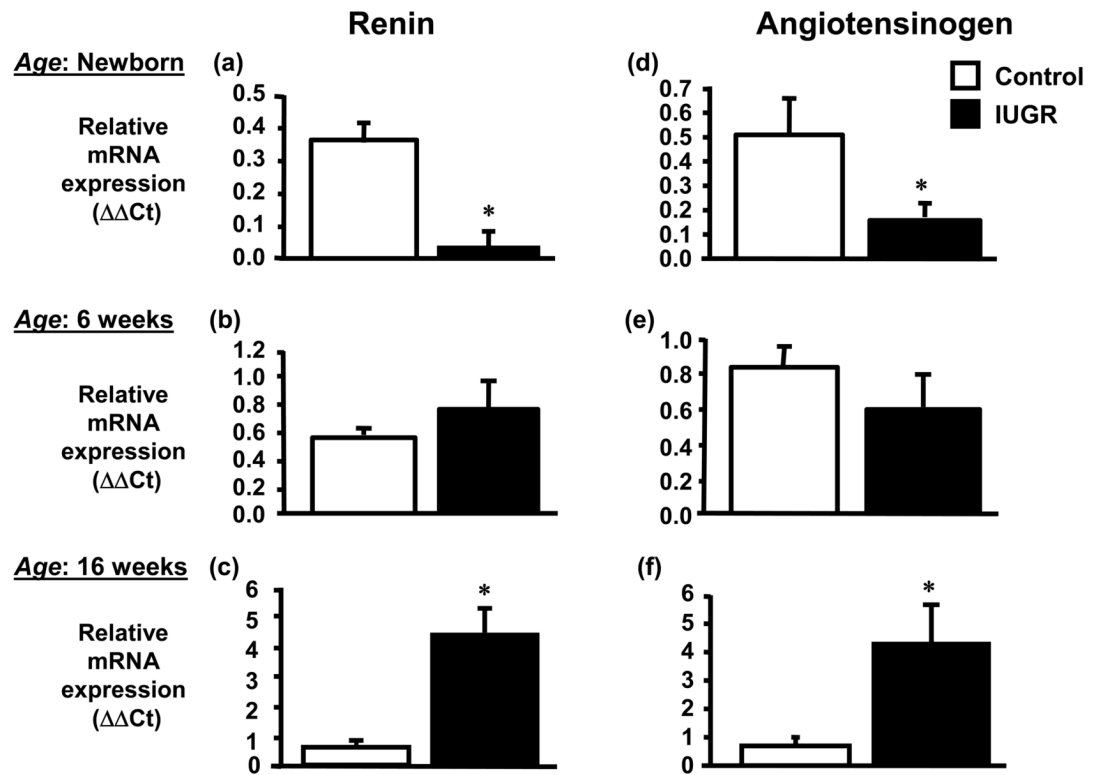


Figure 2.

Temporal changes in renal renin and angiotensinogen in intrauterine growth restricted (IUGR) offspring. Real time PCR was utilized to assess renal renin mRNA expression in newborn (day 1) (a), 6 weeks of age (b), and 16 weeks of age (c) or renal angiotensinogen mRNA expression of in newborn (day 1) (d), 6 weeks of age (e), and 16 weeks of age (f). Quantitation of renal RAS components was normalized relative to renal β -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 weeks of age; 7 IUGR and 8 control offspring at 16 weeks of age. Standard error is calculated from at least 3 determinations from at least three independent experiments. * $P < 0.05$ vs. control. All data are expressed as mean \pm SEM.

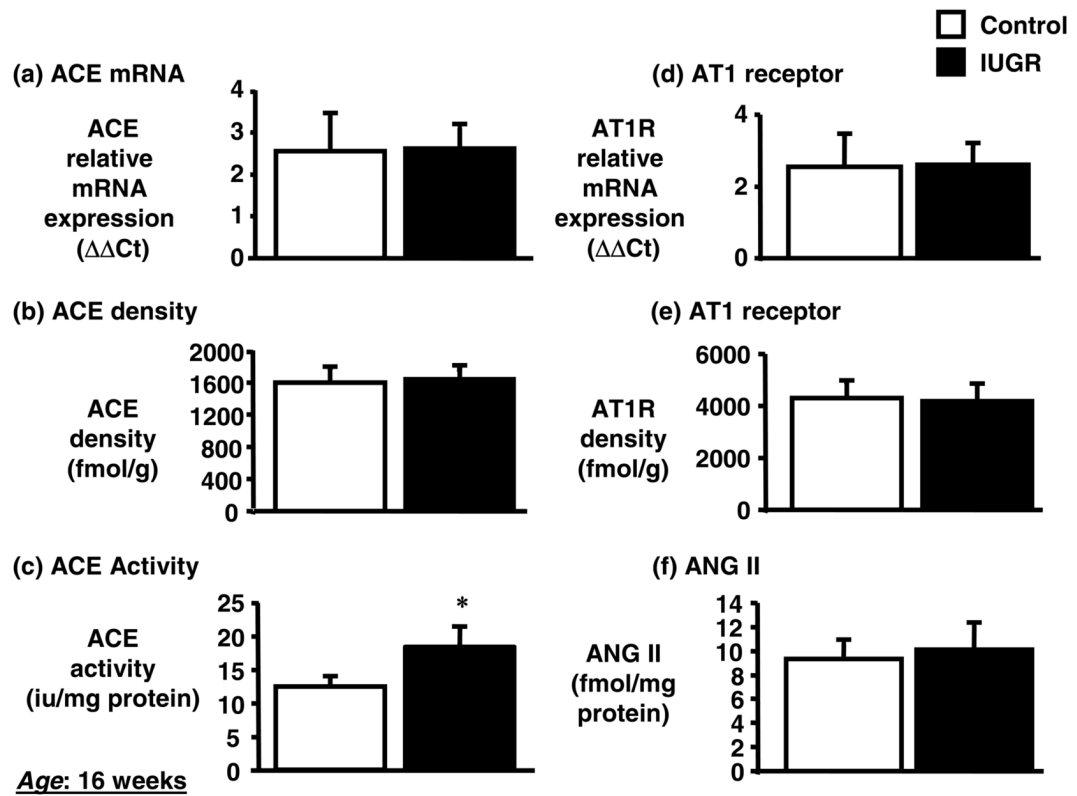


Figure 3.

Renal ACE, AT₁ receptor, and angiotensin II in intrauterine growth restricted (IUGR) offspring at 16 weeks of age. Real time PCR was utilized to assess renal ACE (a) and AT₁ receptor (d) mRNA expression. Quantitation of renal RAS components was normalized relative to renal mRNA β -actin mRNA expression levels. Individual cortical sections were analyzed from 7 IUGR and 8 control offspring at 16 weeks of age. Standard error was calculated from at least 3 determinations from at least three independent experiments. Quantitative binding of renal ACE (b) and AT₁ receptor (e) was determined in 8 IUGR and 8 control offspring. Renal ACE activity (c) and renal ANG II (f) was determined in 6 IUGR and 5 control offspring. All data are expressed as mean \pm SEM.

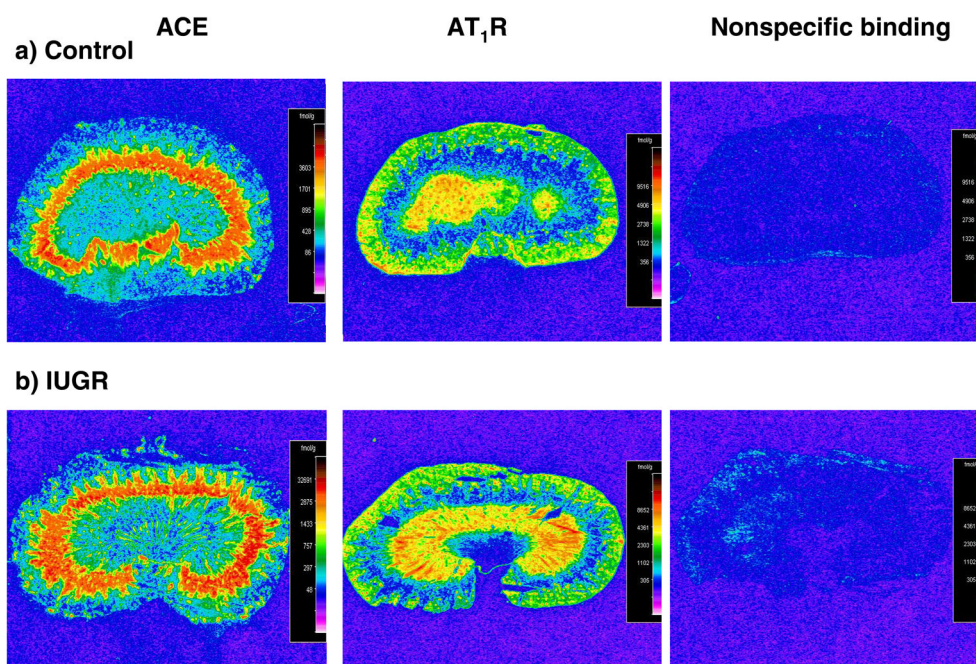


Figure 4. Quantitative autoradiography of angiotensin type 1 (AT₁R) receptors and angiotensin converting enzyme (ACE) in whole kidney from control and IUGR offspring at 16 weeks of age. Representative autoradiographs are shown of ACE (a), AT₁R (b), and non-specific binding (c) of ¹²⁵I-SI ANG II.