Maternal glucocorticoids and prenatal programming of hypertension

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Woods, Lori L. Maternal glucocorticoids and prenatal programming of hypertension. Am J Physiol Regul Integr Comp Physiol 291: R1069–R1075, 2006. First published April 27, 2006; doi:10.1152/ajpregu.00753.2005.—Maternal glucocorticoids have been postulated to play an important role in prenatal programming for adult hypertension in the offspring. However, we have shown previously that offspring hypertension caused by maternal dexamethasone subcutaneous administration at 100 μg·kg⁻¹·day⁻¹ can be accounted for by the corresponding reduction in food intake that these mothers experience. The present studies were designed to determine whether there is a lower dose of dexamethasone that does not reduce maternal food intake yet still causes hypertension in the adult offspring. Pregnant rats were treated with subcutaneous dexamethasone at 50 (D50) or 25 (D25) μg·kg⁻¹·day⁻¹ on days 15–20 of pregnancy. An additional group was untreated or received vehicle injections (control). D25 and D50 dams reduced their food intake by 17% during and after treatment and gained 31% less weight than control over the course of gestation. In adulthood (∼21 wk), chronically instrumented male offspring of D50 and D25 had normal blood pressures (D50: 131 ± 2 mmHg and D25: 127 ± 3 mmHg vs. 127 ± 2 mmHg in control). Qualitatively similar results were found in female offspring. Thus neither dexamethasone per se at these doses nor the accompanying modest reductions in maternal food intake and weight gain have blood pressure programming effects. As far as has been tested, there does not appear to be a dose of dexamethasone that, given over this time period in the rat, programs offspring hypertension without reducing maternal food intake and weight gain. These data do not support the hypothesis that maternal glucocorticoids program offspring hypertension directly.

METHODS

Female Sprague-Dawley rats (∼250–300 g) were obtained from Simonsen and bred at Oregon Health and Science University. Animals were housed in a room with a controlled temperature and a 12:12-h light-dark cycle. They were maintained on a normal-protein (19%), normal-sodium (0.20%) diet (Purina basal diet 5755) throughout pregnancy and lactation. Females were individually housed with a male, and the day sperm were seen in a vaginal smear was designated as day 1 of pregnancy. Dams were injected with dexamethasone (50 or 25 μg·kg⁻¹·day⁻¹ in 4% ethanol/0.9% saline, 100 μg/ml) subcutaneously on days 15–20 of pregnancy (D50, n = 5 and D25, n = 7, respectively). These doses are one-half and one-fourth of the dose our own, have shown that restriction of dietary protein or total food intake in pregnant rats leads to a reduced number of nephrons and hypertension in the adult offspring (14, 19, 25, 30, 32, 34, 36). We have also shown that this is associated with, and likely mediated by, suppression of the intrarenal renin-angiotensin system during development (36).

A second theory regarding the mechanisms by which maternal environment during pregnancy programs for adult disease invokes a role for maternal glucocorticoids (2). The fetus is normally protected from maternal glucocorticoids, which are deactivated by the placental enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD). Under conditions in which maternal corticosteroid levels are elevated or placental enzyme activity is reduced, the fetus would be exposed to higher levels of these corticoids that might, in turn, participate in programming. In support of this idea, administration of dexamethasone, a synthetic corticosteroid not metabolized by this enzyme, to pregnant rats leads to offspring of low birthweight that are hypertensive later in life (2, 21, 22). However, we have recently shown that dexamethasone at the doses used previously impairs maternal food intake and weight gain, and that pair-fed controls produce offspring with similar blood pressures to those in offspring from dexamethasone-treated mothers (39). Thus the effect of dexamethasone to reduce maternal food intake may in large part account for the programming of hypertension in the offspring. However, it remains possible that a lower dose of dexamethasone, one that does not impair maternal food intake and weight gain, might still program the offspring for hypertension. If so, this would provide additional support for a direct role for fetal exposure to excess maternal glucocorticoids in programming for adult hypertension.

The purpose of the present study was to further test the hypothesis that maternal glucocorticoids directly program for hypertension in the offspring by determining whether there is a dose of dexamethasone that does not impair maternal food intake or weight gain yet still programs for offspring hypertension.

EVIDENCE FROM HUMAN STUDIES indicates that babies that are born smaller have a greater risk of many adult diseases than do larger babies. In particular, adult blood pressure has been shown to be inversely related to birth weight (1, 8, 9, 20, 31). Emerging evidence in animals has confirmed that factors in the prenatal environment that affect fetal growth can permanently change the structure and physiology of organ systems, thus programming the individual for various diseases, including hypertension, later in life. Yet the nature of these factors and the mechanisms by which they permanently alter morphology and physiology are still not completely understood.

At least two mechanisms have been postulated to be important in prenatal programming for hypertension and other diseases. One theory is that alterations in maternal nutritional factors, particularly reduced protein intake, suppress the fetal intrarenal renin angiotensin system, which leads to impaired renal development, permanent reductions in nephron number, and adult hypertension. Indeed, several laboratories, including the Division of Nephrology and Hypertension, L463, Oregon Health and Science Univ., 3181 S.W. Simonsen Park Rd., Portland, OR 97239–3098 (e-mail: woodsl@ohsu.edu).

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shown in our previous study to impair maternal food intake and weight gain (39). A third group of dams (n = 3) were injected with the vehicle used to administer the larger dose of dexamethasone, and another group (n = 4) were uninjected. There were no differences in the data between these latter two groups, so they were pooled to yield a single control group. Maternal food intake was measured daily, and all pregnant animals were weighed daily. Litters were counted and weighed within 15 h of birth; pups were also counted and weighed at 1, 5, 10, 15, and 21 days. All pups were weaned to the normal diet at 22 days of age and maintained on that diet until adulthood. Before surgery, each animal was housed overnight in a metabolic cage for collection of a 24-h urine sample.

**Surgical Preparation of Adult Animals**

Adult male and female offspring were chronically instrumented at ∼20 wk of age, as described previously (36, 39). Briefly, using sterile technique, we implanted catheters in the femoral artery and vein and the bladder under ketamine/xylazine/acepromazine anesthesia. The animals recovered for at least 6 days after surgery and were trained in wire restrainers at least three times before the study. Vascular catheters were flushed every 2–3 days with 5% dextrose and filled with heparin.

**Experimental Protocol**

Mean arterial pressure and renal function were measured in conscious animals, as described previously (36, 39). Briefly, with the rat in a wire restrainer in the study room, urine was allowed to drain continuously from the bladder into a tube. Arterial pressure was measured through the arterial catheter using a pressure transducer (Abbott Laboratories, North Chicago, IL) connected to a polygraph (Grass Instruments, Quincy, MA), and a reading was taken after at least 30 min, once the pressure had stabilized. Pressures were always measured between 6:00 and 9:00 AM. After these measurements, a small blood sample (200 μl) was taken for measurement of microhematocrit; plasma protein was measured by refractometry (National Instruments, Baltimore MD). As described previously, glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured as the clearances of inulin and PAH, respectively, in four successive urine clearance periods, with a 0.6-ml blood sample taken at the midpoint of each period (36, 39). After centrifugation and removal of the plasma, the red blood cells were resuspended in an equal volume of saline and returned to the animal. Plasma was stored at −20°C until analysis. When all experiments were completed or when the instrumentation was no longer functional, the rats were killed with a commercial euthanasia solution and the kidneys were removed and weighed.

**Analytical Measurements**

Plasma and urine concentrations of inulin and PAH were measured by the methods of Waugh (33) and Brun (6), respectively. Urine protein was measured by precipitation with sulfoisalicylic acid.

**Statistical Analysis**

The data are expressed as means ± SE. Data for the groups were compared using one-way or two-way ANOVA, followed by a post hoc test (Bonferroni). Statistical significance was assumed with a value of P < 0.05 or better.

**RESULTS**

**Effects of Dexamethasone on Maternal Food Consumption and Weight Gain in Pregnancy**

Dexamethasone at 50 or 25 μg·kg⁻¹·day⁻¹ reduced food intake and weight gain in pregnant animals, as shown in Figs. 1 and 2 and Table 1. During and after the dexamethasone administration, from days 15–22 of pregnancy, food intake was significantly reduced by 17% in both D50 and D25 compared with control. Maternal weight gain in both D50 and D25 was also significantly less than in controls. The length of gestation and number of pups per litter were not different among groups.

**Effect of Dexamethasone on Offspring Growth**

Average birthweights were significantly lower in offspring of dams that received dexamethasone at either 50 or 25 μg·kg⁻¹·day⁻¹ (Table 1). These offspring also tended to be smaller at weaning than controls and were numerically, although not always statistically, smaller than control offspring throughout life. Kidney weights were not significantly different among the groups, but the kidney-to-body weight ratios were higher in D50 animals.

**Effects of Dexamethasone on Physiological Variables in the Offspring**

Physiological variables and organ weights in offspring of dexamethasone-treated and control animals are shown in Fig. 3 and Table 2. Neither group exposed to dexamethasone prenatally had arterial pressures that were significantly different from controls. Absolute GFR and ERPF were not significantly altered by either of the treatments, although both were significantly higher in males than in females, roughly in parallel with their differences in body size. GFR and ERPF per body weight were increased in D50 offspring, reaching significance for GFR in females and for ERPF in both genders.

Hematocrit was significantly increased only in D50 females; plasma protein was not significantly different among groups. Urine protein excretion was not significantly different among groups, although it was higher in males than in females. Adrenal and heart weights were generally not different among groups, except that the heart weight in D50 males was lower than that in control males. This difference disappeared when normalized to body weight.

**DISCUSSION**

Our laboratory and others (2, 39) have shown previously that dexamethasone, administered at 100 μg·kg⁻¹·day⁻¹ during the latter portion of rat pregnancy, caused hypertension in the adult offspring. However, at this dose, dexamethasone also...
reduced maternal food intake and weight gain, and the results of pair-feeding experiments in our study indicated that the impaired maternal food intake itself could, in large part, account for the later offspring hypertension. The goal of the present study was to determine whether there was a lower dose of dexamethasone that, when given to pregnant rats over the last third of gestation, programmed the offspring for hypertension without reducing maternal food intake and weight gain. We found that at both 50 and 25 μg·kg⁻¹·day⁻¹, dexamethasone still modestly reduced maternal food intake (although by only half as much as did the 100 μg·kg⁻¹·day⁻¹ dose) but failed to program for hypertension in the offspring. Thus, on the basis of this set of experiments in which the dose was systematically and gradually reduced, it seems unlikely that there is a dose of dexamethasone that, when given over 6 days in late gestation in the rat, programs offspring hypertension without reducing maternal food intake and weight gain. These findings do not support the hypothesis that maternal glucocorticoids program offspring blood pressure through a direct mechanism but suggest that fetal programming for hypertension in this model may occur indirectly, possibly through impaired maternal nutrition.

The existence of an inverse relationship between early growth patterns and adult blood pressure in humans is now widely accepted (1, 8, 9, 20, 31), but the exact physiological mechanisms responsible for these associations are still not well understood. The two major theories that have been proposed invoke either fetal undernutrition (due to maternal undernutrition or other causes) and impaired renal development, or exposure of the fetus to excess glucocorticoids from the mother. Studies from our laboratory, as well as others, have suggested that maternal nutritional factors, in particular protein intake, and impaired renal development play a critical role in this programming for adult hypertension (19, 32, 36). Modest maternal protein restriction during rat pregnancy leads to programming for adult hypertension (19, 36). Moderate neonatal blockade of the renin-angiotensin system and consequent impairment of renin development (36, 38). Renal renin mRNA, renin protein, and ANG II are reduced during development, and nephron number is permanently decreased in these offspring (36). This programming is associated with and likely occurs through suppression of the fetal/newborn intrarenal renin-angiotensin system and consequent impairment of renal development (36, 38). Furthermore, neonatal blockade of the renin-angiotensin system with losartan in normal animals leads to a decreased nephron number and hypertension in adulthood, supporting a cause-and-effect relationship among the findings in low-protein animals (38).

The second major theory is that fetal programming for hypertension occurs through excess exposure of the fetus to glucocorticoids from the mother (2). Under normal circumstances, the placental enzyme 11β-HSD inactivates maternal corticosteroids before they reach the fetus. But under conditions in which maternal glucocorticoid levels are elevated and/or placental 11β-HSD activity is reduced, the fetus could be exposed to excess maternal glucocorticoids, which have been proposed to program for hypertension. Several lines of evidence support this hypothesis. First, 11β-HSD activity is

Table 1. Growth in control dams and dams treated with dexamethasone at D50 or D25 in late pregnancy, and their offspring

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>D50</th>
<th>D25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy weight gain, g</td>
<td>148±1</td>
<td>102±7*</td>
<td>102±13*</td>
</tr>
<tr>
<td>Number of pups per litter</td>
<td>12±1</td>
<td>11±2</td>
<td>12±1</td>
</tr>
<tr>
<td>Birthweight, g</td>
<td>6.22±0.15</td>
<td>5.24±0.21*</td>
<td>5.37±0.18*</td>
</tr>
<tr>
<td>Gestation length, d</td>
<td>24.3±0.3</td>
<td>24.2±0.2</td>
<td>24.0±0.3</td>
</tr>
<tr>
<td>Food intake, days 1–14, g</td>
<td>251±8</td>
<td>248±9</td>
<td>248±13</td>
</tr>
<tr>
<td>Food intake, days 15–22, g</td>
<td>144±4</td>
<td>120±5*</td>
<td>120±8*</td>
</tr>
<tr>
<td>Weaning weight, g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>67±2</td>
<td>51±3*</td>
<td>55±2*</td>
</tr>
<tr>
<td>Females</td>
<td>58±3</td>
<td>54±2</td>
<td>51±1*</td>
</tr>
<tr>
<td>Weight at study, g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males, 21 wk</td>
<td>446±8</td>
<td>380±9*</td>
<td>427±8†</td>
</tr>
<tr>
<td>Females, 21 wk</td>
<td>277±6</td>
<td>260±9</td>
<td>264±8</td>
</tr>
<tr>
<td>Kidney wt at study, g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>3.03±0.07</td>
<td>3.03±0.08</td>
<td>2.95±0.08</td>
</tr>
<tr>
<td>Females</td>
<td>1.86±0.08</td>
<td>2.14±0.09</td>
<td>1.94±0.08</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE, n = 7 control litters, n = 5 D50 litters, n = 7 D25 litters. Numbers of male and female offspring are the same as indicated in Figure 3. *P < 0.05 compared to control. †P < 0.05 compared to D50. D50, 50 dexamethasone μg·kg⁻¹·day⁻¹; D25, 25 dexamethasone μg·kg⁻¹·day⁻¹.
been higher than necessary to produce physiological/patho-
physiological fetal levels of glucocorticoids. Maternal weight
increase was frequently impaired (2, 22), and maternal health may
have been significantly compromised (11). Although one study
did report that carbenoxolone treatment failed to reduce ma-
ternal weight gain or food intake while still programming for
offspring hypertension (16), the average value of mean mater-
al food intake in rats treated with carbenoxolone throughout
or during the latter portion of pregnancy in that study was more
than 10% lower than controls, even though this did not reach
statistical significance. In our laboratory, pregnant rats injected
with the same dose of carbenoxolone used by others (16, 22)
also showed a reduction in maternal weight gain (unpublished
observations), thus concurring with the findings of Lindsay et
al. (22).

The results of the present study and those of our previous
study (39) indicate that the effects of maternal dexamethasone

reduced in placentas of protein-restricted rat dams that also
produce growth-restricted and hypertensive offspring (18).
Second, it is known that administration of dexamethasone (a
synthetic glucocorticoid that is not inactivated by the placental
enzyme) to pregnant rats either throughout or in the latter part
of pregnancy leads to offspring of reduced birth weight that
come hypertensive later in life (2, 21). Third, pharmacolog-
ical blockade of maternal glucocorticoid synthesis with me-
tyrapone has also been reported to prevent programming of
hypertension by maternal protein restriction (15). However,
interpretation of this finding is complicated by the time win-
dow during which metyrapone was given (early gestation).
Several laboratories, including our own, have shown that the
window of sensitivity to maternal protein restriction or glu-
cocorticoid administration in programming offspring blood
pressure in the rat is in late gestation (28, 32, 39, 41). Me-
tyrapone also had effects on food intake, offspring growth, and
male offspring blood pressures that were not prevented by
administration of corticosterone, further confounding inter-
pretation of this study. Finally, administration of carbenoxolone,
an 11β-HSD inhibitor, to normal pregnant mothers causes
hypertension in the offspring (16, 22).

An important concern with many previous studies is that the
doses of dexamethasone and carbenoxolone used may have

Table 2. Physiological variables and organ weights in
adult offspring of control dams and dams treated with D50
or D25 in late pregnancy

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>D50</th>
<th>D25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney/body wt, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>0.688±0.020</td>
<td>0.768±0.024*</td>
<td>0.683±0.022†</td>
</tr>
<tr>
<td>Females</td>
<td>0.684±0.020</td>
<td>0.813±0.024*</td>
<td>0.746±0.022</td>
</tr>
<tr>
<td>GFR/KW, ml·min⁻¹·g⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>1.13±0.05</td>
<td>1.07±0.06</td>
<td>1.07±0.06</td>
</tr>
<tr>
<td>Females</td>
<td>1.17±0.05</td>
<td>1.10±0.06</td>
<td>1.03±0.05</td>
</tr>
<tr>
<td>ERPF/BW, ml·min⁻¹·100 g⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>3.41±0.14</td>
<td>3.50±0.19</td>
<td>3.16±0.19</td>
</tr>
<tr>
<td>Females</td>
<td>3.62±0.15</td>
<td>3.46±0.18</td>
<td>3.04±0.17*</td>
</tr>
<tr>
<td>GFR/BW, ml·min⁻¹·100 g⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>0.766±0.025</td>
<td>0.830±0.035</td>
<td>0.734±0.030</td>
</tr>
<tr>
<td>Females</td>
<td>0.787±0.026</td>
<td>0.896±0.032*</td>
<td>0.781±0.030†</td>
</tr>
<tr>
<td>Adrenal weight, g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>0.060±0.002</td>
<td>0.059±0.003</td>
<td>0.060±0.003</td>
</tr>
<tr>
<td>Females</td>
<td>0.063±0.002</td>
<td>0.069±0.003</td>
<td>0.066±0.003</td>
</tr>
<tr>
<td>Adrenal/body weight, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>0.014±0.001</td>
<td>0.015±0.001*</td>
<td>0.014±0.001</td>
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<tr>
<td>Females</td>
<td>0.023±0.001</td>
<td>0.026±0.001</td>
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</tr>
<tr>
<td>Heart weight, g</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Males</td>
<td>1.517±0.030</td>
<td>1.378±0.034*</td>
<td>1.497±0.034†</td>
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<tr>
<td>Females</td>
<td>1.042±0.031</td>
<td>1.086±0.038</td>
<td>1.039±0.036</td>
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<tr>
<td>Heart/body weight, %</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Males</td>
<td>0.344±0.018</td>
<td>0.320±0.020</td>
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<tr>
<td>Females</td>
<td>0.383±0.019</td>
<td>0.376±0.023</td>
<td>0.398±0.021</td>
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</tbody>
</table>

Values are expressed as means ± SE. Numbers of male and female offspring are the same as indicated in Fig. 3. *P < 0.05 compared to control. †P < 0.05 compared to D50.
on maternal food intake and weight gain, as well as on offspring blood pressure, are dose related. At 100 μg·kg⁻¹·day⁻¹, all three variables were significantly affected (39). At 50 or 25 μg·kg⁻¹·day⁻¹, food intake and weight gain were still significantly reduced, albeit by only half as much as with the 100 μg·kg⁻¹·day⁻¹ dose. However, at the lower doses, offspring blood pressure was no longer significantly increased. Importantly, as the dose was reduced, the effects on the blood pressure of the offspring disappeared before the effects on food intake and weight gain disappeared. Because the threshold dose for effects on maternal nutrition and growth (somewhere below 25 μg·kg⁻¹·day⁻¹) is lower than the minimum dose required to program hypertension (between 50 and 100 μg·kg⁻¹·day⁻¹), it appears likely that hypertension in rat offspring is only programmed by 6 days of dexamethasone treatment in late pregnancy when reduced maternal food intake and weight gain are also present. These findings strongly support the idea that dexamethasone administered in this way programs offspring blood pressure, at least in part, through an indirect mechanism, likely involving a reduced maternal food intake. It also appears that a relatively severe effect on maternal food intake and weight gain is required to program the offspring for hypertension; in the present study, food intake was reduced by 17% and weight gain by 31% with no effect on offspring blood pressure.

In our previous study, when we used dexamethasone at 100 μg·kg⁻¹·day⁻¹, food intake was reduced by 30% and weight gain by 60%, and both male and female offspring were then programmed for hypertension (mean arterial pressure ~10 mmHg above control). This is consistent with other models of hypertension induced by global food restriction in the mother, in which food intake has been reduced by 30–70% (30, 34). The present findings that lower doses of dexamethasone do not cause increased blood pressure in the offspring are also consistent with a study in the sheep, in which maternal administration of low-dose dexamethasone (20 μg·kg⁻¹·day⁻¹) failed to program for postnatal hypertension (26). In that study, maternal food intake and weight gain were not reported. Fetal weight was significantly reduced by 22% at 130 days gestation. Body weights in dexamethasone-exposed lambs at 45 days gestation, birth, and 2 mo of postnatal age were not significantly different from controls; however, the mean values were 7–13% lower, further suggesting a trend toward reduced growth. Thus it appears that in the sheep, as well as in the rat, the dose of dexamethasone required to program offspring hypertension is greater than the minimum dose that can impair maternal and/or fetal weight gains. Interestingly, a considerably larger dose of dexamethasone (~240 μg·kg⁻¹·day⁻¹) infused into pregnant sheep on days 26 and 27 of gestation did not significantly alter the weights of fetuses at 130 days gestation (27). Using a similar dose regimen, Dodic et al. (10) showed that birth weight was not significantly different and reported that weights at 100, 300, and 560 days of postnatal age were “similar” to controls (data not given). Maternal weights were not measured in either study, and offspring weights were only available at and beyond 130 days gestation, so differences in maternal intake and weight gain, or transient differences in early fetal growth, cannot be ruled out. However, these studies do suggest that a very short exposure to glucocorticoids in sheep pregnancy may program hypertension without affecting offspring growth.

The stage of gestation at which we chose to administer dexamethasone in the present study was based on our previous findings that the window of sensitivity of offspring blood pressure to prenatal dexamethasone exposure or maternal protein restriction was the latter part of gestation; exposure during early pregnancy in the rat failed to program for offspring hypertension (39, 41). The fact that this critical period coincides with the prenatal window of nephrogenesis in the rat is consistent with the idea that permanent alterations in the kidney may play an important role in this programming. Indeed, dexamethasone has been shown to reduce the number of nephrons in offspring of mothers treated during pregnancy (7). As indicated above, in the sheep, short-term administration of dexamethasone very early in pregnancy programs the offspring for hypertension in adulthood (10). Because the window of nephrogenesis occurs much earlier in gestation in this species, this finding is also consistent with an important role for the kidney in this programming.

It must be acknowledged that the present data are limited to application in a model in which large doses of steroid are given over approximately the last third of gestation. We chose to give dexamethasone for 6 days in the present study because that was the duration of administration used in the original study reporting that glucocorticoid exposure in utero programmed adult hypertension (2). Although our present and previous work indicate that dexamethasone administration of this duration at this stage of gestation in the rat likely programs offspring hypertension indirectly, through its effects on maternal food intake, it is possible that a shorter exposure to elevated maternal glucocorticoid levels could program hypertension without significant effects on maternal nutrition, as indicated above (10). Even in the absence of maternal food intake and weight gain data, the fact that only a 2-day exposure is necessary to cause the programming in the sheep makes it seem less likely that the effect of a corticosteroid in this species is due purely to its effects on maternal food intake. Thus glucocorticoids might play a more direct role in programming hypertension in other models. Although a 2-day exposure to maternal dexamethasone (200 μg·kg⁻¹·day⁻¹) in late pregnancy has also been reported to cause hypertension in the rat (28, 29), this represents a much greater proportion of pregnancy in the rat, with a gestation length of ~23 days, than in the sheep with a gestation of ~145–150 days. Again, maternal food intake and weight gain were not reported in these rat studies, so a possible contribution of maternal nutritional factors to this programming cannot be assessed. Birth weights were not significantly reduced; however, nutritional programming has been shown to occur even in the absence of measurable intrauterine growth retardation (17). On the basis of our previous study, it seems likely that a dose of 200 μg·kg⁻¹·day⁻¹ (twice the maximum dose we used) would indeed cause a reduction in maternal food intake and weight gain at least during the period of administration and possibly for some period beyond that (39).

It is possible that the effects of reduced maternal food intake and weight gain cannot be separated from the effects of increased maternal glucocorticoids in programming for offspring hypertension. The present study suggests that, at least under these experimental conditions (species, duration of treatment, stage of gestation), the threshold for effects on maternal nutrition and growth is lower than the threshold for programming of hypertension. Thus it is unlikely that administered
glucocorticoid programs independently of its effects on maternal nutrition. Although we have shown previously that reduced maternal food intake programs independently of administered glucocorticoid, we did not measure endogenous glucocorticoids in that study. Thus the possibility remains that reduced maternal food intake programs hypertension by altering endogenous maternal glucocorticoid levels. More studies will be needed to determine whether the effects of these two stimuli can be separated.

Emerging evidence from our laboratory and others indicates that there are a number of gender differences in fetal programming. Usually, but not always, females tend to be relatively protected from prenatal insults compared with males (13, 30, 37). For example, we have shown that modest maternal protein restriction programs male, but not female, offspring for hypertension; a more severe protein restriction programs both male and female offspring for increased adult blood pressures (36, 37, 41). We found a similar trend in the present study: although males and females generally exhibited similar responses to maternal dexamethasone, effects tended to be more profound in the males. The mechanisms responsible for this relative protection in females are presently unknown, but likely involve the sex steroids, with androgens promoting renal dysfunction and hypertension and/or estrogens providing some protective value.

In summary, the data from our laboratory call into question the conclusions from some previous rat studies in which maternal food intakes and weight gains were either not measured or the observed reductions not addressed. In the sheep, some protocols have yielded at least some indications of impaired fetal growth. Others have not noted any effect on offspring growth, but growth has not been assessed at early ages, and maternal variables have not been measured at all. The present study does not prove that glucocorticoids program hypertension solely through indirect mechanisms, but it does suggest that the possibility needs to be considered. More studies are needed to determine the precise mechanisms by which maternally derived glucocorticoids program for offspring hypertension, as well as the extent to which species differences in these mechanisms may exist. In particular, it will be important to quantify maternal food intakes and weight gains, as well as offspring growth indices, in future studies.

Perspectives

In humans, babies that are born smaller have a higher risk for developing hypertension later in life than do larger babies. This relationship holds across the normal range of birthweights and is not related to prematurity. Brenner and colleagues (3–5, 24) have postulated that essential hypertension in humans may be due to a reduced nephron endowment at birth, and this idea is supported by a recent report indicating that patients with primary hypertension have fewer nephrons than normotensive patients (12). Indeed, we have shown in rats that a surgically reduced nephron number from birth (uninephrectomy) causes hypertension (35, 40). The physiological mechanisms leading to a reduced nephron endowment in humans are not well understood, but animal studies by our laboratory and others indicate that fetal undernutrition and a suppressed fetal/newborn intrarenal renin-angiotensin system are likely important factors (19, 32, 36). Other laboratories have proposed that exposure of the fetus to excess maternal glucocorticoid programs the offspring for hypertension, but our previous work and the present study do not support a direct role for maternal corticosteroids in programming for hypertension. However, this study does not rule out the possibility that maternal corticosteroids may participate directly in programming for other disease processes.

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