Fetal renal and blood pressure responses to steroid infusion after early prenatal treatment with dexamethasone

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Submitted 29 April 2004; accepted in final form 11 August 2004

Moritz, Karen M., Andrew Jefferies, E. Marelyn Wintour, and Miodrag Dodic. Fetal renal and blood pressure responses to steroid infusion after early prenatal treatment with dexamethasone. Am J Physiol Regul Integr Comp Physiol 288: R62–R66, 2005; doi: 10.1152/ajpregu.00282.2004.—Maternal infusion of dexamethasone for 48 h early in gestation results in upregulation of mRNA for mineralocorticoid and glucocorticoid (MR and GR) receptors and angiotensin II receptors in ovine fetal kidneys late in gestation. This study sought to determine whether dexamethasone exposure results in changes in renal function and blood pressure responsiveness to infused cortisol or aldosterone in the late-gestation fetus. Merino ewes carrying single fetuses were infused with isotonic saline (Sal; n = 9) or dexamethasone (Dex, 0.48 mg/h; n = 10) for 48 h between days 26 and 28 of gestation (term = 150 days). At 115–122 days, renal function and blood pressure were measured in fetuses during a 4-h infusion of saline, cortisol (100 μg/h), or aldosterone (5 μg/h). Infusions were given in random order at least 2 days apart. Basal blood pressure and renal function were similar in Sal and Dex groups and did not change over the course of saline infusion. Cortisol infusion caused similar increases in blood pressure, urine flow, and glomerular filtration rate (GFR) in the groups. Aldosterone infusion caused a significantly different GFR response between the groups [P(treatment x time) < 0.05], but increase in K excretion and decrease in Na-to-K ratio were similar in the groups. The similar results obtained with cortisol and aldosterone infusion suggest no increased renal functional maturity to those hormones after early prenatal dexamethasone exposure. This suggests that changes in mRNA for MR and GR in kidneys of dexamethasone-exposed fetuses do not result in functional differences and highlights the renin-angiotensin system, as reported previously, as more important in this model.

fetal programming; cortisol; aldosterone

THE SUSCEPTIBILITY OF AN INDIVIDUAL to development of some adult diseases, including hypertension and coronary artery disease, has been linked to a suboptimal intrauterine and/or early postnatal environment (1). Many animal models have been developed to investigate possible mechanisms underlying this phenomenon, with the two most common being maternal undernutrition and exposure to high levels of glucocorticoids. The development of the kidney has been highlighted as appearing to be consistently affected in both of these experimental models of fetal programming (9, 13). Changes in the kidney include alterations in gene expression, impairment of nephrogenesis, and increased apoptosis (24). The effect on the kidney of various perturbations appears to depend on the stage of renal development, the species used, as well as the nature and duration of the treatment. Impaired nephrogenesis resulting in a decreased nephron number predominantly results when perturbations occur early in kidney development (18, 26, 27). In many models, a low nephron number is associated with hypertension in adult life (3–5, 10, 19). Other differences include changes in renal gene expression for numerous genes, including components of the renin-angiotensin system (14, 25). Although numerous studies have shown alterations in nephron number and renal gene expression, studies showing alterations in renal function are limited. Long-term regulation of blood pressure is highly dependent on the kidney’s ability to regulate salt and fluid homeostasis. Thus, if hypertension results from in utero programming events, it is likely that changes in renal function are present independent of changes in nephron number. In the rat, the glomerular filtration rate (GFR) of offspring was reduced after exposure to a maternal low-protein diet (27).

In sheep, treatment of the ewe early in gestation (days 26–28; term = 150 days) with the synthetic glucocorticoid dexamethasone or the natural glucocorticoid cortisol results in offspring with elevated blood pressure from 4 mo of age (3–5). At 130 days of gestation the kidneys of fetuses of ewes treated with dexamethasone had significantly higher mRNA expression of angiotensinogen and angiotensin receptor types 1 and 2 (AT1 and AT2) (14). In response to infused angiotensin II, fetuses exposed to dexamethasone had a blunted response in terms of GFR and urine flow, indicating functional maturity of the kidney (14). The mRNA for the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR) was also higher in the dexamethasone-exposed fetuses compared with saline-treated controls, although protein levels for these receptors were not altered (8). Finally, adult offspring at 7 yr of age who had been exposed to dexamethasone in utero had a significantly lower number of glomeruli than control animals, although rates of glomerulosclerosis were similar (26). This suggests that there may have been impaired nephrogenesis during fetal development, because formation of new nephrons is complete before birth in the sheep (17). Together these results indicate that the kidney is significantly affected by early dexamethasone exposure. It would appear that the kidney of fetuses exposed to early prenatal dexamethasone may have “matured” prema-

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ture, which may have prevented normal nephron endowment. In this study, we aimed to test whether alterations in gene expression levels for MR and GR resulted in changes in fetal renal function in response to the ligands for these receptors, aldosterone and cortisol.

Increased cortisol levels cause both diuresis and natriuresis in the immature ovine fetus (<125 days of gestation) (6, 23); however, this response disappears in late gestation (>130 days) (22). In addition, cortisol can increase fetal blood pressure in fetuses <120 days of gestation but not in fetuses >130 days (22). We therefore chose to examine fetuses exposed to dexamethasone early in gestation at ~115–120 days of gestation. At this age, we hypothesized, the normal increases in blood pressure and renal function that normally occur in response to infused cortisol may be blunted in fetuses exposed to dexamethasone, making their response similar to that of a more mature fetus.

Studies examining the effects of infused aldosterone in the fetal sheep have been more limited but suggest that the effect is less than in the adult kidney. This may be due in part to the level of gene expression for MR in the ovine fetal kidney being less than half that in the adult (8). After early dexamethasone exposure, there was an upregulation of the mRNA for MR in the late-gestation fetal kidney, although this was not reflected in changes in protein (8). Infusions of aldosterone may cause increases in potassium excretion in the fetus (21) or no change in changes in protein (8). Infusions of aldosterone may cause increases in potassium excretion in the fetus (21) or no change (20). Infusion of aldosterone (5 μg/h) in the ovine fetus after 100 days of gestation causes a decline in the Na-to-K ratio, which occurs more rapidly and to a greater degree in more mature fetuses, but does not cause any change in urine flow rate (11). Adrenalectomy of the fetus at 120 days does not significantly alter urine production rate or composition until 140 days, when the Na-to-K ratio increases (2). In this study we hypothesized that the response in the dexamethasone-exposed “programmed” fetuses would differ from control animals in that a shorter time would be required to reduce the Na-to-K ratio, providing another indication of functional maturity.

METHODS

Animals. All experiments were approved by the Animal Ethics Committee of the Howard Florey Institute before commencement of experimental protocols. Nineteen merino ewes at ~3 yr of age and weighing 48 ± 2 kg were serviced by a single ram. Between days 23 and 25 of gestation, a cannula (inner diameter 0.58 mm, outer diameter 0.97 mm) was inserted into the jugular vein under local anesthetic. Starting on day 26 of gestation, ewes were infused intravenously with saline (0.19 ml/h; n = 9) or dexamethasone (Decadron, 0.48 mg/h; Merck Sharp and Dohme; n = 10) for 8 h. During the infusion, ewes were housed in individual cages with free access to food and water. At completion of the infusion protocol, the cannula was removed and the ewes were returned to pasture. Only ewes carrying single fetuses as confirmed by ultrasound were used in this study. At 110 days of gestation, ewes underwent general anesthesia, at which time cannullas were placed in the fetal carotid artery, jugular vein, and bladder as described previously (15). A cannula was also placed in the amniotic fluid as the uterus was closed. Vascular cannullas were flushed daily with heparinized saline to maintain patency.

Infusion protocols. After 5 days of recovery from surgery, blood pressure and renal function (urine flow rate, GFR) were measured as fetuses received an intravenous infusion of isotonic saline (2 ml/h), cortisol (100 μg/h), or aldosterone (5 μg/h). GFR was measured with $^{51}$Cr-labeled EDTA as described previously (16, 23). The protocol consisted of a 1-h equilibration period, 1 h of control measurements, 4 h of saline, cortisol, or aldosterone infusion, and a 2-h recovery period. Blood samples (3 ml) were taken at the midpoint of each urine collection. Infusions were carried out in random order at least 2 days apart. Because of cannula failure and two fetal deaths, not all protocols were carried out in all fetuses; the numbers of fetuses for each experiment are indicated in RESULTS. Experiments were performed only when the basal fetal urine osmolality was <180 mosmol/kgH2O, indicating that the fetus was in an unstressed state.

Sample analysis. Fetal blood gases (pH, P CO$_2$, and PO$_2$) were measured on a Ciba Corning 278 blood gas machine (Australian Diagnostics). Maternal plasma was analyzed for glucose, and samples of fetal plasma and urine were assayed for sodium and potassium with a Synchrotn CX5 clinical system (Beckman, Fullerton, CA). Osmolality of these fluids was measured by freezing point depression with an Advanced osmometer (Advanced Instruments, Needham Heights, MA). $^{51}$Cr was counted in duplicate 500-μl samples of plasma or urine on a gamma counter.

Blood pressure measurement. Fetal mean arterial blood pressure and heart rate were measured as described previously (15, 22). Amniotic fluid pressure was subtracted from the recorded blood pressure. Results were averaged for each hour of infusion.

Aldosterone concentrations. Plasma aldosterone concentrations were measured before and at the completion of the 4-h aldosterone infusion. Concentrations were measured with a previously validated radioimmunoassay (7). The sensitivity of this assay was 10 pmol/l, and the intra- and interassay coefficients of variation were 11% and 7.5%, respectively.

Statistics. Data are presented as means ± SE. A repeated-measures ANOVA was used to test for differences in GFR, blood pressure, and renal parameters between the groups over time with each infusion protocol. If a significant difference was found, a Tukey post hoc test was used to define which time points were different. Statistics were analyzed with SigmaStat.

RESULTS

The infusion of dexamethasone in the ewe caused a significant increase in maternal plasma glucose concentration (from 4.0 ± 0.2 to 7.7 ± 0.6 mmol/l, P < 0.001), whereas there was no change in the saline-infused group (4.0 ± 0.4 to 3.8 ± 0.6 mmol/l). We reported previously (5) that this dose of dexamethasone causes a complete suppression of maternal ACTH. Fetal blood gases (pH, P CO$_2$, and PO$_2$) and hematocrit were in the normal range for our laboratory for all fetuses. There were six females and three males in the saline-exposed group and six females and four males in the dexamethasone-exposed group.

Saline infusion. Mean age at infusion was 117 ± 2 days of gestation in the dexamethasone-exposed group (n = 7) and 116 ± 1 days of gestation in the saline-exposed group (n = 9). Infusion of saline for 6 h did not cause any significant changes in any parameter of either treatment group. GFR and blood pressure results are shown in Fig. 1. Basal values for all parameters are shown in Table 1. There were no differences between the two groups in any basal cardiovascular or renal parameter measured.

Cortisol infusion. Mean age at infusion was 117 ± 2 (dexamethasone, n = 7) and 119 ± 1 (saline, n = 7) days of gestation. Basal parameters were similar in the groups. Cortisol infusion caused a significant increase in fetal arterial blood pressure in both groups (P < 0.01 for both groups over time); however, the increase was similar in the two groups (Fig. 2). Urine flow and GFR increased in both groups to the same...
extent. The change in GFR, plotted as percent change from basal, is shown in Fig. 2. Cortisol caused a significant natriuresis in both groups (Fig. 2). There was also a similar decrease in free water clearance and fractional reabsorption of sodium in the groups (data not shown). Plasma electrolytes were unaltered.

Aldosterone infusion. Mean age at infusion was 119 ± 1 (dexamethasone, n = 7) and 120 ± 1 (saline, n = 7) days of gestation. There was no change in blood pressure over the course of the aldosterone infusion in either group (data not shown). Basal GFR values were similar in the two groups. Over the aldosterone infusion period, GFR increased in the saline-infused group but remained unchanged in the dexamethasone-infused group, resulting in a significant difference between the groups [P(treatment × time) < 0.05]. Post hoc analysis showed that GFR was significantly different after 3 and 4 h of infusion and during the 2 h of the recovery period. The results are plotted as percent change from basal in Fig. 3. The Na-to-K ratio was decreased in both groups because of an increase in urinary potassium excretion (Fig. 3), but this was similar in the two groups. Plasma electrolytes were unaltered.

To ensure that the differences observed in the GFR response were not due to differences in infusion rates, plasma concent-

Table 1. Basal urinary and cardiovascular parameters in fetuses from ewes infused with saline or dexamethasone

<table>
<thead>
<tr>
<th></th>
<th>Sal</th>
<th>Dex</th>
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<tbody>
<tr>
<td>Fetal age, days of gestation</td>
<td>116±1</td>
<td>117±2</td>
</tr>
<tr>
<td>Urine flow, ml/h</td>
<td>16±2</td>
<td>18±3</td>
</tr>
<tr>
<td>GFR, ml/h</td>
<td>119±7</td>
<td>116±12</td>
</tr>
<tr>
<td>Sodium, mmol/l</td>
<td>27.1±4.2</td>
<td>23.3±1.9</td>
</tr>
<tr>
<td>Potassium, mmol/l</td>
<td>5.3±1.4</td>
<td>6.1±1.5</td>
</tr>
<tr>
<td>UNaV, µmol/h</td>
<td>457±113</td>
<td>428±100</td>
</tr>
<tr>
<td>UKV, µmol/h</td>
<td>82±23</td>
<td>104±35</td>
</tr>
<tr>
<td>Osmolality, mosmol/kgH₂O</td>
<td>148±10</td>
<td>139±9</td>
</tr>
<tr>
<td>Mean arterial blood pressure, mmHg</td>
<td>41±1</td>
<td>41±2</td>
</tr>
<tr>
<td>Free water clearance, ml/h</td>
<td>9.6±1.2</td>
<td>9.8±2.1</td>
</tr>
</tbody>
</table>

Values are means ± SE for n = 10 (saline; Sal) or 7 (dexamethasone; Dex) ewes infused between days 26 and 28 of gestation. GFR, glomerular filtration rate; UNaV, urinary sodium excretion rate; UKV, urinary potassium excretion rate.
trations of aldosterone were measured in the fetus and ewe before and at the completion of the 4 h of infusion. There was no change in maternal aldosterone concentrations in either group (data not shown). Fetal aldosterone concentrations increased to a similar degree in the two groups (saline: basal 50 ± 12 pmol/l, 4 h 661 ± 114 pmol/l; dexamethasone: basal 36 ± 6 pmol/l, 4 h 586 ± 304 pmol/l).

**DISCUSSION**

This study demonstrates that basal renal function and blood pressure between 115 and 120 days of gestation are normal in fetuses that have been exposed to dexamethasone between 26 and 28 days of gestation. We showed previously (14) in slightly older fetuses (127–130 days of gestation) that, although basal renal parameters were similar, the renal response to infused angiotensin II was significantly altered after early dexamethasone exposure. This response indicated that the kidney may be more functionally mature in the dexamethasone-infused group and led us to hypothesize that in response to infused cortisol or aldosterone the renal responses would also be those of a mature fetus. It further suggested that changes in renal function to an infused ligand can occur without measurable changes in the receptor protein. However, the response to cortisol, in terms of both blood pressure responsiveness and renal function, was identical in the two groups. There was a significant increase in mean arterial blood pressure of ~4–5 mmHg with the 4-h infusion of cortisol. This was similar to the effect observed when cortisol was administered to fetuses (<120 days) over 24 h (22) and slightly less than the increase of 6–7 mmHg observed when a higher dose (250 μg/h) was given over 48 h to fetuses around 110 days (6).

In the study by Tangalakis et al. (22), the increase in blood pressure was not observed in fetuses over 130 days, possibly because of increased basal blood pressures at this age and increased maturity of tissues. The increased blood pressure in this study in response to cortisol suggests that early prenatal exposure to dexamethasone has not caused premature maturation of normal development. Furthermore, the renal responses were similar in the two groups. The natriuresis and diuresis observed were also typical of the response of an immature fetus rather than a more mature one (23).

Infusion of aldosterone in the ovine fetus has been shown to have little effect on urine flow rate, although it does cause a decrease in urinary Na-to-K ratio (11). In late gestation (>130 days) infusions of aldosterone into the amniotic fluid compartment, sufficient to raise fetal plasma aldosterone from 17 to >700 pg/ml, did not cause changes in GFR or urine flow but did increase sodium excretion and decrease potassium excretion (12). In contrast, other studies in two groups of fetuses aged <115 days or >125 days also showed that infusion of aldosterone has no effect on urine flow or potassium excretion (9, 20). There are few data on the effect of aldosterone infusions on GFR in the immature fetus (<120 days). In this study, in response to the aldosterone infusion, there was a significant difference in the change in GFR. The increase in GFR in response to infused aldosterone observed in the saline group has not been reported previously in the ovine fetus. It must be noted that the increase in GFR in response to aldosterone was small (15–20%) compared with the much larger increase (30–40%) seen in both groups with cortisol infusion. The mechanism leading to an increase in GFR is not known. However, both groups of fetuses showed an increase in urinary potassium excretion and a decrease in sodium excretion to the same extent, suggesting that the effect of aldosterone on renal function was not significantly different. The finding that the changes in urinary potassium and sodium excretion were not different between the groups was also observed with angiotensin II infusion, where there were similar large increases in sodium excretion in both the saline and dexamethasone groups, even though there were significant differences in the GFR response (14). In the present study it is noteworthy that, despite changes in mRNA for MR and GR in the kidneys of late-gestation fetuses as a result of earlier exposure to dexamethasone, the lack of change in protein levels is reflected in minimal differences in renal function in response to infusion of the ligands for these receptors. This differs from the results
obtained previously, where infusion of angiotensin II caused significant functional differences and increased renal growth in the dexamethasone fetuses despite the fact that upregulation of mRNA for the AT1 receptor was not reflected by changes in protein levels as measured by Western blot.

**Perspectives**

Experimental evidence has shown reduced nephron endowment, altered renal development, and significant changes in receptor expression in many models of fetal programming. As yet, most studies have not assessed whether these changes result in impaired or altered renal function. Such studies are critical, as it is only through changes in renal function that long-term increases in blood pressure are likely to occur. We speculate that changes in the renin-angiotensin system in the developing kidney of ovine fetuses exposed to dexamethasone, including upregulation of the AT1 receptor late in gestation (14), may have important long-term consequences for renal development and function. However, other changes such as upregulation of MR and GR in the late-gestation kidney of these fetuses do not result in marked changes in renal function, suggesting that these “programming” effects may not have long-term deleterious effects. Renal function studies have not yet been conducted in adult sheep exposed to this dexamethasone treatment, but it would be interesting to speculate that there may be differences, especially when the animals are exposed to challenges that alter the renin-angiotensin system.

**ACKNOWLEDGMENTS**

The authors thank Angela Gibson and Melinda Goga for assaying samples and Alan McDonald for help in surgery.

**GRANTS**

This work was supported by a Block Grant from the National Health and Medical Research Council of Australia to the Howard Florey Institute (983001).

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