Thyroid hormone modulates renin and ANG II receptor expression in fetal sheep


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Chen, Kai, Luke C. Carey, Nancy K. Valego, Jingfang Liu, and James C. Rose. Thyroid hormone modulates renin and ANG II receptor expression in fetal sheep. Am J Physiol Regul Integr Comp Physiol 289: R1006–R1014, 2005. First published May 26, 2005; doi:10.1152/ajpregu.00046.2005.—Fetal renin-angiotensin system (RAS) activity is developmentally regulated, increasing in late gestation toward term. At the same time, fetal hemodynamic parameters change, with blood pressure increasing and heart rate decreasing. During this period, fetal plasma thyroid hormone concentrations also increase significantly. In this study we utilized the technique of thyroidectomy (TX), which removes thyroid hormone from the circulation, to investigate the importance of thyroid hormone on the developmental changes in the RAS (in plasma, kidney, heart, and lung) and hemodynamic regulation in fetal sheep. TX was performed at 120 days of gestational age (dGA), and control fetuses were sham operated. Immediately before necropsy (~137 dGA), fetuses were infused with isoproterenol and the hemodynamic responses were noted. TX significantly decreased plasma thyroid hormone concentrations and renal renin mRNA and renal active renin levels but did not change fetal plasma active renin levels. TX decreased both angiotensin II receptor subtype 1 (AT1) mRNA and protein levels in kidney and lung but not in the left ventricle. TX was also associated with increased ANG II receptor subtype 2 (AT2) mRNA and protein at the 44-kDa band in kidney, whereas AT2 protein was decreased at the 78-kDa level in kidney and lung tissue only. TX fetuses had significantly lower basal mean arterial blood pressures (MAP) and heart rates than controls. Isoproterenol infusion decreased MAP in TX fetuses. These findings support the hypothesis that thyroid hormone is important in modulating maturation of RAS and cardiovascular function in the late-gestation fetal sheep.

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THE RENIN-ANGIOTENSIN SYSTEM (RAS), composed of renin, angiotensinogen, ANG I-converting enzyme (ACE), ANG II, and the ANG II receptor subtypes, plays an important role in regulating fetal growth and development, particularly with respect to the cardiovascular system, and electrolyte homeostatic mechanisms (2, 22, 48). It is well established that components of the fetal RAS are developmentally regulated and exhibit increased activity in late gestation (12, 56, 57, 63). Changes in expression of the levels of ANG II receptor subtypes 1 and 2 (AT1 and AT2) are opposite, with AT1 mRNA low in early gestation and rising to a plateau later, before increasing rapidly close to term, whereas AT2 expression is highest during midpregnancy and then decreases gradually thereafter (46, 47). Tissue-dependent differences also are apparent (18, 40, 66). The mechanisms underlying these ontogenetic patterns of expression are not clear.

In fetal sheep, the changes in components of RAS activity appear temporally related to changes in thyroid hormone concentrations, which are low before 130–135 days of gestational age (dGA) and then markedly increased toward parturition (19). A similar pattern of change is seen in humans (44). These observations suggest that thyroid hormone may be important in regulating ontogenetic changes in renin and ANG II receptors.

Thyroid hormone also exerts broad effects on the developing cardiovascular system, increasing cardiac contractility and output, inducing arterial relaxation, and reducing systemic vascular resistance (35). Hypothyroidism, conversely, is associated with decreased stroke volume, cardiac output, and intravascular volume (34). In adult rats, thyroidectomy (TX) significantly decreases both heart rate and blood pressure (14, 20). Likewise, in fetal sheep, TX performed at 80 dGA is associated with lower blood pressure but no change in heart rate (61). These findings suggest that thyroid hormone exerts positive regulatory effects on cardiovascular function in both adult and fetus.

The mechanisms through which thyroid hormone influences cardiovascular system activity are currently unclear. However, previous studies in the rat have confirmed that thyroid hormone alters the cardiovascular response to adrenergic agonists (14), suggesting that this interaction may be at least, in part, responsible.

Therefore, by using the technique of TX, the present studies were designed to assess the importance of thyroid hormone in regulating the components of RAS activity, including renal renin, plasma renin, and tissue ANG II receptor subtype expression in late-gestation fetal sheep. Our rationale for this was that the normal ontogenic change in the RAS would be attenuated in the absence of thyroid hormones in late gestation. In addition, we evaluated the impact of fetal hypothyroidism on cardiovascular function and responsiveness to β-adrenergic stimulation in the late-gestation fetal sheep.

MATERIALS AND METHODS

Animals. Cross-bred pregnant ewes with known insemination dates were obtained from a local supplier. Ewes were housed in individual pens with food and water provided. After 5 days of acclimation, surgery was performed. After surgery, ewes were returned to their pens, where they remained until fetuses were delivered. All procedures were approved by the Wake Forest University Animal Care and Use Committee. A total of 12 fetuses were used: 6 TX (3 male, 3 female) and 6 controls (4 male, 2 female).
Surgical procedures and blood pressure and heart rate measurements. TX was performed similarly to the procedure described by Hopkins and Thorburn (25) at ~120 dGA. Briefly, the fetal neck was exposed and stabilized on a sterile tray, and the skin was opened at the midline. The fetal thyroid gland was exposed and removed. Control fetuses were sham operated, where the thyroid was exposed but not touched. Catheters previously filled with sterile saline were placed in fetal carotid arteries and jugular veins. Additional catheters were inserted into the femoral arteries and veins and advanced to the descending aorta and inferior vena cava. Catheters also were placed in the amniotic sac to measure intrauterine pressure, and in the maternal femoral artery and vein. Gentamicin and ampicillin were administered to the ewe at the time of surgery and for the next 3 days through the maternal venous catheter. Fetal plasma samples were collected after 3 days of postsurgery recovery and just before necropsy for measurement of triiodothyronine (T3) and thyroxine (T4). Fetuses were delivered by cesarean section between 137 and 139 dGA (term in our flock is ~145 days), and tissue samples (kidney cortex, heart, and lung) were collected and stored at ~80°C until assay. These specific tissues were sampled because the RAS is thought to play developmental and/or functional roles in all (11, 50–55).

Basal fetal hemodynamic and plasma active renin and prorenin levels and their responses to β-adrenergic stimulation were studied in both TX and control fetal sheep immediately before necropsy. Fetal arterial blood pressures, heart rate, and amniotic fluid pressure were measured with DMSI system integrators (Digi-Med; Micro-Med Enterprises, Tustin, CA). All blood pressure data were corrected to corresponding amniotic pressure. Data collection was begun after a 30-min stabilization period. The data were averaged and recorded every 1 min automatically throughout the experiment. Responses to β-adrenergic stimulation were determined by measuring the fetal blood pressure and heart rate changes before, during, and after 10 min of isoproterenol infusion (0.06 μg·kg⁻¹·min⁻¹). Mean arterial blood pressure (MAP), amniotic fluid pressure, and heart rate were measured throughout the experiment and for 50 min after the infusion. Blood samples were obtained before isoproterenol infusion, immediately after the isoproterenol infusion was complete, and at the end of the experiment to examine plasma renin levels.

Plasma active renin measurements. T3 was measured by enzyme immunoassay assay using a kit from Diagnostic Systems Laboratories (Webster, TX). The minimum detectable amount of T3 was 0.4 ng/dl. Coefficients of variation were 5.7% intra-assay and 6.7% interassay.

Plasma T4 was measured by RIA using a kit from ICN Pharmaceuticals (Costa Mesa, CA) that measures total T4. The minimum detectable amount of T4 was 0.76 ng/dl. Coefficients of variation were 5.3% intra-assay and 7.9% interassay.

Plasma active renin concentration was measured as a function of the amount of ANG I generated from angiotensinogen by using a kit (Perkin-Elmer Life and Analytical Sciences, Boston, MA). To measure renin concentration independently of endogenous angiotensinogen, we slightly modified the method from that described for renin activity. Excess renin substrate (0.5 ml of adult nephrectomized sheep plasma) was added to each aliquot (0.1 ml) of plasma along with the enzyme inhibitors, dimercaprol, 8-hydroxyquinoline, and maleate buffer (pH 6.0, to assure a constant pH at the optimum for renin activity). One milliliter of this cocktail was then incubated at 37°C, whereas the rest was kept at 4°C for 1 h. The ANG I generated was measured by RIA with the kit. All samples from an animal were analyzed simultaneously and in duplicate, and all assays included samples from control and TX animals. Results are expressed as nanograms of ANG I (37°C ng/ml − 4°C ng/ml) per milliliter of plasma per hour of incubation.

Tissue active renin concentration measurement. Approximately 100 mg of renal cortex were homogenized on ice for 45 s in 4 ml of saline; the homogenate was then centrifuged at 2,100 g for 10 min, and the supernatant was collected. An aliquot was taken for protein determination, and the remainder was frozen and stored at ~80°C until assayed. For the assay, the sample was diluted with saline containing 5.2 mM 2,3-dimercapto-1-propanol, 0.59 mM 8-hydroxyquinoline, and 10 mM disodium EDTA. Active renin concentration (ARC) was determined as for plasma and is expressed as nanograms per milligram of protein per hour of incubation.

Prorenin concentration measurement. Prorenin concentration was determined by measuring active renin before and after treatment of plasma or kidney cortex homogenate with bovine pancreatic trypsin at a concentration designed to yield maximum renin activation. Each dose of trypsin was tested by constructing a dose-response curve with pooled plasma or kidney homogenate. Once the optimal dose of trypsin was established for each, this dose was used for subsequent assays. Trypsin activation was initiated at 3°C and pH 7.3 for 30 min. The activation was stopped by addition of trypsin inhibitor at room temperature for 15 min. The total renin concentration represented the sum of active renin and prorenin.

RNA extraction. Total tissue RNA was extracted using standard procedures. Briefly, the tissue was homogenized in Trizol regent (GIBCO BRL, Carlsbad, CA) with a high-speed Polytron for 30–60 s, chloroform was added (0.2 ml/ml Trizol), and the mixtures were centrifuged at 12,000 g for 15 min at 4°C after 5 min of incubation at room temperature. The aqueous phase was transferred to a fresh tube, and the RNA was precipitated by the addition of isopropanol (0.5 ml/ml Trizol) and recentrifuged at 7,500 g for 5 min. The ethanol was removed, and the RNA pellets were allowed to air dry before being redissolved in RNase-free water. RNA concentrations were determined by measuring absorbance at 260 nm. RNA sample integrity was determined by electrophoresis on a 1.0% agarose gel containing 6.6% formaldehyde.

Synthesis of antisense RNA probes. The probe used for sheep renin mRNA is a partial sheep renin cDNA from coordinates 117–983 cloned into pGEM-T easy (Promega, Madison, WI) and cut with the restriction enzyme EcoRI to linearize the plasmid in preparation for in vitro transcription. The probe used for sheep AT1 mRNA is a partial sheep AT1 cDNA from coordinates 114–783 cloned into pGEM-T easy (Promega) and cut with the restriction enzyme SpeI to linearize the plasmid in preparation for in vitro transcription. The probe used for sheep AT2 mRNA is a partial sheep AT2 cDNA from coordinates 142–921 cloned into pT7/T3U18 (Ambion, Austin, TX) and cut with the restriction enzyme HindIII to linearize the plasmid in preparation for in vitro transcription.

In vitro transcription was performed by adding the following items in order: 4 μl of 5× transcription buffer, 2 μl of 100 mM dithiothreitol, 1 μl of RNasin Rnase inhibitor, 4 μl of ATP, GTP, and CTP mix (25 mM each), 2.4 μl of 100 μM UTP, 5 μl of [α-32P]UTP (3,000 Ci/mmol; Perkin-Elmer), and 1 μl of SP6 (for renin) or T7 (for AT1 and AT2) polymerase and incubating for 2 h at room temperature. RQ1 RNase-free DNase (1 μl) was added, and the reaction was incubated for an additional 15 min at 37°C to remove the DNA template. Unincorporated nucleotides were removed using G-50 Sephadex column chromatography (Roche Molecular Biochemicals, Indianapolis, IN). The purified probe (1 μl) was placed into a scintillation vial to determine counts per minute. Sense strand RNA used for the standard was synthesized with linearized plasmid by in vitro transcription similarly to that described above; however, [α-32P]UTP and 100 μM UTP were replaced with 25 mM UTP.

Rnase protection assay. Renin, AT1, and AT2 mRNAs were quantified by RNase protection assay (RPA) (RPA kit III; Ambion). Briefly, 20-μg RNA samples from kidney cortex, left ventricle, and lung were mixed with 10 μl of hybridization buffer and 100,000 cpm of the renin, AT1, or AT2 probe. Samples were then heated at 95°C for 4 min and placed in a 48°C water bath for overnight hybridization. RNase A/T1 (1:150 dilution in RNase digestion buffer) was then added to the samples to digest unhybridized probe and RNA. Digestion was stopped, and the hybridized RNA was precipitated by the addition of RNase inactivation/precipitation buffer and incubation for...
30 min at −20°C. Hybridized RNA was pelleted by centrifugation at 14,000 g for 15 min. Samples were then run on a 5% polyacrylamide/8 M urea denaturing gel at 250 V for 1 h. Gels were then exposed to film (Biomax-MR; Kodak, Cealex, France) with an intensifying screen. Known quantities of sense RNA were used to construct a standard curve for each assay. Hybridization signals from the protected fragments of the unknown were quantitated by comparison with the standard curve established from the signals of the sense standards. A standard curve was made for each assay, and samples from both control and TX animals were analyzed in the same assay.

Immunoblotting. Western blot analysis for AT1 and AT2 receptors was performed as previously described (51). Briefly, protein concentrations of samples harvested from TX and control animals were determined using a modified Bradford method. A standard curve was produced using known concentrations of bovine albumin, and the protein concentrations of samples were determined by comparing their optical density (OD) value at 595 nm with values from the standard curve. Both AT1 and AT2 receptor-specific polyclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) have been used to detect the ovine AT1 and AT2 receptors, respectively (51). Protein (40 μg/lane) was electrophoresed on a 12% polyacrylamide gel containing sodium dodecyl sulfate for 1.5 h and then blotted onto a polyvinylidene difluoride membrane (Immobilon; Millipore, Marlborough, MA) by semidry electroblotting. The blot was blocked overnight at 4°C with 6% nonfat milk in 0.05% Tween 20 Tris-buffered saline (TTBS) and then incubated with the primary antibody using 1:2,000 (AT1) or 1:6,000 (AT2) dilutions in 6% dry milk/TTBS for 1 h. Blots were then washed and incubated with the secondary antibody for 1 h. The membrane was then washed and exposed to X-ray film. Values are means ± SE; n = 6 for both groups. *P < 0.05, control vs. TX.

Table 1. Arterial blood gases in control and TX fetal sheep during development after surgery

<table>
<thead>
<tr>
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<th>&lt;130 dGA</th>
<th>131–136 dGA</th>
<th>&gt;137 dGA</th>
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<tbody>
<tr>
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</tr>
<tr>
<td>pH</td>
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<td>7.33±0.01</td>
<td>7.31±0.01</td>
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<tr>
<td>Pco₂, mmHg</td>
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<td>51.00±2.80</td>
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<tr>
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<tr>
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Values are means ± SE; TX, thyroidectomized; dGA, days of gestational age.

Fig. 1. Plasma triiodothyronine (T3) and thyroxine (T4) levels in control and thyroidectomized (TX) sheep fetuses just before necropsy [137–139 days of gestational age (dGA)]. Values are means ± SE; n = 6 for both groups. *P < 0.001, control vs. TX.

Fig. 2. Renal renin mRNA expression in 137–139 dGA control and TX fetuses. Values are means ± SE; n = 6 for both groups. *P < 0.05, control vs. TX.

Fig. 3. Renal active and prorenin levels in 137–139 dGA control and TX fetuses. Values are means ± SE; n = 6 for both groups. *P < 0.05, control vs. TX.
2 h at room temperature. Blots were then rinsed, washed, and incubated with a 1:4,000 dilution of monkey anti-rabbit horseradish peroxidase-conjugated antibody in 6% dry milk/TTBS for 1 h at room temperature. Binding of the secondary antibody was detected using a chemiluminescent system consisting of horseradish peroxidase-hydrogen peroxide oxidation of luminol (ECL Plus; Amersham, Arlington Heights, IL). Blots were then exposed to film for 5–10 min before densitometric analysis was performed.

**Densitometry.** Films were scanned and analyzed using DayOne software (PDI Imageware Systems, San Diego, CA). Sense RNA standards were used to calibrate the system for RPA data. The data were converted from optical density readings to picograms of mRNA per microgram of total RNA for RPA data. Western blot data are reported in OD units.

**Data analysis.** Renal renin mRNA, renal prorenin and active renin, fetal plasma renin concentrations, fetal plasma T3 and T4 concentrations, and levels of AT1 and AT2 protein in TX and control animals were compared using the unpaired Student’s t-test. AT1 and AT2 mRNA expression in different tissues was compared using analysis of variance followed by the Newman-Keuls multiple comparison procedure. Blood pressure and heart rate measures before the isoproterenol infusion, throughout the experiment, and for 50 min after the infusion were compared using ANOVA followed by the Newman-Keuls multiple comparison procedure. All values are expressed as means ± SE, with P < 0.05 considered significant.

**RESULTS**

**Confirmation of TX and fetal health.** The completeness of TX was confirmed by visual inspection at necropsy and by the measurement of plasma T4 and T3 levels. Plasma T4 levels were significantly lower in TX than in control fetuses. Plasma T3 concentrations were below the level of assay detection (<0.004 ng/ml) in all TX fetuses, whereas plasma T3 levels were as expected and significantly higher in controls (Fig. 1). Fetal health, as assessed using arterial blood gas and pH measurements, was normal throughout the duration of the studies in both TX and control fetuses (Table 1).

**Effect of TX on renal and plasma renin.** Renal renin mRNA expression was significantly decreased by TX compared with the control values (Fig. 2). TX also significantly decreased the total renal renin (3,661 ± 1,009 vs. 2,481 ± 402 ng ANG I·min⁻¹·mg⁻¹, P = 0.03) and renal active renin concentrations (P < 0.05) but had no effect on renal prorenin levels (Fig. 3).

Just before necropsy, basal concentrations of plasma active and prorenin were similar, whereas isoproterenol infusion increased plasma active renin concentrations equally in both groups (P < 0.05) (Fig. 4). Prorenin levels did not change (Fig. 4).

**Effect of TX on ANG II receptor subtype expression.** AT1 and AT2 mRNA were expressed in kidney cortex, lung, and left ventricle in TX and control fetuses. In all tissues, and in both groups, expression of AT1 mRNA was greater than that of AT2 (F = 9.14, P < 0.001, AT1 mRNA vs. AT2 mRNA). TX...
significantly decreased AT1 mRNA expression in kidney and lung \((F = 4.29, \ P < 0.05)\) but did not affect left ventricular expression (Fig. 5). TX was associated with increased AT2 mRNA levels in kidney only \((F = 5.39, \ P < 0.05)\) (Fig. 6).

AT1 and AT2 receptor proteins were detected in all three tissues, with bands of expected size (51). The major AT1 band, at 67 kDa, tended to decrease in kidney \((P = 0.06)\) and was significantly decreased in lung \((P < 0.05)\) after TX (Fig. 7). AT2 bands were detected at 44 and 78 kDa, considered to be the primary bands expressed in fetal kidney and adult adrenal, respectively (51). TX led to increased AT2 44-kDa band protein expression in the kidney \((P < 0.05)\), whereas at the 78-kDa band, TX was associated with decreased expression in kidney and lung \((P < 0.05)\). Neither AT1 nor AT2 protein levels were altered in the heart after TX (Fig. 8).

Effect of TX on cardiovascular system. Fetal arterial blood pressure and heart rate recordings are presented in Figs. 9 and 10. TX fetuses had significantly lower basal heart rate values than controls \((F = 268.99, \ P < 0.001)\), whereas isoproterenol infusion significantly increased heart rate in both groups \((F = 44.43, \ P < 0.001)\) with similar magnitude (Fig. 9). Basal MAP values were significantly lower in TX fetuses \((F = 3.528.71, \ P < 0.001)\). Isoproterenol infusion decreased MAP in TX fetuses \((F = 29.44, \ P < 0.001)\) (Fig. 10).

DISCUSSION
This study examined changes in RAS activity and cardiovascular function in the late-gestation sheep fetus after TX. We found that renal renin mRNA, renal total and active renin concentrations, and ANG II receptor subtype expression in different tissues, as well as basal blood pressure and heart rate, were altered as a consequence of TX. These findings indicate that thyroid hormone plays an important role in regulating maturation of components of the RAS and cardiovascular system in late gestation.

We and others have previously noted that renal renin mRNA and renin content increase significantly in the late-gestation fetal sheep (9, 10, 45) and that this is a consequence of increased number and content of renin-containing cells (63). In fetal sheep, the concentration of thyroid hormone also increases in late gestation, suggesting that this may be the driving force behind these changes (19). The findings from our present study support this assertion. We observed that fetuses lacking thyroid hormone (i.e., TX fetuses) exhibited decreased renal renin mRNA expression and renin content in late gestation.
Several in vitro studies have shed further light on exactly how thyroid hormone may be influencing renin gene expression. For instance, in transgenic mice carrying extra copies of the Ren2 gene, thyroid hormone has been demonstrated to increase renin mRNA accumulation by directly stimulating transcription and/or stabilizing precursor renin mRNA (29, 59). Additional studies using Calu-6 cells have confirmed that thyroid hormone directly stimulates the promoter region of the human renin gene through thyroid hormone response element-dependent mechanisms (36).

Contrary to expectations, TX had no effect on either basal or isoproterenol-increased plasma renin concentrations. Renal renin is considered to be the main source of the circulating plasma renin, and it has been shown that concentrations also increase in later gestation (45). Plasma renin levels also increase significantly in response to isoproterenol infusion during this period (41, 64). In adult rats, TX significantly decreases plasma renin activity (6, 15, 40) and the renin responses to isoproterenol (5). In our studies, although renal renin mRNA and content were decreased by TX, basal plasma renin concentrations were maintained and isoproterenol infusion increased plasma active renin concentrations similarly in both control and TX fetuses. These findings suggest that there are species differences with respect to how renin secretion is regulated or that factors other than thyroid hormone help maintain basal and isoproterenol-stimulated renal renin secretion in thyroidectomized fetal sheep. One possibility toward this end is increased sympathoadrenal activation, as has been observed in hypothyroid human subjects (17). There is some evidence in fetal sheep suggesting that sympathetic tone is altered after TX; for example, fetal sheep thyroidectomized at 80 dGA had significantly higher plasma norepinephrine concentrations compared with controls when assessed at 125–135 dGA (61). Alternatively, the ~30% decline in active renal
stimulation of lung ACE activity, which, in turn, usually decreases lung size (4) and functional immaturity at term (13). This is most likely due, in part, to the lack of thyroid hormone receptors are expressed earlier in the brain and that thyroid hormone per se has the capacity to influence maturational expression of thyroid hormone receptors may vary between tissues; for example, in the human fetus, thyroid hormone receptors are expressed earlier in the brain than in liver (16).

Renal AT1 mRNA expression is lower in early and higher in late gestation, whereas the reverse is true for AT2 mRNA (46, 47). What regulates these developmental patterns of expression is not known. We found that after TX, renal AT1 receptor expression was decreased at both the mRNA and protein level. In adult human and animal models of hypothyroidism, renal function is also altered, resulting in decreased renal blood flow, glomerular filtration rate, and sodium reabsorption (7, 31, 32, 43), as well as impaired concentrating and diluting capacity (24, 60). These outcomes are likely to be mediated in part by associated changes in AT1 receptor expression. Indeed, AT1 receptor blockade also exerts similar effects with reference to sodium and water reabsorption in adult rats (42). Similarly, in the late-gestation fetal sheep, ANG II acts via the AT1 receptor to regulate salt and water excretion, hence maintaining fetal fluid sac volumes and ensuring normal growth and development (65), and inhibition of the system leads to anuria and oligohydramnios (1, 49). Our findings imply that thyroid hormone may be an important mediator of this phenomenon and, indeed, of AT1-mediated reabsorption in general.

Some work also has been undertaken examining the relative importance of the RAS and sympathetic nervous system in hyperthyroid-induced cardiac hypertrophy. To date, the results of these studies have been inconsistent (37, 38), although there may be a role for both systems in this phenomenon (26).

Fig. 10. Mean arterial blood pressure (BP) before, during (10th to 20th min), and after isoproterenol infusion in 137–139 dGA control and TX fetuses. BP was lower in TX fetuses ($P = 3.528.71, P < 0.001$ vs. control) and declined with isoproterenol infusion ($P = 29.44, P < 0.001$ vs. basal BP). Isoproterenol infusion had no effect on BP in control fetuses. Values are means ± SE; $n = 6$ for both groups.

Prior investigation has confirmed that ANG II receptor subtype expression in the kidney is developmentally regulated. Renal AT1 mRNA expression is lower in early and higher in late gestation, whereas the reverse is true for AT2 mRNA (46, 47). What regulates these developmental patterns of expression is not known. We found that after TX, renal AT1 receptor expression was decreased at both the mRNA and protein level. In adult human and animal models of hypothyroidism, renal function is also altered, resulting in decreased renal blood flow, glomerular filtration rate, and sodium reabsorption (7, 31, 32, 43), as well as impaired concentrating and diluting capacity (24, 60). These outcomes are likely to be mediated in part by associated changes in AT1 receptor expression. Indeed, AT1 receptor blockade also exerts similar effects with reference to sodium and water reabsorption in adult rats (42). Similarly, in the late-gestation fetal sheep, ANG II acts via the AT1 receptor to regulate salt and water excretion, hence maintaining fetal fluid sac volumes and ensuring normal growth and development (65), and inhibition of the system leads to anuria and oligohydramnios (1, 49). Our findings imply that thyroid hormone may be an important mediator of this phenomenon and, indeed, of AT1-mediated reabsorption in general.

Some work also has been undertaken examining the relative importance of the RAS and sympathetic nervous system in hyperthyroid-induced cardiac hypertrophy. To date, the results of these studies have been inconsistent (37, 38), although there may be a role for both systems in this phenomenon (26).

Until recently, little was known about the function of the AT2 receptor. In the kidney, it has been suggested that AT2 may play an important role in early-gestation nephrogenesis (21). In the present study, we noted that after TX, expression of AT2 mRNA, as well as protein at the 44-kDa band, was elevated, whereas protein was decreased at the 78-kDa band. It has previously been found that the 44-kDa band is the primary band in fetal kidney, whereas the 78-kDa band is the primary band in adult adrenal (51). The present pattern of expression suggests that TX may have resulted in delayed renal maturation and that thyroid hormone per se has the capacity to influence AT2 expression in the fetus.

Thyroid hormone also is implicated in fetal lung development and maturation (3, 27, 33). In fetal sheep, TX results in decreased lung size (4) and functional immaturity at term (13). This is most likely due, in part, to the lack of thyroid hormone stimulation of lung ACE activity, which, in turn, usually facilitates RAS development in preparation for postnatal life (62). TX is associated with significantly decreased lung ACE concentrations in the fetal sheep (18). ANG II, through the AT1 receptor, also is believed to play an important role in fetal lung development and maturation. Strong evidence for this assertion comes from human and animal studies in which fetal exposure to AT1 receptor blockers has been associated with lung hypoplasia (49) and decreased lung liquid flow (58). We found that TX led to decreased expression of AT1 mRNA and protein in the fetal sheep lung, thus indicating that the previously observed impaired lung development following TX (4, 13) also may be related to retarded AT1 receptors, as well as ACE expression.

TX had no effect on the expression of pulmonary AT2 mRNA. Protein expression, however, was decreased at the 78-kDa band. Again, this may be indicative of delayed tissue maturation.

Although TX significantly altered AT1 and AT2 receptor expression in both kidney and lung, no changes were evident in fetal sheep heart. These differential tissue-specific effects also have been observed after thyroid hormone treatment in different species. Adult rats treated with T3 exhibited decreased adrenal but increased heart, liver, and kidney ANG II receptor densities (40). Dogs similarly exposed to T3 had increased ANG II receptor density in the left ventricle and liver but no change in adrenal expression (52). The mechanisms underlying these tissue differences are not clear.

In a prior investigation (40), it was found that hypothyroid adult rats had increased cardiac AT2 but unchanged AT1 receptor density. These changes are contrary to what we observed in fetal sheep. Apart from the species difference, there are several plausible explanations for the different findings. First, basal ANG II receptor subtype expression may be different between adult and fetal animals. In rats, AT2 mRNA expression is higher in fetal tissues, whereas AT1 mRNA is higher in adults (23). Second, the mechanisms that regulate ANG II receptor expression may be different in adults and fetuses. This is apparent with respect to another RAS component, renal renin, the expression of which is decreased after renal denervation in the adult but not in the fetus (28). Finally, the maturational expression of thyroid hormone receptors may be different between tissues; for example, in the human fetus, thyroid hormone receptors are expressed earlier in the brain than in liver (16).

Our studies demonstrate that in the late-gestation TX fetal sheep, both basal blood pressure and heart rate are decreased.
The hypothyroid-induced hypotension may be due to decreased blood volume, peripheral vascular resistance, myocardial Na+-K+ ATPase activity and myocardial contractility, and/or changing adrenergic receptor number and subtype. In a previous investigation, TX at 80 dGA was also associated with decreased MAP at 125–135 dGA (61). Our studies also demonstrate that MAP declined after the isoproterenol infusion in TX fetal sheep. This response suggests that TX inhibits or decreases the inotropic effect of isoproterenol and may be related to decreased cardiac contractile protein ATPase, reduced calcium uptake by cardiac sarcoplasmic reticulum, altered expression of vascular adrenergic receptors, and/or failed augmentation of left ventricular rate of dP/dt (an index of left ventricular contractility) in response to the isoproterenol challenge (14).

The mechanisms underlying the decreased basal heart measures in TX fetuses are not clear, although it appears that the timing of TX is an important determinant. In a previous study in which TX was performed at 80 dGA, there was no effect on heart rate at ~125–135 dGA (61), whereas in two other investigations, TX performed at ~130 dGA (8) or at term, following caesarian section delivery (5), was associated with decreased and normal postnatal heart rate measures, respectively.

In contrast to blood pressure, isoproterenol infusion increased heart rate equally in both TX and control fetuses in late gestation. This suggests that the positive chronotropic effects of adrenergic stimulation are somehow maintained in TX fetuses. Hypothyroid-induced increased sympathetic and adrenal activity (17) may help maintain the normal positive chronotropic responsiveness to adrenergic stimulation. Another possible contributing factor is the expression level of the ANG II receptors, which mediate the positive chronotropic effects of ANG II (30). As previously noted, we found that neither AT1 nor AT2 receptor mRNA or protein was altered in the left ventricle as a consequence of TX in fetal sheep.

In summary, we have found that in the late-gestation fetal sheep, renal renin mRNA and renal total renin and active renin concentrations were decreased as a consequence of TX, whereas expression of the ANG II receptor subtype mRNA and protein were altered in a tissue-specific manner. Basal blood pressure and heart rate measures were decreased after TX, and MAP declined after isoproterenol infusion. These findings demonstrate that thyroid hormone plays an important role in mediating some of the developmental changes in the RAS and in cardiovascular function in the late-gestation sheep fetus.

GRANTS

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REFERENCES


AJP-Regul Integr Comp Physiol • VOL 289 • OCTOBER 2005 • www.ajpregu.org
R1014  THYROID HORMONE AND RAS ACTIVITY IN FETAL SHEEP


