Dexamethasone and Corticosterone Administration to Pregnant Rats: Effects on Pituitary-Adrenocortical Function in the Newborn[1] (36674)

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Although there is convincing evidence that the fetal pituitary–adrenocortical system of the rat is fully operative in late stages of gestation, the question of whether maternal corticosteroids traverse the placenta to influence fetal hypophyseal function has not been satisfactorily resolved. Enlargement of the fetal adrenal occurs if pregnant rats are either adrenalectomized (1–3) or treated with adrenocortical inhibitors (4, 5). Administration of cortisone to either intact or adrenalectomized pregnant rats causes reduction in fetal adrenal weight (1, 6) and compensatory hypertrophy of the remaining adrenal following fetal unilateral adrenalectomy is inhibited by injections of cortisone or hydrocortisone to the fetus (6–8). Knobil and Briggs (2), however, reported no increase in fetal adrenal weight if pregnant rats were hypophysectomized prior to adrenalectomy and concluded that the fetal adrenal hypertrophy reflects increased maternal rather than fetal ACTH secretion. Others have demonstrated that stimulation of the maternal adrenal (by cold or with exogenous ACTH) suppressed rather than stimulated fetal adrenal function and these investigators (9) maintain that maternal corticosteroids, but not ACTH, are capable of crossing the placenta. It is alleged (10) that the source of the relatively high plasma corticosterone levels found in newborn rats is the fetal adrenal itself but other investigators maintain that maternal corticoids normally traverse that placenta at term to exert a restraining influence on fetal ACTH secretion (1, 6, 11).

The possibility that placental transmission of maternal corticoids may result in inhibition of ACTH secretion by the fetal hypophysis has close relevance to accurate interpretation of early postnatal changes which occur in the rat's pituitary–adrenocortical axis. The adrenal gland fails to respond to certain noxious stimuli in early postnatal life. It is not yet clear whether this "stress nonresponsive" period (12) in the neonate is due to immaturity of the pituitary ACTH secretory mechanism (13), failure of CRF release from the hypothalamus (10), or prolongation of corticosterone negative feedback potency on brain and/or pituitary (14). Whether or not it may also reflect a new set of hormonal conditions imposed by the external environment remains to be determined. The present study was directed toward elucidation of this problem by ascertaining the status of ACTH secretion and adrenal function in the newborn of intact and hypophysectomized pregnant rats administered dexamethasone or corticosterone during the last several days of gestation. The results obtained are consistent with the hypothesis that deficiency or excess of circulating levels of adrenocorticoids in the mother induces significant change in ACTH secretion by the fetal pituitary.

Materials and methods. Timed-pregnant rats of the CD strain (Sprague–Dawley derivatives) were supplied by the Charles River Breeding Laboratories, Wilmington, MA. Rats arrived at the laboratory on the 14–15th day of gestation and were kept in separate breeding cages under controlled conditions of temperature (23 ± 1°) and artificial illumination (0700–1900 hr). Tap water and Purina Laboratory Chow were allowed in unrestricted amounts. Body weights and water intake were recorded daily. Dexamethasone-

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21-phosphate (Dexa) was given to intact and hypophysectomized (12–14th day gestation) pregnant rats in the drinking water (3–10 μg/ml) over the last 48–72 hr of gestation. Corticosterone acetate (Coac) was administered either in the drinking water (100 μg/ml; 4–6 days) or by sc injection (2 mg every 4 hr, thrice daily for 2–3 days). Drug treatment was discontinued after spontaneous delivery occurred.

Mothers and offspring were killed within 2–18 hr after delivery with mothers routinely sacrificed prior to their neonates. Blood was drawn from mothers by direct cardiac puncture within 80 sec of light ether anesthesia. Blood from neonates was obtained by decapitation and thoracic incision several min after a heparin injection (0.05 ml). At autopsy, pituitary and adrenals of mothers and offspring were removed, weighed on a microtorsion balance and prepared for assay or chemical study. Corticosteroid content of plasma and adrenal was determined by Guillemin's modification of the Silber fluorometric procedure (15). Bioassays of pituitary ACTH were done by modification of the dexamethasone-block method of Purves and Sirett (16). Test rats (female) weighing 125–140 g were anesthesized with pentobarbital (6.5 mg/100 g body, ip) and 2–4 cm^3 of blood was obtained for corticosteroid determinations by cardiac puncture 20 ± 1 min after iv (jugular) injection of saline extracts of 2–4 pituitaries of newborn rats or 0.8–2.0 mg of adult adenohypophyses. Precision (λ) of the assay, based on three-point standard dose response curves, ranged from .136–.228, averaging approximately that (.185) described by Purves and Sirett (16). ACTH potency and 95% confidence limits of the neonatal pituitaries were ascertained by subjecting the data to analysis of variance (17). Endocrine gland weight was related to final body weight and is represented herein as mg/100 g body. Data were also statistically analyzed by the Student's *t* test.

Results. Administration of dexamethasone (Dexa) or corticosterone acetate (Coac) to intact pregnant rats over the last several days of gestation resulted in significant change in adrenal weight and corticosteroid levels of the mother (Table I). Mean intake per rat of 1.89 mg of Dexa (48–72 hr period) resulted in adrenal atrophy (20%), significant diminution in corticoid content of plasma and adrenal and marked reduction in pituitary ACTH stores (60%). Coac treatment given either by injection (15 mg/rat) or in the

TABLE I. Effect of Dexamethasone and Corticosterone Acetate Treatment in Pregnant Rats.

Treatment (no. rats)	Body weight (g ± SE)	Adrenal weight (mg/100 g body ± SE)	Corticosteroids		Pituitary ACTH[a] (mU/gland ± SE)
			Plasma (μg/100 ml ± SE)	Adrenal (μg/g ± SE)	
Intact mothers[b]					
Normal (10)	245 ± 7	26.3 ± 1.1	31.6 ± 3.1	38.1 ± 2.4	22.3 ± 1.9
Dexa (4)[c]	233 ± 6	20.6 ± 1.2[e]	2.3 ± 0.9[e]	5.4 ± 2.1[e]	8.9 ± 0.9[e]
Coac (7)[d]	260 ± 4	21.5 ± 1.4[e]	22.5 ± 4.0	8.5 ± 2.1[e]	19.5 ± 1.3
Hypx mothers[b]					
Normal (10)	276 ± 6	13.2 ± 0.4	4.0 ± 0.3	3.3 ± 0.1	
Dexa (5)[c]	251 ± 20	13.1 ± 1.2	3.0 ± 0.7	3.9 ± 1.1	
Coac (5)[d]	247 ± 7	14.3 ± 0.9	40.0 ± 2.0[e]	3.7 ± 0.8	

[a] Mean ACTH content (mU/mg × mean adenohypophysial wet weight); mean gland weight differed by < 10%.

[b] All mothers killed 2–18 hr after delivery; hypophysectomy done on 12–14th day of gestation.

[c] Dexamethasone administered in drinking water (last 2–3 days of gestation).

[d] Corticosterone acetate given in drinking water (4–6 days) or by sc injection (2–3 days).

[e] Means differ significantly ($p = < 0.05$) from appropriate normal control value.

TABLE II. Dexamethasone and Corticosterone Administration in Pregnancy: Effects on the Pituitary–Adrenal System of the Newborn.

Group (no. rats)	Body weight (g ± SE)	Adrenal weight (mg/100 g body ± SE)	Adrenal corticosteroid (μg/g ± SE)	Pituitary ACTH (mU/mg) (95% conf. limits)
Newborn of intact mothers				
Normal (92)	6.04 ± .11	43.7 ± 1.6	7.3 ± .8	3.46 (2.19– 5.46)
Normal (29)	6.23 ± .10	40.9 ± 1.6	6.6 ± .3	2.81 (2.12– 3.73)
Dexa (29)	4.27 ± .08	27.2 ± 1.9[a]	5.1 ± .7[a]	1.37 (0.85– 2.19)[a]
Coac (46)[b]	6.20 ± .14	35.5 ± 1.0[a]	8.3 ± .5	17.50 (8.50–36.20)[a]
Coac (33)[c]	6.42 ± .11	33.8 ± 0.8[a]	7.3 ± .4	.92 (0.60– 1.40)[a]
Newborn of hypx mothers				
Normal (109)	5.74 ± .08	48.2 ± 1.2	9.3 ± .5	1.68 (1.10– 2.62)
Dexa (52)	5.03 ± .09	37.2 ± 1.2[a]	3.3 ± .8[a]	3.33 (2.01– 5.53)
Coac (56)[c]	6.08 ± .08	36.7 ± 0.8[a]	5.8 ± .2[a]	1.45 (1.06– 1.98)

[a] Means differ significantly ($p = 0.05$) from appropriate control value.
[b] Pups of mothers given Coac in the drinking water.
[c] Pups of mothers injected with Coac.

drinking water (44.7 mg over 94–96 hr period), produced less but significant reductions in weight and corticosteroid concentration of the maternal adrenal. Values for plasma corticosteroid and pituitary ACTH remained within the normal range. As expected, values for adrenal weight and corticosteroids in hypophysectomized rats were substantially below those of intact animals. Neither Coac (43.5 mg/rat over 113–117 hr) nor Dexa (1.64 mg/rat; 48–72 hr) treatment appreciably influenced the atrophic adrenal but maternal plasma corticosteroid level was significantly elevated after Coac administration. The elevation probably reflected not only increased time of treatment but also a slower rate of corticosterone disposal in the hypometabolic, hypophysectomized pregnant rat.

Pups of mothers receiving Dexa were invariably subnormal in weight (Table II). It has already been shown that intra-amniotic injections of dexamethasone produce marked decrease in fetal and placental weight (18). Effects on the pituitary–adrenal system of newborn were also marked. Adrenal weight and corticosteroid content in offspring of Dexa treated mothers were substantially reduced and pituitary ACTH concentration was less than 50% of that found in normal pups. The consequences of Coac treatment on the pituitary–adrenal system of the neonate were less marked and differed according to the route of administration. In newborn of intact pregnant rats given Coac in the drinking water some adrenal atrophy occurred but adrenocorticosteroid concentrations were slightly, yet not significantly, above normal. There was a fivefold increase in ACTH concentration of the pituitary, however. Offspring of rats receiving Coac injections also bore smaller adrenals with nearly normal corticosteroid content but pituitary ACTH levels in these were sharply reduced (70%).

The adrenals in newborn of untreated, or saline injected hypophysectomized rats were consistently heavier and contained more corticosteroid than those from intact mothers (Table II). Pituitary ACTH concentration was reduced (45–50%). These indices of pituitary–adrenocortical function were reversed in neonates of Dexa treated hypophysectomized mothers. Adrenal weight and corticosteroid concentration were substantially reduced and ACTH concentration in the pituitary was almost doubled. Coac treatment of hypophysectomized pregnant rats resulted in newborn with adrenals subnormal in weight and corticosteroid content. Pituitary ACTH

levels remained within the range found in neonates of untreated, hypophysectomized mothers.

Discussion. Most of the earlier studies on maternal–fetal adrenal interrelations in the rat were based largely on fetal adrenal weight change after adrenalectomy, hypophysectomy, and substitution therapy in the mother (1–3). The results of this study, utilizing ACTH and corticosterone determinations, provide more direct evidence for a specific action of maternal adrenocorticosteroids on pituitary–adrenal interaction in the fetus. The findings clearly indicate that deficiency or excess of maternal corticoids produces significant change in the activity of the fetal pituitary–adrenal system. They strongly suggest also that corticoids secreted by the mother's adrenal cross the placenta in the perinatal period to inhibit ACTH secretion by the fetal hypophysis, thereby rendering the neonatal adrenal temporarily unresponsive to various stressing agents (12, 13, 19). The pattern of change in indices of adrenal function observed in neonates of adrenocortical hormone, deficient mothers can best be interpreted as resulting from stimulation of fetal ACTH secretion in the absence of a restraining influence by maternal corticosteroids. There was no evidence, however, to indicate that increased secretion by the fetal adrenal promoted any passage of corticosteroids from fetal to maternal compartments.

Schapiro (20) estimated pituitary ACTH content in the 1-day old rat to be equivalent to 2–3 mU. Our estimated values for ACTH stores in the pituitary at birth are slightly less (1–2 mU ACTH per gland; wet weight of 0.3–0.5 mg). The effectiveness of Dexa on suppression of ACTH secretion and adrenocortical function in rats receiving the drug in drinking water has already been demonstrated (16). The inhibitory effects of Dexa on the pituitary–adrenal system of mother and offspring were more marked than with Coac. The substantial increase in ACTH stores found in pituitaries of newborn from mothers receiving Coac in the drinking water rather than by injection is difficult to explain. Possibly, a critical circulating level is achieved in the fetus which promotes increased synthesis of ACTH while inhibiting release. This phenomenon has been described in the goitrous rat for TSH (21).

Although it appears that temporary blockage of fetal ACTH secretion by maternal and fetal corticosteroids does occur in the perinatal period, it is not yet clear whether the negative feedback effect is mediated directly at the level of the pituitary, or indirectly through hypothalamic CRF. Jost, Dupouy, and Monchamps (22) have demonstrated that encephalectomy of the rat fetus (16–19th day) results in adrenal atrophy; involution of the adrenal could be prevented by exogenous CRF, provided the pituitary was present. Hiroshige and Sato (10) reported that rat fetal hypothalamic tissue responds to stress by an immediate increment in endogenous CRF content. They have suggested that high levels of circulating corticosteroids in early neonates may suppress synthesis or release of hypothalamic CRF through the negative feedback mechanism. The relatively slow rate of corticosterone metabolism characteristic of the newborn rat conceivably could prolong the inhibition on ACTH secretion (14). Separate and opposite effects of adrenocortical steroids on hypothalamus and pituitary in late gestation have not been precluded.

Summary and conclusions. Deficiency or excess of adrenocorticosteroids in the mother is associated with significant alterations in activity of the fetal pituitary–adrenal system of the rat. Hypophysectomy of the pregnant rat results in increased weight and corticosterone content of the adrenal and depletion of pituitary ACTH in the neonate. Administration of corticosteroids to hypophysectomized pregnant rats produces atrophy of the newborn's adrenal and inhibition of ACTH secretion by the neonatal pituitary. It is postulated that maternal adrenocorticosteroids cross the placenta in the perinatal period to exercise a restraining influence on ACTH secretion by the fetal hypophysis.

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