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Dexamethasone glucocorticoids
fetus plasma

Distribution of Dexamethasone between Mother and Fetus after Maternal Administration

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Summary

The adrenal glucocorticoid hormones are believed to have an important role in the definitive biochemical differentiation of a number of fetal organs. This paper presents data for dexamethasone transfer and distribution between mother and fetus using 20–21 days pregnant rats. This synthetic glucocorticoid is rapidly transferred to the fetus and reaches a plateau by 90 min after administration to the mother. Pregnant rats receiving either a high dose (20 mg) or a low dose (2.2 μg) had almost identical concentration distribution ratios with a mean of 0.311 (± 0.013) and 0.332 (± 0.019) between fetal and maternal plasmas, respectively. *In vitro* binding measured by equilibrium dialysis showed that maternal plasma bound only 4–5% more than the fetal plasma. Essentially no radioactivity remained bound to protein after Sephadex G-50 chromatography, indicating no high affinity sites for dexamethasone in either maternal or fetal plasma. Acetylation of ethyl acetate extracts of fetal and maternal plasma followed by paper chromatograph revealed two identical peaks for both the dexamethasone standard and the plasma radioactivity.

This study demonstrates a rapid transfer of administered dexamethasone from the mother to the fetus with a concentration difference between the two plasma pools. The difference in concentration does not appear to be the result of differential protein binding or metabolism.

Speculation

The rat placenta would appear to maintain an appreciable concentration difference between maternal and fetal plasma for the glucocorticoid hormones. The nature of the process which accounts for this phenomenon needs further investigation.

The adrenal glucocorticoid hormones are believed to have an important role in the definitive biochemical differentiation of a number of organs (19). The administration of synthetic glucocorticoids to mothers with threatened premature delivery has been advocated to accelerate pulmonary development in the fetus (1, 13). The site of action has been proposed to be the type II pneumocyte and the action to be an increased rate of synthesis or secretion of surfactant (7, 11).

The rate of transfer to the fetus and the fetal body fluid, as well as the tissue content for a given dose of hormone and the metabolic fate of the synthetic glucocorticoids, has received little attention. This information may be important because the optimal dose of glucocorticoid for accelerating phospholipid synthesis in fetal lung is 10^{-8}–10^{-9} M in organ culture (8). Concentrations above this inhibit growth and phospholipid, DNA, and protein synthesis (8). This paper presents data for dexamethasone transfer and distribution between mother and fetus using 20–21 day pregnant rats.

MATERIALS AND METHODS

Time-bred Sprague-Dawley rats (300–350 g) were obtained from Charles River Laboratories, Wilmington, MA, and acclimated 5 days before study. Dexamethasone phosphate (16β-^{3}H-labeled, spec act 2.58 mCi/mmol) was a gift of Merck Sharp and Dohme, Rahway, NJ. Dexamethasone (1,2,4-^{3}H-labeled spec act 30.7 Ci/mmol) was obtained from New England Nuclear, Boston, MA. The specific dexamethasone antibody used for the radioimmunoassay was kindly given to us by Dr. Dan Tulchinsky, Harvard Medical School, Boston, MA. The antibody was prepared as described by Meikle *et al.* (15).

SAMPLING TECHNIQUE

The hormone was administered to the pregnant rats by two routes, ip and po. Dexamethasone phosphate was used for the ip injections and dexamethasone as the free alcohol was given by gavage in 1 ml saline. The animals were maintained under light anesthesia using ip sodium phenobarbital (4 mg/100 g body wt) supplemented with ether. The fetuses were exposed using a midline incision and removed serially for time points. The incision was covered with moist saline packs between collection times. Blood was obtained by cardiac puncture from the mother and by decapitation from the fetuses. The serum (initial experiment, only) or plasma was separated after centrifugation and either added to counting solution (Aquasol, New England Nuclear, Boston, MA) directly or stored at −70° for analysis.

DEXAMETHASONE ASSAY

The plasma or serum radioactivity was measured by adding aliquots of 100 μl or less to 10 ml Aquasol (New England Nuclear). Counting efficiency was determined by internal standard. For the dexamethasone radioimmunoassay (RIA), the antiserum was diluted 1:1000, and the plasma to be assayed was diluted 1:2000, 1:4000, and 1:8000. The buffer used contained NaH_2PO_4 (0.01 M), NaCl (0.14 M), EDTA-Na_2 (0.01 M), and NaN_3 (0.015 M), pH 7.8. The [^{3}H]dexamethasone (0.1 ml, 20.8 pg), diluted antiserum (0.1 ml), and diluted plasma (0.1 ml) were incubated at 37° for 30 min, were transferred to an ice bath for 15 min, then 0.5% dextran-coated charcoal in 0.5% gelatin solution (0.5 ml) was added, vigorously mixed, and centrifuged at 4° for 20 min. The supernatant solution was decanted into Multisol (Isolab) counting solution for assay of the radioactivity. A standard curve was run with each assay using 1, 2, 4, 8, and 16 μg cold dexamethasone. The percentage of maximum binding (B/B_0) was plotted on logit-log paper against the dexamethasone concentration and the displacement by the plasma calculated directly from the linear plot. Maximal concentrations of corticosterone (300 ng/ml) and progesterone (140 ng/ml) reported for Sprague-Dawley rats during pregnancy (9, 16) caused no displacement of [^{3}H]dexamethasone from the antiserum in the dilutions of plasma used for assay.

PLASMA BINDING STUDIES

High affinity binding of [^{3}H]dexamethasone to fetal and maternal plasma was measured using a 25 × 1.25 cm Sephadex G-50 column equilibrated with 0.05 M NaH_2PO_4 buffer, pH 7.4 (21).

Low affinity binding was measured by equilibrium dialysis (21). Cellulose tubing (Union Carbide) with a pore size of 2.4 mμ was used. The time required for equilibrium was determined by adding labeled hormone both inside and outside tubing which contained plasma diluted 1:10 with 0.05 M NaH_2PO_4 buffer, pH 7.4 in separate baths. Equilibrium was achieved by 22 hr at 37°. The volume of the buffer in the bath was 2 times the sum of the bag volumes.

CHROMATOGRAPHY

The radioactivity in pregnant rat plasma was extracted with 15 vol ethyl acetate and compared to a radioactive dexamethasome standard by paper chromatography (20). Whatman no. 1 paper strips (3.5 × 55 cm) were run in a preequilibrated tank using benzene-methanol-water, 1:1:1, to develop the chromatogram. The experiment was repeated after acetylation of the plasma extract and standard using 0.25 ml pyridine and 0.25 ml acetic anhydride at room temperature for 24 hr (14). The acetate derivatives were chromatographed using the same system. Chromatograms were cut into 1-cm lengths which were placed in 10 ml Aquasol on a shaker for 2 hr; radioactivity was quantitated by scintillation counting.

RESULTS

The time course for the appearance of radioactivity in fetal serum after the ip injection of [^{3}H]dexamethasone phosphate into pregnant rats at 20 and 21 days of gestation is shown in Figure 1 for three animals. The maximum values in the fetal plasma ranged between 5.2 and 8.8 × 10^4 cpm/ml.

The data have been normalized by representing values at each time point as a percentage of the maximal levels attained in the fetal blood. The dexamethasone is rapidly transferred to the fetus and reaches a plateau by 90 min after injection of the mother. This state is maintained for at least 6 hr after the injection.

DISTRIBUTION OF DEXAMETHASONE BETWEEN MATERNAL AND FETAL PLASMA

The distribution of radioactivity between fetal and maternal plasma in four pregnant animals, 20 days of gestation, was measured after 90 min. Two animals were injected with large amounts of [^{3}H]dexamethasone (as the phosphate) and two received the high specific activity hormone by gavage using 10^4 less material. Fetuses were removed at times between 90 and 360 min and the mothers were bled at the end of the experiment (240 or 360 min). In both the animals receiving a high dose (20 mg) and the animals receiving a low dose (2.2 μg), the distribution was almost identical with mean ratios of 0.311 (± 0.013) and 0.332 (± 0.019) between fetal and maternal counts.

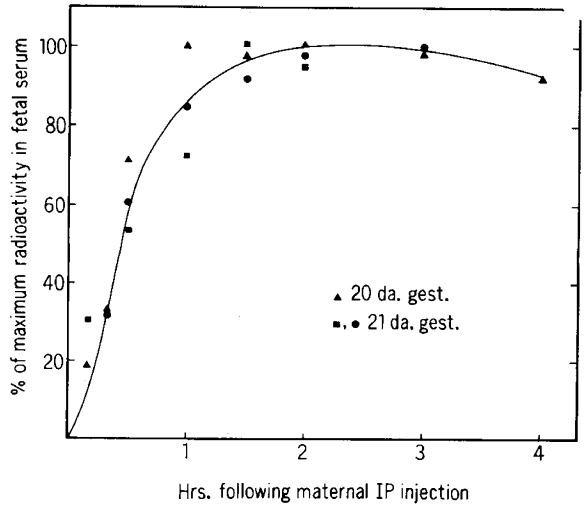

Fig. 1. Dexamethasone (16β-^{3}H, 100 μCi, 20 mg) was injected into three pregnant rats on either the 20th or 21st day of gestation. The radioactivity in the fetal serum was measured at intervals up to 4 hr after injection. The data have been normalized by representing values at each time point as a percentage of the maximal levels attained in the fetal blood from each litter. The initial rate of increase is approximately 3%/min with a plateau being achieved after 90 min.

Table 1. *Dexamethasone concentration in maternal and pooled fetal plasma*

	Conc, μg/ml[1]		
	Fetal	Maternal	Fetal/ maternal
Experiment 1	12.7 ± 0.3	27.9 ± 0.4	0.46
Experiment 2	13.8 ± 0.9	27.2 ± 0.1	0.51
Calculated conc			
Experiment 1	12.0 ± 0.3	31.1	0.38
Experiment 2	12.4 ± 0.2	38.4	0.32

[1] Mean ± SEM for five determinations.

Table 2. *Radioactive dexamethasone distribution between fetal and maternal plasma at equilibrium*[1]

			cpm/ml plasma (×10^{-3})		
Animal	Amount	Time (min)	Fetal	Maternal	Fetal/ maternal
1	20 mg, 100 μCi ip	90	51.2		0.264
		120	52.5		0.352
		180	51.7		0.266
		240	48.3	149.2	0.324
2	20 mg, 100 μCi ip	90	50.1		0.307
		180	53.5		0.328
		240	54.7	163.1	0.335
			51.8[1]		0.311[1]
			±0.7[2]		(±0.013)[2]
3	2.2 μg, 128 μCi po	120	40.9		0.235
		180	59.7		0.343
		240	50.9		0.292
		300	52.2		0.300
		360	50.3	174.2	0.289
4	2.2 μg, 128 μCi po	120	58.2		0.457
		180	42.5		0.334
		240	45.6		0.358
		300	48.5		0.381
		360	42.4	127.3	0.333
			49.12[1]		0.332[1]
			±2.04[2]		(±0.019)[2]

[1] Mean.
[2] SEM.

This apparent concentration difference between fetal and maternal plasma was confirmed by RIA for dexamethasone. Twenty milligrams of dexamethasone (phosphate) were injected into two pregnant animals and all fetuses were removed at 180 min. The fetal blood was pooled and the concentration of dexamethasone in the fetal and maternal plasma measured using RIA. The mean concentrations in five aliquots for the two animals are shown in Table 1. The fetal and maternal concentrations were almost identical in the two animals with an average of 13.2 μg/ml in the fetal and 27.5 μg/ml in the maternal plasma. The fetal to maternal distribution ratio is approximately 0.48.

The data in Table 2 and the specific activity of the injected material, 2.58 mCi/mmol, were used to calculate the concentration of dexamethasone from the average disintegrations per min in the fetal plasma. The counting efficiency in this system for ^{3}H was 38%. The average disintegrations per min per ml for the fetal plasma from animal 1 is 1.33 × 10^5 and for animal 2 is 1.38 × 10^5.

The corresponding disintegrations per min per ml for animal 1 is 3.90×10^5 and animal 2 is 4.28×10^5. The calculated concentrations for the fetal plasma of 12.0 and 12.4 μg/ml are in close agreement with the concentration in fetal plasma measured by the RIA technique. The calculated concentrations for the maternal plasma are higher than the values found by RIA. This may be due to the presence of a radioactive degradation product of the labeled dexamethasone which does not react with the dexamethasone antibody.

DEXAMETHASONE BINDING BY FETAL AND MATERNAL PLASMA

The binding of dexamethasone to fetal and maternal plasma was estimated by equilibrium dialysis using two concentrations in the dialysis buffer, 1.0 and 500 ng/ml. Two maternal and two fetal plasma samples (0.4 ml diluted to 4.0 ml) were placed in each 32-ml bath. The buffer was stirred with a magnetic stirrer and the dialysis performed at 37° for 24 hr in a tissue culture incubator.

The per cent of the hormone bound to the diluted plasma is shown in Table 3. The binding was independent of dexamethasone concentration in the range tested and the maternal plasma bound 4–5% more than the fetal plasma per unit volume. The amount of protein added to each bag was 27.9 mg for the maternal and 9.66 mg for the fetal plasma. When the amount bound was extrapolated to that expected for undiluted plasma using the esperimentally determined protein concentration (21), the maternal plasma binding was 2–12% higher than the fetal.

The binding measured by equilibrium dialysis includes both low and high affinity sites (21). Fetal and maternal plasma (0.2 ml) labeled *in vivo* by administering the radioactive hormone was chromatographed at 4° using Sephadex G-50 to measure the high affinity sites for dexamethasone. The protein (measured by absorbance at 280 nm) and the radioactivity were measured in each 0.3-ml fraction from the column. Essentially no radioactivity remained bound to protein in either the maternal or fetal plasma under these conditions, indicating no high affinity sites for dexamethasone.

CHARACTERIZATION OF PLASMA RADIOACTIVITY

The dexamethasone concentration in fetal plasma calculated by using the specific activity of the injected material was in excellent agreement with the concentration estimated by RIA. The calculated concentration for maternal plasma was higher than the concentration estimated by RIA, suggesting that 7–10 μg of a metabolite not recognized by the dexamethasone antiserum was present.

Plasma from five pregnant animals removed 22–24 hr after injection with [^{3}H]dexamethasone (30.4 Ci/mmol) was extracted with 15 vol ethyl acetate. The mean fetal to maternal ratio of

Table 3. *Equilibrium dialysis of maternal and fetal plasma samples at 37°*[1]

	% Bound	
	1:10 Dilution of plasma	Extrapolated to undiluted plasma[2]
Maternal plasma		
1 ng/ml dexamethasone	14.8	59.3
	14.2	58.9
500 ng/ml dexamethasone	14.3	58.6
	21.3	68.1
Fetal plasma		
1 ng/ml dexamethasone	10.9	51.9
	10.1	50.4
500 ng/ml dexamethasone	12.8	55.8
	12.8	56.1

[1] Each value represents a single determination.

[2] Assumes combining affinity does not change with dilution.

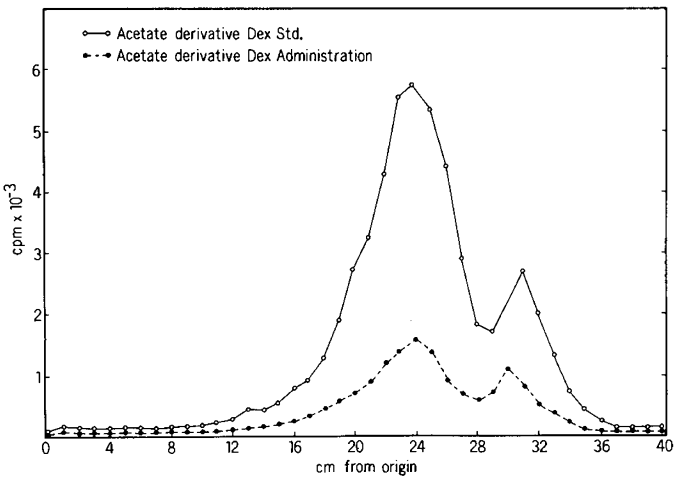

Fig. 2. Standard [^{3}H]dexamethasone added to 3 ml plasma, extracted with 15 vol ethyl acetate, acetylated, and chromatographed using benzene-methanol-water, 1:1:1, is compared with the average values for plasma from five pregnant rats injected with 100 μCi [^{3}H]dexamethasone 22–24 hr before bleeding. The solvent front was 40 cm and the R_f for the two peaks was 0.56 and 0.74. No dexamethasone metabolites are apparent in the maternal plasma extract.

radioactivity in plasma at this time was 0.456 ± 0.066. The mean recovery of total radioactivity in the ethyl acetate extract of the plasma was 84.8%. Recovery of standard dexamethasone added to plasma was 97.5%. The plasma extracts from the five animals and pooled fetal plasma samples from each litter were acetylated and the reaction mixtures evaporated to dryness using nitrogen. Standard radioactive dexamethasone added to plasma was carried through the extraction, acetylation, and the chromatographic procedures. The chromatograms were cut into 1-cm strips and a plot of the radioactivity found is shown in Figure 2. Two identical peaks were found for the standard dexamethasone and the plasma radioactivity with R_f values of 0.56 and 0.74, respectively. Unacetylated samples and standards chromatographed using the same system gave single radioactive peaks with R_f values of 0.1.

DISCUSSION

The adrenal glucocorticoid hormones are believed to cross the plasma cell membrane by free diffusion. This has been shown only for thymocytes where the process of entry into the cell requires no more energy than that of free diffusion (12). One factor which modifies the equilibrium distribution of hormones between plasma and the extravascular space is binding of the hormone to the plasma corticosteroid-binding globulin (CBG) (5). Increased maternal CBG has been used to explain the higher concentration in maternal compared to fetal plasma for the physiologic glucocorticoids (6). The synthetic glucocorticoid dexamethasone is not bound specifically to the high affinity sites on CBG (18). These observations suggest that when the hormone is administered to the mother, dexamethasone should reach an equilibrium between maternal and fetal plasma with no appreciable difference in concentration. This has been reported to be the case for the human delivered at term by cesarean section (17).

The data presented here have demonstrated dexamethasone concentration reached a constant ratio between the maternal and fetal plasma 90 min after administration either as the phosphate ip or the alcohol po. The distribution appeared to be independent of concentration up to 60 mg dexamethasone/kg body wt. Between 90 and 240 min after administration the concentration in fetal plasma over a 10^4-fold dosage range was 0.3–0.5 that found in maternal plasma. Removing the fetuses serially from a single mother did not appear to introduce artifacts in the distribution since there was excellent agreement between the experiments where serial removal was used and where all fetuses were removed at the same time 3 hr after injection.

The explanations considered for the difference in concentration observed between maternal and fetal plasma were as follows: 1) the maternal plasma binds dexamethasone to a higher degree than does fetal plasma; 2) the dexamethasone has been metabolized to a product preferentially retained in the material circulation; 3) the placenta maintains the concentration difference by some active, nonsaturable process; 4) an equilibrium was not achieved during the observation period.

The data presented demonstrated no difference between fetal and maternal plasma in either low affinity, high capacity binding (estimated by equilibrium dialysis) or high affinity, low capacity binding (estimated by Sephadex gel chromatography) for dexamethasone. This held even when the equilibrium dialysis data were extrapolated to undiluted plasma and takes into account the 3-fold difference in protein content of the maternal and fetal plasma (21). Thus, preferential binding by either specific or nonspecific sites appeared to be excluded as an explanation for the concentration difference.

The metabolic fate of dexamethasone has not been studied in detail. The plasma half-time after oral and iv administration is approximately 4 hr in the human (10, 15). The products of dexamethasone metabolism in the urine are predominantly unconjugated with approximately 15% being excreted in conjugated form (10).

Based on ethyl acetate extraction, 15% of radiolabeled dexamethasone appears to be a water-soluble derivative in maternal rat plasma 6 hr after administration. This fraction cannot account for the difference in concentration between maternal and fetal plasma. When the ethyl acetate-extractable material was chromatographed as the acetate derivative, no differences in radioactive peaks were found between the dexamethasone standard and samples from plasma of injected animals. Two peaks were present after acetylation of the standard and plasma samples. The front running peak is assumed to be the diacetate of dexamethasone and the major peak, the 21-monoacetate (20).

This study demonstrates a rapid transfer to the fetus of dexamethasone administered to the mother with a concentration difference between the two pools. The difference in concentration does not appear to be the result of differential binding or metabolism. Similar differences are reported for both natural and synthetic glucocorticoids (2–4). We assume this difference is maintained by an active process in the placenta, but this speculation needs to be confirmed experimentally.

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