Maternal glucocorticoid treatment programs HPA regulation in adult offspring: sex-specific effects

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Maternal glucocorticoid treatment programs HPA regulation in adult offspring: sex-specific effects. Am J Physiol Endocrinol Metab 280: E729–E739, 2001.—Pregnant guinea pigs were treated with dexamethasone (1 mg/kg) or vehicle on days 40–41, days 50–51, and days 60–61 of gestation. Adult offspring were split into two groups. Group 1 guinea pigs were catheterized, and the hypothalamo-pituitary-adrenal (HPA) axis was tested in basal and activated states. Group 2 guinea pigs were euthanized with no further manipulation. In male offspring, prenatal dexamethasone exposure resulted in a significant reduction in brain-to-body weight ratio. Dexamethasone-exposed male offspring exhibited reduced basal and activated plasma cortisol levels, which was associated with elevated hippocampal mineralocorticoid receptor (MR) mRNA and increased plasma testosterone. In females exposed to glucocorticoids in utero, basal and stimulated plasma cortisol levels were higher in the follicular and early luteal phases of the cycle, but this effect was reversed in the late luteal phase, indicating a significant interaction of sex steroids. In female offspring (at estrus), glucocorticoid receptor mRNA levels were lower in the paraventricular nucleus and pars distalis but higher in the hippocampus in animals exposed to dexamethasone in utero. Hippocampal MR mRNA levels were significantly lower (~50%) than in controls. In conclusion, repeated antenatal glucocorticoid treatment programs HPA function in a sex-specific manner, and these changes are associated with modification of corticosteroid receptor expression in the adult brain and pituitary.

dexamethasone; glucocorticoid receptor; mineralocorticoid receptor; limbic system; hypothalamo-pituitary-adrenal axis; programming

IN NORTH AMERICA AND EUROPE, ~10% of pregnant women are treated with synthetic glucocorticoids between 24 and 34 wk of gestation (term is ~40 wk). This promotes fetal lung maturation in fetuses at risk of being delivered prematurely (26). Although such treatment has been shown to be effective in preventing a number of pulmonary complications, very little is known about long-term impact on the brain, particularly in species that give birth to neuroanatomically mature young. This information is critical, because repeated antenatal glucocorticoid treatment has become common clinical practice (29). Development of the fetal hypothalamic-pituitary-adrenal (HPA) axis is critical for normal growth and organ development (6). However, studies have indicated that HPA maturation and its subsequent function can be permanently programmed during fetal life. This has very significant consequences on adult health, as chronic elevation of plasma glucocorticoid concentrations has been shown to predispose to neurological, metabolic, and cardiovascular disease (1, 25, 34). Conversely, a chronic reduction in HPA activity can protect against a number of pathologies associated with aging.

Fetal exposure to synthetic glucocorticoids produces adult male rats that exhibit elevated basal plasma corticosterone concentrations and hypertension (18). These adult male offspring express decreased levels of glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) mRNA in the hippocampus and dentate gyrus (18). To our knowledge, no studies have investigated the impact of prenatal glucocorticoid exposure on HPA function in adult female offspring. Studies in rats have yielded important information; however, this species gives birth to immature young, in which much of neuroendocrine maturation occurs postnatally (28). A single study has investigated pituitary-adrenocortical function in prepubertal rhesus monkeys that had been exposed to glucocorticoids in utero. These offspring (10 mo) also exhibited elevated basal and stress-stimulated cortisol concentrations (30). No studies have assessed HPA function in school-age children or young adults who were exposed to antenatal glucocorticoids during fetal life.

Guinea pigs, unlike rats, give birth to mature young, and peak brain growth occurs around days 50–52 of gestation (75%; birth = 68 days) (11, 12). We have shown that extensive neuroendocrine maturation occurs during late fetal life in this species (22), and this situation is similar to that occurring in primates (8, 12). We have also reported very rapid neuron-specific changes in GR and MR populations in late gestation in the guinea pig (22). Recent studies in our laboratory have indicated that this development can be acutely modified by glucocorticoid exposure in utero and that...
this effect is sex specific (9). In the present study, we examine the hypothesis that repeated fetal glucocorticoid exposure during the phase of maximal brain growth permanently programs HPA function in adult life in a sex-specific manner. We predict that this involves long-term modification of central corticosteroid receptor systems and glucocorticoid negative feedback.

MATERIALS AND METHODS

Animals and Treatment

Female guinea pigs were mated in our animal facility as described previously (9). This method produces accurately timedate pregnant guinea pigs. Animals were maintained on a 12:12-h light-dark cycle for the duration of the experiments (lights on 0700–1900). Studies were performed according to protocols approved by the Animal Care Committee at the University of Toronto, in accordance with the Canadian Council for Animal Care. Pregnant guinea pigs were subcutaneously injected with dexamethasone (1 mg/kg; n = 13) or vehicle [saline (70%)-propylene glycol (30%), 200 µl; n = 12; control] at 0800 on days 40–41, 50–51, and 60–61 of gestation (term = 68 days). After treatment, guinea pigs were left undisturbed and allowed to deliver naturally. At birth, gestation length and the weight and sex of all neonates were recorded. Young guinea pigs were weaned at 30 days of age, placed into one of two study groups, and housed separately. Body weight was measured at 10-day intervals from birth to adulthood (Table 1).

Experiments in Adult Offspring

Group 1. Adult offspring [control males (CM); control females (CF); dexamethasone-exposed males (DM); dexamethasone-exposed females (DF); n = 6–10/group] were surgically implanted with indwelling jugular and carotid catheters at ∼day 65, as described previously (21). A small jacket and a spring were then fitted to the guinea pig, and the catheters were passed up through the spring and attached to a Teflon swivel (Lomir Biomedical, Notre-Dame, QC, Canada). This allowed full rotation of the catheter and unrestricted movement of the guinea pig. Catheters were filled with heparinized saline and were flushed daily. We have previously demonstrated that repeated sampling with this approach does not activate the HPA axis (21). Care was taken to ensure that the female guinea pigs were in the estrous (day 0) phase of the reproductive cycle on the day of surgery. This was established by identification of a fully ruptured vaginal membrane, as described previously (9). The reproductive cycle of the guinea pig is 14–16 days in our colony. After 4 days of recovery from surgery, five separate tests of HPA function were performed. Tests were administered in the same order, because previous studies in the female rat have indicated that there is an interaction of the reproductive cycle with regulation of the HPA axis (4), and we wished to preclude this as a variable in the present study. In test 1, blood samples (250 µl) were collected at 0900, 1300, and 1600 to determine basal plasma ACTH and cortisol concentrations. In test 2, blood samples (150 µl) were drawn before and after (∼30, 0, 5, 15, 30, 60, 90, and 120 min) injection of human corticotropin-releasing hormone (hCRH; 0.5 µg/kg). In test 3, human ACTH (0.5 µg/kg) was administered, and animals were sampled as described for test 2. In test 4, animals were exposed to restraint stress in a clear Perspex tube for 30 min and then returned to their home cage. In test 5, animals were fasted overnight and injected with insulin (5 U/kg) at 0900 to induce hypoglycemia. Blood samples were drawn at ∼30, 0, 5, 15, 30, 60, 90, 120, and 240 min; animals were then provisioned with 3 g of food (240 min), and an additional sample was taken 1 h later (300 min). Plasma glucose was monitored at each blood sample (hypoglycemia test) using a blood glucose meter (One Touch, Lifescan, Milpitas, CA). All hormonal measurements were administered via the jugular vein and were commenced between 1300 and 1400 except for test 5 (see above). All blood samples were taken via the carotid artery. An equal volume of sterile saline was infused after each of the samples was withdrawn. Treatments were administered after the 0-min sample. At least 48 h of recovery were allowed between treatments. Blood samples were centrifuged, and plasma was separated and stored at −20°C until analysis. ACTH (1–24) and hCRH were purchased from Peninsula Laboratories (Belmont, CA), and insulin (Iletin II) came from Eli Lilly (Toronto, ON, Canada).

Mean arterial pressure was measured via the carotid artery catheter in male and female offspring. A small displacement pressure transducer was connected to a MacLab/4e data acquisition system (AD Instruments, Castle Hill, Australia) and a PowerMac Macintosh computer driven by MacLab Chart 3.5.6 software. Blood pressure was measured for 5 min between 1200 and 1300, and approximately three determinations were made on separate days for each animal. For female offspring, measurements were confined to the time of tests 1 and 5 (late follicular phase/early luteal phase) to exclude the possibility of interaction between stage of the reproductive cycle and blood pressure regulation.

Group 2. Adult offspring (CM, CF, DM, DF; n = 6–8/group) were euthanized by rapid decapitation (between 9 and 11 AM), with no further manipulation, at ∼80 days of life. Brains, pituitaries, and major organs (see Table 2) were removed and weighed. Brains and pituitaries were rapidly frozen on dry ice and stored at −80°C until molecular analysis. Care was taken to ensure that the female guinea pigs were euthanized in estrus (day 0) to prevent any confounding influences of the reproductive cycle on changes associated with glucocorticoid exposure in utero.

Plasma Analysis

Plasma ACTH, cortisol, and thyroxine (T4) concentrations were measured by radioimmunoassay as described previously (9, 22). For cortisol analysis, all samples from within each test were run in the same assay to negate interassay bias. Plasma ACTH was only measured in the basal state (test 1). Plasma triiodothyronine (T3), progesterone, and testosterone were measured using established radioimmunoas-
Table 2. Effect of maternal dexamethasone treatment on adult organ-to-body weight ratios

<table>
<thead>
<tr>
<th>Organ</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Dex</td>
</tr>
<tr>
<td>Adrenal (L) ×10</td>
<td>2.03 ± 0.12</td>
<td>2.00 ± 0.07</td>
</tr>
<tr>
<td>Adrenal (R) ×10</td>
<td>1.94 ± 0.18</td>
<td>1.78 ± 0.12</td>
</tr>
<tr>
<td>Brain</td>
<td>6.83 ± 0.14</td>
<td>5.92 ± 0.02*</td>
</tr>
<tr>
<td>Gonad</td>
<td>5.12 ± 0.39</td>
<td>5.59 ± 0.31</td>
</tr>
<tr>
<td>Heart</td>
<td>2.98 ± 0.15</td>
<td>2.98 ± 0.18</td>
</tr>
<tr>
<td>Kidney (L)</td>
<td>3.49 ± 0.14</td>
<td>3.36 ± 0.08</td>
</tr>
<tr>
<td>Kidney (R)</td>
<td>3.48 ± 0.12</td>
<td>3.30 ± 0.07</td>
</tr>
<tr>
<td>Liver</td>
<td>33.00 ± 0.87</td>
<td>32.89 ± 1.19</td>
</tr>
<tr>
<td>Lung</td>
<td>4.67 ± 0.11</td>
<td>4.55 ± 0.14</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.97 ± 0.01</td>
<td>0.93 ± 0.05</td>
</tr>
<tr>
<td>Thymus</td>
<td>1.14 ± 0.11</td>
<td>1.07 ± 0.09</td>
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Values are means ± SE. L, left; R, right. Dex treatment was 1 mg/kg on days 40–41, 50–51, and 60–61 of gestation. *P < 0.05 vs. control.

In Situ Hybridization

The method for in situ hybridization has been described in detail previously (22). Coronal cryosections (12 μm) were mounted onto (poly)-l-lysine (Sigma Chemical, St. Louis, MO)-coated slides, dried, and postfixed with paraformaldehyde (4%). The antisense GR, MR, and CRH oligonucleotide probes were labeled using terminal deoxynucleotidyltransferase (GIBCO, Burlington, ON, Canada) and [35S]deoxyadenosine 5'-[α-thio]triphosphate (1,300 Ci/mmol, NEN, Du Pont Canada, Mississauga, ON, Canada) to a specific activity of 1.0 × 10⁶ cpm/μl. Labeled probes in hybridization buffer (200 μl) were applied to slides at a concentration of 1.0 × 10⁸ cpm/μl. Slides were incubated overnight in a moist chamber at 42°C. After washing in 1× standard sodium citrate (SSC; 30 min at room temperature), and 1× SSC (30 min at 55°C), the slides were rinsed once with 1× SSC and with 0.1× SSC at room temperature, dehydrated in ethanol, and then dried and exposed to autoradiographic film (Biomax, Kodak). The films were developed using standard procedures (exposure: GR 28 days; MR 21 days; CRH 42 days).

Statistical Analysis

Group data are presented as means ± SE. Data were statistically analyzed using multivariate ANOVA, followed by Duncan’s method of post hoc comparison. Gestation length and birth weight were analyzed by one-way (treatment) ANOVA. Initial analysis of basal plasma cortisol concentrations from both male and female treatment groups by threeway (sex × treatment × time) repeated-measures ANOVA revealed a significant (P < 0.02) sex effect and a significant (P < 0.007) interaction between sex and treatment. For this reason, male and female data were considered separately for all further analyses. Organ-to-body weight ratios, plasma free cortisol, testosterone, progesterone, T₄, and T₃ levels were analyzed by one-way (treatment) ANOVA. Body weight and plasma ACTH and cortisol concentrations were analyzed by two-way (treatment × time) repeated-measures ANOVA. In situ hybridization data were also measured by two-way (treatment × region) ANOVA. Statistical significance was set at P < 0.05.

RESULTS

Birth, Growth, and Blood Pressure

Dexamethasone treatment in late gestation had no effect on litter size (control, 3.3 ± 0.2; dexamethasone,
3.2 ± 0.3; not illustrated). There was no significant difference in birth weight, although there was a strong tendency toward an increase in body weight in the dexamethasone-exposed neonates (Table 1). There was a significant (P < 0.05) increase in gestation length in the pregnant animals that had been injected with dexamethasone (control, 69.1 ± 0.4 days; dexamethasone, 70.6 ± 0.4 days; not illustrated). There was no significant difference in body weight gain over the first 60 days of life between the control and dexamethasone-exposed offspring (Table 1). Dexamethasone exposure in utero resulted in adult female offspring with increased adrenal-to-body weight ratio (AW/BW). Interestingly, this effect was confined to the left adrenal (Table 2). There was no difference in adrenal weights in male offspring. However, in male offspring, prenatal glucocorticoid exposure resulted in a significant (P < 0.005) decrease (>10%) in brain-to-body weight ratio (BrW/BW). There was no effect of prenatal treatment on mean arterial pressure in either male (control, 48.7 ± 2.1 mmHg; dexamethasone, 52.4 ± 2.2 mmHg) or female offspring (control, 47.9 ± 1.4 mmHg; dexamethasone, 49.5 ± 4.3 mmHg, not illustrated).

Plasma Hormone Concentrations: Basal

Prenatal dexamethasone exposure resulted in male offspring with significantly reduced basal plasma cortisol levels at 0900 (P < 0.01) and 1300 (P < 0.05) but not at 1600 (Fig. 1C). Although there were similar trends in basal plasma ACTH concentrations, these failed to attain significance (Fig. 1A). In females, basal testing was undertaken 5.3 ± 0.4 days (control) and 5.7 ± 0.6 days (dexamethasone exposed) after surgery. In contrast to males, female offspring that had been exposed to dexamethasone in utero demonstrated significantly (P < 0.02) elevated basal plasma cortisol when all time points were considered (Fig. 1D). However, this effect was most pronounced (P < 0.005) at 1300. Basal ACTH levels showed a similar trend to cortisol overall, but the effect of dexamethasone exposure was significant (P < 0.05) only at 1600 (Fig. 1B).

There were no differences in percentage of free cortisol between dexamethasone and control groups (Fig. 2A). However, basal plasma testosterone concentrations were twofold increased (P < 0.01; control 2.0 ± 0.5 ng/ml; dexamethasone 3.9 ± 0.5 ng/ml) in male
offspring that had been exposed to dexamethasone in utero (Fig. 2B). Testosterone levels in female guinea pigs were below the detection limit of the assay. There were no significant differences in T₄ (CM 37.1 ± 2.6 ng/ml; DM 42.5 ± 3.8 ng/ml; CF 38.0 ± 3.8 ng/ml; DF 43.0 ± 5.2 ng/ml; not illustrated) or T₃ (CM 256.3 ± 85.9 pg/ml; DM 203.5 ± 36.5 pg/ml; CF 146.3 ± 41.9 pg/ml; DF 158.6 ± 47.7 pg/ml; not illustrated) concentrations among any of the treatment groups. Plasma progesterone levels were not significantly different at the time of euthanasia (CF, 1.1 ± 0.4 ng/ml; DF, 1.4 ± 0.4 ng/ml; not illustrated) in group 2 females.

**Plasma Hormone Concentrations: Stimulated**

Adrenocortical responses to CRH and ACTH challenge, restraint stress, and insulin-induced hypoglycemia are shown in Fig. 3. Pretreatment (basal) plasma cortisol concentrations are presented along with net responses to each challenge (i.e., pretreatment values subtracted). For the purposes of analysis, the entire response (5–120 min) was considered initially. The response was subsequently split into an increasing (5-min to peak) and recovery (120-min to peak) phase. Finally, direct comparisons were made at the individual time points. CRH challenge (0.5 μg/kg) resulted in significant increases in plasma cortisol in all treatment groups (CM *P* < 0.01; DM *P* < 0.05, CF *P* < 0.02; DF *P* < 0.002). In DM, there was a strong tendency for reduced cortisol in the pretreatment period (Fig. 3A), and when all pretreatment periods (from all tests) were considered together, this effect became highly significant (*P* < 0.001). The net cortisol response to CRH was
similar in both male groups, with peak cortisol occurring at 60 min. In females, CRH challenge was administered 7.1 ± 0.7 days (CF) and 7.4 ± 0.5 days (DF) after surgery. There was a strong tendency for increased cortisol in the pretreatment period, although this failed to attain significance at this stage of the reproductive cycle (Fig. 3B). The initial net cortisol response to CRH challenge was similar between the DF and CF groups. However, in the CF group, cortisol peaked at 60 min, whereas in the DF group cortisol did not peak until 90 min after injection.

ACTH challenge (0.5 μg/kg) resulted in significant increases in plasma cortisol in all treatment groups (CM P < 0.0005; DM P < 0.001; CF P < 0.002; DF P < 0.01). In male offspring, plasma cortisol levels were significantly (P < 0.05) lower in DM animals in the pretreatment period (Fig. 3C). The net change in cortisol concentrations was significantly (P < 0.05) lower 5 min after ACTH injection, indicating a slower response to the challenge. Plasma cortisol levels took significantly (P < 0.04; 60–120 min) longer to return to baseline in the DM group compared with the CM group. This effect was most pronounced at 90 (P < 0.05) and 120 min (P < 0.02). In females, ACTH challenge was administered 9.9 ± 0.7 days (CF) and 9.9 ± 0.4 days (DF) after surgery. At this stage of the reproductive cycle, there was a striking change in the pattern of pretreatment cortisol levels, such that plasma cortisol concentrations were significantly (P < 0.05) lower in the DF animals compared with controls (Fig. 3D). When the net response to challenge was considered, there were no significant differences between CF and DF, although there was a tendency for a lower peak response in the DF group.

Restraint resulted in significant increases in plasma cortisol in all treatment groups (CM P < 0.04; DM P < 0.007; CF P < 0.04; DF P < 0.02). In DM, there was a strong tendency for reduced cortisol in the pretreatment period. When net responses were considered at 5 min, it was clear that the DM animals responded significantly (P < 0.02) more slowly to the restraint stress than controls (Fig. 3E). This was similar to the situation after ACTH challenge. Subsequently, there were no significant differences in the net response profile. In females, restraint stress was undertaken 12.1 ± 0.7 days (CF) and 12.7 ± 0.5 days (DF) after surgery. There was no difference in cortisol concentrations during the pretreatment period, and the cortisol response to restraint was similar in both groups, with peak cortisol levels occurring at 30 min (Fig. 3F).

Insulin-induced hypoglycemia resulted in significant increases in plasma cortisol in all treatment groups (CM P < 0.005; DM P < 0.005; CF P < 0.001; DF P < 0.002). Animals had been fasted overnight before the challenge, and so cortisol concentrations were elevated above basal values in the pretreatment period. In males, cortisol levels appeared to be lower in the DM animals, although this effect failed to achieve significance (Fig. 3G). Plasma cortisol concentrations increased up to 240 min after the induction of hypoglycemia, at which point animals were given 5 g of feed. There was a rapid decline in cortisol concentrations 60 min after feeding (300-min sample) in the DM group, although levels failed to decrease in controls. There was no difference in net responses between groups. In females, insulin was administered 14.1 ± 0.7 days (CF) and 15.5 ± 0.6 days (DF) after surgery. There was no difference in cortisol concentrations during the pretreatment period, although concentrations were elevated above basal values due to fasting (Fig. 3H). In DF offspring, plasma cortisol levels increased immediately (5 min) after insulin administration, whereas levels did not increase until 30 min in the CF animals. Comparison of the net responses indicated a significantly (P < 0.01) greater cortisol response of the dexamethasone-exposed animals to hypoglycemia (5–240 min). This difference was particularly prominent at 5 (P = 0.055) and 30 min (P < 0.05; Fig. 3H).

In males, fasting blood glucose concentrations in the pretreatment period tended to be lower in the DM group (4.7 ± 0.3 nmol/l; not illustrated) than in the control (5.8 ± 0.5 nmol/l) offspring, although this effect just failed to attain significance (P = 0.08). Injection of insulin resulted in a rapid decrease in glucose in both groups. At 15 min, glucose levels dropped to ~50% of the pretreatment value. At this point blood glucose was significantly (P < 0.04) lower in the DM group (2.4 ± 0.1 nmol/l) compared with controls (3.2 ± 0.3 nmol/l). Levels decreased to 120 min (CM 1.7 ± 0.2 nmol/l; DM 1.5 ± 0.1 nmol/l) and then began to rise. There was a rapid increase in blood glucose after food ingestion in both groups, although blood glucose remained significantly (P < 0.05) lower in the DM group (4.7 ± 0.8 nmol/l) compared with controls (7.5 ± 0.6 nmol/l). In females, blood glucose showed a similar response to that described for males. Fasting plasma glucose concentrations were not different between female groups (CF 6.0 ± 0.3 nmol/l; DF 5.4 ± 0.3 nmol/l; not illustrated). Plasma glucose levels were lowest at 120 min (CF 1.9 ± 0.1 nmol/l; DM 2.0 ± 0.2 nmol/l) and increased 30 min after the presentation of food (CF 7.2 ± 0.5 nmol/l; DM 7.4 ± 0.7 nmol/l). There was no difference in the response profiles between DF and CF animals.

Hypothalamus and Pituitary

Measurement of CRH mRNA levels in the hypothalamic PVN revealed a very strong tendency (P = 0.07) toward reduced CRH mRNA in males that had been exposed to dexamethasone in utero compared with controls. There was no difference between female groups (Fig. 4). GR mRNA was expressed at high levels in the PVN (Fig. 5) and VMH, although expression was apparent in several other areas. Prenatal glucocorticoid exposure had no effect on GR mRNA levels in the male PVN or VMH (Figs. 5 and 6, A-C). In contrast, in female offspring killed at estrus, GR mRNA levels were lower in total (P < 0.007) and parvocellular regions (P < 0.002) of the PVN in the DF group compared with controls (Fig. 6, A-B). There was also a strong trend for reduced GR mRNA levels in the VMH of DF animals (Fig. 6C). In the pituitary, GR mRNA was expressed.
exclusively in the pars distalis. There was no difference between GR mRNA levels in the male treatment groups (Fig. 6D). However, in female offspring, GR mRNA levels were significantly ($P < 0.01$) lower in animals that had been exposed to dexamethasone in utero.

**Limbic System**

GR and MR mRNA was expressed at high levels in the hippocampus and dentate gyrus (MR; Fig. 7). Highest levels of GR mRNA were observed in the hippocampal CA1/2 region and the dentate gyrus (Fig. 8A and B). In male offspring, there were no differences in GR mRNA between the treatment groups in any of the limbic regions measured. However, in female offspring, GR mRNA levels were significantly ($P < 0.0001$) higher in DF animals when all areas were considered. The effect was most apparent ($P < 0.0005$) in the CA1/2 region of the hippocampus.

In males, MR mRNA levels were significantly ($P < 0.007$) elevated in the DM group when all limbic regions were considered together (Figs. 7 and 8C). Greatest elevation ($P < 0.02$) occurred in the CA1/2 region of the hippocampus. In contrast, prenatal dexamethasone exposure resulted in female offspring with a 50% reduction ($P < 0.0001$) in MR mRNA levels compared with control. This effect was highly significant in all of the regions measured (CA1/2 $P < 0.003$; CA3 $P < 0.02$; dentate gyrus $P < 0.00006$) except for the CA4 region of the hippocampus (Figs. 7 and 8D).

**DISCUSSION**

**Glucocorticoids and Organ-to-Body Growth and Blood Pressure**

Repeated glucocorticoid exposure has no significant effect on fetal growth in the guinea pig. However, daily maternal dexamethasone injections over the last week of gestation result in a reduction in birth weight in rats (18). This discrepancy from the present study likely results from differences in the relative duration of exposure as well as species sensitivity to dexamethasone. A regimen of three courses (2 injections/course) of synthetic glucocorticoid in a 70-day pregnancy (guinea pig) is considerably different from 7 daily injections in a 21-day pregnancy (rat). The former is more analogous to the repeated antenatal glucocorticoid therapy used in cases of suspected preterm labor. In addition, the rat is severalfold more sensitive to dexamethasone than the guinea pig (14, 16). A recent study in the sheep has indicated that a single dose of maternal glucocorticoid can inhibit fetal growth (13). However, the sheep is exquisitely sensitive to glucocorticoids, and under normal circumstances preterm delivery occurs soon after injection of synthetic glucocorticoid (19).

Repeated glucocorticoid treatment leads to a consistent 1.5-day extension of gestation length. The mechanisms by which such an increase occurs remain unclear but may involve glucocorticoid-induced modification of fetal HPA activity or alteration in placental function. Interestingly, an early human study reported a strong tendency toward an increase in gestation length in women treated with betamethasone (20). The doses of dexamethasone administered in the present study are comparable to those prescribed to pregnant women (0.2–0.3 mg/kg) (20, 26), since the affinity of the guinea pig GR is approximately three to four times lower than that of the human (14). In the present study, there was no effect of antenatal glucocorticoid exposure on postnatal growth.

In adult female offspring, the increase in AW/BW (removed in estrus) in the DF animals was consistent with the increase in HPA activity noted in this group.
this time. It is likely that this increase in size results from increased cortical mass, although this remains to be determined. In males, the 10% reduction in BrW/BW in the DM group indicates that brain growth is significantly altered by antenatal glucocorticoid exposure but that this effect is confined to males. It is unclear whether the decrease in brain growth occurs in utero, after antenatal glucocorticoid exposure, or whether it develops during postnatal life. In this connection, exposure of rhesus monkeys to a single course of glucocorticoids in late gestation caused significant damage to pyramidal neurons and resulted in a 30% reduction in hippocampal volume in prepubertal offspring.

There was no significant effect of antenatal glucocorticoid exposure on mean arterial pressure in either male or female offspring. A previous study in the rat has indicated that prenatal glucocorticoid exposure, which leads to elevated plasma corticosterone concentrations, also results in an elevation of systolic and diastolic blood pressure (18). It is unclear whether this effect of prenatal glucocorticoid exposure is mediated by increased vascular exposure to glucocorticoids throughout life or by a direct effect on the developing cardiovascular system. In female offspring that had been exposed to glucocorticoids in utero, we observed elevated basal plasma cortisol concentrations around the time of estrus and in the early luteal phase, but no associated elevation in blood pressure. Although guinea pig offspring in this study were of a similar chronological age to the rats used in the previous study (18), rats attain puberty earlier than guinea pigs. It is therefore possible that, as the guinea pigs age, differences in blood pressure may develop between the prenatal treatment groups.

Glucocorticoids and Altered Endocrine Function: Males

In male guinea pigs, antenatal glucocorticoid exposure results in mature adults that exhibit reduced basal and activated HPA activity. The effect on basal HPA activity appears to be confined to the morning and early afternoon and does not involve alterations in the proportion of free cortisol in the plasma. Interestingly, decreased HPA activity was associated with a strong tendency toward lower fasting blood glucose, as well as
a greater reduction in glucose levels after insulin-induced hypoglycemia. Glucocorticoids are critical in the regulation of blood glucose, particularly during stress (25), and it has been shown in rats that a reduction in plasma glucocorticoid is linked to lower blood glucose (33). The precise interaction between plasma glucocorticoids and glucose homeostasis in adult male offspring that were exposed to synthetic glucocorticoids in utero requires further investigation, but this study indicates that glucose regulation may be different in this group compared with controls. To our knowledge, no clinical studies have considered HPA activity and/or glucose homeostasis in children whose mothers were treated with glucocorticoid in late gestation. In the present study, we found considerable variability in plasma ACTH concentrations, which is consistent with our previous studies (21). However, the trend for a decrease in plasma ACTH concentrations reflected the reduced adrenocortical activity.

The initial cortisol response (after 5 min) to either ACTH or restraint was lower in the DM group compared with controls, indicating that the pituitary-adrenocortical response is delayed in the DM group. However, this effect appears to be stimulus specific, because the rate of adrenocortical response was similar after CRH challenge and insulin-induced hypoglycemia in the DM and CM groups. It is difficult to pinpoint the specific mechanisms that underlie these differences in adrenocortical responsiveness without further focused study of pituitary and adrenal sensitivities in animals exposed to glucocorticoids in utero. However, the delay in adrenocortical recovery (60–120 min) after ACTH challenge likely results from a delay in the initial response and a resultant shifting of the entire response curve to the right. In both CM and DM offspring, the peak response to ACTH occurred earlier than that to CRH, because CRH must first activate the pituitary corticotroph. When all responses in the male are considered, the net responses to challenge are similar, albeit from a lower initial baseline. In rats, an increase in basal but not stimulated HPA activity has been linked to fetal glucocorticoid exposure over the last week of gestation (18). These comparative studies indicate that the treatment regimen and the stage of development at the time of exposure are critical to outcome. Only one study has investigated the long-term impact of fetal environment on neuroendocrine function in the adult guinea pig (3). A single episode of maternal psychological stress (3 h) on day 60 of gestation resulted in adult offspring with reduced basal and stress-activated pituitary-adrenocortical function. The sex of the offspring tested was not reported in that study (3). In guinea pigs, prenatal stress increases transfer of maternal cortisol to the fetus (7). Together, these studies would indicate that antenatal exposure to synthetic glucocorticoids (present study) may have a similar impact to increased exposure to maternal cortisol after prenatal stress (3).

MR mRNA levels in the hippocampus and dentate gyrus were higher in the DM group than in control. Previous studies have linked a reduction in MR mRNA levels with reduced MR binding and an increase in basal HPA activity (4, 10, 18). Specifically, variations (25%) in MR mRNA associated with stage of reproductive and diurnal cycles have been shown to be mirrored by changes in receptor binding in adult rats (4). It is likely that a similar relationship between mRNA and active receptor exists in the adult guinea pig. During basal HPA activity, the hippocampal MR is ~80% occupied, whereas the GR is largely unoccupied, a result of a higher affinity of the hippocampal MR for endogenous glucocorticoid (10). Therefore, the higher
levels in hippocampal MR mRNA observed in the DM group in the present study may be directly responsible for the reduction in basal HPA activity. In contrast to the MR, and because of its lower affinity for endogenous glucocorticoid, the hippocampal GR has been associated with the regulation of activated HPA activity (10). De Kloet et al. (10) have elegantly demonstrated that activation of the hippocampal GR is important in maintaining drive to the parvocellular CRH neuron, and that GR in the PVN and pituitary pars distalis is important in feedback inhibition of activated HPA activity. In the present study, no differences in GR mRNA levels were observed between CM and DM groups, and this may explain why the rates of HPA recovery after challenge were similar in the two groups.

Plasma testosterone concentrations were 100% increased in the DM group. To our knowledge, this is the first time that such a substantial alteration in gonadal function has been observed in adult animals after any prenatal manipulation. The mechanism underlying this elevation is not clear, although it is possible that there was substantial interaction of synthetic glucocorticoid with the developing hypothalamo-pituitary-gonadal axis. In this connection, sexual differentiation of the guinea pig brain occurs in the second half of gestation (27), and fetal plasma testosterone levels are known to be elevated around this time (2). Increased testosterone has been shown to inhibit pituitary-adrenocortical activity in the adult rat (32), and it is likely that the same relationship exists in the guinea pig. Testosterone appears to interact with the HPA axis at the level of parvocellular AVP regulation (32). In the present study, AVP mRNA levels were not measured because of a lack of sequence information. However, there was a strong tendency for a reduction (\( P = 0.07 \)) in CRH mRNA in the PVN in the DM group, and this is consistent with the reduced HPA activity. Because testosterone is central to male behavior, it is likely that males exposed to glucocorticoids in utero will exhibit altered reproductive behavior (i.e., aggression) and associated fertility.

Glucocorticoids and Altered Endocrine Function: Females

The endocrine outcome of repeated antenatal glucocorticoid exposure in the adult female offspring was very different from that in males. In catheterized females (group 1), specific tests of HPA activity were carried out at the same stage of the reproductive cycle in DF and CF animals. It was not feasible to undertake the same HPA challenges at different stages of the reproductive cycle in the present study design. Results of the individual tests indicate that the nature of HPA programming changes as the animal progresses through the reproductive cycle. In the early luteal phase (basal test + CRH challenge), when progesterone levels are low or rising, respectively (5), basal pituitary-adrenocortical activity appeared greater in the DF group compared with controls. Of note, there was a discrepancy between basal plasma ACTH and cortisol concentrations in the afternoon or evening in DF offspring. The mechanisms that underly this diurnal difference in adrenal sensitivity are not clear and, as indicated for males, further focused investigation of adrenocortical function is required in these animals. In the late luteal phase (ACTH challenge + restraint), when progesterone levels were high or decreasing from peak values (5), basal adrenocortical activity was lower (ACTH challenge) or not different (restraint) in the DF group compared with controls. In all cases, the cortisol response to challenge was consistent with the modification of basal function (i.e., there was no difference in net responses). In the hypoglycemia test (animals in the late follicular phase), the net cortisol response to insulin was significantly greater in the DF group than in controls, suggestive of an increased sensitivity of the axis to challenge at this time. To our knowledge, this is the first time that a link between prenatal programming of HPA function and reproductive cycle has been described. A single study has compared the HPA programming effects of prenatal stress in adult male and female rat offspring (24). In this study, adult females that were born to mothers that had been exposed to stress exhibited elevated HPA function, whereas males exhibited lower adrenocortical activity (24).

In the present study, analysis of corticosteroid receptor levels and CRH mRNA was undertaken at estrus. There was a 50% reduction in MR mRNA levels in the hippocampus and dentate gyrus in the DF group compared with controls. There was also a 30% reduction in GR mRNA in the parvocellular PVN and in the pituitary pars distalis. This is consistent with the increased basal HPA activity measured at this time in animals that had been exposed to dexamethasone in utero. In contrast to the hypothalamus and pituitary, GR mRNA levels were higher in the hippocampus and dentate gyrus at this time in the DF group. It has recently been proposed that activation of the hippocampal GR is important in maintaining drive to the parvocellular PVN during periods of stress (10). The increased pituitary-adrenal activity observed in the follicular phase and early luteal phase, particularly after hypoglycemic challenge, would be consistent with this hypothesis. Although it appears that glucocorticoid negative feedback is reduced in this group, it is also possible that prenatal treatment has a long-term effect on adrenal sensitivity. This is currently under investigation. We had not anticipated such a striking interaction between glucocorticoid-induced HPA programming and status of the reproductive cycle. Further experiments in which the same challenge is repeated at different stages of the cycle are required to further investigate this fascinating phenomenon.

In summary, this study provides the first evidence for permanent programming of HPA function by antenatal glucocorticoid exposure in a species in which extensive neuroendocrine development takes place during fetal life. Furthermore, these changes are associated with significant modification in the systems that regulate central glucocorticoid negative feedback.
These alterations will have a major impact on accumulated body exposure to cortisol throughout the animal’s life, and this has been associated with altered susceptibility to a number of pathologies in adulthood (1, 25, 34). Because repeated treatment with synthetic glucocorticoid has become common practice in the clinical management of women presenting in suspected preterm labor, it is critical that we fully understand the potential long-term neuroendocrine and physiological consequences of such therapy.

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REFERENCES


