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IN VITRO STEROIDOGENESIS OF THE FETAL ADRENAL GLANDS AFTER DEXAMETHASONE ADMINISTRATION TO PREGNANT RATS*

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SUMMARY

The *in vitro* synthesis of corticoids from [4-^{14}C] progesterone by the adrenal glands of rat fetuses after treatment of intact and adrenalectomized females with dexamethasone was investigated. Dexamethasone strongly inhibited fetal adrenal growth and fetal adrenal synthesis of corticosterone. Fetal adrenal glands converted [4-^{14}C] progesterone to deoxycorticosterone (DOC), corticosterone, 18-hydroxy-11-deoxycorticosterone (18-OH-DOC), 18-hydroxy-corticosterone (18-OH-B) and aldosterone. Dexamethasone markedly suppressed transformation of [4-^{14}C] progesterone *in vitro* to corticosterone, DOC, and 18-OH-DOC and increased production of 18-OH-B and aldosterone. These results indicate that fetal adrenal growth and fetal adrenal synthesis of DOC, corticosterone and 18-OH-DOC, but not of 18-OH-B and aldosterone, are controlled by fetal ACTH during the last days of intrauterine development.

INTRODUCTION

The factors involved in the development of fetal steroidogenesis are insufficiently understood. While adrenalectomy of the pregnant rat increases the size of the fetal adrenals and their corticosterone production[1–6], high concentration of maternal plasma corticosterone inhibits fetal adrenal growth and fetal adrenal steroidogenesis [2–9]. Fetal ACTH deficiency after fetal encephalectomy [4] or implantation of ACTH secreting tumor to pregnant rats [3, 10] results in retardation or cessation of fetal adrenal growth and differentiation [3, 4, 6, 10, 11].

In the experiments reported in this paper dexamethasone which markedly suppresses both maternal and fetal pituitary-adreno-cortical endocrine system [8, 9, 12, 13] was given to pregnant rats from the 15th day of pregnancy. Fetal adrenal growth and fetal adrenal steroidogenesis *in vitro* were examined.

MATERIALS AND METHODS

Pregnant rats of the Fischer strain and their fetuses were used. The animals were kept in an air conditioned animal room at temperature of 24–25°C on a daily regimen of 14 h light and 10 h darkness. Water and standard laboratory diet were continuously available. 3-month-old females were caged with males overnight and examined each morning for spermatozoa in the vagina. The day when spermatozoa were found was designated day 1 of pregnancy. Intact and adrenalectomized females were used. On the 12th day of pregnancy one group of females was adrenalectomized by the dorsal approach under ether anesthesia. Intact rats and one group of adrenalectomized females were given dexamethasone 21 phosphate sodium salt (Lek Yugoslavia) in the drinking water (10 μg/ml) from the 15th day to the 22nd day of pregnancy. The intake of dexamethasone per rat was approximately 230–260 μg per 24 h. All females were killed under ether anesthesia between the 17th and the 22nd day of pregnancy. To test reversibility of inhibition of fetal adrenal growth by dexamethasone a group of pregnant rats drank dexamethasone in drinking water (10 μg/ml—approximately 230–260 μg/rat per 24 h) on the 15th and 16th day, and the 17th and 18th day of pregnancy. The rats were killed on the 20th day and 22nd days of pregnancy. Fetuses were removed, dried on filter paper and weighed on an analytical balance. The fetuses were decapitated and their blood was collected for corticosterone analyses according to the method of Zenker and Bernstein[14]. Adrenal glands from one or two fetuses were cleaned of adhering tissues under a stereomicroscope, dried with filter paper and weighed. Each pair of fetal adrenal glands was incubated in 2 ml of Krebs–Ringer bicarbonate buffer pH 7.4 containing 4 mg of glucose and 0.4 μCi of [4-^{14}C] progesterone (Amersham, Great Britain, 60.6 mCi/mmol) in a meta-

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The following trivial names are used: 18-hydroxy-11 deoxycorticosterone (18-OH-DOC) (18,21-dihydroxy-4-pregnene-3,20-dione). 18-hydroxycorticosterone (18-OH-B) (11β, 18,21-trihydroxy 4-pregnene-3,20-dione).

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bolic shaker over a period of 3 h at 37°C in an atmosphere of O_2–CO_2 (95–5%). Progesterone conversion per pair of fetal adrenals accounts for 28% of the added substrate [11]. The radioactivity that remained in the incubation tissue after washing it with 2 ml of Krebs–Ringer bicarbonate buffer accounted for 100–600 d.p.m./pair of fetal adrenals. ^3H labeled progesterone and corticosterone were added to the incubation medium for the determination of recovery after extraction. The incubation medium was extracted 3 times with 10 ml of a mixture of chloroform–methanol (2:1), and evaporated to dryness in vacuum at 35°C. The dry residue was dissolved in ether and transferred to a thin layer chromatography plate coated with 250 μm thick layer of silica gel GF_{254} (Merck, West Germany), and then developed in two solvent systems: I Methylene dichlorede–*n*-heptane–methanol (15:4:1 by vol) and II benzene–acetone–*n*-heptane–methylene dichloride (4:4:2:1 by vol) at 20–24°C. In addition to labelled steroids the following unlabelled corticoids were subjected to thin layer chromatography: progesterone, corticosterone (B), 11-deoxycorticosterone (DOC), 18-hydroxy-11-deoxycorticosterone (18-OH-DOC), 18-hydroxy-corticosterone (18-OH-B) and aldosterone. After developing the thin layer plates steroids hormones were detected on the chromatograms using U.V. light and iodine vapor. The radioactive spots were detected following autoradiography on X-ray films. All radioactive steroids were identified by recrystallization to constant S.A. [15] as described previously [6, 16].

The steroids were scraped off the plates into scintillation vials and the radioactivity of samples was determined by the liquid scintillation counter Mark II. The overall recovery of radioactive steroids including loss in chromatography was 75–80%. The radioactivity obtained in d.p.m. was converted by mean of S.A. into ng per 2 ml incubation medium [6]. After analysis of variance and the test of homogeneity of variance, Student's *t*-test or Kramer's multiple range test [17] were used to calculate the significance of differences between groups.

RESULTS

Fetuses from pregnant rats drinking dexamethasone from the 15th day of pregnancy had lighter body and adrenal weights and lower concentration of plasma corticosterone during the last 4 days of intrauterine development (Table 1). There was no difference in absolute adrenal weights and plasma corticosterone concentration between fetuses from intact and adrenalectomized dexamethasone treated pregnant females. Reversibility of dexamethasone inhibition of fetal adrenal growth is demonstrated by the results presented in Table 2.

Fetal adrenal glands converted [4-^{14}C] progesterone added to the incubation medium to DOC, corticosterone, 18-OH-DOC, 18-OH-B and aldosterone (Fig. 1). Radioactive DOC, corticosterone and 18-OH-DOC were found in the incubation medium following the incubation of adrenal glands of 17-day-old fetuses of all three groups of females. The amounts of those compounds were increased more than twice after the incubation of adrenals of 18-day-old fetuses reaching the maximal values by incubating the adrenals of 20-day- and 21-day-old fetuses and decreased amounts following the incubation of adrenals of 22-day-old fetuses of control females. The ability of fetal adrenal glands, of dexamethasone treated females, to convert [4-^{14}C] progesterone to DOC, corticosterone and 18-OH-DOC was practically the same over the period of 17th to 22nd day of intrauterine development. Small amounts of radio-

Table 1. Body weights, adrenal gland weights, and plasma corticosterone concentrations of 17–22-day-old fetuses from intact females (INT), and intact (INTD) and adrenalectomized (ADXD) rats treated with dexamethasone from the 15th to the 22nd day of intrauterine development

Fetal age (days)	Body weight (g)			Adrenal weight (mg)			Plasma corticosterone (μg/100 ml)		
	INT	INTD	ADXD	INT	INTD	ADXD	INT	INTD	ADXD
17	0.45 ±0.01	0.34 ±0.01	0.39 ±0.01	0.35 ±0.01	0.18ˢ ±0.01	018ˢ ±0.01	—	—	—
18	0.63 ±0.01	0.62 ±0.01	0.59 ±0.01	0.76 ±0.02	0.24ˢ ±0.01	0.23ˢ ±0.01	—	—	—
19	1.25 ±0.02	0.94ˢ ±0.01	0.96ˢ ±0.02	1.17 ±0.02	0.39ˢ ±0.02	0.34ˢ ±0.02	17.6 ±1.8	5.8ˢ ±0.4	6.0ˢ ±0.3
20	1.88 ±0.06	1.33ˢ ±0.01	1.24ˢ ±0.02	1.33 ±0.03	0.41ˢ ±0.02	0.43ˢ ±0.02	23.7 ±1.9	5.1ˢ ±0.3	5.3ˢ ±0.2
21	3.48 ±0.07	1.51ˢ ±0.02	1.54ˢ ±0.05	2.16 ±0.05	0.52 ±0.03	0.54ˢ ±0.02	21.3 ±1.6	3.2ˢ ±0.1	3.5ˢ ±0.2
22	4.08 ±0.02	1.65ˢ ±0.04	1.86ˢ ±0.03	1.78 ±0.04	0.49ˢ ±0.01	0.48ˢ ±0.02	12.6 ±0.7	2.2ˢ ±0.1	2.1ˢ ±0.1

Mean ± S.E.; 8 fetuses per group from 4–5 females. s = significant difference vs intact group date. Adrenalectomy was performed on the 12th day of gestation.— Not measured. The intake of dexamethasone per rat was approximately 230–260 μg per 24 h.

Table 2. Influence of 2-day dexamethasone treatment of the pregnant rats upon the fetal adrenal weight 2, 4 and 6 days later

Dexamethasone treatment Age of fetuses:	Adrenal weights (mg) 20	22
0	1.81 ± 0.09	2.05 ± 0.09
I	1.05 ± 0.12	1.43 ± 0.06
II	0.58 ± 0.06	1.11 ± 0.06

Mean ± S.E., 4–5 females—24–30 fetuses per group. I. The rats drank dexamethasone in drinking water on the 15th and 16th days of pregnancy. II. The rats drank dexamethasone in drinking water on the 17th and 18th days of pregnancy.

active 18-OH-B and aldosterone were found in the incubation medium following the incubation of adrenal glands of 19-day- and 20-day-old fetuses, respectively, of all three groups of females. These two compounds were found in increasing amounts in the incubation medium after the incubation of adrenal gland of 20-day-, and 21-day- and 22-day-old fetuses. It seems that dexamethasone treatment of pregnant rats stimulates the fetal adrenal glands to produce more 18-OH-B and aldosterone, particularly on the last day of fetal development.

DISCUSSION

Reduction in body weight gain of fetuses from dexamethasone treated intact and adrenalectomized pregnant rats from the 19th day of intrauterine development onward is in agreement with the results of Chamberlain and Kasahara[12]. More pronounced retardation of adrenal gland growth of fetuses from dexamethasone treated females as compared to a degree of reduction of adrenal weight of fetuses with totally blocked pituitary adrenocorticotrofic activity [3] indicates a possibility that some other factor(s), except fetal ACTH but susceptible to dexamethasone, influences fetal adrenal growth. An alternative explanation would be nonspecific inhibitory effect of dexamethasone on fetal growth in general. However, the reversibility of that inhibition argues against such a possibility (Table 2). The ability of the adrenal glands to convert [4-^{14}C] progesterone to DOC, corticosterone and 18-OH-DOC 2–3 times more on the 18th day than on the 17th day of intrauterine development is in accord with the earlier data on the synthesis of corticosterone in the fetal adrenal glands and of the fetal pituitary ACTH activity from 18th day of the intrauterine life on [3, 6, 10, 18]. It is well known that a high concentration of maternal plasma corticosterone on the last day of pregnancy partially inhibits fetal adrenal growth and secretion [3, 6, 10], which seems to be a plausible explanation for the decreased ability of the fetal adrenal gland to convert [4-^{14}C] progesterone to corticosterone, DOC and 18-OH-DOC on the 22nd day of intrauterine development. The inhibition of adrenal glands of fetuses from dexamethasone treated intact and adrenalectomized females to produce corticosterone, DOC and 18-OH-DOC from [4-^{14}C] progesterone shows that this process is at least partially controlled by fetal ACTH over a period from the 17th to the 22nd day of fetal development. Conversion of [4-^{14}C] progesterone to 18-OH-B and aldosterone by fetal adrenal glands was initiated on the 19th and 20th day of intrauterine development, respectively. Contrary to the decreased production of corticosterone, DOC and 18-OH-DOC, conversion of progesterone to 18-OH-B and aldosterone is high even on the last day of fetal life. Failure of dexamethasone to inhibit production of 18-OH-B and aldosterone indicates that this process is probably not controlled by the fetal pituitary ACTH. Perhaps significantly greater capacity of adrenal glands from fetuses of dexamethasone treated females in production of 18-OH-B and aldosterone results from reduced synthesis of other corticoids during this period of fetal life.

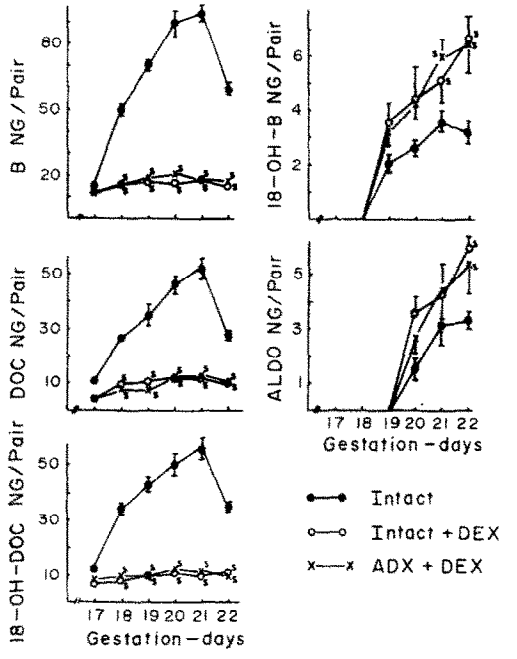

Fig. 1. *In vitro* conversion of [4-^{14}C] progesterone by the adrenal glands of 17–22-day-old fetuses from intact mothers, intact and adrenalectomized dexamethasone treated mothers from 15th day of gestation. Mean ± S.E. ng/ pair of adrenal glands in the incubation medium. Adrenalectomy was performed on the 12th day of gestation.

Our results show a parallelism between trophic and secretory activities of fetal ACTH, both being similarly inhibited by dexamethasone. This is even more evident when fetal adrenal gland capacity to convert [4-^{14}C] progesterone to corticosterone, DOC and 18-OH-DOC is expressed as corticoid production per mg of gland. In other words, production of the mentioned corticoids per mg of gland is similar in all three groups of fetuses. On the other hand, fetal adrenal production of 18-OH-B and aldosterone per

mg of tissues is several times greater in groups of fetuses belonging to dexamethasone treated females. The stimulatory effect of dexamethasone in this process cannot be ruled out on the basis of results presented in this paper.

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