Ultrastructural Changes in Adrenocortical Cells Following Injection of Corticotropin Releasing Factor (CRF) in Fetal Rats

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It is well known that steroidogenesis occurs both in mitochondria with vesicular or tubular cristae and in smooth endoplasmic reticulum (sER) in adrenocortical cells [6]. In fetal rats, these organelles gradually develop during late period of gestation [6, 9, 10]. Concurrently with the development of these organelles, steroidogenic enzymes become functional [8] and plasma corticosterone concentrations are increased [2]. By administration of ACTH to a fetal rat on late gestational days, development and differentiation of fetal adrenocortical cells are accelerated, accompanied by an enlargement of mitochondria with an increased number of vesicular cristae and by a marked expansion of sER [9]. By administration of progesterone to a pregnant mother, similar developmental acceleration can be induced in fetuses retained beyond the normal gestation [3, 10]. Such a developmental acceleration in prolonged gestation seems to be due to a constant or augmented release of ACTH from the fetal pituitary gland. On the contrary, it has been reported that dexamethasone induces a retardation of differentiation of fetal adrenocortical cells [5]. Previously, the authors studied the effect of fetal encephalectomy on the degree of hypertrophy of fetal adrenals to be induced by maternal treatment with metopirone during the late gestational period. As a result, few days before birth adrenal hypertrophy occurred to some extent without the fetal brain, but close to the end of gestation, it did not occur without the fetal brain [13]. Furthermore, it has been reported that the fetal hypothalamus partially regulates the pituitary-adrenal system between days 17 and 19 of gestation and completely regulates it thereafter [1, 4].

The present study was designed to extend studies of chronological development of the fetal hypothalamo-pituitary-adrenal axis, comparing CRF-treated, saline-treated and control fetuses, on the basis of electron microscopic observation

of adrenocortical cells.

Rats of the Wistar strain were used. They were given a commercial diet (Labo MR Breeder) and water, both *ad libitum*. Females were placed with males overnight and examined the next morning for the presence of sperm in the vaginal smear. The day on which sperm were detected was counted as day 0 of gestation. The pregnant rats were subjected to mid-ventral laparotomy under ether anesthesia on day 17, 18, 19 or 20 of gestation. Then 2 fetuses in each litter were given 2 μ g ovine-CRF (Peptide Institute, Inc.) dissolved in 0.04 ml saline by subcutaneous injection through the uterine wall. Similarly 2 other fetuses in the same litter were given saline alone. These injections were performed at 10 A.M. of each gestational day. Each treated fetus was marked with a piece of white thread (for CRF injection) or black thread (for saline injection) stitched on its back skin for identification at autopsy. The animals were autopsied 8 hrs later. At autopsy the treated fetuses were randomly matched with intact littermates of the same sex as much as possible and the animals were weighed. The left adrenal gland of each fetus was weighed and discarded. The right gland was cut into small pieces, fixed in 2.5% glutaraldehyde in 0.2 M cacodylate buffer, pH 7.4, at 4°C for 2 hrs, dehydrated in ethanol, and embedded in SPURR resin (TAAB Laboratories Equipment Ltd.). Ultrathin sections were double-stained with uranyl acetate and lead citrate and examined with a JEM-100CX electron microscope (Japan Electron Optic Lab.). Electron micrographs of 10 to 15 cortical cells from each gland were prepared to measure area ratios of mitochondria/cells (M/C) and lipid droplets/cell (L/C) with the aid of a Figure-Analyzer (KONTRON). These area ratios were expressed as percentages and averaged by the number of cells observed in each gland. From statistical theory, percentages form a binominal distribution, the deviation from normality being great for small or large percentages (0-30% and 70-100%) [14]. Therefore, the

Table 1. Weight of the left adrenal, and "mitochondria/cell" and "lipid droplets/cell" ratios in CRF-treated, saline-treated and control fetuses.

Group	Day of gestation	No. of fetuses*	Adrenal wt (mg)	Area		Ratio	
				Mitochondria Cell		Lipid droplets Cell	
				(%)	$\sin^{-1} \sqrt{\%}$	(%)	$\sin^{-1} \sqrt{\%}$
CRF	17	6(5) ^{a)}	0.49±0.02 ^{b)}	17.0	24.3±0.6*	4.3	10.2±1.9*
Saline	17	6(5)	0.47±0.02	18.2	25.2±0.7*	4.8	12.6±0.4*
Control	17	7(5)	0.49±0.03	16.0	24.7±0.5*	4.8	12.5±0.7*
CRF	18	7(3)	0.75±0.01	16.3	23.8±0.5*	4.5	12.0±0.9*
Saline	18	7(3)	0.67±0.03	15.1	22.7±1.0*	4.5	12.1±0.7*
Control	18	7(3)	0.68±0.04	16.4	23.9±0.4*	4.5	12.1±0.5*
CRF	19	7(4)	1.21±0.12	22.8	28.5±0.5*	4.9	12.6±0.9*
Saline	19	8(4)	1.30±0.12	19.9	26.5±0.6**	5.1	12.9±0.5*
Control	19	7(4)	1.06±0.08	18.3	25.2±0.8**	5.4	13.3±1.0*
CRF	20	10(5)	1.44±0.06	24.2	29.5±0.4*	5.5	13.3±0.7*
Saline	20	8(5)	1.47±0.05	18.8	25.6±0.7**	4.8	12.4±0.1*
Control	20	10(5)	1.39±0.06	20.0	26.5±0.6**	5.3	12.8±1.3*

a) Number of litters in parenthese.

b) Mean and standard error.

Significantly different at $P < 0.05$ between * and **

square root of each area ratio was transformed to its arcsine in order to analyze the resultant data with Duncan's new multiple range test.

The adrenal weight of CRF group was almost equal to those of saline and control groups on every experimental day (Table 1). CRF on day 17 or 18 did not induce a significant change in the M/C ratio, but induced a significant increase on days 19 and 20. However, CRF did not alter the L/C ratio on every experimental day.

Cells of the zona fasciculata on day 19 are shown in Figs. 1 and 2. Adrenocortical cells of control fetuses contained round nuclei and prominent nucleoli (Fig. 1). In the cytoplasm, numerous mitochondria packed with vesicular cristae were found. Usually in close association with mitochondria, numerous lipid droplets and arrays of compact sER were found. Short profiles of rough ER (rER) and numerous polysomes were also observed. In CRF-treated fetuses, mitochondria appeared somewhat enlarged and the network of sER was developed (Fig. 2). Also, the Golgi complex was well developed and polysomes were increased in number, but

mitochondria did not alter in number.

In this study, fetal rats were directly given ovine-CRF by subcutaneous injection in order to assess at what time the fetal pituitary-adrenal system would begin to react to CRF. The negative feedback mechanism between the pituitary and the adrenal glands in fetal rats was reported to begin to operate between days 15 and 17 of gestation [11, 12]. If the fetal pituitary gland can react to exogenous CRF to release ACTH, then the fetal adrenal cortex would show some stimulated signs in the morphological aspect. Actually in the present study, CRF caused a significant increase of the M/C ratio on days 19 and 20, but caused no significant increase on days 17 and 18 of gestation. As regards the ACTH action on the amount of lipids in adrenocortical cells, there have been published two conflicting reports; one says the lipid amount is increased [7] and the other says it is decreased [9]. In the present study, however, ACTH caused changes neither in L/C ratio nor in number of lipid droplets. The present experimental period of 8 hrs might be too short to induce changes in the

Fig. 1. A cortical cell of a control fetus on day 19 of gestation, containing numerous mitochondria (M) and polysomes (arrowheads). N: nucleus, L: lipid droplets. $\times 11700$.

Fig. 2. A cortical cell of a CRF-treated fetus on day 19 of gestation, having numerous mitochondria (M) with vesicular cristae. A number of polysomes (arrowheads), well-developed Golgi complex (G) and an abundance of tubular elements (arrows) of sER are seen. $\times 11700$.

amount of lipid. Therefore, it is not clear how ACTH acts on the amount of lipid droplets in fetal adrenocortical cells, at least from the present study.

In conclusion, the present result indicates that the fetal pituitary gland begins to react to CRF on day 19 of gestation.

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要 約

副腎皮質刺激ホルモン放出因子 (CRF) 投与ラット胎仔副腎皮質細胞の微細構造 (短報) : 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) —— ラット胎仔に、胎齢17~20日の午前10時においてCRFを直接皮下注射し、8時間後に副腎皮質細胞を電顕的に観察した。胎齢17日例と18日例では変化はみられなかったが、胎齢19日以降の例においては、ミトコンドリア/細胞面積比が有意に増加し、同時に、滑面小胞体の拡張、ポリゾームの増加、ゴルジ体の発達が観察された。