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Prenatal Exposure to Dexamethasone Alters Leydig Cell Steroidogenic Capacity in Immature and Adult Rats

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ABSTRACT: This study examines the effects of prenatal exposure to dexamethasone (DEX) on postnatal testosterone production in male rats. Pregnant female rats were treated on gestation days 14–19 with DEX (100 μg/kg body weight per day; n = 9) or vehicle (n = 9). Results show that 35-day-old male offspring from DEX-treated pregnant females (n = 42) had decreased levels of serum testosterone (45.6% lower, $P < .05$) compared with control offspring (n = 43), although serum luteinizing hormone (LH) levels were not significantly altered. These findings suggest that a direct programming of developing gonadal cells occurs in response to high levels of maternal glucocorticoid. Indeed, testosterone production was significantly reduced in Leydig cells isolated from immature offspring of DEX-treated pregnant females compared with controls (48.3%, $P < .001$), and LH stimulation of these cells did not compensate for the lowered steroidogenic capacity. The hypothalamic-pituitary-adrenal axis was also affected, because significant reductions in both serum adrenocorticotropic hormone (ACTH; 26.2%, $P < .001$) and corticosterone (CORT; 32.3%, $P < .001$) were measured in DEX-exposed immature male offspring. In contrast, adult male offspring from DEX-treated dams had significantly higher levels of serum ACTH (39.2%, $P <$. 001) and CORT (37.8%, $P < .001$). These same animals had higher serum testosterone (31.6%, $P \leq .05$) and a significant reduction in serum LH (30.8%, $P < .001$). Moreover, Leydig cells isolated from these adult offspring exhibited an increased capacity for testosterone biosynthesis under basal (38.6%, $P < .001$) and LH-stimulated conditions (33.5%, $P < .001$). In summary, sustained changes in steroidogenic capacity were observed in male rats exposed to high levels of glucocorticoid during prenatal development. More specifically, DEX exposure in utero perturbed Leydig cell testosterone production in both pubertal and adult rats.

Key Words: Testis, neuroendocrine, luteinizing hormone, testosterone, corticosterone.

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In animals, it has been proposed that glucocorticoid exposure during adulthood acts on the brain and pituitary to inhibit testosterone production. For example, stress levels of glucocorticoid (100 nM and higher) inhibit the release of gonadotropins from the pituitary by acting on receptors in the hypothalamus (Veldhuis, 1997). In contrast, low serum testosterone concentrations observed in males following chronic elevations in serum glucocorticoid due to military combat training, population stress, or male competition are not associated with low serum luteinizing hormone (LH; Boonstra and Singleton, 1993; Bernton et al, 1995; Blanchard et al, 1995). Moreover, restraint stress had no effect on the binding capacity or affinity constants of testicular interstitial cell LH/human chorionic gonadotropin receptors (Orr and Mann, 1990). These studies suggest that in addition to action at sites in the central nervous system, glucocorticoids act at other levels of the reproductive axis. Consistent with this hypothesis, glucocorticoids are known to act directly on Leydig cells via a glucocorticoid receptor (GR)-mediated mechanism that results in reduced testosterone synthesis (Stalker et al, 1989). It has also been demonstrated that acute restraint stress disrupts testicular steroidogenesis in adult male rats by inhibiting the activities of 17α-hydroxylase and 17,20-lyase without affecting Leydig cell LH receptors (Orr et al, 1994).

Maternal stress and its accompanying rise in circulating glucocorticoids adversely affects the intrauterine milieu and has been shown to predispose offspring to health problems such as hypertension, diabetes, and reproductive dysfunction in adult life (Langley, 1997; Seckl, 1998). Maternal stress affects the reproductive axis, including the brain, pituitary, and gonads, and significant increases in glucocorticoid levels in dams have been associated with a delay or impairment of male sex organ development in offspring. For example, restraint stress applied to pregnant rats during gestation days (GDs) 14–21 resulted in reduced testis weights and a shortened anogenital distance in the male pups at birth (Dahlof et al, 1978); changes in adult male sexual behavior were also correlated with prenatal dexamethasone (DEX) exposure (Ward et al, 1994).

In addition to possible effects on the developing hypothalamic-pituitary-gonadal HPG axis, it has been

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shown that the fetal hypothalamic-pituitary-adrenal (HPA) system is disturbed by high levels of maternal corticosterone (CORT; Henry et al, 1994; Takahashi et al, 1998). It is not clear, however, that high serum CORT is directly responsible for perturbations in these feedback systems during prenatal development. For example, treatment with DEX, CORT, or restraint stress during the last trimester of rat gestation results in differential effects on the male offspring's sexual behavior (Holson et al, 1995). Because DEX is commonly used in clinical settings to suppress preterm labor and promote fetal lung development, it is important to examine the long-term effects of this drug on postnatal development of the offspring. The present study was designed to examine the effects of a high level of maternal glucocorticoid activity on adrenal and testicular steroid output in male offspring. This output was assessed during pubertal development and adulthood. In addition, Leydig cell function was examined in vitro in order to determine whether prenatal exposure to DEX directly affects the steroidogenic capacity of these cells. Our data demonstrate that exposure to DEX in utero alters serum CORT and testosterone concentrations, and changes testosterone biosynthesis in both pubertal and adult rat Leydig cells.

Materials and Methods

Animals

Eighteen female Sprague Dawley rats (250–300 g) purchased from Charles River Laboratories (Wilmington, Mass) were maintained under conditions of controlled lighting (lights on from 0700 to 1900 hours) and temperature (23°C) and given free access to food. The rats were time-mated and administered either DEX (100 μg/kg per day; n = 9; Sigma Chemical Company, St Louis, Mo) or vehicle (controls = saline + 0.4% ethanol, n = 9) subcutaneously each day during days 14–19 of gestation. (Day 0 is defined as the morning of appearance of the vaginal plug. Gestation in the rat lasts 21–22 days.) We based the 100 μg/kg dose on numerous studies reported in the literature (Bakker et al, 1995; Holson et al, 1995; Nyirenda et al, 1998; Ahlbom et al, 2000), including an extensive dose-response study conducted by Slotkin et al (1992). The male offspring were weaned on postnatal day 21 and housed 5–6 per group according to litter. Immature male offspring from DEX-treated (n = 42) and control dams (n = 43) were taken from each litter and studied at 35 days of age. Adult males were killed and prepared for study at 90 days of age (DEX-treated, n = 26; control, n = 27). All animals killed were rotated in sets of control and DEX-treated litters between the hours of 10:00 and 11:00 AM by asphyxiation with CO_2 in a precharged chamber. This protocol is in accordance with a procedure that was approved by the Animal Care and Use Committee of Rockefeller University (protocol 412000R2).

Radioimmunoassays

On day 19 of pregnancy, maternal tail tip blood samples were collected to determine serum CORT levels. Trunk blood was collected from each individual offspring at 35 or 90 days and analyzed for serum concentrations of adrenocorticotropin (ACTH), CORT, testosterone, and LH. These hormones were analyzed using specific radioimmunoassay procedures (Chandrashekar et al, 1988; Shan and Hardy, 1992; Monder et al, 1994).

Isolation and Purification of Leydig Cells

Leydig cells from 35- and 90-day-old animals were isolated according to previously published protocols (Shan and Hardy, 1992). The immature (1.0×10^6 cells/mL) or adult Leydig cells (0.1×10^6 cells/mL) were incubated in culture medium consisting of Dulbecco modified Eagle medium (DMEM)/Hams F-12 (1:1), 15 mM Hepes, 14 mM $NaHCO_3$, and 1% bovine serum albumin. The cells were maintained in a shaking water bath for 3 hours at 34°C. Incubations of triplicate samples were conducted in medium alone (basal) or in medium plus a maximally stimulating dose of ovine LH (100 ng/mL). At the end of 3 hours, the samples were centrifuged at $500 \times g$ and the supernatants were analyzed for testosterone concentration by radioimmunoassay. All testosterone values were normalized according to the cell count that was determined using a hemacytometer.

Statistics

In each experiment, data were obtained from triplicate assays on serum from each individual animal, and the results are expressed as the mean ± SEM. The overall experimental design was performed twice to ensure that the results were repeatable. The data were analyzed using analysis of variance followed by Newman-Keuls multiple comparisons testing to identify significant differences between groups.

Results

Prenatal Exposure to Dexamethasone: Effects on Serum Hormones

Treatment with 100 μg DEX/kg per day during the final week of rat pregnancy resulted in a 1-day delay of parturition, because control offspring were born on GD 21 and the DEX-treated offspring were born on GD 22. Prenatal exposure to DEX did not affect body or testis weights in male offspring at 35 days or 90 days compared with offspring from untreated pregnancies (data not shown). However, DEX treatment sharply decreased maternal serum CORT (DEX, 13.33 ± 1.2 nm/mL vs control 121.25 ± 27.8 ng/mL, $P < .001$; Figure 1) consistent with a negative feedback response to the high levels of circulating DEX. The low levels of maternal CORT indicated that the treated dams and their fetuses were pre-

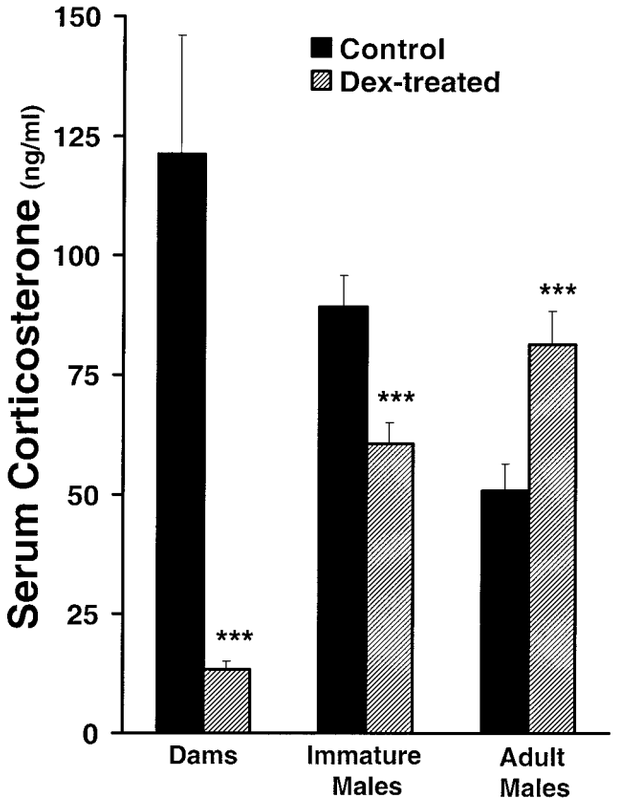

Figure 1. Serum CORT in dams and male offspring prenatally exposed to DEX. Eighteen female Sprague Dawley rats were time-mated and injected with either DEX (100 μg/kg · day^{-1}, n = 9) or vehicle (controls; saline + 0.4% ethanol, n = 9) subcutaneously on days 14–19 of gestation. On day 19, tail vein blood was collected from the dam and analyzed for CORT using a specific radioimmunoassay. Immature male offspring from DEX-treated (n = 45) and control dams (n = 47) were taken from each litter and killed at 35 days of age. Males from the same litter were maintained until 90 days (adult DEX-treated n = 23, adult control n = 24). Trunk blood was analyzed for hormone levels using a specific radioimmunoassay. ***Denotes a difference compared with controls at $P < .001$.

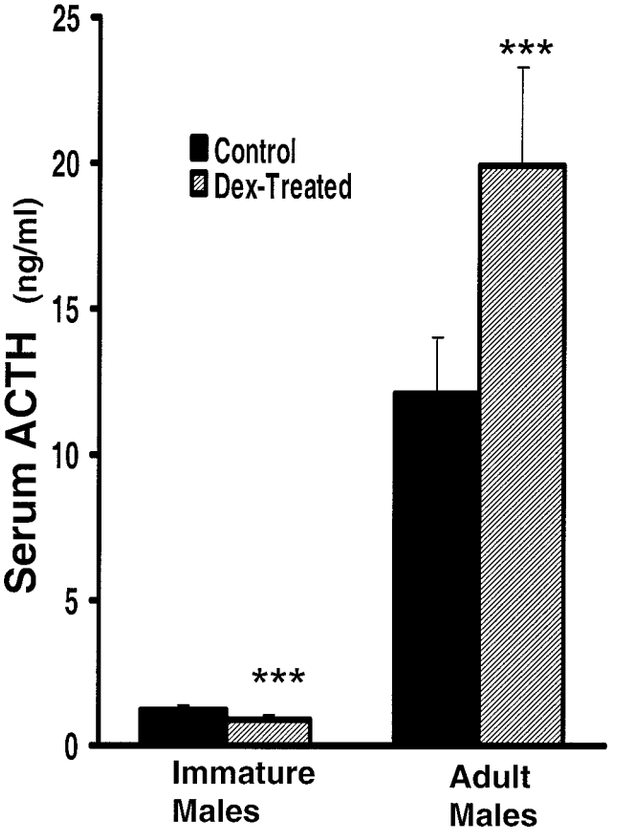

Figure 2. Serum ACTH levels in male offspring prenatally exposed to DEX. Male littermates from control and DEX-treated pregnant rats were killed on days 35 and 90. Trunk blood was analyzed for hormone levels using a specific radioimmunoassay. ***Denotes a difference compared with controls at $P < .001$.

dominantly exposed to DEX as the major circulating glucocorticoid during the last trimester of gestation. Because the half-life for DEX is approximately 9 hours (Charles et al, 1993) and DEX treatment in this study ceased on GD 19, direct effects of this steroid beyond parturition are unlikely.

Male offspring from DEX-treated females had pronounced changes in both serum CORT and ACTH levels in the offspring from the two age groups (Figures 1 and 2, respectively). In the DEX-exposed immature offspring (35 days old), serum CORT levels were significantly reduced (DEX, 60.67 ± 4.5 ng/mL vs control, 89.29 ± 6.7 ng/mL; $P < .001$) compared with controls. This finding was associated with a decreased serum ACTH level (DEX, 0.91 ± 0.07 ng/mL vs control, 1.23 ± 0.12 ng/mL; $P < .001$). In contrast, the adult offspring (90 days old) exposed to DEX in utero had markedly higher serum CORT (DEX, 81.27 ± 7.1 ng/mL vs control, 50.91 ± 5.6 ng/mL; $P < .001$) and circulating ACTH (DEX, 19.93 ± 3.41 ng/mL vs control, 12.26 ± 1.92 ng/mL; $P < .001$).

Serum testosterone levels were altered in the DEX-exposed offspring at both ages (Figure 3). At 35 days, the prenatally exposed males had twofold lower values for serum testosterone (DEX, 0.32 ± 0.04 ng/mL vs control, 0.57 ± 0.06 ng/mL; $P < .05$) and at 90 days, testosterone levels were 1.5-fold higher in DEX-exposed males (DEX, 3.26 ± 0.19 ng/mL vs control, 2.23 ± 0.17 ng/mL; $P < .05$). Serum LH levels were not significantly different from controls in the immature animals (DEX, 0.25 ± 0.08 ng/mL vs control, 0.35 ± 0.10 ng/mL; $P > .37$); however, the adult male offspring from treated dams exhibited a marked reduction in LH (DEX, 0.31 ± 0.09 ng/mL vs control, 0.44 ± 0.11 ng/mL; $P < .001$; Figure 4). These data suggest that male offspring exposed to high levels of glucocorticoid in utero are subject to perturbations in both the HPA and the HPG systems during fetal development. Moreover, the alterations in steroid output following prenatal exposure to DEX are sustained, with be-

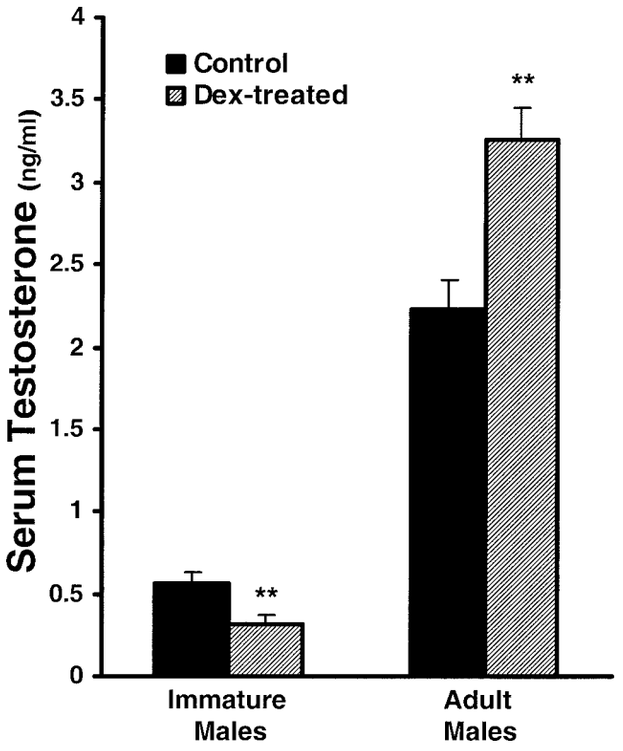

Figure 3. Serum testosterone levels in male offspring prenatally exposed to DEX. Male littermates from control and DEX-treated pregnant rats were killed on days 35 and 90. Trunk blood was analyzed for hormone levels using a specific radioimmunoassay. **Denotes a difference compared with controls at $P < .05$.

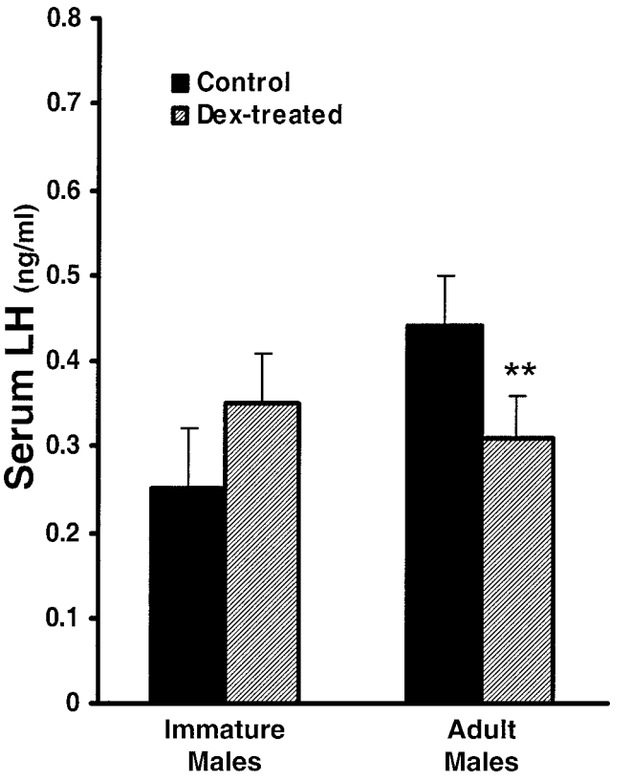

Figure 4. Serum LH levels in male offspring prenatally exposed to DEX. Male littermates from control and DEX-treated pregnant rats were killed on days 35 and 90. Trunk blood was analyzed for hormone levels using a specific radioimmunoassay. **Denotes a difference compared with controls at $P < .05$.

low-normal levels in immature males at 35 days and elevated levels in adults at 90 days.

Prenatal Exposure to Dexamethasone: Effects on Leydig Cell Steroidogenic Capacity

In vitro analysis of Leydig cell function indicated that age-dependent changes in Leydig cell steroidogenic capacity correlated well with changes in serum testosterone concentrations in offspring from both age groups. Cells purified from 35-day-old DEX-exposed males had lower rates of testosterone production under both basal (3.81 ± 0.25 vs 7.37 ± 0.33 ng/10^6 cells per 3 hours, respectively; $P < .001$; Figure 5) and LH-stimulated conditions (48.97 ± 4.2 vs 82.33 ± 5.2 ng/10^6 cells per 3 hours; $P < .001$; Figure 6). This demonstrated that although LH responsiveness was intact, LH stimulation did not compensate for the reduction in testosterone biosynthesis observed in the immature Leydig cells. In contrast, cells isolated from adult DEX-exposed males had higher rates of testosterone production compared with controls under basal (DEX, 27.49 ± 1.7 vs control, 16.89 ± 0.81 ng/10^6 cells per 3 hours; $P < .001$; Figure 5) and LH-stimulated conditions (DEX, 275.05 ± 10.4 vs control, 182.96 ± 10.4 ng/10^6 cells per 3 hours; $P < .001$; Figure 6). Moreover, the increased steroidogenic capacity of purified adult Leydig cells was consistent with the increased serum testosterone values observed at 90 days. It is possible that prenatal exposure to DEX alters gene expression for key steroidogenic components in Leydig cells during fetal development in an attempt to oppose perturbations in the fetal HPA and HPG systems. The findings reported here provide evidence that fetal compensation does occur, and that prenatal exposure to DEX directly affects Leydig cell postnatal development and function.

Discussion

The data presented herein support the hypothesis that male offspring exposed to high levels of glucocorticoid in utero exhibit sustained changes in both the HPA and HPG axes during pubertal development and adulthood. Studies to date suggest that the stress-induced increases in maternal glucocorticoid during pregnancy alter genetic programming of the fetus (reviewed in Langley, 1997; Weinstock, 1997; Seckl, 1998). Moreover, this developmental imprinting disturbs adrenal and reproductive function in the adult animal (Anderson et al, 1986; Kerchner and Ward, 1992; Maneoka et al, 1997). Our experiments support this hypothesis. At 35 days of age, significant reductions in serum ACTH, CORT, and testosterone occurred in the DEX-exposed males compared with control offspring at the same age, although no significant change was observed in serum

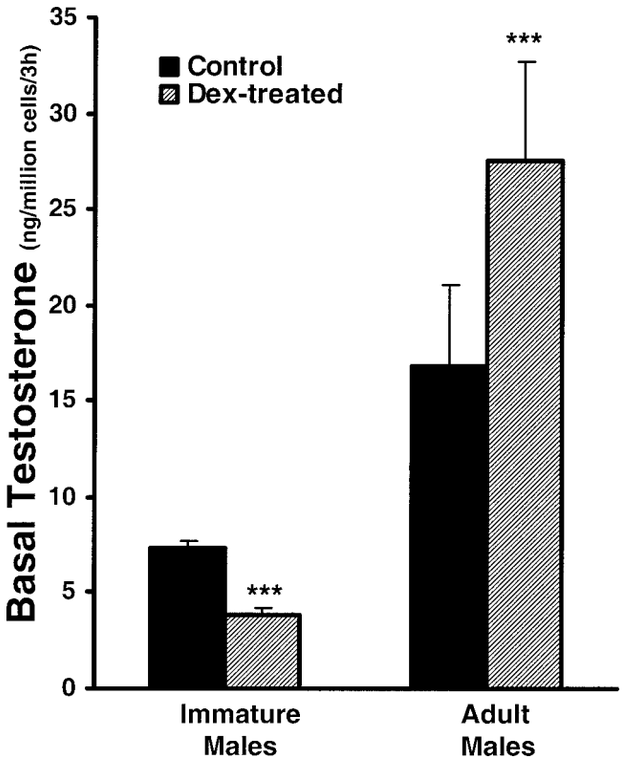

Figure 5. Basal testosterone production by Leydig cells in vitro. Purified Leydig cells from 35- and 90-day old male offspring borne of DEX-treated or control rat dams were incubated in DMEM/F12 medium at a concentration of 1.0×10^6 cells/mL and 0.25×10^6 cells/mL, respectively. ***Denotes a difference compared with controls at $P < .001$.

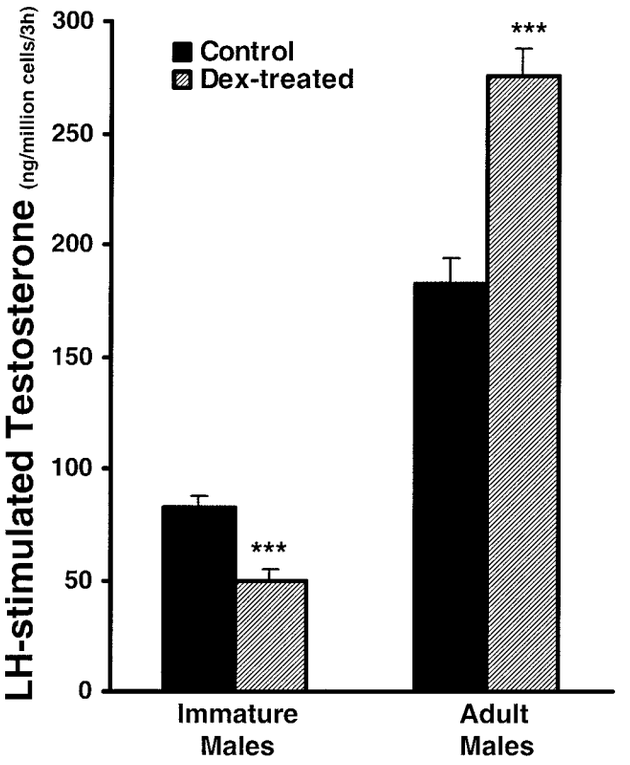

Figure 6. LH-stimulated testosterone production by Leydig cells in vitro. Purified Leydig cells from 35- and 90-day old male offspring borne of DEX-treated or control rat dams were incubated in DMEM/F12 medium in the presence of ovine LH (100 ng/mL) at a concentration of 1.0×10^6 cells/mL and 0.25×10^6 cells/mL, respectively. ***Denotes a difference compared with controls at $P < .001$.

LH. In contrast, adult males from the same DEX-exposed litters had significantly higher serum ACTH, CORT, and testosterone levels compared with control offspring, as well as a significant reduction in serum LH.

The developmental change from low serum ACTH, CORT, and testosterone in the pubertal males to high serum ACTH, CORT, and testosterone in the adult male offspring from treated dams may indicate that output from the HPA and HPG axes was lowered due to a maturational delay. Evidence supporting the hypothesis that prenatal stress and its accompanying glucocorticoid excess results in a developmental delay has been reported (Barlow et al, 1978, Weinstock et al, 1988). Although glucocorticoid hormones are necessary for organogenesis, excessive exposure to DEX during adrenal development results in desensitization of the GRs and a consequent atrophy of both the medulla and cortex in immature 35-day-old offspring (Betito et al, 1993; Hristic et al, 1997; Manojlovic et al, 1998). Additional studies have shown that prenatal DEX exposure delays biochemical differentiation and cardiac development in rats. (Torres et al, 1997). The reproductive axis is also affected; offspring from dams exposed to DEX display a pubertal delay in development of the external genitalia (Smith and Waddell, 2000). These reports support the possibility that the significant reductions in ACTH, CORT, and testosterone and LH measured in the present study are also due to a maturational delay in the 35-day-old males.

Because the hormonal parameters of immature males are in developmental flux, it was necessary to conduct measurements on 90-day-old adult rats in which these parameters are considered to be more stable and thus more reliably indicative of sustained changes in neuroendocrine function. The levels of circulating ACTH, CORT, and testosterone in the adult males were, interestingly, all significantly higher compared with controls. However, the serum LH in adult offspring from DEX-treated dams was significantly lower compared with controls. These data suggest that a long-term change in adrenal and testicular steroidogenesis occurred. The increased capacity of adult Leydig cells for testosterone biosynthesis was not due to an overproduction of circulating gonadotropin; in fact, the down-regulation of LH in the treated group indicates that the negative feedback system at the level of the brain and pituitary is intact. Conversely, the higher levels of ACTH and CORT in the DEX-exposed offspring suggest that the negative feedback in the HPA system has been perturbed.

It is possible that these two findings can be interrelated via a down-regulation of GR gene expression in feedback target tissues such as the hippocampus, hypothalamus, and pituitary of the HPA system as well as a reduced GR gene expression in Leydig cells.

Numerous studies have demonstrated that the GR is integrally involved in the negative feedback regulation of the stress response (reviewed in Keller-Wood and Dallman 1984; Sapolsky, 1985). Exposure to high levels of maternal glucocorticoid during critical windows of organogenesis could affect both the HPA and HPG by altering the expression of GR in order to compensate for the excess levels of glucocorticoid presented to the differentiating target tissues, most notably brain, pituitary, and testis (Pollard, 1985; Barbanges et al, 1996; Takahashi, 1998; Dean and Matthews, 1999). Moreover, a recent study has shown that maternal exposure to DEX results in a significant decrease in hippocampal GR concentrations in male offspring (Levitt et al, 1996). It is possible that this programmed reduction in GR level diminishes the effectiveness of circulating CORT as a negative feedback component. This change would then blunt HPA feedback termination of the stress response, resulting in higher levels of circulating effector molecules, most notably ACTH and CORT, as is shown in our study. In contrast to the hippocampal studies, the effects of maternal DEX on the developing hypothalamus and pituitary have not been examined; however, exogenous cortisol has been shown to alter GR messenger RNA in the developing ovine pituitary (Matthews, 1995).

It is possible that the effects of excess glucocorticoid on the HPG axis during gestation also result from changes in GR regulatory systems, particularly in the testis. It has been shown that glucocorticoids act directly on adult Leydig cells via a GR-mediated mechanism that results in reduced testosterone synthesis (Ge et al, 1997). Moreover, males exposed to excess glucocorticoids after reaching adulthood experience the inhibitory actions of these hormones on testicular steroidogenesis (Charpenet et al, 1981; Welsh et al, 1982; Orth et al, 1983; Orr and Mann, 1992; Orr et al, 1994). However, just as glucocorticoid has been shown to modulate testicular function, there is also testosterone action on the adrenal gland. Testosterone inhibits HPA activity, thus maintaining reproductive competence during chronic stress in the adult (Handa et al, 1994a,b). Interaction between the two systems is also prominent during GDs 14 through 19, when simultaneous differentiation of both the HPA and HPG axes occurs (Setalo and Nakane, 1976; Wilson et al, 1983). Because central and peripheral homeostatic regulatory systems are being established and matched to the existing milieu at this time, any environmental factors that significantly alter the timing of fetal testosterone synthesis, adrenal steroid production, or both may result in long-term endocrine changes. For example, exposure to excess glucocorticoid in utero results in reduced levels of testosterone and LH in the male fetus (Ward and Weisz, 1980, 1984; Salisbury et al, 1989). In contrast, adult male offspring from restraint-stressed dams had a significantly higher level of serum testosterone, whereas LH levels remained relatively constant (Ward et al, 1996). Moreover, the adult offspring from the restraint-stressed dams also exhibited a marked tendency to display lordosis (Ward, 1972), although the effects on copulatory response were less clear (Ward et al, 1994). The increased lordosis potential (Ward et al, 1994) was, surprisingly, associated with a significantly higher serum testosterone level in adult males (Ward et al, 1996). These studies suggest that decreased fetal LH and testosterone levels in response to excess glucocorticoid exposure during development may in part be predicates of a change in target tissue sensitivity in the adult offspring. Our data support this model because, despite high levels of circulating CORT in adult males from DEX-exposed dams, the serum testosterone levels were significantly higher compared with controls. The expected down-regulation of testosterone by CORT was not observed, and this finding suggests that the responsiveness of the Leydig cell to circulating glucocorticoid was diminished. It is possible that an in utero reduction in testicular GR gene expression leads to a consequent decrease in GR-mediated inhibition of Leydig cell steroidogenesis and higher levels of circulating testosterone in the adult.

Although it is possible that the effects of glucocorticoid on testicular steroidogenesis may operate indirectly at the level of the brain and pituitary gonadotropes, studies have shown that the reduction of testosterone in stressed adult animals exposed to high circulating CORT has not been associated with consistent changes in LH concentrations (Pollard et al, 1980; Orr and Mann, 1990; Srivastava et al, 1993). In fact, various forms of chronic stress have been shown to decrease (Tache et al, 1980), increase (Briski et al, 1984), or have no effect (Charpenet et al, 1982) on serum LH levels in the male rat despite the suppression of circulating testosterone. In our study, the negative feedback system between serum testosterone and LH appears to be operating in the DEX-exposed adult offspring because the high levels of testosterone were associated with the expected lowering of LH. These findings suggest that prenatal exposure to DEX may act directly on components regulating testosterone biosynthesis in the differentiating Leydig cells rather than at higher centers of the HPG axis. In order to determine whether the steroidogenic response in the prenatally exposed offspring was a result of a sustained change in Leydig cell function, we examined the capacity for testosterone biosynthesis in Leydig cells isolated from the 35- and 90-day-old males. Following short-term 3-hour culture, testosterone production in cells isolated from the immature

offspring was lower under both basal and LH-stimulated conditions. In contrast, Leydig cells isolated from the adult littermates were capable of producing significantly higher levels of testosterone. These data demonstrate that Leydig cell steroidogenic capacity had been directly affected by prenatal exposure to DEX.

Our results suggest that the HPA axis is in part altered at the level of the brain and pituitary, because the high levels of ACTH and CORT indicate a reduction in negative feedback. Moreover, the HPG axis appears to be affected primarily at the level of testicular Leydig cells because serum testosterone levels are significantly higher than in controls even though the negative feedback on LH is maintained. It is possible that the GR regulatory systems may be central to the observed output in circulating steroids. Currently, this hypothesis is being explored in our laboratory. Studies designed to investigate the postnatal effects of prenatal exposure to DEX are important because this steroid is used clinically as a therapeutic in women at risk for preterm labor and delivery. However, the long-term consequences of prenatal exposure to this glucocorticoid have not been thoroughly investigated. Our study demonstrates that high levels of maternal glucocorticoid perturb the steroid output from both the HPA and HPG axes. More specifically, we have shown that the effects of DEX on Leydig cell steroidogenesis in utero persist into postnatal development. Unlike the transient effects experienced following adult exposure to stress levels of glucocorticoid, prenatal exposure exerts a sustained perturbation in male steroidogenesis.

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References

Ahlbom E, Gogvadze V, Chen M, Celsi G, Ceccatelli S. Prenatal exposure to high levels of glucocorticoid increases the susceptibility of cerebellar granule cells to oxidative stress-induced cell death. *Proc Natl Acad Sci USA.* 2000;97:14726–14730.

Anderson R, Fleming DE, Rhees RW, Kinghorn E. Relationships between sexual activity, plasma testosterone, and the volume of the sexually dimorphic nucleus of the preoptic area in prenatally stressed and nonstressed rats. *Brain Res.* 1986;370:1–10.

Bakker JM, Schmidt ED, Kroes H, Kavelaars A, Heijnen CJ, Tilders FJH, van Rees EP. Effects of short-term dexamethasone treatment during pregnancy on the development of the immune system and the hypothalamo-pituitary-adrenal axis in the rat. *J Neuroimmunol.* 1995; 63:183–191.

Barbanges A, Plazza PV, Le Moal M, Maccari S. Maternal glucocorticoid secretion mediates long-term effects of prenatal stress. *J Neurosci.* 1996;16:3943–3949.

Barlow SM, Knight AF, Sullivan FM. Delay in postnatal growth and development of offspring produced by maternal restraint stress during pregnancy in the rat. *Teratology.* 1978;18:211–218.

Bernton E, Hoover D, Galloway R, Popo K. Adaptation to chronic stress in military trainees. Adrenal androgens, testosterone, glucocorticoids, IGF-I, and immune function. *Ann NY Acad Sci.* 1995;774:217–231.

Betito K, Diorio J, Meaney MJ, Boksa P. Glucocorticoid receptors in bovine adrenal medullary cells in culture: regulation by cyclic nucleotides. *J Neurosci.* 1993;54:263–273.

Blanchard DC, Spencer RL, Weiss SM, Blanchard RJ, McEwen B, Sakai RR. Visible burrow system as a model of chronic social stress: behavioral and neuroendocrine correlates. *Psychoneuroendocrinology.* 1995;20:118–134.

Boonstra R, Singleton GR. Population declines in the snowshoe hare and the role of stress. *Gen Comp Endocrinol.* 1993;91:126–143.

Briski KP, Quigley K, Meites J. Endogenous involvement of opiate in acute and chronic stress induced changes in plasma LH concentration in the male rat. *Life Sci.* 1984;34:2485–2493.

Chandrashekar V, Bartke A, Wagner TE. Endogenous human growth hormone (GH) modulates the effect of gonadotropin-releasing hormone on pituitary function and the gonadotropin response to the negative feedback effect of testosterone in adult male transgenic mice bearing human GH gene. *Endocrinology.* 1988;123:2717–2722.

Charles B, Schild P, Steer P, Cartwright D, Donovan T. Pharmacokinetics of dexamethasone following single-dose intravenous administration to extremely low birth weight infants. *Dev Pharm Ther.* 1993;20:205–210.

Charpenet G, Tache Y, Bernier M, Ducharme JR, Collu R. Stress induced testicular hyposensitivity to gonadotropin in rats. Role of the pituitary gland. *Biol Reprod.* 1982;27:616–623.

Charpenet G, Tache Y, Forest MG, Haour F, Saez JM, Bernier M, Ducharme JR, Collu R. Effects of chronic intermittent immobilization stress on rat testicular androgenic function. *Endocrinology.* 1981;109: 1254–1258.

Dahlof LG, Hard E, Larsson K. Influence of maternal stress on development of fetal genital system. *Physiol Behav.* 1978;20:193–195.

Dean F, Matthews SG. Maternal dexamethasone treatment in late gestation alters glucocorticoid and mineralocorticoid receptor mRNA in the fetal guinea pig brain. *Brain Res.* 1999;846:253–259.

Ge RS, Hardy DO, Catterall JF, Hardy MP. Developmental changes in glucocorticoid receptor and 11 beta-hydroxysteroid dehydrogenase oxidative and reductive activities in rat Leydig cells. *Endocrinology.* 1997;138:5089–5095.

Handa RJ, Burgess LH, Kerr JE, O'Keefe JA. Gonadal steroid hormone receptors and sex differences in the hypothalamo-pituitary-adrenal axis. *Horm Behav.* 1994a;28:464–474.

Handa RJ, Nunley KM, Lorens SA, Louie JP, McGivern RF, Bollnow MR. Androgen regulation of adrenocorticotropin and corticosterone secretion in the male rat following novelty and foot shock stressors. *Physiol Behav.* 1994b;55:117–124.

Henry C, Kabbaj M, Simon H, Le Moal M, Maccari S. Prenatal stress increases the hypothalamo-pituitary-adrenal axis response in young and adult rats. *J Neuroendocrinol.* 1994;6:341–345.

Holson RR, Gough B, Sullivan P, Badger T, Sheehan DM. Prenatal dexamethasone or stress but not ACTH or corticosterone alter sexual behavior in male rats. *Neurotoxicol Teratol.* 1995;17:393–401.

Hristic M, Kalafatic D, Plecas B, Manojlovic M. The influence of prolonged dexamethasone treatment of pregnant rats on the perinatal development of the adrenal gland of their offspring. *J Exp Zool.* 1997; 279:54–61.

Keller-Wood M, Dallman M. Corticosteroid inhibition of ACTH secretion. *Endocrinol Rev.* 1984;5:1–24.

Kerchner M, Ward IL. SDN-POA volume in male rats is decreased by prenatal stress, but is not related to ejaculatory behavior. *Brain Res.* 1992;581:244–259.

Levitt NS, Lindsay RS, Holmes MC, Seckl JR. Dexamethasone in the last week of pregnancy attenuates hippocampal glucocorticoid receptor gene expression and elevates blood pressure in the adult offspring in the rat. *J Neuroendocrinol.* 1996;64:412–418.

Langley SC. Intrauterine programming of hypertension by glucocorticoids. *Life Sci.* 1997;60:1213–1221.

Maneoka K, Mikuni M, Ogawa T, Kitera K, Kamei K, Takigawa M, Takahashi K. Prenatal dexamethasone exposure alters brain monoamine metabolism and adrenocortical response in rat offspring. *Am J Physiol.* 1997;273:R1669–R1675.

Manojlovic M, Kalafatic D, Hristic M, Plecas B, Virag A, Cakic M. Treatment of pregnant females with dexamethasone influences postnatal development of the adrenal medulla. *Ann Anat.* 1998;180:131–135.

Matthews SG, Yang K, Challis JRG. Changes in glucocorticoid receptor mRNA in the developing ovine pituitary and the effects of exogenous cortisol. *J Endocrinol.* 1995;144:483–490.

Monder C, Sakai RR, Miroff Y, Blanchard DC, Blanchard RJ. Reciprocal changes in plasma corticosterone and testosterone in stressed male rats maintained in a visible burrow system: evidence for a mediating role of testicular 11 beta-hydroxysteroid dehydrogenase. *Endocrinology.* 1994;134:1193–1198.

Nyirenda MJ, Lindsay RS, Kenyon CJ, Burchell A, Seckl JR. Glucocorticoid exposure in late gestation permanently programs rat hepatic phosphoenolpyruvate carboxykinase and glucocorticoid receptor expression and causes glucose intolerance in adult offspring. *J Clin Invest.* 1998;101:2174–2181.

Orr TE, Mann DR. Effects of restraint stress on plasma LH and T concentrations, Leydig cell LH/hCG receptors, and in vitro testicular steroidogenesis in adult rats. *Horm Behav.* 1990;24:324–341.

Orr TE, Mann DR. Role of glucocortioids in the stress-induced suppression of testicular steroidogenesis in adult male rats. *Horm Behav.* 1992;26:350–363.

Orr TE, Taylor MF. Bhattacharyya AK, Collins DC, Mann DR. Acute immobilization stress disrupts testicular steroidogenesis in adult male rats by inhibiting the activities of 17α-hydroxylase and 17,20-lyase without affecting the binding of LH/hCG receptors. *J Androl.* 1994; 15:302–308.

Orth JM, Weisz J, Ward OB, Ward IL. Environmental stress alters the development pattern of 3β-HSD activity in Leydig cells of fetal rats: a quantitative cytochemical study. *Biol Reprod.* 1983;28:625–631.

Pollard I, Bassett JR, Joss JMP. Plasma T levels and 3β-HSD activity in the testis of the rat following prolonged exposure to stress. *J Reprod Fertil.* 1980;59:101–106.

Pollard I. Effects of stress administered during pregnancy on the development of fetal testes and their subsequent function in the adult rat. *J Endocrinol.* 1985;107:241–245.

Salisbury R, Reed J, Ward IL, Weisz J. Plasma luteinizing hormone levels in normal and prenatally stressed male and female rat fetuses and their mothers. *Biol Reprod.* 1989;40:111–117.

Sapolsky RM, Meaney MJ, McEwen BS. The development of the glucocorticoid receptor system in the rat limbic brain. III. Negative-feedback regulation. *Dev Brain Res.* 1985;18:169–173.

Seckl JR. Physiologic programming of the fetus. *Clin Perinatol.* 1998; 25:939–962.

Setalo G, Nakane PK. Functional differentiation of the fetal anterior pituitary cells in the rat. *Endocrinol Exp.* 1976;10:155–166.

Shan LX, Hardy MP. Developmental changes in the levels of luteinizing hormone receptor and androgen receptor in rat Leydig cells. *Endocrinology.* 1992;131:1107–1114.

Slotkin TA, Seidler FJ, Kavlock RJ, Gray JA. Fetal dexamethasone exposure accelerates development of renal function: relationship to dose, cell differentiation and growth inhibition. *J Dev Physiol.* 1992;17:55–61.

Smith JT, Waddell BJ. Increased fetal glucocorticoid exposure delays puberty onset in postnatal life. *Endocrinology.* 2000;141:2422–2428.

Srivastava RK, Taylor MF, Mann DR. Effect of immobilization stress on plasma luteinizing hormone, testosterone, and corticosterone concentrations and on 3β-hydroxysteroid dehydrogenase activity in the testes of adult rats. *Soc Exp Biol Med.* 1993;204:231–235.

Stalker A, Hermo L, Antakly T. Covalent affinity labeling, radioautography, and immunocytochemistry localize the glucocorticoid receptor in rat testicular cells. *Am J Anat.* 1989;186:369–377.

Tache Y, DuRuisseau P, Ducharme JR, Charpenet G, Haour F, Saez J, Collu R. Effect of chronic intermittent immobilization stress on hypophyso-gonadal function of rats. *Acta Endocrinol.* 1980;93:168–174.

Takahashi LK, Turner JG, Kalin NH. Prolonged stress-induced elevation in plasma corticosterone during pregnancy in the rat: implications for prenatal stress studies. *Psychoneuroendocrinology.* 1998;23:571–581.

Takahashi LK. Prenatal stress: consequences of glucocorticoids on hippocampal development and function. *Int J Dev Neurosci.* 1998;16: 199–207.

Torres A, Belser WW, Umeda PK, Tucker D. Indicators of delayed maturation of rat heart treated prenatally with dexamethasone. *Pediatr Res.* 1997;42:139–144.

Veldhuis JD. Male hypothalamic-pituitary-gonadal axis. In: Lipshultz LI, Howards SS, eds. *Infertility in the Male.* 3rd ed. St Louis, Mo: Mosby; 1997:23–58.

Ward IL. Prenatal stress feminizes and demasculinizes the behavior of males. *Science.* 1972;175:82–84.

Ward IL, Ward OB, Mehan D, Winn RJ, Bielawski D. Male and female sexual behavior potential of male rats prenatally exposed to the influence of alcohol, stress, or both factors. *Behav Neurosci.* 1994;108: 1188–1195.

Ward IL, Ward OB, Mehan D, Winn RJ, French JA, Hendricks SE. Prenatal alcohol and stress interact to attenuate ejaculatory behavior but not serum testosterone or LH in adult male rat. *Behav Neurosci.* 1996; 110:1469–1477.

Ward IL, Weisz J. Maternal stress alters plasma testosterone in fetal males. *Science.* 1980;207:328–329.

Ward IL, Weisz J. Differential effects of maternal stress on circulating levels of corticosterone, progesterone, and testosterone in male and female rat fetuses and their mothers. *Endocrinology.* 1984;114:1635–1644.

Weinstock M. Does prenatal stress impair coping and regulation of hypothalamic-pituitary-adrenal axis? *Neurosci Behav Rev.* 1997;21:1–10.

Weinstock M, Fride E, Hertzberg R. Prenatal stress effects on functional development of the offspring. *Prog Brain Res.* 1988;73:319–331.

Welsh TH, Bambino TH, Hsueh AJW. Mechanism of glucocorticoid induced suppression of testicular androgen biosynthesis in vitro *Biol Reprod.* 1982;27:1138–1146.

Wilson JC, Griffin JE, George FW, Lesbin M. The endocrine control of male phenotypic development. *Aust J Biol Sci.* 1983;36:101–128.