Immunohistochemical Study on the Fetal Rat Pituitary in Hyperthermia-Induced Exencephaly

Author: Watanabe, Yuichi G.

Source: Zoological Science, 19(6) : 689-694

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.19.689

ZOOLOGICAL SCIENCE **19**: 689–694 (2002)

Immunohistochemical Study on the Fetal Rat Pituitary in Hyperthermia-Induced Exencephaly

Yuichi G. Watanabe*

Department of Biology, Faculty of Science, Niigata University, Igarashi, Niigata 950-2181, Japan

ABSTRACT—Hyperthermia of fetal rats is known to cause malformations of various organs including brain. The present study was carried out to investigate the effect of the hyperthermia-induced brain damages on the development of the adenohypophysis. Mother rats of Day 9.5 of pregnancy were anesthetized and immersed in hot water (43°C) for 15 min. At Day 21.5 of gestation, fetuses were removed by caesarian section and examined for exencephaly. Hyperthermal stress induced varying degrees of exencephaly in 36% of surviving fetal rats. In extreme cases a considerable part of head was lost. Even in those fetuses with severe brain deformities, the hypophysial stalk and neural lobe were present though they were markedly underdeveloped. In exencephalic fetuses, no immunoreactive vasopressin was detected in the neural lobe of the hypophysis. Immunohistochemical examination of the adenohypophysis showed that exencephaly caused a marked decrease in the number of growth hormone (GH)-producing cells. Other types of hormone-producing cells appeared to be unaffected by brain anomaly. The reason for a decreased population of GH cells in exencephalic fetuses is discussed in relation to their adrenocortical hypotrophy.

Key words: hyperthermia, exencephaly, pituitary, adrenal atrophy, fetal rat

INTRODUCTION

The mammalian adenohypophysial primordium arising as an invagination of the oral ectoderm of the stomodeum maintains an intimate anatomical relationship with the brain floor from its early developmental stage. Recently we encountered an anomalous rat fetus where the adenohypophysis was ill-developed probably because of its failure to get contact with the brain. Although congenital malformations of this category are of interest from a viewpoint of fetal endocrinology, their incidence is quite low. In utero experiments to elucidate the effect of the fetal brain on the developing adenohypophysis have been performed along several different lines of approach. One is surgical removal of the fetal diencephalon (Jost, 1966; Daikoku, 1966; Daikoku *et al*., 1973), but difficulty of surgical encephalectomy in rat fetuses before Day 16.5 of gestation has hindered application of this method to investigate the early effect of the hypothalamus on the pituitary development. A second method has been hypothalamic destruction induced by hypervitaminosis A (Eguchi *et al*., 1971a, b, 1973). Although vitamin A treatment can be done as early as on days 9–11 of fetal age, it is known to cause a strong teratogenic effect on a wide range of developing organs (For reviews, see Geelen, 1979; Collins and Mao, 1999). The present study relied on a third method: fetal rats were subjected to hyperthermal stress *in utero* on day 9.5 of pregnancy. Hyperthermia at this early stage of fetal period is known to cause brain malformations including anencephaly or exencephaly in rats (Edwards, 1968, 1986; Mirkes, 1985; Webster *et al*., 1985; Aoyama and Yamashina, 1994) and mice (Webster and Edwards, 1984; Chernoff and Golden, 1988; Seller and Perkins-Cole, 1987; Shiota,1988; Shin and Shiota, 1999). The results reported in this article will show the evidence that hyperthermia-induced exencephaly causes marked growth retardation of the adrenal cortex and a decreased population of somatotropes in the adenohypophysis.

MATERIALS AND METHODS

Rats of the Sprague-Dawley strain were used. The animals were kept under a control of light (lights on 6:00–18:00) and temperature (20±2°C). Adult males and females were mated at night. Pregnancy was confirmed by the presence of sperms in the vaginal smears of females. The morning of sperm detection was designated as Day 0.5 of pregnancy.

On Day 9.5 of pregnancy, mother rats were anesthetized by ketamine hydrochloride. The animals were then tied to a wooden board and immersed in hot water (43°C) except their head for 15 min. After the hyperthermal treatment, the rats were wiped with dry towels and kept at 37°C up to recovery. Control mothers were sim-

* Corresponding author: Tel. +81-25-262-6184;
FAX. +81-25-262-6116.
E-mail: watayu@bio. sc.niigta-u.ac.jp

ilarly anesthetized, fixed and immersed in water (38°C). In this study, 16 pregnant rats were subjected to heat stress. Of these 6 rats died. On Day 21.5 of pregnancy, the animals were killed by cervical dislocation and the fetuses were removed for inspection.

After weighing all fetuses with or without brain anomalies, the head was fixed in Bouin's solution for a short time. Except in the cases of severe brain deformities, lateral sides of the head were cut to attain good penetration of fixative into the basal diencephalon. The left adrenal and kidney were separated and weighed, while the right adrenal was fixed for histological observation. After 24 hr of fixation, all tissues were dehydrated in ethanol and embedded in Paraplast. The heads were cut mostly in the sagittal plane at a thickness of 3 μm, whereas a few were also cut transversely. For ordinary observation, sections were stained with hematoxylin and eosin. Quantitative measurements of the hypophysis were performed by using sagittal sections. The volume of the pars distalis was compared using a volume index, which was calculated based on a three-dimensional measurement of the gland. The antero-posterior length was measured on a median sagittal sections of the hypophysis. The mean thickness was the average of the two different levels of dorso-ventral lengths; one was measured in a middle part of a median sagittal section and the other in the thickest lateral portion of the pars distalis. For these measurements, digitized images were analyzed with the aid of NIH image software. Whereas the width of the pars distalis was estimated from the number of sections.

Immunohistochemical staining was performed by using the peroxidase anti-peroxidase (PAP) method. To ascertain the damage of the hypothalamo-hypophysial axis, sections were immunostained with an antibody to vasopressin (Chemicon, CA., USA). While the hormone-producing cells of the adenohypophysis were stained with antisera to human adrenocorticotropin (hACTH), α-melanotropin (αMSH), rat growth hormone (rGH), β-subunit of human thyrotropin (hTSHβ), β-subunit of bovine luteinizing hormone (bLHβ) and rat prolactin (rPRL). The anti-αMSH was a gift from Dr. S. Tanaka, Shizuoka University, and other antibodies were provided by Dr. Parlow (NIDDK, USA). Since data are available to show a heterogenous distribution of LH cells along the medio-lateral axis (Watanabe and Daikoku, 1979), the numbers of the different types of immunoreactive cells were counted on the three different sagittal planes of the pars distalis, i.e., 1) a medial part, 2) a paramedian part including the lateral end of the neural lobe, and 3) a lateral part where the profile of the anterior lobe attains maximum. The easiness in identifying these three profiles in the sagittal plane was also the reason for this choice. For actual cell counting, digitized photographs of the selected areas were used. Immunoreactive cells were counted irrespective of the presence of the nucleus and staining intensity. In this study, the incidence of immunostained cells observed were expressed as "density", or the number of cells per 10, 000 μm^2 of section. For this purpose, the numbers of immunoreactive cells from three different levels were summed and it was divided by summed areas of section profiles which were measured with an image analyzing system (IBAS, Germany). The data were analyzed by Student's T-test.

RESULTS

Effect of Hyperthermia

As reported by other investigators, hyperthermia was found to cause anomalies in some fetuses, but in others the effect was so little that no apparent malformation was recognized (Table 1). In this study, the fetuses subjected to hyperthermal stress were divided into two groups according to the external appearance of the head region. "Group Bm+"

Table 1. Effects of hyperthermia on fetal rats on day 9.5 of gestation

Mother	Number of fetuses	Brain malformations –*	Brain malformations +**	Absorbed or dead
A	7	1	6	0
B	14	14	0	0
C	15	14	0	1
D	15	0	2	13
E	17	9	6	2
F	16	16	0	0
G	15	1	14	0
H	13	13	0	0
I	14	0	8	6
J	8	8	0	0
Total	134	76	36	22

* designated as Group BM–, ** designated as Group BM+

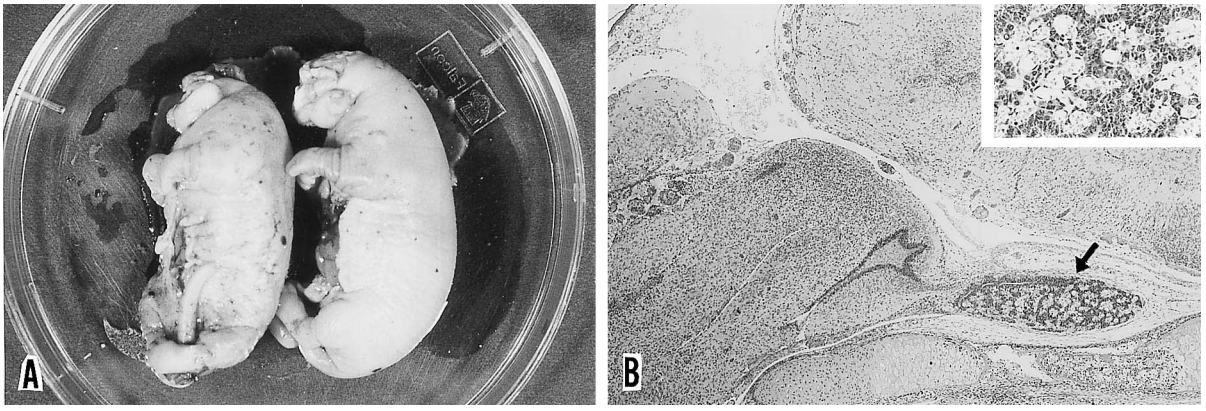

Fig. 1. Representative 21.5-day-old rat fetuses subjected to hyperthermia at Day 9.5 of gestation. A: External appearance of exencephalic fetuses induced by heat stress. ×1.8. B: Sagittal section through the hypophysis (*arrow*) of an exencephalic fetus shown in A. Note marked destruction of the diencephalon. Inset: High magnification of adenohypophysial tissue showing enlarged blood sinusoids. HE stain. ×30, inset ×100.

consisted of 36 fetuses with externally recognizable brain malformations including anencephaly, exencephaly or encephalocoele, whereas "Group Bm–" consisted of 76 fetuses without apparent brain deformity. As far as the simple histological examination was concerned, there could be found little difference between the fetuses of Group Bm–

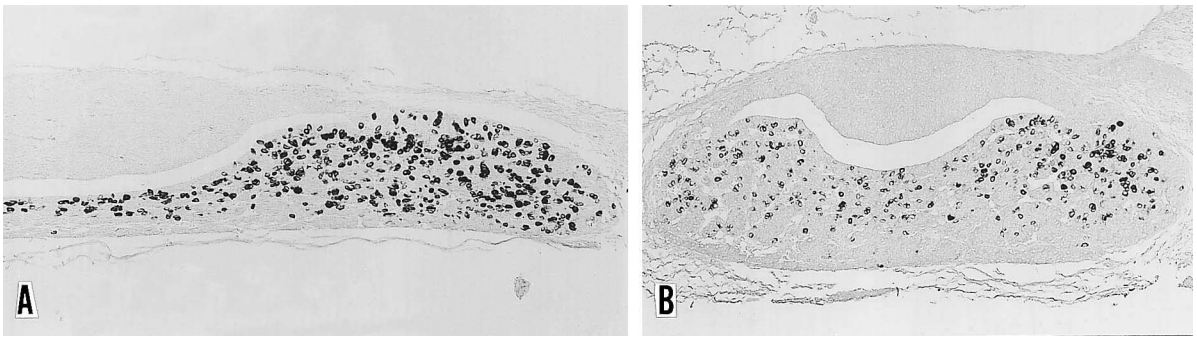

Fig. 2. Transverse sections through the hypophysis immunostained for GH. A: Hypopysis of 21.5-day-old intact control showing many intensely stained GH cells. B: Hypophysis of exencephalic fetus. The shape of the pars distalis differs from that of the control. GH cells are less strongly stained and fewer in number. ×75.

Table 2. Comparison of the size of the pars distalis in different groups of fetal rats

	Rostro-Caudal	Width (mm)	Thickness (×0.1 mm)			Volume
(n)	Length (mm) (a)	(b)	Medial (c)	Lateral (d)	Mean (e)	Index (a×b×e)
Intact (15)	1.05±0.034	1.81±0.041	1.45±0.11	3.30±0.16	2.38±0.12	4.95±0.46
Bm– (21)	1.03±0.029	1.79±0.031	1.52±0.096	3.02±0.076	2.26±0.069	4.57±0.15
Bm+ (34)	1.59±0.11**	0.80±0.024***	2.96±0.35***	4.12±0.17**	3.58±0.024**	4.13±0.29

Values are means±S.E. **P<0.01 and *** P<0.001 vs. Intact and Bm– fetuses

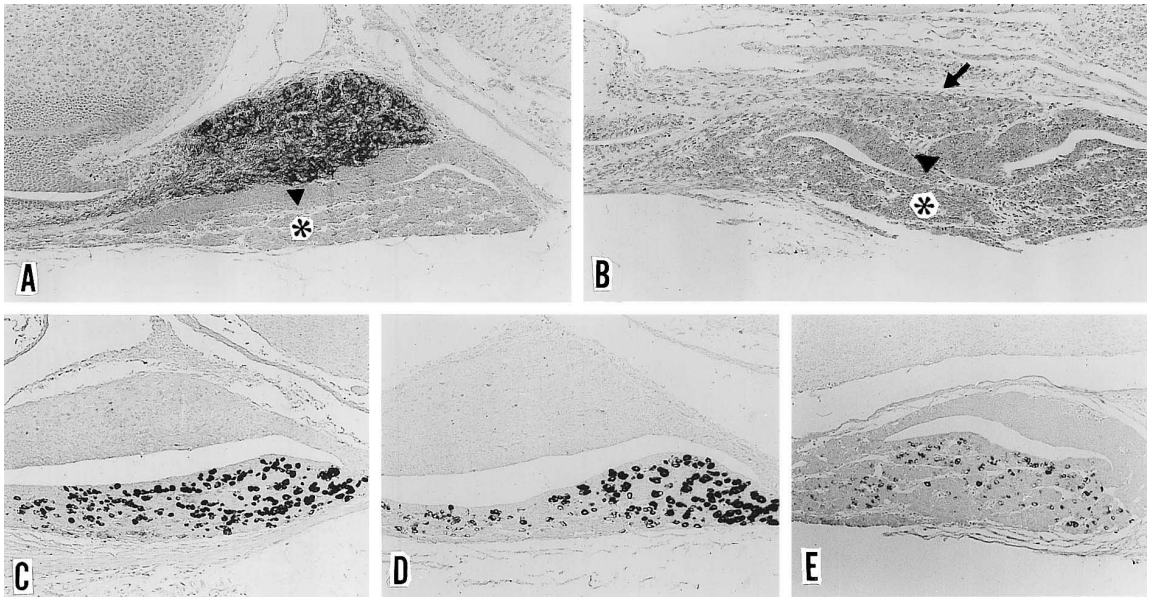

Fig. 3. Sagittal sections through the hypophysis immunostained for vasopressin (A, B) and GH (C–E). Anterior is to the left. Arrowhead indicates the pars intermedia. A: Hypophysis from heat-stressed but nonexencephalic 21.5-day-old fetus (Group Bm–) showing the presence of strongly immunoreactive vasopressin in the pars nervosa. The profile of the pars distalis (∗) is rather flat. ×75. B: In hyperthermia-induced exencephaly (Group Bm+), no vasopressin was observed in the small-sized pars nervosa (*arrow*). Flattening of the pars distalis (∗) is not conspicuous. ×75. C: Normal hypophysis containing many GH cells. ×75. D: GH cells are also abundant in number in the hypophysis of Group Bm–. ×75. E: In exencephalic fetus of Group Bm+, GH cells are markedly fewer in number. ×75.

and intact controls.

General Histology

Fig. 1A shows representative fetuses of Group Bm+ where the upper par of the head is lacking. Histological examination of these anomalies revealed that the brain including the diencephalic floor was destroyed to variable degrees. In many fetuses of this group, the hypophysis was completely open to the amniotic fluid due to a deep cleft running through the malformed diencephalon (Fig. 1B). On occasions, the blood sinusoids were distended in the adenohypophysis of Bm+ (Fig. 1B, inset). In spite of severe damage of the diencephalon, a thin infundibular tissue was observed in all the fetuses of Group Bm+. The shape of the adenohypophysis in exencephalic fetus was notably different from that in normal animals. As shown in Fig. 2, this morphological difference was obvious when the hypophysis was cut transversely. In Group Bm+ fetuses, the lateral growth of the pars distalis was suppressed. Instead, the entire pars distalis was rather thicker in this group (Fig 2B). The rostro-caudal length of the adenohypophysis was also greater in Group Bm+ (Fig. 3B). Table 2 summarizes the size of the pars distalis of the three groups. Although the pars distalis of Group BM+ fetuses was thicker and had a longer rostro-caudal axis, their volume index was almost similar when compared to intact control and Group Bm– fetuses.

Immunohistochemistry of the hypophysis

First of all, immunoreactivity of vasopressin was examined since this substance reflects the functional relationship between the hypothalamus and hypophysis. At the end of gestational period, intensely immunostained vasopressin was observed in the pars nervosa of both intact and Group Bm– fetuses (Fig. 3A). In contrast, no vasopressin immunoreactivity was recognizable in the pars nervosa of Group Bm+ animals (Fig. 3B).

The pars distalis of intact, Group Bm+ and Bm– fetuses was immunostained with antisera against GH, PRL, ACTH, TSH, LH. Among the cells immunoreactive for these hormones, only GH cells were significantly fewer in number in hyperthermia-induced exencephaly. The immunohistochemical results illustrating this decrease of GH cells are given in Figures 2 and 3C-E. The average number of GH cells in group Bm+ (337.2±26.3) were fewer than in intact control (485.0±19.2). Whereas the average area measured in group Bm+ ($35.6\pm1.9\times10^4$ μm^2) was rather greater than in control ($20.5\pm0.9\times0^4$ μm^2). Fig. 4 summarizes the number of different types of cells per area in different groups of fetuses. The population density of immunoreactive GH cells was reduced to a half of that in the control pituitaries. A slight reduction in GH cell density was also seen in the fetuses of Group Bm– but this was statistically insignificant.

Adrenal weight

The body weight was generally more or less reduced in the hyperthermia-induced exencephalic fetus, which was

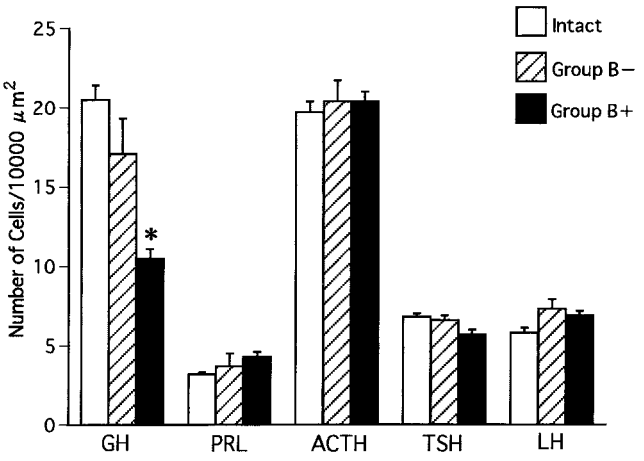

Fig. 4. Number of different types of hormone-producing cells of the adenohypophysis in Intact (n=8), Group Bm– (n=6) and Bm+ (n=28) fetal rats. * p<0.001 vs. Intact controls.

Table 3. Comparison of adrenal and kidney weight in different groups of feta rats

Group (n)	(n)	Body weight (g)	Adrenal (mg)	Kidney (mg)
Intact	19	5.2±0.08	0.8±0.08	16.5±0.6
Bm–	23	5.3±0.09	0.9±0.04	17.9±0.9
Bm+	36	4.1±0.09***	0.4±0.03***	17.8±0.5

Values are means±S.E. *** P<0.001 vs. Intact and Bm– fetuses

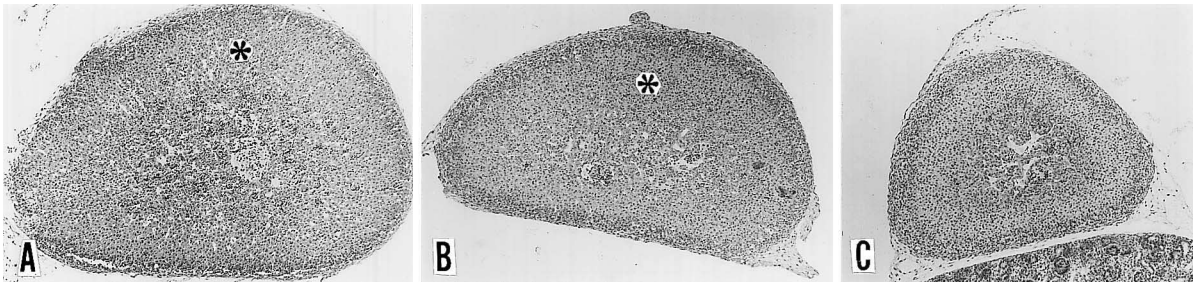

Fig. 5. Section through a medial part of adrenal gland of 21.5-day-old rat fetus. A: Intact normal fetus has the well-developed fetal cortex (∗). ×45. B: Fetal cortex (∗) is also abundant in volume in Group Bm–. ×45. C: Atrophic adrenal of Group Bm+. ×45

partly due to the loss of the head region. In spite of such decrease in body weight, no difference was seen in the kidney weight among the Bm+, Bm– and intact groups (Table 3). In contrast, there was a marked change in the weight of adrenal gland in the exencephalic fetuses. The adrenal in the fetuses of Group Bm+ was almost half as heavy as that of intact controls. No difference was seen in the adrenal weight between Bm– and intact controls. During the fetal period, the zonation of the adrenal cortex was still incomplete and it is roughly divided into the outer dense (permanent) and inner pale (fetal) cortex (Fig. 5A, B). Although the decrease in the entire size of the adrenal in Group Bm+ resulted from the reductions in the volume of both cortical layers, atrophy of the inner layers was more conspicuous (Fig. 5C).

DISCUSSION

Previous investigations on human anencephaly (Angevine, 1938; Tuchmann-Duplessis,1959; Hatakeyama, 1969; Salazar *et al*., 1969; Osamura, 1977;) were carried out with the purpose of determining the effect of the brain on the development of adenohypophysial cells. Experimental data are available to show the importance of a direct contact between the diencephalic floor and the adenohypophysial primordium. We showed that organ culture of Rathke's pouch in the absence of the brain resulted in a marked decrease in the number of ACTH cells (Watanabe, 1982a, b). In frogs too, ACTH cells failed to differentiate (Kawamura and Kikuyama, 1995) or were markedly decreased in number (Matsumoto and Watanabe, 2000) without the infundibulum. The contact between the hypophysis and brain, however, is essential only at the early stage of development. Watanabe (1982) has shown that the effect of the diencephalic floor on the differentiation of ACTH cells was remarkable at day 12.5 of gestation, but not at day 13.5. Since hyperthermia employed in this study apparently failed to destroy the diencephalic floor including the infundibulum and neurohypophysis, cell differentiation and proliferation of the adenohypophysis at the early developmental period are thought to occur rather normally. In this connection, the adenohypophysis of exencephalic fetuses shown in this study differs from that of the congenitally anomalous rat in which completely dislocated adenohypophysis has markedly suppressed differentiation of hormone-producing cells (Watanabe, 1996).

In human anencephaly the neural lobe was reported to occur in 20–40% of fetuses (Covel, 1927; Angevine, 1938). Although the number of fetuses are insufficient, Osamura (1977) reported that 3 out of 4 anencephalies possessed the pars nervosa. From these data, the adenohypophysial primordium in anencephalic fetuses may have a chance to get contact with the neurohypophysial primordium. In fact, Vogel (1961) stated that the brain of human anencephalies may develop normally through the first 6 or 7 weeks of fetal life, after which abnormal angiogenesis causes the destruction of neural tissue. In the present study, too, all heat-stressed fetal rats had both the infundibulum and pars nervosa. Owing to the presence of these neural elements, the differentiation of ACTH cells could occur almost normally irrespective of subsequent brain malformations. In human anencephaly, on the other hand, Osamura (1977) provided immunohistochemical data showing a marked decrease in the number of ACTH cells. At present it is not known whether the discrepancy between his and our data is due to the difference in the developmental manner of ACTH cells between the two species or in the methodologies employed.

Other than the direct tissue contact, hormones from target organs have been known to be necessary for the cytodifferentiation of the adenohypophysis. There is good evidence that the development of GH cells is under the influence of adrenal corticosteroid. Thus GH cells appear only when cortisol (Hemming *et al*., 1984, 1988) was added to serum-free cultures of the adenohypophysial primordium. Sato and Watanabe (1998) further showed a correlation between the number of GH cells in culture explants and the cortisol concentrations administered. This direct effect of glucocorticosteroid on developing GH cells well accounts for the marked decrease in the number of this type of cell in hyperthermia-induced exencephaly in this study. The adenohypophysis in exencephalic fetuses apparently received a insufficient amount of corticosteroids from the ill-developed adrenal glands. The question then arises as to the reason for the adrenal atrophy of the brain-malformed rats where ACTH cells appeared to differentiate almost normally. In view of the reports on congenital anencephaly in human babies (Angevine, 1938; Tuchmann-Duplessis, 1959; Osamura, 1977; Parker *et al*., 1983; Young *et al*., 1989) or experimentally decapitated fetal rats (Jost, 1966; Jost *et al*., 1974), the underdevelopment of the adrenal has been attributed to the decreased production rate of hypothalamic CRH or hypophysial ACTH. For the functional maturation of the hypothalamic-pituitary-adrenal axis, however, not only the normal formation of each organ itself but also vascular development is absolutely necessary. As already stated, anencephaly is usually accompanied by abnormal angiogenesis of the head region (Vogel, 1961). In view of the abnormally distended blood sinusoids filled with blood cells in the adenohypophysis in anencephalic human babies (Covel, 1927; Salazar *et al*., 1969) and exencephalic fetal rats (Fig. 1B, inset) in the present study, blood circulation is believed to be considerably lower than normal in the hypophysis as well. In addition to this disturbed blood flow, brain damage presumably lead to a subnormal level of CRH as suggested by Eguchi *et al*. (1971b). Thus a reduced amount of ACTH release was insufficient for the normal production of glucocorticoids, which in turn caused maldevelopment of GH cells in the adenohypophysis.

In this study, GH cells were also found to decrease in number in heat-stressed nonexencephalic fetuses though this change was not statistically significant. It cannot be excluded that hyperthermia suppressed the function of the

fetal adrenals without remarkable change of their morphology. Further studies are needed to investigate the effect of thermal stress on the rate of hormone release from the hypothalamus, hypophysis and adrenal cortex.

REFERENCES

Angevine DM (1938) Pathologic anatomy of hypophysis and adrenals in anencephaly. Arch Pathol 26: 507–518

Aoyama N, Yamashina S (1994) Cardiac and great vessel abnormalities in rat fetuses with external malformations induced by hyperthermia. (in Japanese) Acta Anat Nippon 69: 34–41

Chernoff GF, Golden JA (1988) Hyperthermia-induced exencephaly in mice: effect of multiple exposures. Teratology 37: 37–42

Collins MD, Mao GE (1999) Teratology of retinoids. Ann Rev Pharmacol. Toxicology 39: 399–430

Covel WP (1927) A quantitative study of the hypophysis of the human anencephalic fetus. Am J Pathol 3: 17–28

Daikoku S (1966) A method of deencephalon in the fetal rat. Okajimas Fol Anat Jap 42: 39–49

Daikoku S, Kinutani M, Watanabe YG (1973) Role of hypothalamus on development of adenohypophysis: an electron microscopic study. Neuroendocrinology 11: 284–305

Edwards MJ (1968) Congenital malformations in the rat following induced hyperthermia during gestation. Teratology 1: 173–178

Edwards MJ (1986) Hyperthermia as a teratogen: a review of experimental studies and their clinical significance. Terat Carc Mutag 6: 563–583

Eguchi Y, Suzuki S, Morikawa Y, Hashimoto Y (1971a) Experimental formation of goiter in exencephalic fetal rats subjected maternal hypervitaminosis A. Endocrinology 88: 261–263

Eguchi Y, Morikawa Y, Hashimoto Y (1971b) Lack of hypertrophy of the adrenal in exencephalic fetal rats subjected to hypervitaminosis A and then to maternal adrenalectomy. Endocrinology 88: 1526–1528

Eguchi Y, Hirai O, Morikawa Y, Hashimoto Y (1973) Critical time in the hypothalamic control of the pituitary-adrenal system in fetal rats: observations in fetuses subjected to hypervitaminosis A and hypothalamic destruction. Endocrinology 93: 1–11

Geelen JA (1979) Hypervitaminosis A induced teratogenesis. CRC Crit Rev Toxicol 6: 351–375

Hatakeyama S (1969) Electron microscopic study of the anencephalic adenohypophysis with the functional differentiation of the hypothalamus and their correlation with the functional differentiation of the hypothalamus during the foetal life. Endocrinol Jpn 16: 187–203

Hemming FJ, Begeot M, Dubois MP, Dubois PM (1984) Fetal rat somatotropes in vitro: effects of insulin, cortisol, and growth hormone-releasing factor on their differentiation: a light and electron microscopic study. Endocrinology 114: 2107–2113

Hemming FJ, Aubert ML, Dubois PM (1988) Differentiation of fetal rat somatotropes in vitro: effects of cortisol, 3,5,3'-triiodothyronine, and glucagon, a light microscopic and radioimmunological study. Endocrinology 123: 1230–1236

Jost A (1966) Problems of fetal endocrinology: the adrenal glands. Recent Prog Horm Res 22: 541–574

Jost A, Dupouy J-P, Rieutort M (1974) The ontogenetic development of hypothalamo-hypophyseal relations. Prog Brain Res 41: 209–219

Kawamura K, Kikuyama S (1995) Induction from posterior hypothalamus is essential for the development of the pituitary proopiomlanocortin (POMC) cells of the toad (*Bufo japonicus*). Cell Tissue Res 279: 233–239

Matsumoto M, Watanabe YG (2000) Differential ability of the pars intermedia in hypthalectomized frog tadpoles. Gen Comp Endocrinol 119: 37–42

Mirkes PE (1985) Effects of acute exposures to elevated temperatures on rat embryo growth and development in vitro. Teratology 32: 259–266

Osamura RY (1977) Functional prenatal development of anencephalic and normal anterior pituitary glands in human and experimental animals studied by peroxidase-labeled antibody method. Acta Pathol Jpn 27: 495–509

Parker CRJr, Carr BR, Winkel CA, Casey ML, Simpson ER, MacDonald PC (1983) Hypercholesterolemia due to elevated low density lipoprotein cholesterol in newborns with anencephaly and adrenal atrophy. J Clin Endocrinol Metab 57: 37–43

Salazar H, Macauley MA, Charles D, Pardo M (1969) The human hypophysis in anencephaly. I. Ultrastructure of the pars distalis. Arch Pathol 87: 201–211

Sato K, Watanabe YG (1998) Corticosteroids stimulate the differentiation of growth hormone cells but suppress that of prolactin cells in the fetal rat pituitary. Arch Histol Cytol 61: 75–81

Seller MJ, Perkins-Cole KJ (1987) Hyperthermia and neural tube defects of the curly-tail mouse. J Craniofac Genet Dev Biol 7: 321–330

Shin JH, Shiota K (1999) Folic acid supplementation of pregnant mice suppresses heat-induced neural tube defects in the offspring. J Nutrition 129: 2070–2073

Shiota K (1988) Induction of neural tube defects and skeletal malformations in mice following brief hyperthermia in utero. Biol Neonate 53: 86–97

Tuchmann-Duplessis H (1959) Etude des glandes endcrines des anencephales. Biol Neonate 1: 8–32

Vogel FS (1961) The anatomic character of the vascular anomalies associated with anencephaly. Am J Pathol 39: 163–174

Watanabe YG (1982a) Effects of brain and mesenchyme upon the cytogenesis of rat adenohypophysis in vitro. I. Differentiation of adrenocorticotropes. Cell Tissue Res 227: 257–266

Watanabe YG (1982b) An organ culture study on the site of determination of ACTH and LH cells in the rat adenohypophysis. Cell Tissue Res 227: 267–275

Watanabe YG (1996) An immunohistochemical study of an anomaly of the fetal rat: adenohypophysis developing without contact with the brain. Arch Histol Cytol 59: 381–387

Watanabe YG, Daikoku S (1979) An immunohistochemical study on the cytogenesis of adenohypophysial cells in fetal rats. Dev Biol 68: 557–567

Watanabe YG (1982) Effects of brain and mesenchyme upon the cytogenesis of rat adenohypohysis in vitro. I. Differentiation of adrenocorticotropes. Cell Tissue Res 227: 257–266

Webster WS, Edwards MJ (1984) Hyperthermia and the induction of neural tube defects in mice. Teratology 29: 417–425

Webster WS, Germain MA, Edwards MJ (1985) The induction of microphthalmia, encephalocele, and other head defects following hyperthermia during the gastrulation process in the rat. Teratology 31: 73–82

Young, MC, Lurence KM, Hughes IA (1989) Relationship between fetal adrenal morphology and anterior pituitary function. Horm Res 32: 130–135

(Received February 12, 2002 / Accepted April 3, 2002)