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Analysis of Metopirone-Induced Hypertrophy of Adrenals in Fetal Rats Encephalectomized *in Utero* (41889)

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Abstract. Pregnant rats were treated with 30 mg metopirone (M) each day for 2 days and autopsied on the third day in various gestational periods (Days 18–20, 19–21, and 20–22). Control rats were treated with saline alone (S). The adrenals of intact fetuses in M-treated dams were significantly heavier than those of intact fetuses in S-treated dams in every experimental period. In both M- and S-treated dams, the adrenals of encephalectomized (E) fetuses were lighter than those of intact littermates. However, in the experimental period of Days 18–20 and 19–21, the adrenals of E fetuses in M-treated dams were slightly but significantly heavier than those of similar E fetuses in S-treated dams. In contrast, in the experimental period Days 20–22, there was no significant difference in the weight of adrenals of E fetuses of M- and S-treated dams. These changes in fetal adrenal weight were reflected histologically in parallel changes in the size of adrenocortical cells. The observations suggest that the fetal adrenal hypertrophy following maternal treatment with metopirone can occur to some extent independent of the fetal brain, but that close to the end of gestation the hypertrophy occurs completely under the control of the fetal brain.

In our previous studies, in which we examined the effect of adrenalectomy and/or metopirone injection performed at different stages of gestation in the rat, the fetal pituitary–adrenal negative feedback mechanism was assessed to begin to operate between Days 16 and 18 of gestation (1, 2). Furthermore, it seems that the hypothalamus partially begins to regulate the pituitary–adrenal system between Days 18 and 20 of gestation, followed by the complete regulation toward the end of the gestation (3).

In the present experiment, pregnant rats were given metopirone and some fetuses were encephalectomized *in utero* for the purpose of removing the hypothalamic area. Metopirone, which is said to inhibit 11β-hydroxylation and corticosterone production, can cross the placenta to reach the fetus and shows an inhibitory effect on the fetal adrenals as well as on the maternal adrenals (4–7), resulting in a marked hypertrophy of the fetal adrenals. This work was designed to assess the degree to which the fetal hypothalamus is involved in the metopirone-induced hypertrophy of fetal adrenals, using chronological observations with gravimetric and histologic methods.

Materials and Methods. Rats of the Wistar strain were used. They were given commercial diet (Labo MR Breeder) and water, both *ad libitum*. Females were placed with males overnight and were examined the next morning for the presence of sperm in the vaginal smear. The day on which sperm was observed was counted as Day 1 of pregnancy.

Pregnant rats were used for fetal encephalectomy under ether anesthesia on Days 18, 19, or 20 of gestation. Fetal encephalectomy was performed by the method of Eguchi *et al.* (8). The rats were divided into two groups, metopirone-injected (M) and saline-injected (S). Rats in the metopirone group received a subcutaneous injection of 30 mg metopirone (Ciba) dissolved in 2 ml saline, each day for 2 days, the first on the day of fetal encephalectomy and the second on the following day (the total dose being 60 mg). Rats of the saline group received 2 ml saline only, in a similar fashion. All rats were autopsied on the day following the second injection.

After the mothers were killed by decapitation, the fetuses were quickly removed from the uterus and weighed. The encephalectomized fetuses were randomly matched to intact littermates of the same sex. The head of each

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encephalectomized fetus was fixed in Bouin's fluid for later histologic examination for the presence of the pituitary. Both adrenals of each fetus were removed and weighed. The right adrenal was fixed in Bouin's fluid, embedded in Paraplast (Sherwood Medical, Ind.) and sectioned serially at 5 μm. The sections were stained with hematoxylin and eosin in order to count the number of adrenocortical nuclei per unit sectional area (4900 μm^2). The counts were made on two areas in each of five successive fifth sections, including the largest section of the series, and were averaged to estimate the size of cortical cells. Larger counts mean that the cells are smaller, and smaller counts mean that the cells are larger.

The data were tested with one-way analysis of variance. The test calculated an overall F value and then calculated a corrected significance level between groups. A probability less than 0.05 was considered to be statistically significant. Analysis of the fetal body weights was not carried out, as an appropriate allowance could not be made for the absence of the brain in the encephalectomized fetuses. Therefore, any conclusions regarding the relationship between adrenal weight and body weight would not be warranted.

Results. In the saline-treated (S) dams, the adrenals of encephalectomized (E) fetuses were significantly lighter than those of intact littermate controls in every experimental period (Table I). Similarly, in the metopirone-treated (M) dams, the adrenals of E fetuses were markedly lighter than those of control (C) fetuses. In particular, the adrenals of C fetuses in M-treated dams were significantly heavier than those of C fetuses in S-treated dams, showing a marked hypertrophy in response to maternal metopirone administration.

Comparison of adrenals of E fetuses of S- and M-treated dams was of particular interest. In the experimental period Days 18–20, the E fetal adrenals in M-treated dams were heavier than those in S-treated dams. The difference in the adrenal weight between these two E groups appeared reduced in the experimental period Days 19–21. Finally, in the experimental period Days 20–22, the adrenals of E fetuses in M-treated dams were almost equal in weight to those of E fetuses in S-treated dams.

The trend of counts of cortical cell nuclei per unit sectional area (Table II) paralleled the weights of the fetal adrenals. In both groups, S and M, the counts were significantly greater in E fetuses than in C fetuses, except for the experimental period Days 20–22 in Group S. Especially to be noted is the fact that in the experimental periods Days 18–20 and 19–21 the counts in E fetuses of Group M were less than those of the corresponding

TABLE I. ADRENAL WEIGHTS OF ENCEPHALECTOMIZED FETUSES (E) AND THEIR INTACT LITTERMATE CONTROLS (C) FROM SALINE-TREATED DAMS (S) AND METOPIRONE-TREATED DAMS (M)

Mothers	Fetuses	Day at surgery	Day at autopsy	Number of fetuses examined	Body wt (g) (means ± SEM)	Adrenal wt (mg) (means ± SEM)
S	C	18	20	9 (6)	2.26 ± 0.11	2.13 ± 0.11[a]
	E	18	20	9 (6)	1.94 ± 0.16	1.43 ± 0.08[b]
M	C	18	20	8 (4)	2.31 ± 0.09	2.68 ± 0.12[c]
	E	18	20	8 (4)	2.11 ± 0.09	1.88 ± 0.15[a]
S	C	19	21	9 (3)	3.55 ± 0.06	2.37 ± 0.15[a]
	E	19	21	9 (3)	3.15 ± 0.09	1.82 ± 0.08[b]
M	C	19	21	16 (8)	3.16 ± 0.08	3.29 ± 0.09[c]
	E	19	21	16 (8)	2.65 ± 0.11	2.15 ± 0.17[d]
S	C	20	22	8 (3)	4.88 ± 0.22	2.53 ± 0.20[a]
	E	20	22	8 (3)	3.69 ± 0.12	1.99 ± 0.15[b]
M	C	20	22	8 (5)	4.50 ± 0.08	3.93 ± 0.22[c]
	E	20	22	8 (5)	3.74 ± 0.13	2.00 ± 0.10[b]

Note. (N): Number of litters. Figures not sharing the same letter (a, b, c, d) are significantly different at $P < 0.05$.

TABLE II. THE NUMBER OF FETAL ADRENOCORTICAL CELL NUCLEI PER UNIT SECTIONAL AREA (4900 μm^2) OF ENCEPHALECTOMIZED FETUSES (E) AND THEIR INTACT LITTERMATE CONTROLS (C) FROM SALINE-TREATED DAMS (S) AND METOPIRONE-TREATED DAMS (M)

Mothers	Fetuses	Day at surgery	Day at autopsy	Number of fetuses examined	Number of cell nuclei/unit area (means ± SEM)
S	C	18	20	9 (6)	27.7 ± 1.1^a
	E	18	20	8 (6)	32.9 ± 1.3^b
M	C	18	20	8 (4)	22.3 ± 0.9^c
	E	18	20	8 (4)	29.0 ± 1.0^a
S	C	19	21	9 (3)	29.4 ± 1.0^a
	E	19	21	9 (3)	37.8 ± 1.7^b
M	C	19	21	16 (8)	23.5 ± 0.6^c
	E	19	21	16 (8)	32.0 ± 1.7^a
S	C	20	22	8 (3)	32.0 ± 1.4^a
	E	20	22	8 (3)	$34.7 \pm 1.2^{a,*}$
M	C	20	22	7 (5)	20.5 ± 1.0^b
	E	20	22	8 (5)	$36.2 \pm 1.4^{c,*}$

Note. (*N*): Number of litters. Figures not sharing the same letter (*a, b, c, d*) are significantly different at $P < 0.05$.
* Not significantly different between the figures marked.

E fetuses of Group S. However, in the experimental period Days 20–22 the count in E fetuses of Group M did not differ from that in E fetuses of Group S.

Discussion. The fetal pituitary regulates the fetal adrenal cortex as early as Day 17 of gestation (9, 10). During this early period, it seems that the fetal pituitary has an autonomous ability to stimulate the adrenal without the participation of the brain, since the destruction of the hypothalamus does not interfere with the growth of the adrenal glands (5). The critial time of onset of hypothalamic control of the pituitary–adrenal system seems to be between Days 18 and 20 of gestation, and during this period fetal encephalectomy slightly retards adrenal growth (3, 9). Such a slight retardation of adrenal growth is accounted for by the lack of the hypothalamus, which begins to release CRH during this time. Subsequently, between Days 20 and 22, fetal encephalectomy retards adrenal growth to an extent very similar to that induced by fetal decapitation, which removes the fetal pituitary (3). Thus, at late gestational period the adrenocortical stimulating activity of the fetal pituitary appears to be largely dependent on hypothalamic control (3, 9, 10).

Similarly, evidence shows that the fetal pituitary–adrenal feedback response is established between Days 16 and 18 of gestation, since the earliest time at which maternal adrenalectomy causes a significant hypertrophy of the fetal adrenals 2 days later is Day 16 (1). According to Dupouy and Jost (11), in rat fetuses encephalectomized on Day 19.5 postcoitum (equivalent to Day 20 of gestation in the present study) and autopsied at 21.5 days, the adrenals are stunted in growth even if the mother has been adrenalectomized, but the degree of stunting is less than that in decapitated fetuses in the same adrenalectomized mother. Therefore, it may be that the compensatory hypertrophy of the fetal adrenal following maternal adrenalectomy is largely dependent on the presence of the fetal hypothalamus, but involves in part the autonomous short feedback between the pituitary and the adrenal.

Metopirone blocks corticosteroid biosynthesis both in the mother and in the fetus, and hence metopirone-induced fetal adrenal hypertrophy is of a greater degree than the hypertrophy induced by maternal adrenalectomy. In this sense, metopirone treatment would convey more exact information about the chronological development of the negative feedback mechanism of the pituitary–adrenal system involving the hypothalamic regulation.

In the present study, the adrenals of intact

C fetuses in M-treated dams were considerably heavier than those of similar C fetuses in S-treated dams, in every experimental period, as a compensatory response. Encephalectomy of fetuses in both M- and S-treated dams resulted in a stunting of fetal adrenal growth. However, careful examination of the results reveals different degrees of stunting between the two groups. In the experimental periods Days 18–20 and 19–21 the adrenals of E fetuses in M-treated dams were heavier than those of similar E fetuses in S-treated dams. Nevertheless, in the experimental period Days 20–22, the adrenals of E fetuses in M-treated dams were almost equal, in weight as well as in cortical cell size, to those of similar E fetuses of S-treated dams. Thus, the difference between E fetuses of M and S groups is gradually lessened toward the end of gestation. These findings are in good agreement with the previous reports which indicate a chronologically strengthened activity of the hypothalamus on pituitary activity (9, 10).

Similar results have been presented by Dupouy (4), who reported that the fetal adrenal hypertrophy following maternal treatment with metopirone represented a larger influence of the hypothalamus as gestational age advanced. However, those results showed that E fetuses in M-treated dams still had adrenals slightly heavier than those of E fetuses in sucrose-treated rats at the end of gestation, in contrast to our results, where the adrenals of E fetuses in M-treated dams are almost equal in weight to those of similar E fetuses of S-treated dams at the end of gestation. The discrepancy between our results and those of Dupouy is difficult to explain, but it may be due to the difference in the duration of treatment (1 day by Dupouy versus 2 days by us) and in the total amount of metopirone given to the pregnant dams (100 mg by Dupouy versus 60 mg by us). An excessive amount of metopirone brings about a toxic and fatal effect on the fetus itself, as well as on the mother, and eventually may alter the endocrine status of the fetus. In any event, the present results strongly support the view that the pituitary–adrenal feedback response comes largely under the control of the fetal hypothalamus close to the end of gestation in the rat.

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