Developmental effect of antenatal exposure to betamethasone on renal angiotensin II activity in the young adult sheep

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Contag SA, Bi J, Chappell MC, Rose JC. Developmental effect of antenatal exposure to betamethasone on renal angiotensin II activity in the young adult sheep. Am J Physiol Renal Physiol 298: F847–F856, 2010. First published January 13, 2010; doi:10.1152/ajprenal.00497.2009.—Antenatal corticosteroids may have long-term effects on renal development which have not been clearly defined. Our objective was to compare the responses to intrarenal infusions of ANG II in two groups of year-old, male sheep: one group exposed to a clinically relevant dose of betamethasone before birth and one not exposed. We wished to test the hypothesis that antenatal steroid exposure would enhance renal responses to ANG II in adult life. Six pairs of male sheep underwent unilateral nephrectomy and renal artery catheter placement. The sheep were infused for 24 h with ANG II or with ANG II accompanied by blockade of the angiotensin type I (AT1) or type 2 (AT2) receptor. Baseline mean arterial blood pressure among betamethasone-exposed sheep was higher than in control animals (85.8 ± 2.2 and 78.3 ± 1.0 mmHg, respectively, \( P = 0.003 \)). Intrarenal infusion of ANG II did not increase systemic blood pressure (\( P > 0.05 \)) but significantly decreased effective renal plasma flow and increased renal artery resistance (\( P < 0.05 \)). The decrease in flow and increase in resistance were significantly greater in betamethasone-exposed sheep compared with vehicle-exposed sheep (betamethasone \( P < 0.05 \), vehicle \( P > 0.05 \)). This effect appeared to be mediated by a heightened sensitivity to the AT1 receptor among betamethasone-exposed sheep. Sodium excretion initially decreased in both groups during ANG II infusion; however, a rebound was observed after 24 h. AT1 blockade was followed by a significant rebound after 24 h in both groups. AT2 blockade blunted the 24-h rebound effect among the vehicle-exposed sheep compared with the betamethasone-exposed sheep. In conclusion, antenatal corticosteroid exposure appears to modify renal responsiveness to ANG II by increasing AT1- and decreasing AT2 receptor-mediated actions particularly as related to renal blood flow and sodium excretion.

The information regarding the long-term effect of antenatal glucocorticoid exposure on the renin-angiotensin system (RAS) is limited. This is complicated by the several levels at which RAS functions. Not only is there a systemic RAS effect as documented by fluctuations in plasma renin or angiotensin levels, but there are also organ-specific differences as well as intracellular differences within a given organ. The acute changes related to antenatal corticosteroid exposure shortly after birth include increased plasma renin and ANG II levels and increased expression of renal AT1 and AT2 receptor mRNA (32, 35). The long-term phenotypic changes in the sheep model include increased angiotensin-converting enzyme (ACE) to ACE2 activity and upregulation of the AT1 receptor in the kidney (21, 47).

There is no evidence regarding the effect of ANG II on renal vascular tone and sodium excretion in adult sheep previously exposed to antenatal corticosteroids. Therefore, the objective of this study was to evaluate the impact of antenatal betamethasone exposure during nephrogenesis on renal responses to direct intrarenal infusion of ANG II. We wanted to establish whether steroid exposure alters renal functional responses to the peptide. We hypothesized that antenatal betamethasone at 80–81 days (0.6) of gestation would increase responsiveness to ANG II in adult rams.

MATERIALS AND METHODS

Animal Preparation

All procedures were approved by the Institutional Animal Care and Use Committee of Wake Forest University School of Medicine. Time-dated pregnant ewes were randomly assigned to receive either two 0.17 mg/kg intramuscular injections of a 1:1 mixture of beta-

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methasone acetate and betamethasone phosphate (Celestone Soluspan, Schering, Kenilworth, NJ) or vehicle alone, which contained 3.4 mg of monobasic sodium phosphate, 7.1 mg of dibasic sodium phosphate, 0.1 mg of sodium EDTA, and 0.2 mg of benzalkonium chloride/ml given 24 h apart at days 80 and 81 of gestation. The ewes were allowed to deliver naturally at term (term is ~145 days in our flock).

Six pairs of male offspring were transferred to the laboratory at 12–18 mo of age. The research team was blinded to the sheep allocation. The temperature in the laboratory facilities and in the sheep pens was kept stable at 20 ± 2°C, and the humidity at ~35–40%. Sheep were fed a standard commercial diet (PMI Rumilab, Brentwood, MO) containing 0.75% NaCl and had ad libitum access to clean tap water. Throughout the experiment, 12:12-h day-night cycles were kept constantly.

Surgical Procedure

A midline suprapubic laparotomy was made in the lower abdomen to expose the bladder. A 14 French silicone Foley catheter (Bard Medical, Covington, GA) was placed through the left flank. One polyvinyl catheter was placed in the left carotid artery, and two catheters were inserted into the left external jugular vein. These were tunneled subcutaneously and merged through a left anterior neck incision. Bilateral groin incisions were made, and the femoral vessels were exposed. Each animal had one right femoral artery, one left femoral artery, and two left femoral vein polyvinyl catheters placed. All vascular catheters were fastened to their corresponding vessels, and the distal vessels were all ligated. The femoral catheters were tunneled subcutaneously to the left flank region.

A paravertebral left (n = 10) or right (n = 2) flank incision was made to expose the corresponding renal artery. A modified, Teflon-coated, 22-gauge endovascular catheter, 25 mm long and with a 0.9-mm external diameter, was placed into the right renal artery. The catheter was tunneled a short distance to emerge adjacent to the spine. We then removed the contralateral kidney.

All the animals received daily doses of ampicillin (1,000 mg), gentamicin (80 mg), and ketorolac (100 mg iv) until 48 h after surgery. All vascular catheters were flushed with the heparin solution immediately after collection. The hematocrit was also measured. Blood and urine samples were centrifuged at 3,200 rpm, 4°C, for 10 min immediately after collection. The hematocrit was also measured. After all experiments had concluded, the animals were euthanized and organ harvesting was performed.

Blood Pressure Measurement

Arterial blood pressure was monitored continuously during experiments by a Cobe transducer connected to a DigiMed analyzer, which digitized the pressure signal that was then recorded by a computer. Pressure was sampled at 100 Hz and averaged over 1-min intervals. The reported blood pressure values are the averaged value over 1 h before the time point reported.

Preparation Infusates

PAH. PAH was obtained from Sigma (St. Louis, MO). A priming dose of PAH using 225 mg dissolved in 40 ml of normal saline was given over 1 min, followed by 7.66 mg/min (0.23 ml/min of a 3.33% solution) that was infused continuously.

Inulin. Inulin was provided by Sigma. A priming dose of inulin using 850 mg dissolved in 40 ml of normal saline was given over 1 min, followed by 6.96 mg/min (0.23 ml/min of a 3.03% solution) that was infused continuously.

Lithium. Lithium chloride was obtained from Sigma. On the day of the study, a priming dose of lithium using 580 mg dissolved in 40 ml of normal saline was given over 1 min, followed by 278 µg/min (0.23 ml/min of a 0.17% solution) that was infused continuously.

ANG II. ANG II was provided by Bachem BioScience (H-1705.0025, Torrance, CA). The weight-based dosage for ANG II was 1 ng·kg⁻¹·min⁻¹. The solution was infused at the rate of 2.08 ml/h.

Candesartan. Candesartan (CV11974) was generously provided as a salt from AstraZeneca (Möndal, Sweden). The dosage for candesartan was 0.3 mg/kg given as a single dose once each day of the experiment, given its extended half-life of ~10 h.

PD 123319. PD 123319 was provided by Sigma-Aldrich (P186). The AT₂ antagonist came as a powder. PD 123319 stock was diluted in sterile isotonic saline, and an initial loading bolus of 500 µg was followed by an infusion at 10 ng·kg⁻¹·min⁻¹. The solution was infused at the rate of 2.08 ml/h.

Renal Function Tests

Plasma inulin. Plasma inulin was measured using an anthrone method and a colorimetric assay for determining the concentration of inulin as previously described (9, 25). Renal inulin excretion was used to derive the glomerular filtration rate (GFR) according to the following formula: 

\[ \text{GFR} = \frac{\text{rate of inulin infusion mg/min} \times (\text{plasma inulin mg/ml}) - 1}{\text{GFR ml/min}} \]

Plasma PAH assay. Plasma PAH was measured using a previously described colorimetric assay (14). Renal PAH excretion was used to derive effective plasma flow rate (EPFR) according to the following formula: 

\[ \text{ERPF} = \frac{\text{rate of PAH infusion mg/min} \times (\text{plasma PAH mg/ml}) - 1}{\text{ERPF ml/min}} \]
**Plasma and urinary electrolytes.** Plasma and urinary concentration of electrolytes were determined using a Medical Easylyte instrument (Bedford, MA). The Easylyte margin of error for plasma electrolytes is 2% and for urinary electrolytes, 5%. All results were reported as millimoles per liter. The analyzer was used to measure Na, K, Cl, and Li in plasma, and Na, K, and Cl in urine.

**Sodium excretion.** Sodium excretion was the product of the urine sample Na concentration multiplied by the urine volume obtained during the hour before the sampling time (Na meq·lt⁻¹×1,000⁻¹×urinary volume 1 ml/h = Na meq). These values were indexed to the sheep weight: meq·h⁻¹·kg⁻¹.

**Plasma ANG II.** We used a commercially available kit (Bühlmann Angiotensin II radioimmunoassay) to measure immunoreactive ANG II. Extracted EDTA plasma samples, calibrators, and controls is first preincubated for 16 h with an anti-ANG II antibody. ANG II-¹²⁵ is added and competes with ANG II present in samples, calibrators, and controls for the same antibody binding sites in a second 6-h incubation step. After this incubation, a solid-phase second antibody is added to the mixture. The antibody-bound fraction is precipitated and counted in a gamma counter.

**Renal artery resistance.** Hemodynamically, renal artery resistance (RAR) would be a ratio of the difference between the renal mean arterial pressure (MAP) and the renal venous pressure to the renal blood flow. Renal artery resistance can be roughly approximated by the ratio of the mean arterial pressure to the renal blood flow according to the following formula: mean arterial pressure/RBP = RAR mmHg·min·ml⁻¹·kg⁻¹.

**Statistical Analysis**

The study data were tabulated and graphed using Graph Prism software. Statistical analysis was accomplished with the use of a paired Student’s t-test and two-way analysis of variance for repeated measurements. Means between the betamethasone- and vehicle-exposed sheep were compared with a two-sample t-test followed by Bonferroni’s post hoc test for multiple t-tests. A P value of <0.05 was considered statistically significant. The data are reported as means ± SE.

**RESULTS**

**MAP**

Baseline MAP was higher in the betamethasone- compared with the vehicle-exposed sheep (85.8 ± 2.2 and 78.3 ± 1.0 mmHg, respectively, P = 0.003). The intrarenal infusion of ANG II did not significantly change systemic MAP over time. Furthermore, the combined infusions of the antagonists and ANG II did not cause significant changes in blood pressure (Fig. 1).

**GFR**

GFR at baseline was not significantly different between betamethasone- and vehicle-treated sheep (1.51 ± 0.05 and 1.59 ± 0.1 ml·min⁻¹·kg⁻¹, respectively, P = 0.39), and this did not change after 24 h of ANG II infusion. Concurrent AT₁ or AT₂ receptor blockade during ANG II infusion did not produce any difference in the GFR over time regardless of the treatment group (Fig. 2).

**ERPF**

The ERPF decreased by the third hour and continued throughout the 24 h of ANG II infusion in both the vehicle- and betamethasone-treated sheep, with a decrease observed by 3 and 24 h compared with baseline (P < 0.05 for both comparisons), but no difference between the treatment groups (P > 0.05) (Fig. 3A). The decrease in the ERPF secondary to ANG II infusion was abrogated during AT₁.
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Fig. 2. A: effect of angiotensin II infusion on glomerular filtration rate (GFR; ml·min⁻¹·kg⁻¹). B: effect of angiotensin II infusion and AT₁ blockade with candesartan on GFR. C: effect of angiotensin II infusion and angiotensin receptor 2 (AT₂) blockade with PD 123319 on GFR.

Fig. 3. A: effect of angiotensin II infusion on effective renal plasma flow (ERPF; ml·min⁻¹·kg⁻¹). B: effect of angiotensin II infusion and AT₁ blockade with candesartan on ERPF. C: effect of angiotensin II infusion and AT₂ blockade with PD 123319 on ERPF.

receptor blockade, with no difference in the ERPF between the treatment groups over time (P > 0.05) (Fig. 3B). AT₂ receptor blockade led to a significant decrease in the ERPF over 24 h among betamethasone (2-way ANOVA: F = 4.12, P = 0.04, baseline compared with 3 h: P < 0.05, and 3 h compared with 24 h: P < 0.05) - but not the vehicle-exposed sheep (2-way ANOVA: F = 1.09, P = 0.37) (Fig. 3C).
ANG II infusion raised the RAR in both groups within 3 h of the infusion, and this persisted during the 24 h of infusion (Fig. 4A). The increased RAR was only significant in the betamethasone group (baseline compared with 3 h: \( P < 0.05 \) and 24 h: \( P < 0.05 \)). The ANG II-induced increase in RAR was blocked by the AT1 receptor antagonist candesartan (Fig. 4B). AT2 receptor blockade was followed by an increase in RAR in both groups, with a greater response in the betamethasone group where the RAR continued to increased over time (betamethasone baseline compared with 3 h: \( P < 0.05 \), 3 compared with 24 h: \( P < 0.05 \), vehicle baseline compared with 3 h: \( P < 0.05 \), and 3 compared with 24 h: \( P > 0.05 \) (Fig. 4C).

Urinary Sodium Excretion

The continuous infusion of ANG II led to a significant decrease in urinary sodium excretion that at 24 h remained markedly suppressed in the betamethasone animals (baseline compared with 24 h: \( P < 0.05 \), and baseline compared with 24 h: \( P < 0.05 \) but not in the vehicle animals (baseline compared with 3 h: \( P < 0.05 \), and baseline compared with 24 h: \( P > 0.05 \)). There was a tendency for sodium excretion to be less in the betamethasone sheep (Fig. 5A). With AT1 receptor blockade, there was an initial suppression of sodium excretion followed by an increase in sodium excretion after 24 h of ANG II infusion in both groups (3 compared with 24 h: \( P < 0.05 \) in both betamethasone- and vehicle-exposed sheep) (Fig. 5B). When ANG II was infused with AT2 blockade, sodium excretion decreased after 3 h and was significant in the vehicle (baseline compared with 3 h: \( P < 0.05 \)) but not in the betamethasone (baseline compared with 3 h: \( P > 0.05 \)) exposed animals (betamethasone: \( F = 0.29, P = 0.75 \), vehicle: \( F = 4.38, P = 0.03 \)). In both groups, excretion returned to baseline after 24 h of infusion with AT2 blockade (Fig. 5C).

Urinary Lithium Excretion

Urinary excretion of lithium followed a pattern similar to that observed for sodium excretion (Fig. 6A). There was lower lithium excretion in the betamethasone compared with the vehicle sheep, which was most noticeable after 3 h (baseline compared with 3 h: \( P < 0.05 \) for both groups) of infusion followed by a rebound in lithium excretion by 24 h of infusion (3 compared with 24 h: \( P < 0.05 \)) especially in the vehicle-exposed sheep (vehicle- compared with betamethasone-exposed sheep at 24 h: \( P < 0.05 \)). This temporal pattern in the lithium excretion was also observed during AT1 and AT2 receptor blockade. AT1 receptor blockade abrogated the suppressive effect of betamethasone exposure on lithium excretion at 24 h with no difference in excretion compared with the vehicle sheep (\( P > 0.05 \)) (Fig. 6B). AT2 receptor blockade did not affect the excretion pattern observed with ANG II (Fig. 6C).

Plasma ANG II

The plasma concentration of ANG II increased in vehicle compared with the betamethasone sheep during the ANG II infusion (1-way ANOVA for betamethasone: \( F = 0.31, P = 0.74 \) and for vehicle: \( F = 6.56, P = 0.03 \), t-test of baseline compared with 24-h levels in betamethasone sheep; \( P > 0.05 \) and vehicle sheep: \( P < 0.05 \) (Fig. 7A). There was an increase in the ANG II concentration over time in both groups during AT1 blockade and peptide infusion (Fig. 7B). The increase over time was not observed during AT2 receptor blockade, but there was a treatment effect evident at 24 h of infusion (t-test of...
mean ANG II plasma concentration between betamethasone and vehicle sheep at 24 h: $P < 0.05$ (Fig. 7C).

**Kidney Weights**

The mean weights of kidneys at the time of uninephrectomy in the vehicle and betamethasone sheep were similar (88.8 ± 5.2 and 89.4 ± 4.1 g for vehicle- and betamethasone-exposed sheep, respectively, $P = 0.93$). After completing all experiments, the sheep were euthanized and the remaining kidney was harvested for further analysis. There was a significant increase in the size of the remaining kidney. The mean renal weight was 142 ± 5.1 and 142.6 ± 6.5 g for the vehicle and betamethasone sheep, respectively ($P = 0.94$). The mean increase in renal weight was 53.1 ± 7.6 (38%) and 53.2 ± 7.4 g.
g (37%) for the vehicle and betamethasone groups, respectively. This increase was significant in both the vehicle (P < 0.01) and betamethasone (P < 0.01) groups but not between the two groups (P = 0.94).

**DISCUSSION**

The purpose of these studies was to determine whether antenatal betamethasone exposure altered renal responses to intrarenal infusions of ANG II. Our working hypothesis was that responses mediated by activation of the AT1 receptor would be enhanced in adults that were exposed to clinically relevant doses of betamethasone during fetal life. We chose to use intrarenal infusions in an attempt to avoid some of the systemic effects of elevations in plasma levels of ANG II that can affect the kidney. Our data suggest that antenatal steroid exposure does upregulate some of the AT1 receptor-mediated renal responses to ANG II.

Intrarenal ANG II infusion decreased ERPF and increased RAR. The changes observed secondary to antenatal betamethasone exposure appeared to be mediated by increased AT1 activity in the betamethasone-exposed compared with the vehicle-exposed sheep and were evident within 3 h after the infusion was started.

Under normal conditions, GFR and ERPF are autoregulated through a mechanism that controls afferent and efferent arterial tone. Increased perfusion pressure and GFR lead to a decreased efferent artery vascular tone and increased renal blood flow, with subsequent decrease in the filtration fraction. Conversely, a decrease in the GFR causes efferent artery vasoconstriction. This will increase perfusion pressure, filtration fraction and decrease renal blood flow (5, 20).

We did not observe a significant decrease in GFR associated with betamethasone exposure under basal conditions or during the ANG II infusions. The latter is consistent with a previous report of ANG II infusion in fetal sheep that reported no change in GFR after 5 days of infusion, but does not agree with earlier studies in dogs (31, 50). The explanation for this difference is not clear but could be related to species differences in responses to the peptide. Long-term studies in adolescent individuals who were antenatally exposed to betamethasone documented a lower GFR (18). Tang et al. (51) have demonstrated a decrease in GFR and sodium excretion by 18 mo of age among male but not female sheep antenatally exposed to betamethasone. This difference may be due to our experimental design using uninephrectomized animals with its associated renal hypertrophy.

Although decreased renal blood flow with ERPF suppression mediated by glomerular mesangial cell contraction has been described after ANG II infusion, the most likely mechanism would appear to be efferent artery vasoconstriction (4, 24). Our finding of increased RAR without modifying the GFR is consistent with an effect on both efferent and afferent arterial vascular tone, which has been reported in other species (29).

During AT1 receptor blockade, the effect of ANG II infusion on ERPF in both groups was abrogated, suggesting that the effect on ERPF is mediated through the AT1 receptor. Candesartan, which was used for the AT1 receptor blockade, can also induce changes in ERPF through an antioxidant effect. This is supported by evidence that bioinactivation of nitric oxide by free oxygen radicals in the kidneys of spontaneously hypertensive rats can be blunted by candesartan (55).

In contrast, ERPF decreased significantly during ANG II infusion on ERPF in both groups was abrogated, suggesting that the effect on ERPF is mediated through the AT1 receptor. Candesartan, which was used for the AT1 receptor blockade, can also induce changes in ERPF through an antioxidant effect. This is supported by evidence that bioinactivation of nitric oxide by free oxygen radicals in the kidneys of spontaneously hypertensive rats can be blunted by candesartan (55).

Fig. 7. A: effect of angiotensin II infusion on plasma angiotensin II concentrations. B: effect of angiotensin II infusion and AT1 blockade with candesartan on plasma ANG II concentrations. C: effect of angiotensin II infusion and AT2 blockade with PD 123319 on plasma ANG II concentrations.
demonstrated by the treatment (group) effect seen with AT1 blockade in vehicle- compared with betamethasone-exposed sheep. This might be a consequence of increased expression of AT1 receptors in the betamethasone animals (21).

With AT2 blockade, an increase in RAR was observed in both groups, supporting the concept of AT2 activation countering AT1 activation. Several mechanisms for AT2 function have been postulated, including AT1/AT2 dimerization with downregulation of AT1, and AT2/AT2 dimerization with subsequent production of nitric oxide, a known vasodilator. (16, 34, 45) Furthermore, ANG III is derived from ANG II, which activates the AT2 receptor and can increase nitric oxide production. Other potential pathways of AT2 activation include bradykinin, prostaglandin, and cGMP release, all of which can impact renal vascular resistance (48, 49). Betamethasone could possibly induce changes in any of the above mechanisms, through decreased AT1 or AT2 dimerization, decreased nitric oxide availability, or changes in the cytokine milieu. The enhanced response among betamethasone-exposed sheep compared with vehicle sheep supports our suggestion regarding a heightened response to AT1 activation in betamethasone- compared with vehicle-exposed sheep.

The MAP in betamethasone-exposed sheep is significantly higher than in vehicle-exposed sheep. The mean difference of 7.5 ± 2.4 mmHg is similar to that previously reported for betamethasone-exposed sheep (17) and would not be accounted for by unilateral nephrectomy.

These results are compatible with our hypothesis that antenatal corticosteroids administrated to sheep at a time and dose similar to that used during human pregnancies for prevention of prematurity related complications can program renal and cardiovascular function (17, 19, 30).

The betamethasone sheep had consistently higher blood pressure, but there was no significant increase in systemic MAP during the ANG II infusion regardless of the use of AT1 or AT2 blockade. Previous studies in a sheep model (50) did not observe any difference in systemic MAP with a short infusion of considerably higher doses of ANG II into the renal artery, suggesting that there is little spillover into the systemic circulation. This is consistent with observations that the kidney clears a large proportion of ANG II (33, 40, 54) and with the very modest increase in plasma levels of ANG II during the peptide infusions (discussed below).

ANG II infusion led to a decrease in sodium excretion. This decrease persisted at 24 h and correlates with the increase in RAR observed during ANG II infusion. AT1 blockade interfered with the suppression of sodium excretion, which was low at 3 h of infusion and rebounded significantly by 24 h in both treatment groups. Candesartan is highly selective for AT1 receptors, with tight binding and slow dissociation (9). Evidence derived from ANG II infusions in a rat model has shown that the natriuretic response observed after candesartan-induced AT1 blockade is due to conversion of ANG II into ANG III with direct stimulation of the AT2 receptor by ANG III (42–44). The process would require time for the upregulation and translocation of AT2 receptors to occur and thus could account for the time-dependent effects of blockade that we observed.

During AT2 blockade, no rebound effect was seen by 24 h, suggesting that it is the AT2 receptor that upregulates sodium excretion. AT2 blockade led to a decreased sodium excretion at 3 h in the vehicle- but not in the betamethasone-exposed sheep. This suggests that the steroid exposure reduces the counterbalancing effects of AT2 receptor activation on AT1 receptor-mediated responses. The decrease in AT2 activity in betamethasone-exposed sheep could be secondary to decreased conversion of ANG II to ANG III, decreased AT2 expression, or decreased transfer of AT2 to the apical tubular cell membrane (42, 43).

The biphasic responses observed for both lithium and sodium excretion appear to be mediated by an initial response to increased RAR and decreased ERPF, followed by early activation of AT1 receptors and delayed activation of AT2 receptors in the tubular epithelium.

Lithium is filtered by the glomeruli, and 80% is reabsorbed in the proximal tubule (52, 53). Lithium reabsorption is relatively stable during short-term infusions in ovine models (8). Although a low salt intake resulting in low tubular concentration of sodium will increase lithium reabsorption, it has been previously reported that renal lithium excretion does not vary with changes in renal perfusion pressure (22).

During the ANG II infusion, we observed a significant decrease in lithium excretion at 3 h and a return to preinfusion levels by 24 h. The same pattern was observed during AT1 blockade, analogous to that observed with urinary sodium excretion, suggesting that AT2 receptor activity may be involved in lithium excretion by decreasing absorption from the proximal tubule. During AT2 blockade, we also observed a decrease in lithium excretion by 3 h of infusion. The rebound in lithium excretion observed after 24 h during AT1 blockade was only seen in the vehicle sheep during AT2 blockade. Lithium excretion remained suppressed in the betamethasone sheep after 24 h of AT2 blockade, similar to what was seen for sodium excretion. This is consistent with increased AT2 receptor activity in the proximal tubule compared with the distal tubule and collecting duct of vehicle- compared with betamethasone-exposed sheep.

The effects of ANG II infusion and subsequent AT1 and AT2 blockade on lithium excretion closely paralleled the changes observed in sodium excretion. This would suggest that the initial suppression is secondary to decreased ERPF and increased RAR, that the effects on renal sodium and lithium excretion are both regulated at the level of the proximal tubule through AT1 and AT2, and that the AT2 receptor appears to be less active in betamethasone- compared with vehicle-exposed sheep. The functional assessment of renal function among the betamethasone- compared with the vehicle-exposed sheep is consistent with our measurement of AT1 and AT2 receptors in the cortex and medulla of betamethasone-exposed sheep, which showed an increased AT1/AT2 ratio in both nuclear and plasma membrane of cortical renal tissue (21).

Plasma concentrations of ANG II increased slightly during the infusion of the peptide alone; however, ANG II concentrations essentially doubled during the infusion and AT1 blockade. Elevated ANG II levels in both groups in the AT1 receptor blockade experiments may be the result of the loss of the normal inhibitory feedback on renin production induced by ANG II (23). The plasma concentrations are the result of an increase in endogenous levels and a small contribution from the infused peptide. Alternatively, if the AT1 receptor is important for clearance of the peptide, then its blockade would allow larger amounts of ANG II to appear in the peripheral
circulation. During AT₂ blockade, although the concentration of ANG II did not increase over time, betamethasone-exposed sheep had consistently higher ANG II concentrations. Increased plasma ANG II levels are consistent with our previously published data reporting decreased plasma and renal cortical tubule concentration of ACE2 among betamethasone-exposed sheep (47).

In conclusion, our findings support the notion of fetal renal programming secondary to antenatal betamethasone exposure, leading to long-term changes in renal function of adult male sheep that not only affect renal vascular resistance but affect renal sodium excretion.

Intrarenal ANG II exerts its effects through activation of AT₁ and AT₂ receptors within the renal vasculature and cortical tubular epithelium. Our findings suggest that although ANG II exerts a stimulatory effect on RAR with a reduction in ERPF, it does not appear to alter GFR secondary to compensatory mechanisms including afferent arteriolar vasoconstriction. The decreased ERPF with increased RAR seems to be under AT₁ receptor control and is stronger in betamethasone-exposed sheep. Supporting the concept of an increased AT₁ to AT₂ activity ratio in betamethasone sheep compared with vehicle sheep.

The increased MAP we have documented among the betamethasone-exposed sheep is consistent with previous reports and demonstrates that unilateral nephrectomy does not abolish the hypertensive effect of antenatal steroid exposure, at least in the short term. Our findings suggest there is a shift in the balance of AT₁ to AT₂ receptor activity among adult male sheep exposed antenatally to betamethasone compared with the vehicle-exposed sheep. The shift tends to favor AT₁ over AT₂-mediated responses.

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DISCLOSURES

No conflicts of interest are declared by the authors.

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