Angiotensin AT$_1$ receptor blockade fails to attenuate pressure-overload cardiac hypertrophy in fetal sheep

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Angiotensin AT$_1$ receptor blockade fails to attenuate pressure-overload cardiac hypertrophy in fetal sheep. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1501–R1508, 1997.—We examined the hypothesis that endogenous angiotensin II and angiotensin type 1 (AT$_1$) receptors participate in the development of fetal right ventricular hypertrophy by studying the effects of AT$_1$ receptor blockade on cardiac growth in fetal sheep subjected to constrictive banding of the pulmonary artery (PA). Seven pairs of twin fetuses were studied beginning at 126 ± 1 days gestation (term = 145 days). One twin was given losartan (10 mg·kg$^{-1}$·day$^{-1}$ iv) for 7 consecutive days after PA banding, and the other twin served as a saline-treated, PA-banded control. Four additional pairs of twins served as sham-operated controls. Fetal heart rate (HR) and mean arterial blood pressure (MABP) were similar in the two groups of PA-banded animals before treatment and remained unchanged in the PA-banded control group. Losartan resulted in a significant decrease (P < 0.05) in MABP between days 0 and 7, whereas HR was not affected. Total body weight of the losartan-treated animals was significantly less (P < 0.05) than twin PA-banded controls and nonbanded fetuses. Right ventricle weight-to-body weight ratios were similar in saline (2.29 ± 0.34 g/kg) and losartan-treated (2.11 ± 0.15 g/kg) PA-banded animals and significantly greater than that in nonbanded fetuses (1.52 ± 0.07 g/kg). Similar differences were seen in the right ventricle weight-to-left ventricle weight ratios. Right and left ventricle AT$_1$ receptor mRNA and protein expression were also similar among the three groups, as were AT$_2$ receptor mRNA levels. These data suggest that endogenous angiotensin II does not contribute to the development of pressure overload-induced right ventricular hypertrophy during fetal life and that expression of angiotensin receptors is not altered by increased afterload in the ovine fetus.

THERE IS AN ABUNDANCE of evidence suggesting that in addition to its inotropic and chronotropic actions, angiotensin II (ANG II) has a growth-promoting effect on the heart (1, 25, 37, 38) and that this effect is mediated, at least in part, by ANG type 1 (AT$_1$) receptors (37, 38). In vitro studies have demonstrated that ANG II stimulates protein synthesis and cell growth in cultured myocytes (1) and isolated perfused rat hearts (38) and causes mitogenesis of cardiac fibroblasts (37). Exposure to ANG II also results in induction of several protooncogenes and growth-promoting genes in cardiac myocytes (16, 37).

In postnatal animals, infusion of ANG II stimulates the development of cardiac hypertrophy and induces a change to the fetal phenotype of cardiac myocytes independent of effects on blood pressure (15, 23). Blockade of the renin-angiotensin system (RAS) with a converting enzyme inhibitor or an AT$_1$-receptor antagonist prevents the development of cardiac hypertrophy in adult rats with aortic constriction (5, 36) and induces regression of ventricular hypertrophy in spontaneously hypertensive rats (24) and adult animals with chronic pressure-overload hypertrophy (9). On the other hand, afterload reduction with hydralazine or administration of an AT$_2$-receptor antagonist fails to alter the development of ventricular enlargement (9, 16). Finally, Beinlich and co-workers (6) demonstrated in newborn piglets that treatment with enalapril or losartan attenuates the rapid growth of the left ventricle (LV) that normally occurs in the first 3 days of life in these animals. Taken together, these results suggest that the effects of endogenous ANG II on cardiac growth are mediated by AT$_1$ receptors and are independent of changes in cardiac afterload.

The hypertrophic effect of endogenous ANG II may be mediated by circulating and/or locally produced hormones. Regulatory components of the RAS, including renin, angiotensinogen, and ANG II receptors, as well as their precursor genes, have been detected in all four cardiac chambers (25). In adult models of pressure-overload hypertrophy, angiotensinogen and angiotensin-converting enzyme mRNA significantly increases (5), whereas AT$_1$ receptors are either downregulated (26) or upregulated (16). AT$_1$ receptor mRNA is also upregulated in both a spontaneously hypertensive rat model and a renovascular hypertensive model of LV hypertrophy (41), suggesting that AT$_1$ receptor expression during ventricular hypertrophy is regulated by a number of factors.

The cardiac ventricles function in a hemodynamic environment quite dissimilar during fetal development compared with postnatal life. In the fetal lamb, the right ventricle (RV) is exposed to similar filling and arterial pressures as the LV and provides two-thirds of the biventricular output (42). Morphological and functional responses to increases in arterial pressure are also different between the left and right fetal ventricles (30, 42). However, the mechanisms regulating the fetal cardiac hypertrophic response have not been studied. Accordingly, the purpose of this study was to test the hypothesis that endogenous ANG II, through occupation of the AT$_1$ receptor, participates in regulating the morphological response to RV pressure overloading in fetal sheep. Specifically, we sought to 1) determine whether the development of fetal RV enlargement in response to pulmonary artery (PA) banding is attenuated by administration of the AT$_1$-receptor antagonist...
Isosartan and 2) ascertain whether expression of fetal cardiac AT₁ and AT₂ mRNA is altered by increased ventricular load.

METHODS

Animals and surgical preparation. Studies were performed in fetal sheep of Dorset and Suffolk mixed breeding obtained from a local source. The gestational ages of the fetuses were based on the induced ovulation technique (22). Fetal body weight was estimated according to the following formula: weight (kg) = 0.0961 × gestational age (days) – 9.2228, r = 0.85 (35). All procedures were performed within the regulations of the Animal Welfare Act and the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of Iowa Animal Care and Use Committee.

Only pregnant ewes at 125–126 days gestation (term 145 days) with twin fetal pregnancies were used for the study (n = 7 ewes). Anesthesia was induced with 12 mg/kg of thiopental sodium (Pentothal Sodium, Abbott Laboratories) and maintained with a mixture of halothane (1%), oxygen (33%), and nitrous oxide. Under sterile conditions, the uterus was opened over the fetal hindlimbs, and polyethylene catheters were placed into the fetal femoral arteries and veins. A catheter for measurement of amniotic pressure was secured to the fetal skin. Fetal skin incisions were closed, and the fetus was returned to the uterus.

Through a separate uterine incision, the fetal head and thorax were exposed. Via a third interspace thoracotomy, the main PA was exposed proximal to the ductus arteriosus and double-wrapped with an umbilical tape ligature to constrict the diameter of the artery by 50%. The fetal chest was closed and the hysteroscopy repaired. These procedures were then repeated on the second twin. At the completion of surgery, maternal incisions were closed in separate layers and all catheters were exteriorized through a subcutaneous tunnel and placed in a doth pouch on the ewe’s flank. Amiprilin sodium (Wyeth Laboratories, Philadelphia) was administered intra-amniotically at the completion of surgery (2 g) and to the ewe before surgery (2 g) and daily for 3 days. Pregnant ewes were returned to individual pens and allowed free access to food and water. A separate group of twin fetuses (n = 8 fetuses) obtained from time-dated pregnancies was used as a non-PA-banded control.

Experimental protocol. The physiological studies were begun 24 h after surgical preparation. Fetal mean arterial blood pressure (MABP) and amniotic pressure were obtained using Statham P23 Db pressure transducers (Spectramed, Critical Care Division, Oxnard, CA) and a Gould recorder (Gould, Valley View, OH). Fetal MABP was corrected relative to concomitant amniotic pressure. Heart rate was monitored with a cardiotachometer triggered from the arterial pressure wave. Arterial blood was obtained from each fetus for determination of arterial blood gases and pH and of plasma ANG II, vasopressin, epinephrine, and norepinephrine levels. After the fetal monitoring period, one twin was randomly assigned to receive losartan (10 mg/kg iv, 50 mg/ml) daily for 7 days while the second twin received an equivalent volume of vehicle (0.9% NaCl). At the end of 7 days (day 7), fetal blood pressure, heart rate, and arterial blood samples were again obtained on both the losartan-treated and control animals. The ewe was then returned to the surgical area and, under general anesthesia, the fetuses were exteriorized individually. RV systolic and diastolic pressures were obtained by direct puncture of the chamber with an 18-gauge needle. The fetus was then euthanized with an overdose of pentobarbital sodium, and the body weight was recorded. Hearts were removed for determination of total weight and RV and LV free wall weights. Tissue from LV and RV free wall, obtained approximately midway between the apex and the atrioventricular groove and kidney were snap frozen in liquid nitrogen and stored at −70°C. The non-PA-banded control twin fetuses from which hearts were obtained were of postconceptional ages similar to PA-banded animals.

Analytic procedures. Arterial blood for pH, P CO₂, and P O₂ was collected anaerobically in heparinized syringe, and measurements were immediately determined at 39.5°C using a BGM 1302 pH/blood gas analyzer (Instrumentation Laboratory, Lexington, MA). Measurements of plasma ANG II, vasopressin, epinephrine, and norepinephrine were performed by radioimmunoassay (University of Iowa Cardiovascular Center RIA Core Facility, Donna B. Farley, Director).

Isolation of RNA and preparation of probes. Total cellular RNA was isolated using Tri-Reagent (Molecular Research Center, Cincinnati, OH). RNA was quantified spectrophotometrically by absorbance at 260 nm. RNA samples were stored as an ethanol precipitate at −70°C until further analysis.

The sheep AT₁ receptor partial cDNA sequence from nucleotides 114 to 783 and AT₂ receptor partial cDNA sequence from nucleotides 142 to 921 were isolated as previously described (32, 33). The plasmids pAT₁ and pAT₂ were then treated with the restriction enzymes Nde I and Hind III, respectively, and purified by phenol extraction and ethanol precipitation. The resultant linear plasmids were used to generate labeled antisense RNA probes using T7 RNA polymerase (United States Biochem, Cleveland, OH) and [32P]UTP (Amersham, Arlington Heights, IL).

An 18S rRNA probe was used to confirm equal loading and transfer of RNA. The 18S rRNA probe was prepared from an 18S cDNA done corresponding to a 82-bp fragment of a highly conserved region of human 18S rRNA (Ambion, Austin, TX).

Northern blot hybridization. Aliquots of 10 µg of RNA as measured by absorbance at 260 nm were fractionated by 1% formaldehyde-agarose gel electrophoresis. After electrophoresis, RNA was transferred to a 0.45-mm Nytran filter. The filters were prehybridized for 1 h at 60°C in a solution of 50% deionized formamide, 5% saline-sodium phosphate-EDTA (SSPE), 5× Denhardt’s solution, 0.5% sodium dodecyl sulfate (SDS), and 200 µg/ml denatured sheared salmon sperm DNA. Hybridization of filters was carried out with fresh hybridization buffer solution containing 2 × 10⁶ counts·min⁻¹·µl⁻¹ of the appropriate radiolabeled probe. The hybridization reaction was carried out at 60°C for 12–18 h.

Filters were washed with three low-stringency washes (1× SSPE, 0.5% SDS) at 68°C and a high-stringency wash (0.1× SSPE, 0.5% SDS) at 65°C. Hybridization signals were detected and quantitated using an AMBIS 4000 Radioanalytic Imaging System (AMBIS, San Diego, CA). The AMBIS 4000 simultaneously images and quantitates the radioisotopic signal generated by 32P on the filters as previously described (33). Background counts above each lane were determined and subtracted from the total counts generated in each region of interest to yield a net count value. The washed filters were additionally exposed to Kodak XAR film at −70°C.

Immunoblotting. Tissue samples were homogenized in 50 mM tris(hydroxymethyl)aminomethane (Tris), 10 mM EDTA, 150 mM NaCl, 0.1% (vol/vol) Tween-20, 0.1% (vol/vol) 2-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride, 5 µg/ml leupeptin, and 5 µg/ml aprotinin, pH 7.5, and sonicated on ice for 15–20 s. One-milliliter aliquots were centrifuged in a microfuge at 12,000 g for 3 min, at 4°C. The pellet was discarded, and samples of supernatant were diluted in a sample buffer, the final composition of which was 47 mM...
Tris-HCl, 7.5% glycerol, 1.5% (wt/vol) SDS, 15% 2-mercaptoethanol, and 0.038% (wt/vol) Bromphenol blue, pH 6.8. Protein concentrations were determined by the method of Lowry, as modified by Peterson (29). Samples were heated in a water bath at 95°C for 4 min, fractionated by SDS-polyacrylamide gel electrophoresis (9% polyacrylamide), and then transferred to nitrocellulose sheets by blotting at 30 V overnight in a ice water bath (20 µg protein/lane). The transfer buffer consisted of 25 mM Tris, 192 mM glycine, and 20% methanol, pH 8.2. The nitrocellulose blots were blocked for 2 h at room temperature in a 5% nonfat milk, Tris-buffered saline (TBS) solution containing 10 mM sodium azide and 0.1% Triton X-100. Two quick rinses in 0.1% Tween-20-TBS (TTBS) followed, and the blots were then washed twice for 10 min each in TTBS at room temperature. To verify transfer of protein to nitrocellulose, the outside lane of every blot contained colored standards (Kaleidoscope Prestained Standards; Bio-Rad, Hercules, CA). In addition, each gel was Coomassie stained after transfer to verify that no protein remained. Blots were then incubated with the primary antibody (rabbit anti-rat AT1A-receptor antibody; Santa Cruz Biotechnology, Santa Cruz, CA) at a 1:1,000 dilution in 1% gelatin-TTBS for 2 h at room temperature. Blots were again rinsed and washed as described above, then incubated with a 1:8,000 dilution of goat anti-rabbit horseradish peroxidase (HRP-horseradish peroxidase conjugated antibody) (Sigma) in 1% gelatin-TTBS at room temperature for 1 h. The blots were again rinsed and washed as described above. Binding of the secondary antibody was detected using a chemiluminescent system consisting of HRP-hydrogen peroxide oxidation of luminol (ECL, Amersham). Blots were then exposed to Fuji RX X-ray film for 5 min. Preliminary experiments using this protocol with ovine adrenal cortex showed three bands of the expected size.

Immunoblots were quantitated using densitometric analysis. To verify that the immunoblot densitometric signal was a linear function of the amount of AT1 receptor present in the sample, we performed preliminary experiments plotting densitometric absorbance against protein concentration. This relationship was linear between 5 and 40 µg/lane (r = 0.89); 5 concentrations within this range were measured. Therefore, under the conditions we used, the antisera provide quantitative measurements of AT1 receptor abundance.

Data analysis. For quantitation of mRNA abundance, all samples were analyzed together on a single Northern blot hybridization to control for day-to-day variations in hybridization efficiency. Northern blots were done in triplicate. Abundance of AT1, AT2, and 18S mRNAs was expressed as total net radioactive counts, after background counts for each sample were subtracted. Expression of AT1 and AT2 mRNA were normalized by corresponding 18S rRNA net counts.

Comparisons among the different groups were performed using one-way analysis of variance. When the analysis of variance indicated significant differences among groups, as indicated by the F statistic, comparison among means was performed by the Duncan multiple-comparison procedure (44). Paired and unpaired t-tests were also used to compare means between two groups. Statistical significance was defined as P < 0.05, and the results are presented as means ± SE.

RESULTS

Effects of PA banding and losartan on fetal hemodynamics and arterial blood values. The effects of PA banding and concomitant treatment with losartan on fetal hemodynamic measurements are summarized in Table 1. Fetal heart rate and MABP were similar in the two groups before treatment and remained unchanged in the control PA-banded group over 7 days. Administration of losartan for 7 days resulted in a significant decrease (P < 0.05) in MABP from 47 ± 2 to 30 ± 3 mmHg, whereas heart rate was not affected. Losartan-treated animals also had significantly lower RV systolic pressures (46 ± 1 mmHg) than the twin PA-banded controls (60 ± 1 mmHg). Arterial blood pH, P O2, P CO2, and AVP were similar in losartan- and saline-treated, PA-banded fetuses on day 0 and day 7. Plasma vasopressin concentrations were higher on day 0 than on day 7 in both groups, although no differences existed between groups.

Effects of PA banding and AT1 receptor blockade on fetal somatic and cardiac mass. Total body weight of the losartan-treated animals (2.68 ± 0.22 kg) was significantly less (P < 0.05) than the twin PA-banded controls (3.08 ± 0.13 kg) and nonbanded fetuses (3.18 ± 0.22 kg). There was no difference in body weight between banded and nonbanded control fetuses. PA banding alone increased (P < 0.05) fetal heart weight and the heart weight-to-body weight ratio compared with nonbanded animals (Table 2). The increase in mass appeared specific for the RV, as demonstrated by the significant increases in the RV weight-to-body weight, RV weight-to-LV weight ratios (Fig. 1). No alteration in the LV weight-to-body weight ratio was seen between saline-treated control PA-banded and nonbanded fetuses (Fig. 1).

We originally postulated that blockade of cardiac AT1 receptors would attenuate the development of pressure overload-induced hypertrophy. However, administration of losartan over the 7-day period failed to prevent the development of increased RV mass in PA-banded animals. Although absolute heart weight and RV weight were not statistically different among the three groups, heart weight-to-body weight, RV weight-to-body weight,

<table>
<thead>
<tr>
<th>Control</th>
<th>Losartan-Treated</th>
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<tr>
<td>Day 0</td>
<td>Day 7</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>182 ± 13</td>
</tr>
<tr>
<td>MABP, mmHg</td>
<td>50 ± 2</td>
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<tr>
<td>RV systolic pressure, mmHg</td>
<td>60 ± 1</td>
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<tr>
<td>pH</td>
<td>7.36 ± 0.01</td>
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<tr>
<td>Pco2, mmHg</td>
<td>51.0 ± 2.0</td>
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<tr>
<td>Po2, mmHg</td>
<td>21.0 ± 2.0</td>
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<tr>
<td>ANG II, pg/ml</td>
<td>10.5 ± 4.13</td>
</tr>
<tr>
<td>AVP, µU/ml</td>
<td>30.4 ± 9.4</td>
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<tr>
<td>Epi, pg/ml</td>
<td>87.8 ± 20.7</td>
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<td>NE, pg/ml</td>
<td>1,010 ± 284</td>
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Values are means ± SE; n = 7 in each group. Day 0 (125–126 days gestation), before start of daily administration of either 0.9% NaCl (control) or losartan (10 mg/kg); day 7, after 7 days of 0.9% NaCl or losartan; HR, heart rate; MABP, mean arterial blood pressure; RV, right ventricle; ANG II, angiotensin II; AVP, arginine vasopressin; Epi, epinephrine; NE, norepinephrine. *P < 0.05 compared with corresponding day 0 value. †P < 0.05 compared with corresponding control value.
and RV weight-to-LV weight ratios were significantly greater in losartan-treated, PA-banded fetuses compared with nonbanded animals (Table 2). Furthermore, no differences in any of the cardiac growth parameters were demonstrated between the losartan-treated and saline-treated PA-banded animals (Fig. 1).

Effects of PA banding on expression of AT₁ receptor mRNA and protein. Northern blot analysis was used to determine the effects of RV pressure overload on expression of fetal cardiac AT₁ and AT₂ mRNA. Pressure overload of the RV was not associated with any significant alterations in the levels of AT₁ or AT₂ mRNA (Fig. 2). AT₁ receptor blockade also failed to produce changes in right and LV AT₁ and AT₂ mRNA levels in PA-banded animals.

Using Western blot analysis, we sought to determine whether AT₁ receptor protein expression was altered with the development of RV hypertrophy. Immunoblots (Fig. 2) probed with a rabbit anti-rat AT₁ receptor antibody showed four bands of the expected size, with the major band at 67 kDa, as previously reported by Marrero et al. (27). Bird and co-workers (8) characterized the antiserum we have used in sheep tissues and showed a similar pattern of bands in the adrenal cortex.

The four bands they detected showed a tissue rank order of AT₁ receptor expression identical to that seen in pharmacological binding studies, supporting the idea that these bands represent specific binding. Consistent with that found for AT₁ mRNA levels, no differences in ventricular AT₁ receptor protein expression were seen among the three groups of animals.

DISCUSSION

Constriction of the proximal PA has previously been shown to be a useful model for the development of pressure-overload RV hypertrophy (7, 10). In the present study, partial occlusion of the proximal PA to ~50% of the normal diameter (75% of the normal cross-sectional area) for 8 days produced RV enlargement, as demonstrated by significant increases in RV mass and RV weight-to-body weight ratio. Although the development of cardiac hypertrophy is an adaptive response to a sustained increase in hemodynamic workload, the mechanisms mediating the development of hypertrophy are not fully understood.

The present study was designed to investigate the contribution of endogenous ANG II to the development of RV hypertrophy in the developing fetus. Contrary to our hypothesis, we found that blockade of AT₁ receptors failed to attenuate the development of RV hypertrophy in PA-banded fetuses. RV weight-to-body weight and RV weight-to-heart weight ratios were similar in the losartan-treated and saline-treated PA-banded fetuses and significantly greater than control values. The finding that endogenous ANG II has little involvement in pressure overload-induced cardiac growth during fetal life is in contrast to most (9, 15, 16, 36) but not all (28, 43) studies performed in postnatal animals. There are several plausible explanations for these discordant results. It is possible that the increase in RV mass in the PA-banded animals was secondary to hyperplasia rather than hypertrophy. The fetus is capable of increasing the number of myocytes during development.
whereas, postnatally, ventricular mass may only increase through cellular hypertrophy (12). Bical et al. (7) found in fetal sheep with PA stenosis (mean duration 42 days) that although RV weight was increased in this group, the transverse diameter of RV free wall myocytes was similar to that of control animals. The myocyte appeared more mature with more numerous myofibrils and mitochondria and little free cytoplasmic area, raising the possibility that both hyperplasia and hypertrophy occur in response to pressure overload early during development. Although we did not perform detailed morphological studies, light microscopy showed that the RV of PA-banded fetuses contained larger nuclei that were surrounded with a greater amount of myofibrillary material compared with that of non-banded control animals. This observation would be consistent with the increase in RV wall mass in the PA-banded fetuses resulting at least in part from cellular hypertrophy, and not strictly myocyte hyperplasia.

Concomitant with the development of load-induced cardiac hypertrophy in the adult are characteristic changes in the expression of fetal cardiac genes (2, 11, 17). Activation of genes coding for contractile proteins (11, 17), natriuretic factors (2), and immediate-early genes (21) in response to pressure overload results in adaptive growth, although the molecular and biochemical mechanisms remain to be elucidated. The presence of ANG II appears important for these responses, because blockade of the RAS inhibits many of these molecular and cellular adaptations to pressure-overload states (24). Furthermore, the molecular and biochemical changes in the heart in response to ANG II, independent of changes in afterload, are similar to those seen with pressure overload (23). However, the genes and proteins that are upregulated in the adult myocardium in response to pressure overload or ANG II are already present and active in the developing fetus. Thus active transformation to this “fetal cellular environment,” which appears vital to the hypertrophic response.

Fig. 2. Effect of PA banding with and without concomitant administration of losartan on fetal right and left cardiac ventricle ANG type 2 (AT2) levels. Top: representative autoradiograms of Northern blots of right and left ventricle RNA hybridized with a sheep AT2 RNA probe and with a human 18S rRNA labeled with 32P. Bottom: abundance of right and left ventricle AT2 mRNA is expressed as total net counts of 32P. Expressed values are normalized to corresponding 18S value. Seven pairs of twin fetuses were studied with PA bands. Four pairs of twins comprised the no-PA band group. Values are means ± SE.

Fig. 3. Effect of PA banding with and without concomitant administration of losartan on expression of fetal right and left cardiac ventricle AT1 receptor protein. Top: representative autoradiogram of Western blots of right and left ventricle homogenates incubated with a rabbit anti-rat AT1A receptor antibody; adrenal is used as a tissue standard. Rat renal proximal tubule brush-border membrane vesicles (BBMV) are included as positive tissue control. Bottom: expression of right and left ventricle AT1 receptor is expressed as densitometric units using chemiluminescence. Seven pairs of twin fetuses were studied with PA bands. Four pairs of twins comprised the no-PA band group. Values are means ± SE.
response, need not occur in the immature animal. As a consequence, blockade of the RAS may have little or no effect on the load-induced myocardial changes in the fetus, as demonstrated in the present study.

The role of AT2 receptors in regulating cell growth is unclear. The predominance of AT2 receptors during fetal life and their abundant expression within fetal parenchymal tissues have led investigators to postulate that these receptors may be involved in protein synthesis and growth (40). Recent reports of mice with targeted disruption of the AT2 gene demonstrate that in this species, the AT2 receptor is involved in cardiovascular regulation but is not required for normal embryonic and postnatal development (18, 19). Furthermore, in vitro studies have shown that treatment with an AT2 receptor antagonist has no significant effect on ANG II induction of hypertrophy or expression of protooncogenes and growth factor genes (37). On the other hand, Poole and co-workers (31) suggest that AT2 receptors are principally involved in the cardiac hypertrophy seen in rats with aorto caval fistulas. Our data neither confirm nor refute a role for AT2 receptors in the development of hypertrophy. However, we have recently shown that ovine cardiac AT2 mRNA levels are high late in fetal development and decrease abruptly after birth (39). Studies using AT2 receptor antagonist are needed to investigate a potential role for these receptors in development of load-induced increases in ventricular mass during fetal life.

The design of the study also allowed us to investigate potential mechanisms regulating the expression of ANG II receptor mRNAs and protein. We found that in the developing fetus, RV pressure overload failed to alter the levels of cardiac AT1 and AT2 mRNA or expression of AT1 receptor protein. This lack of alteration of cardiac AT1 and AT2 mRNA levels with ventricular hypertrophy is consistent with that reported by Iwai and Inagami (20) and Wolf et al. (43). On the other hand, Lopez and co-workers (26) found in aortic-banded rats that LV hypertrophy was associated with down-regulation of the AT1 receptor, whereas Suzuki et al. (41) showed in the experimental rat model of renovascular hypertension that AT1a mRNA and protein were upregulated with cardiac hypertrophy. Everett et al. (16) similarly found that LV AT1 mRNA was upregulated in rats with cardiac hypertrophy induced by abdominal aorta coarctation. Reasons for these discrepancies are unclear but may be related to differences in animal species, the models of hypertrophy, or the maturation stages of cardiac development.

Administration of losartan produced significant effects on fetal hemodynamics and growth. The large decrease in blood pressure in the losartan-treated, PA-banded fetuses supports an important role of the RAS in regulating fetal cardiovascular function. Previous studies have shown smaller but significant decreases in fetal blood pressure in response to acute administration of angiotensin-converting enzyme inhibitors or ANG II receptor antagonists (13, 34). However, in neither of these studies did treatment continue beyond several hours. Because arterial blood pressures were not obtained at intermediate time points in our fetuses treated with losartan, the time course for the development of relative hypotension cannot be determined. We speculate, however, that the greater hypotensive effect seen in our study is likely related to the longer treatment period with subsequent inhibition of both peripheral and centrally mediated vasoconstrictive effects of ANG II.

The etiology of the impaired somatic growth in losartan-treated animals cannot be directly determined, although it is likely related to changes in placental blood flow secondary to the decrease in arterial pressure. Reduction of fetal femoral arterial pressure by 12–15 mmHg is accompanied by a decrease in umbilical blood flow by about one-third (3). Furthermore, reductions in umbilical blood flow result in decreased fetal O2 consumption (4) and growth rates. On the basis of estimated fetal weights at 125 days gestation (day of initial surgery), non-losartan-treated animals grew on average 4%/day, similar to the rate reported with gestational age (3). It is also possible that blockade of AT1 receptors impairs organ growth via influences on the expression of growth-related genes and growth factors (24), independent of effects of blood pressure.

We conclude that in this fetal ovine model of RV pressure overload, AT1 receptor blockade has no effect on the compensatory increase in RV mass. Furthermore, levels of cardiac ventricle AT1 and AT2 receptor mRNA and AT1 receptor protein are not altered by increased RV load during fetal development. These findings suggest that the RAS has little role in regulating load-induced cardiac growth early during development. However, endogenous ANG II appears important for normal fetal somatic growth, most likely by maintaining adequate arterial pressure and placental and organ blood flow.

Perspectives

The roles of circulating ANG II and organ-specific RAS on organ growth and function remain to be defined. Endogenous ANG II levels are high during fetal development and, as suggested in this study, are
important in maintaining arterial and placental perfusion pressures. It is possible that the biological actions of ANG II on organ growth and function change during maturation, in concert with developmentally regulated expression of specific ANG II receptors. Only with further investigation will the physiological functions and mechanisms for regulation of the RAS early during development be elucidated.

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