Corticosteroids, Ornithine Decarboxylase Activity and the Incorporation of Leucine, Uridine, and Thymidine into Mouse Placenta

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Summary

The *in vitro* incorporation into mouse placenta of [¹⁴C]leucine, [³H]uridine, and [³H]thymidine fell between gestational days 14 and 19 by 61, 30, and 72%, respectively; ornithine decarboxylase activity fell by 75%. Injection of dexamethasone resulted on day 14 in values normally found between days 15 and 18. These changes coincided with rising activity in fetal liver of the enzyme 11 β -hydroxysteroid:NADP oxidoreductase, which reduced the abundant 11-dehydrocorticosterone to the active hormone, corticosterone. These events appear to be part of a spectrum of effects produced by corticosteroids on fetal growth at this time, probably mediated primarily by fetal liver.

Speculation

The events induced by corticosteroids in placenta and brain involve changes in the enzyme which interconverts corticosterone and its inactive 11-dehydrometabolite. The same relationship has been reported between this enzyme and maturation of rat and rabbit lung, but at a later state of gestation. This suggests that regulation of these phenomena is not simply due to a rise in circulating hormone level, but involves enzyme activities in tissues which function to some extent independently.

Introduction

Two recent reports have related effects of corticosteroids in fetal tissues to the enzyme which increases corticosterone by reduction of 11-dehydrocorticosterone. The latter is abundant in fetal tissues until late gestation, and reduction to the 11 β -OH configuration increases the amount of active hormone at the expense of the inactive metabolite. Inhibition of this reaction prevented maturation of fetal rat lung, as measured by phospholipid synthesis (5). Rising reductase activity in fetal mouse liver (3) coincided with a decrease in the dehydrogenation reaction in mouse brain, which was considered responsible for a marked decrease in the rate of incorporation of leucine, uridine and thymidine into fetal mouse brain (7). This communication describes similar effects in mouse placenta, indicating an action of corticosteroids on this tissue.

Materials and Methods

The mice used were of the UBC Swiss strain, raised at the Zoology Department Vivarium at this University. The timing of pregnancies, the preparation and incubation of tissues and measurement of incorporation of leucine- 3 H (U), uridine- 3 H and thymidine methyl- 3 H into the acid-insoluble components of tissues have been described earlier (7). The preparation of 11-dehydrocorticosterone-4- 14 C and incubation of it and corticosterone-4- 14 C with tissues and the preparation of autoradiograms has also been described therein. The assay for ornithine decarboxylase activity by collection of 14 CO $_2$ liberated from ornithine-1- 14 C has been described elsewhere (6).

Results

The reduction of 11-dehydrocorticosterone to corticosterone in fetal mouse liver and placenta on gestational days 14 and 17 is illustrated in Fig. 1. In similar experiments the dehydrogenation of corticosterone was also followed in these tissues. The ratio of reductase/dehydrogenase activity increased in liver from 0.1 to 7 between days 14 and 17. In placenta reductase activity remained high at all times, the ratio was over 4.

Table 1 gives the values for the incorporation of radioactive substrates into placenta on gestational days 14, 16 and 19. In all cases there was a significant decrease in the rate of incorporation after day 14, when changes in liver reductase activity began. This continued for several days with the greatest change between days 14 and 16. The values for leucine, uridine and thymidine fell by 61, 30 and 72%, respectively, of those on day 14. Ornithine decarboxylase activity followed a similar pattern (Table 1), declining by 75% on day 19. That these events can be attributed to the steroid is indicated by the fact that injection of dexamethasone on day 13.5 resulted in values on day 14 for all parameters which were reduced to a level found normally on days 15 to 18 (Table 1).

Microscopic examination of sections from treated placentas did not reveal any significant differences from controls.

Discussion

Until gestational day 14, 11-dehydrocorticosterone in fetal-placental tissues predominates over the active hormone by at least eightfold. This was attributed in earlier work (1) to dehydrogenase activity in fetal tissues and placenta. Subsequently, however, using double isotope labelling of substrates to ascertain both dehydrogenase and reductase activities simultaneously, it was found that the ratio of reduction/dehydrogenation in placenta remains around 4 (Fig. 1), while the brain is the major site of dehydrogenation (3,7). Conceivably the placenta functions to reduce the metabolite formed in fetal tissues before returning it to the maternal compartment. By contrast, reductase activity in fetal liver increased greatly, changing the ratio of reduction/dehydrogenation from 0.1 to 7 between days 14 and 17. It is probably this activity which is the major factor in raising corticosterone concentrations in the fetal circulation and in blood entering the placenta from the fetus (3).

The presence of corticosteroid receptors in cytosol and nuclei of placenta has been reported (8), suggesting that this is a "target" organ. The induction of specific biochemical events in placenta by corticosteroids, however, does not appear to have been reported previously. The decrease in the incorporation of leucine, uridine and thymidine observed here (Table 1) is similar to that observed in fetal brain (7), also attributed to an increase in active corticosteroid. In both instances these events could be produced prematurely by the injection of a potent synthetic corticoid, dexamethasone. The synthetic compound was used in these experiments because it escapes to a large extent the metabolic regulation which limits the passage of hormone from mother to fetus, with the result that a more effective dose reaches the fetus (9). It was found that, 16 hours after injection, 6% of the dexamethasone remained in the maternal circulation. After 15 minutes, there was estimated (9) to be 60 ng/mg fetal protein. Since the receptor site concentration in brain (7) and in placenta (8) is less than 1 pmole/mg protein, it seems likely that receptors were saturated and the effect of the dexamethasone maximal. While there was variation in the response, it should be pointed out that the stimulus here is being applied 1 to 2 days earlier than the rise in corticosteroid which occurs in the normal course of development, (3, Fig. 1) and this might modify the magnitude of the response in a tissue. In any case, all treated fetuses responded, with the values obtained being in the range normally observed between gestational days 15 and 18.

The decrease in ornithine decarboxylase activity paralleled that of thymidine incorporation in both placenta (Table 1) and in fetal brain (7). Ornithine decarboxylase is an indicator of change in metabolic or proliferative activity of tissues induced by hormones (6). A similar decline in thymidine kinase activity at this stage has been reported in rat placenta (6). These data suggest a decrease in the rate of several biosynthetic processes in fetal tissues and placenta of which DNA is the most affected. Corticosteroid-induced maturation of fetal rabbit lung was accompanied by decreased growth and decreased cell division both *in vivo* (2) and *in vitro* (4). Normal maturation could be inhibited by a steroid, 11-keto-progesterone, which inhibited 11 β -reductase

activity in fetal rat lung (5). These events appear to be part of a spectrum of effects of corticosteroids on fetal growth, mediated primarily by changes in the activity of the enzyme, 11 β -hydroxysteroid:NADP oxidoreductase, which might function in different tissues somewhat independently.

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Table 1. Ornithine decarboxylase activity and the *in vitro* incorporation of 14 C-leucine, 3 H-uridine and 3 H-thymidine into mouse placenta.

Gestational Day	Ornithine decarboxylase, pmoles CO $_2$ /mg protein	Incorporation, dpm/mg protein of:		
		Leucine	Uridine	Thymidine
14	13008 $\pm$ 1491	759 $\pm$ 96	1017 $\pm$ 44	1040 $\pm$ 132
16	9243 $\pm$ 562	333 $\pm$ 17	633 $\pm$ 83	396 $\pm$ 81
19	3179 $\pm$ 609	304 $\pm$ 85	713 $\pm$ 89	190 $\pm$ 33
14 + DEX*	6170 $\pm$ 1330	465 $\pm$ 40	725 $\pm$ 105	641 $\pm$ 55

*Dexamethasone, 200 μ g, was injected subcutaneously 16 hr earlier.

Ornithine decarboxylase activity was assayed by incubating the 20,000 $\times$ g supernatant of placenta with 50 nCi 14 C-D,L-ornithine for 30 min, following which 14 CO $_2$ was trapped in hyamine. 14 C-leucine, 0.14 μ Ci, 3 H-uridine, 0.46 μ Ci and 3 H-thymidine, 0.46 μ Ci, were incubated in 1 ml bicarbonate buffer pH 7.4 for 1 hr. The tissue was then homogenized, an aliquot removed for protein determination, and 0.1 vol 30% TCA was added. The precipitate was washed 4x with 3% TCA and then solubilized and counted. Each figure is a mean $\pm$ SEM of 4 to 7 values from different litters. The difference between values on day 14 and day 16, and the values on day 14 vs day 14 + DEX are all significant, $P < 0.01$.

Fig. 1. Autoradiograms of chromatographed extracts of mouse fetal liver and placenta incubated with 14 C-11-dehydrocorticosterone.

Liver from fetuses of a litter, or 2 placentas were minced finely and incubated in 1 ml buffer pH 7.4 with 5 nCi 14 C-11-dehydrocorticosterone for 15 min. The mixture was extracted with CH $_2$ Cl $_2$ which was evaporated and spotted onto silica gel TLC sheets, developed in first, hexane:ethyl acetate (4:1) and secondly, toluene:chloroform:methanol:water (120:60:20:1; v/v/v). A: 11-dehydrocorticosterone; B: corticosterone. 1, fetal liver, day 14; 2 fetal liver day 17; 3, placenta, day 14; 4, placenta day 17. Note the increase in the size of the B zone in liver on gestational day 17.