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The Effect of Glucocorticoids on Plasma Insulin-Like Growth Factor I Concentration in the Rat Fetus

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ABSTRACT. Radioimmuno- and radioreceptor-assayable insulin-like growth factor I (IGF-I) concentration was determined in plasma pools of rat fetuses on gestation day 21 following treatment of the pregnant rats with pharmacologic doses of betamethasone, dexamethasone, or cortisone on gestation days 12 and 13. Dose-related reduction of plasma concentration of IGF-I occurred after betamethasone or dexamethasone treatment. There was no change in IGF-I concentration after cortisone treatment. The changes in fetal IGF-I concentration after steroid treatment were parallel between the two assays. Comparison of the results with previous data showing the effects of identical treatment of pregnant rats on fetal body and organ growth suggests that the IGF-I changes correlate better with reduction of liver/body ratio than with reduction of body weight. The findings indicate that growth retardation after steroid treatment in the fetus is in part the result of factors other than IGF-I. This may include a direct effect of the glucocorticoids on skeletal tissue. Reduced IGF-I concentration may contribute to the growth deficit. (*Pediatr Res* 22: 92–95, 1987)

Abbreviations

GH, pituitary growth hormone
IGF-I, insulin-like growth factor I, somatomedin-C
IGF-II, insulin-like growth factor II, MSA
RIA, radioimmunoassay
RRA, radioreceptor assay
SDS-PAGE, polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate
HPM, human placental membrane

Glucocorticoid treatment in the pregnant rat produces dose-related retardation of fetal somatic and visceral growth (1–4). The mechanism of glucocorticoid-induced growth impairment is unclear, although pathologic alteration of chondrocytes has been observed after high dose glucocorticoid administration to the fetal (5) and postnatal rat (6).

The role of IGFs (somatomedins) in glucocorticoid-induced fetal growth retardation is also not clear. The IGFs may serve as mitogens for fetal tissues as suggested by the presence in fetal tissues of specific membrane receptors for the IGFs, and the capability of multiple fetal tissues to synthesize IGF (7).

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The present study was carried out in order to elucidate the role of IGF-I in growth retardation of the glucocorticoid-treated rat fetus. Three different glucocorticoids were used in order to test for possible differences due to molecular specificity. Inasmuch as IGF-I is growth hormone dependent (8) plasma growth hormone was also determined in two of the steroid groups.

METHODS

Long-Evans rats obtained from Simonsen Labs (Gilroy, CA) were housed in the Animal Resource Facility of the University of California, Irvine, CA. Except during breeding, all animals were housed individually in stainless steel cages (hanging type) measuring 7 × 7 × 10 inches in size. The stock diet was Purina Lab Chow, St. Louis, MO. Food and tap water were provided *ad libitum*. Room air was fresh and filtered and was kept at 20.5–23.9° C; light/dark periods of 14/10 h were automatically controlled. Animal handling was carried out by the same attendant.

All experiments were carried out during the first pregnancy. The day of appearance of the vaginal plug was designated day 1 of gestation. All rats were weighed on day 12 of gestation prior to injections and again on day 21. At the time of weighing on day 12 the pregnant rats were sorted into experimental and control groups on the basis of body weight in order to provide similar means and variance of body weight between groups. The pregnant rats were injected subcutaneously on days 12 and 13 of gestation with one of the following: a mixture of betamethasone sodium phosphate and betamethasone acetate suspension (Celestone Soluspan, Schering Corp. Kenilworth, NJ) in a dose of 0.18 or 0.42 mg/injection, dexamethasone acetate suspension (Decardron-LA, Merck, Sharp & Dohme West Point, PA) in a dose of 0.12 or 0.24 mg/injection, or cortisone acetate suspension (Cortone Acetate, Merck, Sharp & Dohme) in a dose of 50 mg/injection. The lower dose of betamethasone and dexamethasone, based on the mean weights of the pregnant rats, was approximately 0.4 and 0.6 mg/kg, respectively. In terms of body weight the lower dose is somewhat greater than that used for betamethasone and dexamthasone in the prevention of respiratory distress syndrome in the human. The dose in that case may be 12 mg/day (9, 10) or 0.18 mg/kg in the case of a 65-kg woman. The cortisone dose, approximately 140 mg/kg, was made relatively large in proportion to the antiinflammatory potencies of betamethasone and dexamethasone doses in order to overcome the well-known resistance of the rat to teratogenic influences by cortisone (11, 12) and to lower the maternal weight gain to the same range resulting from betamethasone and dexamethasone treatments. Control rats were injected with physiologic saline. Fetuses were removed under ether anesthesia on day 21 of gestation and bled from the neck into micro tubes containing

dry heparin. The tubes were promptly centrifuged and the plasma pooled for each litter of fetuses. The plasma samples were stored at −60° C pending assay.

Human IGF-I was purified by the method of Spencer *et al.* (13). Human Cohn fraction IV-1 was extracted with acid-ethanol and the IGFs precipitated by acetone. The acetone precipitate was chromatographed on Sephadex G-75 and G-50, both in 1 M acetic acid. The IGF containing peak was subjected to isoelectric focussing on a flat bed of Sephadex G-75. The IGF focussing at pH 8.3 was eluted and purified to homogeneity by reverse phase high-performance liquid chromatography on a C_{18} column using an acetonitril gradient in 0.1% trifluoracetic acid. Homogenity was established by SDS-PAGE and a single N-terminal residue. The identity of IGF-I with somatomedin-C was verified by an amino acid composition in excellent agreement with the known integral values for this peptide, determination of the N-terminal 20 residues by microsequence analysis, and C-terminal carboxypeptidase digestion. The insulin-like activity in isolated fat cells of the purified IGF-I agreed with that of a pure preparation of IGF-I (courtesy of Herington AC) (14). It was biologically active in several assays: chicken embryo fibroblasts, Balb/c-3T3 cell progression assay, and cartilage sulfation activity performed by, respectively, Nissley *et al.* (15), Stiles *et al.* (16) and Jennings *et al.* (17). In the cell progression assay the maximum effect was achieved at 5 ng/ml which compares favorably with IGF-I purified by others.

The IGF-I antibody was prepared by Reber and Liske (18). Rabbits were immunized with human IGF-I purified by Ritschard and Roncari at Hoffman-La Roche (Basel, Switzerland) from human Cohn fraction IV. The immunizing material contained a single peptide as determined by physicochemical and N-terminal microsequence analysis, performed at a level that would detect greater than 10% contamination. This antibody has been used in our RIA (19) and that of Zapf *et al.* (20). Its cross-reactivity with rat somatomedin-C is 35% and with rat and human IGF-II it is 3%. No cross-reactivity was observed with growth hormone, prolactin, insulin, glucagon, epidermal growth factor, and bradykinin.

IGF-I was iodinated using solid-phase lactoperoxidase beads according to the protocol supplied by BioRad, Richmond, CA. The labeled polypeptide was separated from the free ^{125}I by Sephadex G-50 chromatography in 50 mM Tris-HCl pH 7.4 containing 0.25% human serum albumen. The specific activity averaged 200 $\mu Ci/\mu g$.

Several modifications have been incorporated into the IGF-I RIA (19). Samples were extracted with acid-ethanol to separate the binding protein by a modification of the method of Daughaday *et al.* (21). After extraction, samples were dried in a Speed Vac Concentrator, Savant Instruments, Farmingdale, NY, dissolved in assay buffer, and adjusted to pH 7.4 with 2 M Tris. The RIA buffer was 30 mM phosphate pH 7.4 containing 0.25% human serum albumen and 0.02% sodium azide. The assay volume was 0.4 ml and contained the rabbit anti-IGF-I antiserum in a final dilution of 1:22,000 and 15,000 cpm of ^{125}I-IGF-I. The incubation was carried out at 4° C for 18 h and the free ^{125}I-IGF-I was separated from bound by double antibody plus PEG precipitation. Fifty percent displacement in the assay was at 2 ng; the sensitivity was 0.4 ng. The interassay and intraassay variations were 12 and 15%, respectively. The mean ± SD values for normal adults and acromegalics are 260 ± 80 and 812 ± 90 ng/ml.

Details of the IGF-I HPM radioreceptorassay have been reported except that samples were extracted with acid-ethanol and the IGF-I used as ligand and standard was purified as described above (22). The receptor specificity of the HPM radioreceptorassay is such that it is primarily a measure of IGF-I but other IGFs cross-react and it may be possible to detect large changes in their concentration.

GH was determined in duplicate by RIA (23). All samples in the present study were assayed in the same run. The interassay and intrassay variations for the GH RIA were 6.7 and 6.2%, respectively.

Statistical significance was determined by a one-tailed Student's *t* test.

RESULTS

Plasma IGF-I. The results of determinations of IGF-I by RIA and RRA and of GH are shown in Table 1. The single dose level of cortisone resulted in no significant change by either assay. This was also true of the low dose levels of betamethasone and dexamethasone. The high dose levels of both betamethasone and dexamethasone resulted in a significant decrease of IGF-I by either assay. The changes were approximately proportionate between the two assays. GH concentration was decreased in the two betamethasone pools; however, the result was significant only at the 0.18-mg dose level. Greater variability accounted for the lack of significance at the higher dose level. There was no decrease in GH concentration with either dose of dexamethasone.

DISCUSSION

The present experiments indicate significant and parallel reduction of immuno- and receptor-assayable IGF-I in fetal plasma after treatment of the pregnant mothers with high doses of either betamethasone or dexamethasone. Lower doses of either steroid did not produce significant differences from control levels. Treatment with cortisone produced no change in IGF-I concentration as measured by either assay. Betamethasone treatment resulted in decreased fetal plasma GH concentration; dexamethasone had no effect on GH concentration.

We have previously reported the effects on fetal and organ weights in identically treated pregnant rats (4). Fetal body weight was significantly reduced by betamethasone and dexamethasone in both low and high doses and by cortisone in the same dose used in the present experiments. Cortisone treatment resulted in a quantitatively minor, although significant, reduction of the liver/body weight ratio. Betamethasone and dexamethasone resulted in a greater reduction of liver/body weight ratio. The relative preservation of the liver/body ratio in cortisone treatment may account for the normal levels of IGF-I in the cortisone-

Table 1. *IGF-I concentrations in fetal plasma pools determined by RIA and RRA (mean ± SE)**

Group	Dose (mg/day)	N_P	N_F	RIA (ng/ml)	*p*	RRA (ng/ml)	*p*	GH (ng/ml)	*p*
Control	0	6	7–13	59.5 ± 1.1		223 ± 18		190 ± 21	
Cort	50.0	5	10–14	55.8 ± 2.4	NS	232 ± 23	NS		
Bet	0.18	5	7–11	53.4 ± 5.1	NS	182 ± 17	NS	146 ± 7	<0.05
Bet	0.42	5	7–12	36.8 ± 3.3	<0.005	170 ± 16	<0.05	148 ± 21	NS
Dex	0.12	5	8–12	52.9 ± 6.1	NS	249 ± 9	NS	198 ± 32	NS
Dex	0.24	5	8–12	39.0 ± 3.5	<0.005	151 ± 6	<0.005	182 ± 12	NS

* Cort, cortisone; Bet, betamethasone; Dex, dexamethasone; N_P, number of litters pooled; N_F, number of fetuses/pool, given as a range; *p*, probability by one-tailed *t* test.

treated fetuses. The reduction in IGF-I after betamethasone and dexamethasone correlated with the size of the dose in the present experiments as did the previously reported reduction in liver/body ratio (4).

The fetal skeleton is not uniformly affected at all dose levels of glucocorticoids. Using similar dosage schedules we have found that the lower dose of betamethasone or dexamethasone stimulated maturation of the appendicular skeleton and slowed maturation of the axial skeleton in the fetal rat, while the higher dose retarded maturation in both regions (24). In addition, the effects on fetal organ weight have been shown to be disproportionate, with relatively greater impairment of the liver/body weight ratio than of the corresponding organ/body weight ratios of brain, heart, or kidneys (4). It is evident from those experiments that there are regional and tissue differences in the response to the steroids.

Under certain *in vitro* conditions glucocorticoids stimulate skeletal growth and maturation. Hydrocortisone at 14 μM in the media has been found to extend lifespan and to increase the rate of proliferation of cells and the rate of DNA synthesis in cultures of fibroblast-like human cells. Higher concentrations were suppressive (25). It has been shown that virus-transformed chick embryo fibroblasts lose the inhibitory response to hydrocortisone in concentrations 10^5 times that capable of producing 50% inhibition of normal cells (26). Steroids may directly stimulate cartilage maturation by amplifying the activity of pituitary and brain-derived growth factors (27). That chondrocytes are capable of producing IGF-I *in situ* (28) suggests that regional differences may result from differences in perfusion of the regions with exogenous steroid, differences in numbers of IGF-I-producing chondrocyte clones, or other, as yet undetermined, factors having an influence on the paracrine actions of IGF-I.

Recent findings indicate that growth hormone may directly regulate clonal expansion of differentiated chondrocytes producing IGF-I in the growth plate (28). In the rat fetus at 17 days gestation plasma levels of IGF-I are slightly below the maternal plasma level, while IGF-II levels greatly exceed the maternal concentration (29). High levels of fetal plasma IGF-II relative to maternal concentration of IGF-II and to fetal IGF-I concentration have also been observed in the sheep (30) and guinea pig (31). While it has been shown that somatomedin (IGF-I) has a mitogenic effect in fetal human cartilage (32), the relevance of fetal plasma concentration of IGF to fetal skeletal growth is uncertain in view of the evidence that IGF-I is synthesized in many organs (33). However, radioimmunoassayable plasma IGF-I of human fetuses, aged 15–23 wk, was found to correlate significantly with fetal body weight, placenta weight, crown-rump length, and crown-heel length; no correlation was found between radioimmunoassayable IGF-II and body weight, length, placenta weight, or gestational age (34). Liver and serum IGF-I in fetal rats correlated with fetal weight in growth retardation produced by uterine artery ligation (35). Recent studies have shown divergence patterns of thymidine uptake stimulating activity from immunoassable IGF-I concentration in blood of human fetuses at gestational ages from 21–28 wk (36) and cord blood of human neonates (37) suggesting that factors other than IGF contributing to thymidine activity may also play a role in fetal growth.

The reduction in GH concentration in the betamethasone-treated fetuses has its counterpart in the human exposed in late gestation to betamethasone (38). Comparable human data are lacking for dexamethasone. In an earlier study, we found no difference between the two steroids in their effect on fetal growth and on brain/body and liver/body weight ratios. On the other hand, marked differences existed with regard to heart/body, kidney/body, and adrenal/body weight ratios (4). The differences between the tissue effects of betamethasone and dexamethasone are thus not likely to be related to differences in placental passage but, rather, to modulation of the steroid effect by differences in concentration and affinity of specific tissue steroid receptors. An example of steroid receptor specificity occurs in the fetal lung cell line W1-38 which has high affinity binding sites which are steroid-molecular structure specific (39). A difference in hypothalamic and/or pituitary tissue steroid receptors in the fetal rat could account for the present observations.

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