Gravimetric and Histologic Changes in Fetal Adrenals following Maternal Adrenalectomy and Metopirone Injection in the Rat

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ABSTRACT. Adrenalectomy (ADX) of pregnant rats on day 14 or 15 of gestation did not induce any hypertrophic feature in the fetal adrenals within 2 days. ADX on day 16 significantly increased both the weight of fetal adrenals and the size of fetal adrenocortical cells in 2 days. However, ADX on day 17 induced some increase in the weight of fetal adrenals, not accompanied with any significant cortical cell enlargement within 2 days. ADX on day 18 caused no increase in either the weight of fetal adrenals or the size of cortical cells. ADX on day 19 or later also induced a highly significant increase in both the weight of fetal adrenals and the size of cortical cells. To know more clearly the pattern of the negative feedback mechanism of the fetal pituitary-adrenal axis, metopirone was injected into another series of ADX rats on each of two days after ADX and the animals were autopsied on the third day. Metopirone caused more marked hypertrophy of the fetal adrenals and cortical cells than did ADX alone performed after day 16. These results strongly support the view that the fetal pituitary-adrenal negative feedback mechanism begins to operate between days 16 and 18 of gestation.

Adrenalectomy of pregnant rats results in hypertrophy of the fetal adrenals near the end of gestation and just after birth [6, 11]. This phenomenon is considered to be a compensatory event in the fetus for maternal adrenocortical deficiency.

In our previous study [12], the time of establishment of the fetal pituitary-adrenal negative feedback mechanism was assessed by maternal adrenalectomy performed at different stages of gestation in the rat. It appeared that the mechanism began to operate between days 16 and 18 of gestation. Since the fetal adrenals remain to function after maternal adrenalectomy, however, hypertrophy of fetal adrenals as a result of the feedback response may not occur on a certain stage of gestation. In fact ma-

ternal adrenalectomy on day 17 or 18 failed to induce hypertrophy of the fetal adrenals within 2 days [12].

In the present experiment, pregnant rats received metopirone (Su 4885) that inhibits 11 β -hydroxylation and corticosterone production. Metopirone, crossing the placenta, reaches the fetus and shows the same inhibitory effects on the fetal adrenal glands [4, 5, 8]. This work using metopirone was designed to make clearer the time of establishment and the chronological pattern of the fetal pituitary-adrenal negative feedback mechanism. Interpretation of the results is based on the gravimetric and histologic changes in the fetal adrenals of the adrenalectomized pregnant rats and the adrenalectomized and metopirone-injected rats.

MATERIALS AND METHODS

Rats of the Wistar strain were used. They were given *ad libitum* a commercial diet (Labo MR Breeder) and water. Females were placed overnight with males. The vaginal smear was examined the next morning for sperm. The day of observation of sperm was counted as day 1 of gestation.

Pregnant rats were bilaterally adrenalectomized via the dorsolumbar approach under ether anesthesia on day 14, 15, 16, 17, 18, 19 or 20 of gestation. The adrenalectomized rats were divided into two groups; one was given metopirone injection and the other saline injection. The metopirone group received a subcutaneous injection of 30 mg of metopirone (Ciba) in 2 ml saline on the day of adrenalectomy and the next day. The saline group received 2 ml of saline alone on the same days. All rats were given saline as a drinking water. They were autopsied on the third day after surgery and matched with the non-operated control rats of the same gestational days.

Sham-adrenalectomy was not performed in this study, as it might give stress causing fetal adrenal shrinkage. Various maternal stresses have been reported to reduce markedly the size and weight of the fetal adrenal [1, 3, 10].

After the rats were killed by decapitation, their fetuses were removed quickly from the uterus and weighed. Both adrenals of each fetus were removed and weighed. The right adrenal was fixed in Bouin's fluid, embedded in Paraplast (Sherwood Medical Industry), and sectioned at 5 μm . The sections were stained with hematoxylin and eosin for counting adrenocortical cell nuclei per unit sectional area (4,900 μm^2). Counts in 10 areas in each animal were averaged to estimate changes in the size of cortical

cells; the larger the counts, the smaller the size of the cells.

The data were tested by one-way analysis of variance. An overall F value and a corrected significance level between groups were calculated; *P* smaller than 0.05 was considered to be statistically significant.

RESULTS

Maternal adrenalectomy (ADX) performed on day 14 or 15 of gestation induced no hypertrophy of the fetal adrenals within 2 days (on days 16 and 17, respectively; cf. controls in Table 1.). ADX on day 16 caused a significant increase in the weight of fetal adrenals in 2 days. ADX on day 17 increased only the ratio of "adrenal weight/body weight". ADX on day 18 and autopsy on day 20 however, showed no increase in the weight of fetal adrenals. ADX on day 19 or later caused a highly significant degree of hypertrophy of fetal adrenals.

ADX accompanied with metopirone injection (ADX plus M) on day 14 or 15 did not increase the weight of fetal adrenals as in ADX alone. However, ADX plus M on day 16 or later increased more markedly the weight of fetal adrenals than did ADX alone. Particularly to be noted was that ADX plus M on day 17 or 18 significantly increased the fetal adrenal weight, in contrast to a slight (day 17) or no degree (day 18) increase after ADX alone.

The count of cortical cell nuclei per unit sectional area (Table 2) fairly well paralleled the weight of the fetal adrenals. In ADX rats, the counts on days 15–17, 17–19 and 18–20 were not significantly different from those of corresponding controls, but those on 16–18, 19–21 and 20–22 significantly differed from those of corresponding controls. In ADX plus M rats, the counts were fur-

Table 1. Body weights and adrenal weights of fetuses from intact control, adrenalectomized pregnant (ADX) and adrenalectomized pregnant rats receiving metopirone injections (ADX+M)

Group	Day of adrenalectomy	Day at autopsy	Number of fetuses	Body wt. (g)	Adrenal wt. (mg)	Adrenal wt.
						Body wt. (mg/100 g)
ADX+M	14	16	16(3)	0.25±0.01a	0.21±0.01a	83.4±6.1a
ADX	14	16	18(3)	0.27±0.01a	0.21±0.02a	78.0±5.7a
Control		16	10(2)	0.31±0.01a	0.22±0.02a	70.9±5.0a
ADX+M	15	17	11(2)	0.47±0.02a	0.55±0.05a	115.8±7.3a
ADX	15	17	17(3)	0.52±0.00a	0.52±0.03a	100.0±4.6a*
Control		17	12(2)	0.52±0.01a	0.46±0.02a	87.8±4.1b*
ADX+M	16	18	18(3)	0.87±0.02a	1.10±0.04c	125.5±2.6c
ADX	16	18	21(4)	0.92±0.02a	0.99±0.03b	107.3±2.3b
Control		18	16(3)	0.93±0.03a	0.91±0.02a	98.8±2.7a
ADX+M	17	19	11(2)	1.41±0.03a	1.93±0.04b	137.8±2.7c
ADX	17	19	12(2)	1.47±0.01a	1.69±0.05a	114.9±3.5b
Control		19	12(2)	1.52±0.03a	1.55±0.05a	102.3±3.1a
ADX+M	18	20	12(2)	2.19±0.05c	3.48±0.01c	158.8±3.6b
ADX	18	20	16(3)	2.19±0.04b	2.18±0.06b	99.6±2.6a
Control		20	12(2)	2.52±0.08a	2.45±0.11a	97.1±3.0a
ADX+M	19	21	12(2)	2.69±0.06c	3.78±0.11c	140.9±4.0c
ADX	19	21	12(2)	3.61±0.08b	3.53±0.08b	95.6±2.8b
Control		21	11(2)	4.05±0.09a	2.79±0.19a	70.6±4.8a
ADX+M	20	22	18(3)	3.66±0.05c	5.50±0.20c	150.7±5.8c
ADX	20	22	18(3)	4.65±0.13b	3.50±0.10b	75.6±1.9b
Control		22	12(2)	5.17±0.08a	2.90±0.10b	56.2±1.7a

a, b, c: Figures not sharing the same letter are significantly different at $p < 0.05$.

*: Not significantly different between the figures marked.

Number of litters in parentheses.

Mean±SEM.

further smaller on days 16–18 and later.

In general, in fetuses of the ADX alone or plus M mothers, the adrenocortical cells and sinusoids appeared histologically larger than those in controls. In those of the mothers given ADX alone on day 17 or 18 and autopsied on day 19 or 20, however, neither the size of adrenocortical cells nor that of sinusoids differed from that in the controls (Figs. 1 and 2). Although ADX alone performed on day 17 slightly increased the weight of fetal adrenals (Table 1), the fetal cortical cells were not enlarged, based on the cell nuclear number in a unit sectional area (Table 2). On both days 17–19 and

18–20, however, ADX plus M markedly enlarged the cortical cells and the size of sinusoids of the fetal adrenals (Figs. 2 and 3). Such exceedingly larger sizes of cortical cells were observed also in fetuses of the ADX plus M group on days 19–21 and 20–22 (Figs. 5 and 6; cf. Fig. 4), and the sinusoids located in the inner zone appeared more widened in such fetuses than in those of the ADX alone mothers.

DISCUSSION

The present observations give further evidence for the establishment of the fetal pituitary-adrenal negative feedback

Table 2. The number of fetal adrenocortical cell nuclei per unit sectional area ($4,900 \mu\text{m}^2$) after adrenalectomy (ADX) or that with metopirone injection (ADX+M)

Group	Day of adrenalectomy	Day at autopsy	Number of fetuses	Number of cell nuclei/unit area
ADX+M	15	17	7(2)	$29.7 \pm 1.3a$
ADX		17	9(3)	$26.9 \pm 3.7a$
Control		17	7(2)	$29.6 \pm 2.1a$
ADX+M	16	18	7(2)	$22.9 \pm 0.4c$
ADX		18	11(4)	$25.3 \pm 0.6b$
Control		18	6(3)	$31.2 \pm 1.1a$
ADX+M	17	19	6(2)	$21.9 \pm 0.7b$
ADX		19	6(2)	$27.2 \pm 1.6a$
Control		19	7(2)	$27.6 \pm 0.9a$
ADX+M	18	20	8(2)	$18.7 \pm 0.4b$
ADX		20	11(3)	$25.4 \pm 1.3a$
Control		20	7(2)	$26.2 \pm 1.2a$
ADX+M	19	21	6(2)	$16.9 \pm 0.6c$
ADX		21	7(2)	$24.5 \pm 0.5b$
Control		21	8(2)	$27.8 \pm 0.8a$
ADX+M	20	22	10(3)	$20.8 \pm 0.7c$
ADX		22	9(3)	$25.8 \pm 0.9b$
Control		22	8(2)	$32.0 \pm 1.3a$

a, b, c: Figures not sharing the same letter are significantly different at $p < 0.05$.

Number of litters in parentheses.

Mean $\pm$ SEM.

mechanism between days 16 and 18 of gestation, as stated in our previous paper [12]. The time is in harmony with that reported by Milković *et al.* [9], who found that the fetal pituitary began to regulate the fetal adrenal between days 17 and 18 of gestation.

In our previous study [12], a question was raised why maternal ADX on days 17 and 18 failed to induce hypertrophy of the fetal adrenals. The same question has to be raised in the present study. According to Cohen [2], the plasma corticosterone concentration in fetal rats reaches the peak on day 19.5 postcoitum (equivalent to day 20 of gestation in the present study). According to Chatelain *et al.* [1], the plasma ACTH concentration in fetal rats also reaches the peak on the same day. These observations strongly suggest that the fetal pituitary is

very active around such a period. During the period of the peak, the plasma corticosterone concentrations in fetuses are markedly higher than those in the mother, in which the concentrations remain to be low [6]. If this is also the case in the present study, the lack of fetal adrenal hypertrophy after ADX performed on day 17 or 18 could be explained by the very active status of the fetal pituitary corresponding to the comparatively low level of maternal corticosterone, which caused no apparent hypertrophy of fetal adrenals.

In the present study, ADX plus M on day 17 or 18 caused a highly significant increase in the weight of fetal adrenals as well as the size of cortical cells, though ADX alone induced no clear-cut changes as stated above. Such a marked increase induced by ADX plus M is interpreted as

a result of the augmented production of ACTH in the fetus in response to the drastic decline in the amount of circulating plasma corticosterone caused by the inhibition with metopirone of the corticosterone synthesis in the fetal adrenals. Similarly interpreted is that in all the experimental periods, both the weight of fetal adrenals and the size of cortical cells were significantly larger in the ADX plus M rats than in the ADX alone rats.

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EXPLANATION OF FIGURES

All figures show sections of the fetal adrenals stained with hematoxylin-eosin. Magnification is $\times 345$.

Fig. 1. Adrenal cortex of a fetus on day 20 of gestation from a control mother.

Fig. 2. Adrenal cortex of a fetus on day 20 of gestation from a mother adrenalectomized (ADX) 2 days before (on day 18). The cortical cells are comparable in size with those in Fig. 1.

Fig. 3. Adrenal cortex of a fetus on day 20 of gestation from a mother given ADX 2 days before (on day 18) and metopirone. The cortical cells are larger and the sinusoids more widened

than those in the control (Fig. 1) and the ADX alone rat (Fig. 2).

Fig. 4. Adrenal cortex of a fetus on day 22 of gestation from a control mother.

Fig. 5. Adrenal cortex of a fetus on day 22 of gestation from a mother given ADX 2 days before (on day 20). The cortical cells are enlarged as compared with those in Fig. 4.

Fig. 6. Adrenal cortex of a fetus on day 22 of gestation from a mother given ADX 2 days before (on day 20) and metopirone. The cortical cells are much more enlarged and the sinusoids are more widened than those in Fig. 5.

要 約

母ラット副腎除去およびメトピロン投与後の胎仔副腎重量および組織学的変化: 山本雅子・有嶋和義・江口保暢 (麻布大学獣医学部解剖学第二講座), 望月公子 (東京大学農学部家畜解剖学教室)——妊娠14, 15日のラットの副腎を除去2日後では胎仔副腎は対照とくらべて差がなかったが, 妊娠16日の副腎除去2日後では, 胎仔副腎重量および皮質細胞は対照非除去例にくらべて有意に大きかった。しかし, 妊娠17日あるいは18日で副腎除去した場合には, 2日後の胎仔副腎重量の変化は有意ではなかった。妊娠19日以降に副腎除去すると, 妊娠16日除去例と同様胎仔副腎重量および皮質細胞は対照例とくらべて有意に大きかった。メトピロンを1日に1回2日間投与後3日目に剖検した副腎除去母ラットでは妊娠16日以降除去例全例において, メトピロンを投与しない場合と比較して, 胎仔副腎重量および皮質細胞は著しく大きかった。以上の成績からは, 胎仔下垂体-副腎系のネガティブフィードバック機構が妊娠16日~18日に機能し始めると考えられる。