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DEVELOPMENT OF STEROIDOGENESIS IN THE FETAL RAT ADRENAL GLAND: AN *IN VITRO* STUDY*

R. KLEPAC, KARMELA MILKOVIĆ and S. MILKOVIĆ†
Institute of Biology, School of Medicine, University of Zagreb, Šalata 3, and †Laboratory for Experimental Medicine, University of Zagreb, Domagojeva 2, 41000 Zagreb, Yugoslavia

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SUMMARY

In vitro synthesis of steroid hormones from [4-^{14}C]-progesterone by the adrenal glands of rat fetuses with various pituitary adrenocorticotropic activities has been investigated. The fetal adrenal glands are capable of synthesizing deoxycorticosterone, corticosterone, 18-hydroxy-11-deoxycorticosterone, 18-hydroxy-corticosterone, 11-dehydrocorticosterone and aldosterone. Adrenal corticosterone synthesis begins on the 14th day of fetal life and continues uninterrupted until delivery. On the 18th day corticosterone synthesis sharply increases, because of the activation of fetal ACTH which induces quantitative changes in fetal steroidogenesis. The yield of radioactive conversion products per unit of fetal adrenal weight decreases during the intrauterine development. The biotransformation of hormones per unit of gland weight depends on the age of fetuses and not on the degree of fetal adrenocorticotropic activity.

INTRODUCTION

The importance of the fetal adrenocorticotropic activity in the morphogenesis of the fetal adrenal gland has been demonstrated [1–10]. Also, the secretory activity of the fetal adrenal gland under various experimental conditions *in vivo* has been described [2, 7, 9, 11–18]. However, as yet, the pattern of steroidogenesis *in vitro* of normal, hyperfunctioning and hypofunctioning fetal adrenal glands has not been systematically compared and analyzed. The results of this study show that fetal steroidogenesis *in vitro* begins on the 14th day of intrauterine development and that fetal ACTH begins to participate in this process on the 18th day of fetal life.

MATERIALS AND METHODS

Fischer strain albino rats and their fetuses were used. The animals were raised in an air-conditioned animal unit at 23–24°, on a daily regimen of 14 h light and 10 h darkness. Water and standard laboratory diet were continuously available. Females were caged with males overnight and examined each morning for spermatozoa in the vagina. The day when spermatozoa were found was designated day 0 of pregnancy. In most cases delivery took place early afternoon on the 22nd day of pregnancy.

On the 1st day of pregnancy a group of animals was inoculated subcutaneously with a tumor ($MtTF_4$), as described elsewhere [19], secreting large amounts of ACTH and slightly less prolactin [20]. Another group, consisting of intact females, was adrenalectomized by the dorsal approach under light ether anesthesia on the 12th day of pregnancy. Following adrenalectomy the rats were given saline to drink. In the third group of female rats the left uterine tube was ligated by the dorsal approach 8–10 days before mating. The results of this intervention is reduction of the number of fetuses to about half of the normal number.

All females were killed under light ether anesthesia between the 13th and 22nd day of pregnancy. From each animal one or two fetuses were removed, immediately dried with filter paper and weighed on an analytical balance. The fetal adrenal glands were removed, freed from adhering tissues under a stereomicroscope which magnified 10 times, dried with filter paper and weighed. Each pair of fetal adrenals was incubated in 2 ml of Krebs–Ringer bicarbonate buffer, pH 7.4, containing 4 mg of glucose, 0.032 μCi of [4-^{14}C]-progesterone—190 ng progesterone (Amersham, Great Britain, 52.8 mCi/mmol). The fetal adrenal glands were incubated for 3 h at 37° in an atmosphere of $O_2 + CO_2$ (95 + 5%) in a Dubnoff metabolic shaker. Progesterone conversion per pair of the fetal adrenal gland accounts for 30% of the added substrate [21].

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. per

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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); 11-dehydrocorticosterone (11-dehydro-B) (21-hydroxy 4-pregnene-3, 11, 20-trione); 18-hydroxy-corticosterone (18-OH-B) (11β, 18, 21-trihydroxy 4-pregnene-3,20-dione).

Reprint requests should be addressed to: Dr Ratimir Klepac, Institute of Biology, School of Medicine, Šalata 3, 41000 Zagreb, Yugoslavia.

Table 1. Body weights and adrenal gland weights of fetuses from normal intact and adrenalectomized, and tumor-bearing intact and prenatally uterine tube ligated female rats from the 16th to the 22nd day of fetal development

Fetal age (days)	Normal body wt (g)	Intact mother Adrenal wt (mg)	Adrenalectomized mother		Tumor bearing mother		Mother with reduced number of fetuses	
			Body wt (g)	Adrenal wt (mg)	Body wt (g)	Adrenal wt (mg)	Body wt (g)	Adrenal wt (mg)
16	0.31 ± 0.01	0.13 ± 0.001	0.26 ± 0.01	0.13 ± 0.001	0.31 ± 0.005	0.13 ± 0.001	0.31 ± 0.01	0.13 ± 0.001
17	0.45 ± 0.01	0.34 ± 0.01	0.41 ± 0.01	0.34 ± 0.03	0.49 ± 0.01	0.33 ± 0.03	0.45 ± 0.01	0.37 ± 0.01
18	0.80 ± 0.01	0.76 ± 0.02	0.79 ± 0.03	0.61 ± 0.02	1.00 ± 0.08	0.61 ± 0.07	0.78 ± 0.01	0.72 ± 0.01
19	1.32 ± 0.03	1.29 ± 0.03	1.36 ± 0.03	1.50 ± 0.03[s]	1.50 ± 0.04	0.80 ± 0.04[s]	1.47 ± 0.02	1.30 ± 0.02
20	1.91 ± 0.13	1.51 ± 0.01	2.23 ± 0.17	1.90 ± 0.05[s]	2.34 ± 0.03	0.95 ± 0.05[s]	2.23 ± 0.02	1.86 ± 0.03[s]
21	3.45 ± 0.09	2.11 ± 0.09	3.81 ± 0.22	2·55 ± 0.05[s]	3.41 ± 0.07	1·05 ± 0.04[s]	3.50 ± 0.05	2.40 ± 0.03[s]
22	4.04 ± 0.17	1.81 ± 0.04	3.97 ± 0.09	2.22 ± 0.08[s]	4.35 ± 0.08	0.94 ± 0.03[s]	5.16 ± 0.01[s]	2.57 ± 0.07[s]

Mean ± S.E.; seven fetuses per group; s = significant difference vs control group data. Adrenalectomy performed on the 12th day of gestation.

pair of fetal adrenal glands. Tritium-labeled progesterone and corticosterone were added into the incubation medium for the determination of recovery after extraction. The incubation medium was extracted 3 times with 20 ml of ether. The ether extract was evaporated to dryness in a Büchi rotatory evaporator. The steroids were dissolved in ether and separated using two dimensional chromatography on thin layer plates coated with silica gel GF_{254} (Merck, West Germany) in solvent systems: I. Methylene dichloride–*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°. In addition to labeled steroids, the following unlabeled steroid hormones were subjected to t.l.c.: progesterone, corticosterone (B), 11-deoxycorticosterone (DOC), 18-hydroxy-11-deoxycorticosterone (18-OH-DOC), 18-hydroxycorticosterone (18-OH-B), 11-dehydrocorticosterone and aldosterone. After developing the thin layer plates steroids 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 crystallization to constant S.A. [22] followed by acetylation and oxidation with periodic acid [23] as described earlier [24]. The compounds obtained were subjected to chromatography in the benzene–ethyl acetate (1:10, v/v) and benzene–ether–methylene dichloride (1.3:3:9, by vol) solvent systems.

The steroids identified were scraped from the plates into vials to which 10 ml scintillation fluid had been added. Radioactivity was measured in a Model 3375 Packard Tri-Carb liquid scintillation spectrometer. The overall recovery of radioactive steroids including loss in chromatography was 80–85%. d.p.m. Of each fraction was converted by means of S.A., into ng per incubation medium [25].

After analysis of variance of the data obtained and the test of homogeneity of variance, Student's *t*-test or Kramer's test were used [26].

RESULTS

Fetal body and adrenal gland weights are summarized in Table 1. The conversion of [4-^{14}C]-progesterone by the fetal adrenal glands *in vitro* is presented in Table 2. On the 14th day of intrauterine life only a small amount of radioactive corticosterone could be demonstrated in the incubation medium of the control group. The amounts of radioactive corticosterone in the incubation medium slightly increased during the next three days. However, significant amounts of [4-^{14}C]-corticosterone could be detected only from the 18th day onwards: 14.3 ng on the 18th day and 23.5 ng on the 21st day. Thereafter a decrease set in and on the last day of fetal life the incubation medium contained 15.5 ng of corticosterone. On the 15th day of fetal life in addition to corticosterone the incubation medium contained also DOC and 18-OH-DOC. The amount of these hormones also increased until the 21st day of fetal life and decreased thereafter (Table 2). Radioactive 18-OH-B appeared into incubation medium of the control group on the 20th day of intrauterine life, and of aldosterone as late as the 21st day. These two steroid hormones could be demonstrated in the incubation medium in very small amounts.

Table 2 also shows that the adrenal glands of fetuses from adrenalectomized mothers converted progesterone and released into the incubation medium increased amounts of radioactive corticosterone on the 19th and 22nd day, of DOC on the 22nd day and of 18-OH-DOC on the 19th day of intrauterine life as compared to control groups.

The conversion of [4-^{14}C]-progesterone by the adrenal glands *in vitro* of fetuses from intact mothers and that by the adrenal glands of fetuses from prenatally reduced litters differed only with regard to corticosterone. In the incubation medium of the latter group increased amounts of radioactive corticosterone were found on the 22nd day and 17th day of fetal life.

DISCUSSION

The adrenal glands of fetuses are capable of synthesizing corticosterone (0.33 ng/pair of adrenals) from [4-^{14}C]-progesterone *in vitro* as early as the 14th day of intrauterine life (Table 2), i.e., before the beginning of the adrenocorticotropic activity of the fetal pituitary. Appreciable conversion of radioactive progesterone to corticosterone occurs first time on the 18th day (14.3 ng/pair of adrenals), i.e., exactly at the time of initiation of the fetal pituitary adrenocorticotropic activity [7, 9, 10], and when corticosterone of fetal origin can be found in demonstrable amounts in the adrenalectomized pregnant females [15, 18].

It is well documented that fetal adrenocorticotropic activity plays an important role in the morphogenesis of the fetal adrenal glands [1–10]. Our results show that the enlargement of the adrenal glands is associated with an increased conversion of progesterone, and that lighter adrenal glands accompany the decreased capacity to convert [4-^{14}C]-progesterone to its metabolites. It is possible that these results may indicate the secretory activity of the fetal adrenal glands is a function of the total adrenal weight, i.e. that there exists an almost complete parallel between the tropic and secretory effect of the fetal ACTH. In addition, these results also indicate that the participation of the fetal pituitary in the development of adrenal steroidogenesis is only of quantitative nature, as was shown for fetal adrenal growth obtained, demonstrates, contrary to the results reported by Roos[12] and Cohen[13], and in accordance with a number of other reports [9, 10, 14, 15], that steroidogenesis which has once started increases continuously until the 21st day of fetal life when suppression of the adrenal growth and secretory activity set in.

Table 2. (a) *In vitro* conversion of [4-^{14}C]-progesterone by the adrenal glands of fetuses from normal intact mother (C), normal adrenalectomized mother (ADX), ACTH-secreting tumor bearing mother (MtT), and mother with prenatally reduced number of fetuses (RED)

Steroids fetal age (days)	Corticosterone				11-Deoxycorticosterone				18-Hydroxy-11-deoxycorticosterone			
	C	ADX	MtT	RED	C	ADX	MtT	RED	C	ADX	MtT	RED
14	0.33± 0.01[a]	—	—	—	—	—	—	—	—	—	—	—
15	0.50± 0.04	—	—	—	0.85± 0.18	—	—	—	0.36± 0.08	—	—	—
16	3.88± 0.22	3.01± 0.38	2.82± 0.25	3.50± 0.23	1.98± 1.19	1.42± 0.16	1.28± 0.17	1.33± 0.09	2.21± 0.14	1.84± 0.15	0.95± 0.28	2.21± 0.24
17	3.70± 0.13	5.32± 0.71	4.86± 0.33	5.92 0.46[s]	2.48± 0.12	1.82± 0.04	2.06± 0.45	2.11± 0.24	2.66± 0.19	2.68± 0.37	2.51± 0.25	2.30± 0.22
18	14.3± 1.00	15.4± 1.50	10.8± 1.15	13.5± 1.01	7.25± 0.38	5.74± 0.39	5.87± 0.70	7.24± 0.64	8.67± 0.65	7.14± 0.70	4.98± 0.52[s]	6.33± 0.70
19	18.0± 0.78	24.7± 1.58[s]	12.0± 1.12	19.0± 2.02	9.42± 1.57	11.2± 1.05	8.89± 1.45	11.2± 1.68	10.9± 0.73	14.4± 1.01[s]	5.40± 0.70[s]	10.8± 0.80
20	21.3± 1.77	25.1± 0.62	9.52± 0.72	22.9± 0.86	11.7± 1.06	10.9± 0.67	7.75± 0.83	10.1± 0.39	14.4± 1.08	14.4± 0.53	6.18± 0.93[s]	11.0± 0.49
21	23.5± 0.87	27.6± 2.03	10.5± 0.72[s]	20.6± 0.92	13.4± 1.45	10.6± 0.67	5.57± 0.51[s]	9.78± 0.52	13.2± 0.92	14.1± 0.23	3.41± 0.35[s]	9.91± 0.22
22	15.5± 0.30	26.8± 1.87[s]	10.3± 1.16	22.1± 1.24[s]	5.72± 0.35	13.0± 0.87	7.21± 0.20	8.16± 0.77	7.30± 0.21	10.6± 1.16	5.11± 0.01	9.14± 0.55

Table 2. (b)

Steroids fetal age (days)	11-Dehydrocorticosterone				18-Hydroxycorticosterone				Aldosterone			
	C	ADX	MtT	RED	C	ADX	MtT	RED	C	ADX	MtT	RED
16	0.77± 0.16	0.56± 0.03	0.78± 0.14	0.47± 0.06	—	—	—	—	—	—	—	—
17	0.54± 0.02	0.43± 0.06	0.42± 0.03	0.61± 0.04	0	0.29± 0.03	0.51± 0.06	0.30± 0.05	0	0	0	0
18	0.91± 0.09	0.62± 0.04	0.80± 0.12	0.68± 0.08	0	0.29± 0.02	0.46± 0.05	0.74± 0.08	0	0	0	0
19	1.29± 0.16	1.00± 0.13	0.61± 0.09	0.65± 0.10	0	0.53± 0.07	0.65± 0.07	1.00± 0.11	0	0	0	0
20	1.23± 0.16	1.21± 0.16	0.88± 0.13	0.88± 0.06	0.54± 0.06	1.26± 0.25[s]	0.59± 0.11	0.84± 0.03	0	0.43± 0.02	0	0
21	2.87± 0.19	1.72± 0.17	0.89± 0.05	1.74± 0.14	0.95± 0.07	0.90± 0.07	0.53± 0.05	0.72± 0.07	0.75± 0.07	0.58± 0.06	0.44± 0.04[s]	0.63± 0.20
22	0.98± 0.14	2.10± 0.16	0.65± 0.07	2.17± 0.21	0.88± 0.21	0.95± 0.06	1.20± 0.17	0.85± 0.07	0.80± 0.07	0.75± 0.08	0.76± 0.07	0.84± 0.04

a = Mean ± S.E., ng/pair of adrenal glands in the incubation medium; s = significant difference vs control group data. Adrenalectomy performed on the 12th day of gestation. 0 = Not detectable, — Not measured.

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