Maternal dexamethasone treatment at midgestation reduces nephron number and alters renal gene expression in the fetal spiny mouse

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IT IS NOW WELL ESTABLISHED that early-life environmental factors, such as maternal undernutrition or stress, during critical periods of development can lead to permanent changes in structure and function of organs and tissues. These changes have been shown to significantly increase the risk of developing diseases in adult life such as cardiovascular disease, diabetes, and metabolic syndrome (see reviews in Refs. 12, 25, 46). What has emerged from many of these studies is that there are key windows of development that are more susceptible to particular insults.

Numerous models are currently being used to investigate the mechanisms of the developmental origins of adult disease including maternal undernutrition, both global (14, 15, 47, 52, 54) and nutrient specific such as low protein (19, 45, 53, 55), uterine artery ligation to induce fetal growth restriction (39), and maternal glucocorticoid exposure (7–9, 34, 35, 51). It has been proposed that the effects of maternal dietary alterations such as those described above may be mediated by elevated levels of maternal (and thus fetal) glucocorticoids (32), although this has been debated (54). In rats and sheep, exposure of the pregnant mother to synthetic glucocorticoids during mid and early gestation, respectively, when the kidney is at a preglomerular stage of development, results in a decrease in nephron number and hypertension in the adult offspring (35, 51). The mechanisms by which these effects occur are still largely unknown.

In this study, we examined the effect of maternal glucocorticoid exposure in a third species, the spiny mouse, Acomys cahirinus. The spiny mouse is a small desert-dwelling species, related to Gerbillinae, and native to regions of Egypt and Israel. Spiny mice have a relatively long gestation (38–40 days), deliver few pups (1–5, usually 1–3) and in which a major part of organ development occurs in utero. We previously showed that nephrogenesis begins at day 18 in the spiny mouse and is complete before birth, with glomeruli present from day 24 of gestation (5). This is unlike other rodent species where nephrogenesis is not completed until after birth (23, 42). This developmental trajectory of the kidney, together with the fact that the adrenal gland synthesizes cortisol rather than corticosterone, makes it physiologically relevant to the human. The spiny mice offspring develop rapidly after birth, are sexually mature by 3 mo of age, and have a life span of 3–4 yr.

Our aim was to test the hypothesis that maternal dexamethasone (dex) treatment at a time when fetal kidneys were at a preglomerular stage would result in a reduction in nephron number and increased blood pressure in young adult spiny mice. If this could be shown, it would provide further evidence that it is the timing of glucocorticoid exposure with respect to glomerular development, rather than a time in pregnancy, that is the important variable, as the rat, sheep, and spiny mouse have considerably different gestation lengths, and the time during gestation when the metanephric kidney develops is significantly different. Furthermore, we hypothesized that this nephron deficit would result from changes in the expression of genes known to be involved in branching morphogenesis. Thus we speculated there would be upregulation of genes that are known to inhibit branching morphogenesis (BMP4 and TGF-β1) and a downregulation of genes known to promote nephron formation (Wnt4). We also examined other members of the TGF-β super family [gremlin, an antagonist of BMP4 action (28), and Follistatin, known to increase ureteric bud branching and promote cell growth (22)]. A second potential mechanism
contributing to decreased nephron endowment may be increased rates of apoptosis. This has been demonstrated in the kidneys of rats exposed to a low-protein diet and IUGR (40, 50). Therefore, we further hypothesized that there would be upregulation of the proapoptotic gene (Bax) in the fetal kidney.

As sex differences have been shown in many programming models (11, 21, 26, 27, 56), both male and female offspring were independently examined.

METHODS

Animals. All experiments were approved in advance by Monash University Departments of Physiology and Anatomy and Cell Biology Animal Ethics Committees and conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. The spiny mice used in this study were obtained from our own laboratory colony and bred as previously described (5).

Implantation of mini osmotic pumps. Pregnant spiny mice (at 20 days of gestation) were weighed and briefly (2–4 min) anesthetized by inhalation of Isoflurane mixed with room air (4.5% induction, 2.5–2.8% maintenance dose; Rhodia Australia P/L, Victoria, Australia). A small subscapula incision was made, and hemostats were used to create a pocket under the skin ~3 × 1 cm. A mini osmotic pump (model 1003D; 100–μl capacity, 1-μl/h delivery rate; Alzet, Durect) was filled with either dex (125 μg/kg, n = 19) or saline (sal; n = 19) following the manufacturer’s instructions and then placed into the pocket created under the skin. The wound was closed using wound clips and xylocaine and betadine were placed on the skin to provide local anesthesia and antisepsis, respectively. The animal was allowed to recover for a few minutes, and when observed to have regained posture and motor control it was returned to its cage. At day 23 of gestation (after 60 h), six females from each treatment group were killed by cervical dislocation and fetal kidneys were collected and frozen for subsequent real-time PCR analysis.

The remaining 13 females from each treatment were reanesthetized following the technique as described above, the wound clips were removed, and the osmotic pump was removed (following manufacturer’s recommendations that osmotic pumps not remain inside an animal after delivery of the solution has ceased) through the same wound which was then repaired with surgical silk (5.0; Dynek, Australia). The spiny mouse was weighed and placed back in her cage for the duration of pregnancy.

Each spiny mouse was allowed to deliver naturally. Newborn pups were weighed within 6 h of birth, and their tails were colored with water-based marker to allow future identification. Pups were weaned at 40 days of age and housed in same sex groups of up to four animals until they reached 20 wk of age, where they underwent experimentation as described below.

Surgical preparation and blood pressure measurement. Twenty-week-old male (sal; n = 20, dex; n = 21) and female (sal; n = 21, dex; n = 13) spiny mice were randomly chosen from the 13 litters born in each treatment group. They were anesthetized (30–40 min), Isoflurane mixed with room air via inhalation, 4.5% induction, 2.5–2.8% maintenance; Rhodia Australia P/L) and placed on a water-circulating heat pad to maintain body temperature at 37.5°C. Blunt dissection was used to isolate a length of ~12 mm of the left carotid artery in the midneck region, ~3 mm below the bifurcation of the carotid arteries. The artery was occluded with a suture (5.0 silk), and a small cut was made in the vessel wall with microscissors. A heat-stretched polyethylene catheter (0.58-mm internal diameter and 0.96-mm outer diameter) directed toward the heart was inserted, the distal suture released, and the catheter was advanced until it reached a Silastic rubber droplet placed on the catheter ~10 mm from the tip. The catheter was secured in place with two sutures (5.0 silk), one tied around the Silastic rubber droplet to provide additional support. The neck wound was closed using silk suture and discontinuous stitches. The catheter was tunnelled subcutaneously and passed through a titanium anchor button (AT-3040; Agnths), which was then attached to the skin using discontinuous stitches. A small piece of stockng material was stitched over the skin and button to provide the skin with extra resistance against the weight of the swivel, tether, and strain of the titanium button, as the skin of the spiny mouse is fragile and easily damaged. The catheter was checked for patency before being flushed clean with heparinized saline and occluded. A stainless steel tether (AT-3010; Agnths) was attached to the button by a small hook and a plastic sheath pulled over the catheter and tether to protect it from being chewed. The tether was attached to a balance arm (AT-3010; Agnths) located on top of the cage and the catheter was attached to a Teflon swivel (TCS2–21; Agnths). The weight on the balance arm was adjusted to suit the weight of the animal so that there was minimal force pushing or pulling on the button. The tether and swivel system allowed the animal to move freely within the cage, while protecting the arterial catheter from twisting.

Blood pressure was measured continuously for 1 wk using a pressure transducer (Truwave disposable; Edwards Lifesciences), quad bridge amplifier (Life Sciences, Melbourne, Australia), and a computerized data-acquisition system specifically designed for cardiovascular measurements. The fourth day of recording was considered baseline after recovery from surgery. Catheter patency was maintained by a constant infusion (0.25 μl/min) of heparinized saline. Pulsatile arterial pressure was continuously monitored and sampled at 1,000 Hz and recorded on a computer using an analog-to-digital data-acquisition program (Universal version 2004) created by Geoffrey Head (Baker Heart Research Institute, Melbourne, Australia) and Elena Lukoshkova (National Cardiology Research Centre, Moscow, Russia) using Labview (National Instruments). A special algorithm was used to detect systolic arterial pressure, diastolic arterial pressure, and pulse interval (33). Mean arterial pressure (MAP) was calculated on a beat-to-beat basis and instantaneous heart rate (HR) was calculated from the pulse interval.

Adult postmortem and tissue collection. Differences between the number of animals exposed to the postmortem procedures below and the numbers of animals that underwent and survived 24-h postsurgery were due to loss of catheter patency through blood clots, damage, or removal of the catheter from the animal during the week of blood pressure measurement.

After a complete week of blood pressure measurements (male: sal: n = 7, dex: n = 10; female: sal: n = 10, dex: n = 10), a blood sample (where possible) was taken and the spiny mice were killed by cervical dislocation. Blood samples were centrifuged, and plasma was removed and frozen at −20°C for later analysis of plasma cortisol concentrations. These spiny mice were weighed with the amount of blood taken added to the body weight only, and all organ weights were recorded. The left kidneys of five animals per group were fixed in 10% buffered formalin, processed to glycolmethacrylate resin, and serially sectioned for the determination of nephron number and volume estimates as previously described (5).

Cortisol assay. Male (sal: n = 5, dex: n = 6) and female (sal: n = 9, dex: n = 9) plasma samples were assayed for total cortisol using a modified version of an assay validated for sheep plasma as previously described (3). The volume of plasma used was reduced to 5 μl to facilitate detection of the higher cortisol concentration in the spiny mouse compared with the sheep. The intra-assay coefficient of variation was 9% and the sensitivity of the assay was 0.2 ng/ml.

Real-time PCR. Real-time PCR probe and primer sets that had been optimized for other species (mice, rats, and sheep) within our laboratory were used in this study as very few sequences are available specifically for the spiny mouse. A trial run of three samples, in parallel with three samples of the species, the primer, and probe sequences were designed for, was used to test the genes in the spiny mouse. Those that were expressed above the level of background and the housekeeping gene (18S) in the spiny mouse underwent quantitative analysis within all tissue samples. The following methods
subtracting the CT value of the gene of interest to give the expression level relative to the mean of the saline group. The assay was run twice for each gene.

**RESULTS**

Litters and body and organ weights. Dex treatment (125 μg/kg for 60 h) during midgestation in spiny mice did not alter gestation length (sal: 39.8 ± 0.4 days, dex: 40.1 ± 0.5 days), offspring number (sal: 2.9 ± 0.2, dex: 3.2 ± 0.2), viability of the offspring (100% in both groups), or the sex ratio at birth (male-to-female ratio; sal: 0.46 ± 0.07, dex: 0.61 ± 0.09). Dex treatment had no effect on maternal weight gain between days 20 and 23 of gestation (sal: 1.89 ± 0.45 g, dex: 1.11 ± 0.39 g), on neonatal birth weights (Table 2), or the eventual adult body weight of the male and female offspring (Table 2). There were no significant differences in the weight of individual organs between groups or between males and females when samples were collected at the end of the 1 wk of blood pressure measurement (Table 2).

Adult kidney analysis. At 20 wk of age, male and female offspring had significantly fewer glomeruli following dexamethasone exposure compared with the sal-exposed controls (Table 3). Mean glomerular and corpuscle volumes were significantly greater following dex exposure for both sexes, but total glomerular and corpuscle volumes were not significantly different between the groups in either the males or females (Table 3). Kidney volume was not different between groups or between males and females (Table 3).

**Gene expression using real-time PCR.** Preliminary analysis showed that there was no difference between male and female fetuses in expression levels of any gene in any treatment group. Data from both sexes are thus pooled for each treatment. Exposure of the fetus to excess maternal glucocorticoids from days 20–23 of gestation in the spiny mouse led to significant increases in the expression of BMP4 (P < 0.05; Fig. 1A), TGF-β1 (P < 0.05; Fig. 1B), and gremlin (P < 0.01; Fig. 1D) in 23-day fetal kidneys compared with sal-exposed controls. There was a significant upregulation of the proapoptotic gene Bax in 23-day fetal kidneys of offspring exposed to excess maternal glucocorticoids (P < 0.05; Fig. 1F).

There were no significant differences in Wnt4 and follistatin expression in day 23 fetal kidneys between groups (Fig. 1, C and E).
Table 2. Body and organ weights for male and female spiny mice at 20 wk of age following in utero exposure to dex or saline

<table>
<thead>
<tr>
<th>Weight</th>
<th>Male (n = 7*)</th>
<th>Female (n = 10*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight, g</td>
<td>5.04±0.14</td>
<td>5.04±0.14</td>
</tr>
<tr>
<td>Body, g</td>
<td>32.5±1.0</td>
<td>30.4±1.1</td>
</tr>
<tr>
<td>Total kidney</td>
<td>27.8±1.1</td>
<td>28.8±1.4</td>
</tr>
<tr>
<td>Total kidney/BW</td>
<td>0.86±0.04</td>
<td>0.95±0.04</td>
</tr>
<tr>
<td>Brain</td>
<td>81.4±1.3</td>
<td>81.6±0.8</td>
</tr>
<tr>
<td>Brain/BW</td>
<td>2.51±0.07</td>
<td>2.72±0.10</td>
</tr>
<tr>
<td>Total repro. organs</td>
<td>63.3±3.6</td>
<td>9.60±1.6</td>
</tr>
<tr>
<td>Total repro. organs/BW</td>
<td>1.95±0.09</td>
<td>0.31±0.04</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of mice. All postmortems were performed after the animals had been attached to the blood pressure swivel for 1 wk.

Table 3. Kidney volume and glomerular number estimates in the left kidney for male and female spiny mice, at postnatal age 20 wk, following in utero treatment with dex or saline

<table>
<thead>
<tr>
<th></th>
<th>Kidney Volume, mm³</th>
<th>Glom. Number</th>
<th>Glom. Volume, mm³ × 10⁴</th>
<th>Total Glom. Volume, mm³</th>
<th>Corp. Volume, mm³ × 10⁴</th>
<th>Total Corp. Volume, mm³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (n = 5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>129.8±7.9</td>
<td>7,870±27</td>
<td>3.6±0.2</td>
<td>2.8±0.1</td>
<td>3.7±0.2</td>
<td>3.0±0.1</td>
</tr>
<tr>
<td>Female (n = 5)</td>
<td>127.3±11.7</td>
<td>7,495±73</td>
<td>3.8±0.5</td>
<td>2.9±0.4</td>
<td>4.0±0.5</td>
<td>3.0±0.4</td>
</tr>
<tr>
<td>Dex</td>
<td>151.6±11.8</td>
<td>6,878±173</td>
<td>5.1±0.2</td>
<td>3.5±0.1</td>
<td>5.4±0.1</td>
<td>3.7±0.1</td>
</tr>
<tr>
<td></td>
<td>122.5±12.5</td>
<td>6,206±419</td>
<td>4.6±0.2</td>
<td>2.9±0.3</td>
<td>4.8±0.1</td>
<td>3.0±0.3</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>NS</td>
<td>P&lt;0.05</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Treatment</td>
<td>NS</td>
<td>P&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Interaction</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of mice. Glom, glomerular; Corp, corpuscle; NS, not significant.
ministered earlier (11–12 and 13–14 days) or later (19–20 and 20–21 days) in gestation have no effect on nephron number or blood pressure (34, 35), indicating that there is a particular stage of kidney development that is sensitive to exposure of excess glucocorticoids. Thus the present study not only confirms this for another species and shows that dex exposure at the stage of kidney development when branching morphogenesis begins results in a similar renal phenotype, but we have identified changes in expression of key genes likely to regulate this reduction in nephron number.

While different models of a poor intrauterine environment using pregnant animals have been shown to produce a significant nephron deficit, the mechanisms involved have not been identified. For example, in rats a low-protein diet throughout pregnancy resulted in increased apoptosis of mesenchymal cells at embryonic day 15, but not at embryonic day 13, producing a mild nephron deficit at 2 wk of age (50). The authors proposed that enhanced apoptotic deletion of mesenchymal cells, before glomerular formation, was a likely cause of the nephron deficit (50). Similarly, Pham and colleagues (40) identified increased expression of the proapoptotic gene Bax and a downregulation of the anti-apoptotic gene Bcl-2 at term in a rat model of IUGR. The authors (40) also concluded that increased apoptosis was the likely cause of the reduced nephron number in the IUGR offspring.

Our results support a role for increased apoptosis in the developing fetal kidney following an insult that leads to an eventual nephron deficit but more importantly, we have iden-

Table 4. Numbers of male and female spiny mice entering and surviving anesthesia, followed by carotid artery catheter implantation surgery, at 20 wk of age

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>Dex</td>
</tr>
<tr>
<td>Surgery</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td>Surgery survival</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>24 h Postsurgery survival</td>
<td>17*</td>
<td>12*</td>
</tr>
<tr>
<td>%Survival</td>
<td>85</td>
<td>57†</td>
</tr>
</tbody>
</table>

* Differences between these values and the numbers of animals used for morphological (Table 2) and hemodynamic analysis are due to loss of catheter patency through blood clots, damage, or removal of the catheter from the animal during the week of blood pressure recording. †χ² = 16.18; df = 7; P<0.05, compared with all other groups.
tified altered expression of a number of genes that are known to play a major role in branching morphogenesis. BMP4 mRNA during early kidney development is normally expressed in the mesenchymal cells surrounding the Wolffian duct, comma- and s-shaped bodies, and the ureteric bud (4). During branching morphogenesis, BMP4 acts to inhibit ectopic branching of the ureteric bud and is required for growth of the ureter stalk (13). TGF-β1 has been shown to play a key role in the control of differentiation and morphogenesis in embryonic development. Epithelial expression of TGF-β1 is associated with regions of active morphogenesis involving epithelial-mesenchymal interactions (29). TGF-β1 is an inhibitor of epithelial cell proliferation and is capable of inducing epithelial-to-mesenchymal transformations in a variety of epithelial cells (44).

The upregulation of not only BMP4 and TGF-β1, known inhibitors of branching morphogenesis, but also gremlin, the product of which is an antagonist of BMP4 activity, in the 23-day fetal kidney, suggests that there is an interesting interplay between genes that regulate linear growth and branching in the primordia of the ureteric tree. TGF-β1 is known to selectively inhibit branching morphogenesis in cell culture (41), and it has been suggested that BMP4 restricts the site of ureteric budding along both the Wolffian duct and stalk of the branching ureters by its antagonistic effect on GDNF signaling (30). Gremlin is the primary antagonist of BMP4 activity (28). Gremlin has been identified as the essential extracellular signal that initiates metanephric kidney development by enabling the ureter to invade the metanephric mesenchyme (28). During the onset of metanephric development, gremlin mRNA is rapidly downregulated and restricted posteriorly in the Wolffian duct (28). During branching morphogenesis, gremlin mRNA is expressed locally in the mesenchyme surrounding the invading ureter and BMP4 is expressed in mesenchyme adjacent to the ureter stalk (28). The antagonistic actions of gremlin on BMP4 are thought to regulate the temporal and spatial kinetics of ureter branching, whereas BMP signaling alone promotes ureter stalk elongation (28). Gremlin has also been shown to negatively modulate BMP4 induction of lung branching morphogenesis in the embryonic mouse lung (43).

Thus it is reasonable to hypothesize that increased expression of BMP4 and TGF-β1 leads to inhibition of branching and then to a significant nephron deficit. The upregulation of gremlin may be a consequence of the upregulation of BMP4, required to regulate the large increase in BMP4 expression, thereby preventing a more severe nephron deficit.

Unlike the previous studies in rats and sheep (9, 34), dex exposure did not cause hypertension in young adult spiny mice despite the reduction in nephron number. There are many studies that find an association between low nephron number and subsequent hypertension and conclude that the increased blood pressure is, at least in part, due to the reduced nephron number. Langley-Evans and colleagues (18) found that a maternal low-protein diet throughout pregnancy led to a 13% nephron deficit and systolic blood pressures (determined by tail cuff) ~13 mmHg above that for control animals. It was concluded that the effect of maternal undernutrition, to decrease nephron number, had the eventual impact to increase blood pressure and the risk of renal disease in adult life (18). Similarly Vehaskari and Woods (46) identified a 28% nephron deficit associated with higher systolic blood pressures (determined by tail cuff) by 6 wk of age in offspring exposed to a maternal low-protein diet limited to the second half of pregnancy. These authors (46) also concluded that the mechanisms likely to be involved in the prenatal “programming” of hyper-
tension are an altered renin-angiotensin axis and a deficit in total nephron number.

The absence of hypertension in the spiny mouse after maternal dex treatment, in contrast to these previous studies, may be due to a number of factors, such as the age of study (5 mo) relative to life span of these animals (3–4 yr), differences in the developmental profiles of other organ systems related to blood pressure regulation (e.g., renin-angiotensin system, sympathoadrenal system), and perhaps different methodologies used to record blood pressure. In this study, we took great care to ensure that animals had recovered completely from the stress of surgery and that stable recordings of arterial blood pressure were obtained over a sufficient time to ensure that the readings were not confounded by stress or other disturbances, factors often associated with the use of tail cuff plethysmography. Other factors, such as vascular dysfunction (36), alterations in the renin-angiotensin system (17, 31), and alterations in the activity of sensory unmyelinated C fibres (38), are likely to contribute to the development of hypertension in these animal models, and the reduced nephron number would be likely to exacerbate and perpetuate the disease as these animals aged.

While the nephron deficit seen in the current study is less than that seen in sheep and rat after maternal dex treatment (~30–40% reduction), a loss of ~15% in a species with a constitutively low nephron number (~7,500) might have been expected to have significant consequences for renal and cardiovascular function. The spiny mouse, being native to semiarid desert environments and mesic forest areas (48), may be capable of adapting to a reduction in nephron number that in other species results in increased blood pressure. The spiny mouse kidney is structurally and functionally different to that of the C57BL/6 mouse (5). Although the spiny mouse kidney is relatively smaller and has 36% fewer nephrons than the C57BL/6 mouse, the glomeruli of the spiny mice are significantly larger than C57BL/6, resulting in an equivalent total glomerular volume (5). The functional significance of these differences is currently under investigation, but these differences are likely related to the ability of the spiny mouse to cope with a more physiologically challenging environment than the C57BL/6 mouse, and thus the maintenance of a normal basal blood pressure in the face of a modest nephron deficit could be attributable to its highly efficient kidney. To date, there are no known models of programming of hypertension with or without a low nephron number in other strains of mice.

Other studies have shown dissociation between low nephron number and the development of hypertension. For example, in the rat a low-protein diet maintained throughout pregnancy and the postnatal period reduces nephron number by ~20% yet the offspring actually show a reduced blood pressure in adulthood (16). Also, when maternal protein restriction is imposed throughout pregnancy and early lactation, the offspring are not hypertensive at any age, despite a nephron deficit of ~30% (57). These studies also suggest that a low nephron number is not the sole contributor to the development of hypertension in adult life.

There are a number of models of maternal glucocorticoid exposure that, unlike the present study, also result in a significant reduction in birth weight of the offspring (2, 20, 54). The differences between these and the current study are timing of exposure and mode of delivering the drug. In the present study, a low dose of dex was given for a short time (60 h) in midgestation by continuous infusion, whereas other studies administered dex by multiple bolus injections and the treatments were either given later in gestation (20, 54) and/or over longer periods of time (2). The protocols used in the current study were devised to examine the effects of glucocorticoid excess at a critical time of kidney development, whereas the other protocols are used as models of antenatal glucocorticoid treatment as given to pregnant women at risk of preterm delivery.

In the current study, there were sex differences in the effect that dex had on the capacity of animals to survive anesthesia and surgical stress. Despite the absence of effects on birth weight or adult body weight in either sex, male spiny mice were more likely to die under or shortly after anesthesia compared with males from the saline control group and compared with females from the dex-exposed group. The surviving dex-exposed males had a significantly increased HR for 24 h following surgery. These observations suggest that the dex exposure may have had an impact on the hypothalamic-pituitary-adrenal (HPA) axis of males but not the female spiny mice, although neither sex had elevated plasma cortisol concentrations. It is interesting to consider the large range of cortisol values seen in both groups of female spiny mice. It is likely that these data reflect the different stages of the oestrus cycle that these females may have been in at the time the final collection of blood was made. For example, cortisol concentrations in mares are highest during the late dioestrous phase of the oestrus cycle and lowest during oestrus (1). Prenatal glucocorticoid exposure permanently increases basal plasma corticosterone levels in adult rats (20, 49); however, control and dex-treated rats showed similar elevations in corticosterone levels following a restraint stress (20). There is no evidence of altered activation of the HPA axis in sheep following dex treatment in early pregnancy (10).

The blood pressures obtained by day 4 are representative of basal blood pressure as seen by the gradual and consistent decline over the first 3–4 days (recovery from surgery) and the plateau reached by days 4–6 after surgery. The present study also demonstrates for the first time that the spiny mouse has a significantly lower MAP and HR than the C57BL/6 mouse (24). It is known that the regulation of HR in the spiny mouse heart is different to that of the C57BL/6 mouse (37). It was proposed that the differences in the mechanical responses of isolated C57BL/6 (negative force-frequency relationship) and spiny mouse (positive force-frequency relationship) hearts to high calcium concentrations were due to the effects of the specific glucocorticoid hormone (corticosterone in mouse, cortisol in spiny mouse) on the development of the sodium-calcium exchanger (37). Specifically, it was proposed that the effects of the glucocorticoid hormones are expressed by de novo protein synthesis of specific components of the contractile apparatus of the heart, responsible for the regulation of storage and delivery of calcium in the heart (37). This suggests that the basic regulation of blood pressure in the spiny mouse is different to that of other rodents and may more closely resemble that of the human where a similar (positive) force-frequency relationship is seen and where cortisol is the glucocorticoid secreted by the adrenal gland.
Perspectives

In a third animal model, the spiny mouse, which differs significantly from the rat and the sheep in the timing of metanephric kidney development in relation to the length of gestation, we provide evidence that the “critical period” for the effect of glucocorticoid excess on nephron number is the preglomerular stage of kidney development. This adds considerably to the concept that this stage of metanephrogenesis is particularly vulnerable to excess glucocorticoid exposure, irrespective of whether it occurs very early (sheep), in the middle (spiny mouse), or late (rat) in pregnancy, or whether nephrogenesis is completed before (sheep and spiny mouse) or after (rat) birth. While we have shown that excess glucocorticoids suppress branching morphogenesis through changes in expression of genes known to inhibit branching morphogenesis in the developing kidney, the possibility that this occurs in other organs where epithelial branching is also a feature of development (e.g., lung and pancreas) should be considered. Relevance of the time of treatment to placental development, particularly in relation to the capacity of the placenta to regulate transfer of endogenous maternal adrenal steroids to the fetus, needs to be considered. The capacity of dexamethasone to cross the spiny mouse placenta is not yet known.

The fact that the spiny mouse normally maintains a lower blood pressure than another rodent of comparable size (C57BL/6 mouse) yet has a constitutively lower number of nephrons suggests that the relationship between renal function and cardiovascular regulation in this species is different from that of conventional laboratory rodents. The spiny mouse provides evidence that a modest, although significant, nephron deficit does not necessarily lead to the development of high blood pressure, at least in relation to the capacity of the placenta to regulate transfer of endogenous maternal adrenal steroids to the fetus, needs to be considered. The capacity of dexamethasone to cross the spiny mouse placenta is not yet known.

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GRANTS

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REFERENCES


