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Testosterone contributes to marked elevations in mean arterial pressure in adult male intrauterine growth restricted offspring

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Ojeda, Norma B., Daniela Grigore, Licy L. Yanes, Radu Iliescu, Elliot B. Robertson, Huimin Zhang, and Barbara T. Alexander. Testosterone contributes to marked elevations in mean arterial pressure in adult male intrauterine growth restricted offspring. Am J Physiol Regul Integr Comp Physiol 292: R758 –R763, 2007. First published August 17, 2006; doi:10.1152/ajpregu.00311.2006.—Our laboratory uses a model of intrauterine growth restriction (IUGR) induced by placental insufficiency in the rat to examine the developmental origins of adult disease. In this model only male IUGR offspring remain hypertensive in adulthood, revealing sex-specific differences. The purpose of this study was to determine whether testosterone with participation of the renin-angiotensin system (RAS) contributes to hypertension in adult male IUGR offspring. At 16 wk of age a significant increase in testosterone (346 ± 34 vs. 189 ± 12 ng/dl, P < 0.05) was associated with a significant increase in mean arterial pressure (MAP) measured by telemetry in IUGR offspring (147 ± 1 vs. 125 ± 1 mmHg, P < 0.05, IUGR vs. control, respectively). Gonadectomy (CTX) at 10 wk of age significantly reduced MAP by 16 wk of age in IUGR offspring (124 ± 2 mmHg, P < 0.05 vs. intact IUGR) but had no effect in control (125 ± 2 mmHg). A significant decrease in MAP in intact IUGR (111 ± 3 mmHg, P < 0.05 vs. untreated intact IUGR) and castrated IUGR (110 ± 4 mmHg, P < 0.05 vs. untreated CTX IUGR) after treatment with enalapril for 2 wk suggests a role for RAS involvement. However, the decrease in blood pressure in response to enalapril was greater in intact IUGR (∆36 ± 1 mmHg, P < 0.05) compared with CTX IUGR (∆15 ± 2 mmHg), indicating an enhanced response to RAS blockade in the presence of testosterone. Thus these results suggest that testosterone plays a role in modulating hypertension in adult male IUGR offspring with participation of the RAS.

hypertension; rat; gonadectomy; renin; angiotensin

HISTORICALLY, THE ETIOLOGY of hypertension has included two components, a genetic and an environmental component. However, recent epidemiological studies report an inverse relationship between birth weight and hypertension (21, 28, 39). These findings suggest an adverse fetal environment may also contribute to the etiology of hypertension (2, 3, 14, 26, 27, 31, 32, 49). Specifically, alterations in the fetal environment may result in fetal adaptive changes to ensure fetal survival. However, these changes may also result in permanent structural and physiological changes that lead to long-term consequences and increased risk for development of disease later in life (2, 3, 14, 26, 36). Animal models confirm that an adverse fetal environment during a critical period of fetal development results in IUGR, hypertension, and alterations in the regulatory systems that regulate the long-term control of blood pressure (1, 12, 33, 37, 48). Thus, in response to an insult in utero, the mechanisms for long-term control of blood pressure (17) may be programmed for an abnormal response leading to hypertension later in life.

Sex differences in the cardiovascular response to an adverse fetal environment are reported in many different animal models of adult disease (1, 5, 10, 19, 24, 38, 48). Vascular dysfunction is observed in male, but not female, offspring exposed to hypoxia (19) or moderate global undernutrition (38) in utero. Programming of neuroendocrine regulation in response to in utero exposure to cytokines is evidenced by decreased insulin sensitivity and locomotor activity in male offspring but increased locomotor activity and hyperandrogenism in female offspring (5). Moderate levels of gestational protein restriction in the rat result in reductions in nephron number and hypertension in male offspring only (38, 48), suggesting a role for sex hormones in modulating cardiovascular responses to an adverse fetal environment. Testosterone is an important regulator of growth and differentiation during fetal development (8, 47). Human and animal studies report that the hypothalamus-pituitary-adrenal (HPA) axis is an endocrine system sensitive to in utero insults (6, 30, 35), and subsequent HPA axis alterations may lead to changes in the pituitary-gonadal axis (29, 44, 45). Thus an insult during fetal life may alter testosterone leading to adverse adaptations later in life. Another important regulatory system, the renin-angiotensin system (RAS), is also affected in animal models of prenatal programming of adult disease and hypertension (43, 46, 48).

In a model of placental insufficiency initiated at day 14 of gestation in the pregnant rat, intrauterine growth restriction (IUGR) is associated with development of marked elevations in mean arterial pressure (MAP) in the IUGR offspring (1). However, only male IUGR offspring remain hypertensive in
adulthood, suggesting that sex-specific differences occur in this model of programmed hypertension (1). Thus the purpose of this study was to determine whether the sex hormone testosterone with participation of the RAS plays a role in defining sex-specific differences in this model of IUGR-associated hypertension induced by placental insufficiency.

**METHODS**

**Animals**

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-h light-dark cycle with food and water available ad libitum. Timed pregnant Sprague-Dawley rats were purchased from Harlan (Indianapolis, IN). At day 14 of gestation, rats destined for reduced uterine perfusion were clipped as described below. All dams were allowed to deliver at term with offspring birth weight recorded within 12 h. At this time, the number of pups in the control and reduced uterine perfusion litter was trimmed with a size of 8 pups per dam to ensure equal nutrient access for all offspring. Animals were weighed twice weekly. Pups were weaned at 3 wk of age. Male offspring from 8 control pregnant and 12 reduced uterine perfusion pregnant litters were randomly assigned into four groups: control intact (n = 17), control gonadectomized (CTX) (n = 16), IUGR intact (n = 16), and IUGR CTX (n = 15). Implantation of telemetry probes and initiation of either CTX or sham CTX were performed at 10 wk of age. MAP was determined continuously by telemetry from 12 to 16 wk of age. A subset of animals from each group was randomly assigned into four additional groups for an angiotensin-converting enzyme (ACE) inhibition protocol initiated at 14 wk of age: control intact treated (n = 8), control CTX treated (n = 8), IUGR intact-tREATED (n = 8), and IUGR CTX treated (n = 8).

**Reduced Uterine Perfusion in the Pregnant Rat**

Reduced placental perfusion, as previously described (1), was utilized for induction of IUGR. Briefly, rats undergoing surgical procedures were anesthetized with 2% isoflurane. At day 14 of gestation, the lower abdominal aorta was isolated, and a silver clip (0.203 mm ID) was placed around it above the iliac bifurcation. Because compensation of blood flow occurs through an adaptive increase in ovarian blood flow, a silver clip was slipped around both branches of the ovarian artery (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 noted in offspring from pregnant rats undergoing a sham operation or offspring from pregnant rats unexposed to a sham surgical procedure. These findings result from a large number of procedures performed in our laboratory over the past several years (Alexander, unpublished observation).

**Castration in Male Offspring**

All rats undergoing surgical procedures were anesthetized with 2% isoflurane. At 10 wk of age, a small median incision was made at the distal tip of the scrotum. Subcutaneous connective tissue was cleared, and the testes were visualized. The muscular sac of the testes was excised and exposed by gently pulling on the cauda epididymis. The blood vessels were tied and the vas deferens with the testes was removed. The muscle and the skin were closed with a suture. The sham operation involved exposure of the testes without isolation.

**Measurement of mean arterial pressure by radiotelemetry**

Animals were anesthetized with 2% isoflurane, and a flexible catheter attached to a radio transmitter (Data Sciences, Minneapolis, MN) was inserted in the abdominal aorta just below the renal arteries. The transmitter was secured to the abdominal muscle and remained in the abdominal cavity for the duration of the experiment. After surgery, rats were housed in individual cages positioned over an RLA-3000 radiotelemetry receiver. Rats received food and water ad libitum. Blood pressure measurements obtained with a 10-s sampling period were averaged and recorded every 10 min, 24 h a day in unrestricted animals housed in the same room.

**Renin Angiotensin System Blockade**

The ACE inhibitor, enalapril (250 mg/l), was administered in the drinking water, from 14 to 16 wk of age. All treated groups received a similar dose of enalapril of ~39 mg·kg⁻¹·day⁻¹; based on average daily water consumption, 65 ml/day, and average daily body weight, 0.42 kg; no significant difference between groups.

**Measure of Testosterone Levels**

Blood samples were collected following decapitation at the end of the study to determine serum testosterone using a commercially available radioimmunoassay kit (Coat-A-Count Total Testosterone Assay kit, Diagnostics Products). Blood samples collected following decapitation at the end of the study were used to determine plasma renin activity (PRA) measured by radioimmunoassay (RIA) using a modification of the method by Haber (16) with ANG I standards, tracer, and antibody from National Bureau of Standards New England Nuclear, and Arnel, respectively.

**Measure of Plasma Renin Substrate**

Blood samples collected following decapitation at the end of the study were used to determine plasma renin substrate (PRS) measured by RIA, as previously described (25).

**Statistics**

GraphPad Prism (ver. 4) was used for all statistical analyses. For comparison made between groups, ANOVA, with adjustments for multiple comparisons was used. A value of P < 0.05 was considered statistically significant.

**RESULTS**

**Birth and Body Weight**

Weight at birth was significantly reduced in IUGR offspring from reduced uterine perfusion dams compared with control offspring from control dams (Table 1). At 16 wk of age, body weight did not differ upon comparison of IUGR to control offspring (Table 1). Thus IUGR offspring exhibited catch-up

*Table 1. Comparison of body weight between control and IUGR offspring*

<table>
<thead>
<tr>
<th></th>
<th>Birth</th>
<th>16 wk of Age</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Intact-UT</td>
</tr>
<tr>
<td>Control</td>
<td>6.3±0.1</td>
<td>408±10</td>
</tr>
<tr>
<td>IUGR</td>
<td>5.3±0.1*</td>
<td>422±11</td>
</tr>
</tbody>
</table>

Weight at birth was measured within 12 h. Control intact untreated (n = 9), control gonadectomized (CTX) untreated (n = 8), control intact treated (n = 8), control CTX treated (n = 8), intrauterine growth restriction (IUGR) intact untreated (n = 8), IUGR CTX untreated (n = 7), IUGR intact treated (n = 8), and IUGR CTX treated (n = 8). UT, animals treated with vehicle; T, animals treated with enalapril. *P < 0.05 vs. control offspring. All data are expressed as means ± SE.
growth, as differences in body weight disappeared by 16 wk of age. Neither castration nor ACE inhibition affected weight gain in control or IUGR offspring, as body weight did not differ upon comparison of intact offspring to their castrated counterparts or treated offspring to their enalapril-treated counterparts (Table 1).

**MAP**

MAP was significantly elevated in intact IUGR offspring compared with intact control offspring from 12 to 16 wk of age (Fig. 1). This marked increase in MAP in IUGR offspring as measured by telemetry is consistent with previous observations made by a single direct measurement of MAP via indwelling carotid arterial catheter (1). Castration abolished the marked increase observed in IUGR offspring (Fig. 1). However, castration did not have a significant effect on blood pressure in control offspring (Fig. 1). Two-week treatment with the ACE inhibitor enalapril initiated at 14 wk of age also abolished hypertension by 16 wk of age in intact IUGR offspring; ACE inhibition also decreased MAP in castrated IUGR offspring (Fig. 2). However, the depressor response to ACE inhibition was greater in intact IUGR offspring compared with castrated IUGR offspring (Fig. 3). ACE inhibition also decreases blood pressure in control offspring intact or castrated; however, this effect did not reach statistical significance (Fig. 4).

**Serum Testosterone Levels**

Serum testosterone measured at 16 wk was significantly increased in intact IUGR offspring compared with control offspring (Fig. 5), and testosterone levels were markedly reduced following castration in both control and growth-restricted offspring compared with their intact counterparts (190 ± 12 vs. 3.4 ± 0.5 ng/dl and 347 ± 34 vs. 1.2 ± 0.1 ng/dl control and IUGR offspring, respectively).

**PRA and PRS.** No significant difference was observed at 16 wk of age in either PRA (3.5 ± 1 vs. 4 ± 1 nmol·angiotensin I(-1)·h(-1)) or PRS (29 ± 8 vs. 23 ± 7 ng angiotensin I·ml(-1)·h(-1)) on comparison of control vs. IUGR offspring, respectively.

**DISCUSSION**

Sex-specific differences are observed in many animal models that examine the developmental origins of adult disease (1,
Influences during fetal life may lead to long-term consequences on physiological and endocrine function. Thus alterations in physiological and endocrine function. Thus alterations in physiological and endocrine function. Thus alterations in physiological and endocrine function. Thus alterations in physiological and endocrine function.

Testosterone plays an important role in the regulation of growth and differentiation during fetal development (8, 47). Influences during fetal life may lead to long-term consequences on physiological and endocrine function. Thus alterations in the development of the fetal HPA axis induced by adverse fetal conditions, such as undernutrition and placental insufficiency, may lead to changes in endocrine systems and promote pathophysiological changes later in life.

The mechanism by which testosterone contributes to hypertension in IUGR offspring may involve a regulatory system important in sodium and water homeostasis and arterial pressure control, the RAS (4, 9, 42). Treatment with the ACE inhibitor enalapril abolished hypertension in intact IUGR offspring. Thus involvement of the RAS in the maintenance of hypertension is demonstrated in this model of IUGR. ACE inhibition also resulted in a significant decrease in MAP in IUGR offspring. However, the decrease in blood pressure in response to ACE inhibition was greater in intact IUGR \((\Delta 36 \pm 1 \text{ mmHg}, P < 0.05)\) compared with CTX IUGR \((\Delta 15 \pm 2 \text{ mmHg}), \) indicating the response to ACE inhibition was greater in the presence of elevated levels of testosterone.

A relationship between testosterone and RAS expression is reported by numerous animal models of hypertension (4, 9, 13, 42). Angiotensinogen mRNA expression in kidney and liver are testosterone dependent (4, 9). Testosterone can influence the RAS and the degradation of vasopressin at the level of the HPA axis (13). In addition, testosterone can augment renal proximal tubule transport by stimulation of the local RAS, thus contributing to higher blood pressure (40). Therefore, activation of the RAS mediated via augmented levels of testosterone may contribute to hypertension in adult male IUGR offspring induced by placental insufficiency.

Alterations in components of the RAS are observed in animal models of prenatal programming of adult disease and hypertension. Increased renal expression of the angiotensin type 1 (AT-1) receptor is observed in offspring from protein-restricted dams (43, 46). PRA is also increased in offspring from protein-restricted dams but only after establishment of the hypertension (33). Thus, in the low-protein model of fetal programming, inappropriate activation of the RAS occurs despite significant elevations in arterial pressure, suggesting a role for RAS involvement in the pathogenesis of hypertension produced in response to a fetal insult.

In this model of fetal programming induced by placental insufficiency, plasma levels of RAS components were similar to control values.
between control and IUGR offspring after establishment of hypertension at 16 wk of age. However, our laboratory has previously reported that intrarenal levels of RAS components are significantly elevated by 12 wk of age in adult male IUGR offspring but not in young IUGR offspring (15). Tissue-specific overexpression of renal angiotensinogen in transgenic mice results in marked elevations in blood pressure despite unchanged levels of peripheral angiotensin, suggesting a role for intrarenal contributions to blood pressure control (7).

Therefore, activation of the intrarenal RAS occurs in adult male IUGR offspring and is associated with increased levels of testosterone. In addition, activation of the intrarenal RAS occurs at the same time sex-specific differences in blood pressure are observed in IUGR offspring. Thus these findings indicate elevated levels of testosterone may contribute to hypertension in adult male IUGR offspring. Furthermore, activation of the intrarenal RAS may be the mechanism by which elevated testosterone levels lead to hypertension in adult male IUGR offspring.

Perspectives

Sex differences are demonstrated in the prevalence and severity of human hypertension, and androgens appear to play an important role in this sexual dimorphism. Sex differences are also apparent in several models of fetal programmed hypertension. Findings from this study suggest intrarenal activation of the RAS may be the mechanism whereby augmented levels of testosterone mediate hypertension in adult male IUGR offspring. However, hypertension in this model of IUGR induced by placental insufficiency occurs before puberty in both male and female offspring. Thus mechanisms responsible for early development of programmed hypertension in male and female IUGR offspring may differ from mechanisms that mediate sex-specific programmed hypertension in the adult animal. Results from this study highlight the complexity of the fetal origins of adult disease, and future studies are needed to clarify the effects of an adverse fetal environment on regulatory control systems for blood pressure control and interactions with sex hormones.

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GRANTS

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REFERENCES


