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Hypothalamic CRF Immunoreactivity in Genetically Hypothyroid (*hyt/hyt*) Mice (42553)

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Abstract. The induction of hypothyroidism in young rats by feeding thiouracil to their mothers during pregnancy has been shown to depress hypothalamic content of bioactive and immunoactive corticotropin-releasing factor (CRF). The present study was done to determine whether genetically hypothyroid young mice (*hyt/hyt*) born to euthyroid mothers (+/*hyt*) exhibited a similar depression in hypothalamic CRF immunoreactivity. Young euthyroid and hypothyroid littermate mice were examined by radioimmunoassay for hypothalamic CRF content at 15, 20, 25, or 30 days of age. Mean CRF content was depressed insignificantly (to about 80% of normal) by hypothyroidism, at 15–25 days of age. However, after weaning by the mother, 30-day-old hypothyroid pups demonstrated significantly depressed hypothalamic CRF levels (71%). It is suggested that maternal factors may be assisting in the maintenance of hypothalamic CRF until after weaning. Furthermore, genetic hypothyroidism does not appear to have nearly as marked an influence as thiouracil feeding on hypothalamic CRF levels.

Previous studies have shown that induction of hypothyroidism by administration of thiouracil via the maternal diet delays maturation of the hypothalamus–pituitary–adrenal (HPA) axis response to a general stressor in young rats (1, 2) and mice (3). More recent studies have found the hypothalamic content of corticotropin-releasing factor (CRF) bioactivity (4) and immunoreactivity (5) to be depressed in 15-day-old pups of thiouracil-fed rats, while the *in vitro* reactive potentials of pituitary and adrenal components of the axis are not altered. A question which remains unanswered in these studies is whether depressed hypothalamic CRF activity is the result of a direct influence of thiouracil on the development of hypothalamic secretory neurons, or whether hypothyroidism induced by thiouracil produces the maturational delay. With the development of the genetically hypothyroid mouse model (*hyt/hyt*) (6), it has been possible to separate hypothyroidism from the confounding effects of thiouracil administration. The *hyt/hyt* mouse possesses a rudimentary thyroid gland which is insensitive to thyroid-stimulating hormone, resulting in retardation of growth and the low serum thyroxine (T_4) levels of clinical hypothyroidism. Heterozygous littermates (+/*hyt*) are phenotypically normal. The present study measured hypothalamic CRF content in 15-, 20-, 25-, or 30-day-old hypothyroid mice and their euthyroid littermates.

Materials and Methods. Original stock animals consisted of 10 breeding pairs of C·RF/J (*hyt*) mice purchased from Jackson Labs (Bar Harbor, ME). Homozygous (*hyt/hyt*) hypothyroid mice were made reproductively competent by incorporation of 0.025% desiccated thyroid powder (Sigma Chemical Co., St. Louis, MO) into a Lab Blox Mash diet (Continental Grain Co., Chicago, IL) as previously described (7). Offspring of these pairs were selected as parents of young mice used in the present study. In all the experimental mating pairs, males were of the genotype *hyt/hyt* and females were +/*hyt*, producing litters of which approximately half the young were euthyroid and half were hypothyroid. A thyroid-supplemented diet was fed in the mating cage, and females were removed from the mating cage when visibly pregnant. Pregnant and lactating dams were fed standard pelleted Lab Blox for the remainder of the study.

All pups of a given litter were handled so as to minimize HPA axis activation. At either 15, 20, 25, or 30 days of age they were weighed to the nearest 0.1 g and killed by decapitation. Blood was collected and serum was extracted for determination of circulating thyroxine (T_4) levels. Hypothalamic fragments were rapidly excised and stored frozen in 0.1 *N* HCl (25 μl/hypothalamus) for subsequent extraction of CRF. T_4 was determined in individual serum samples by radioimmunoassay (RIA), using kits purchased from Micromedic Systems, Inc.

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(Horsham, PA) to determine the thyroid status of the pups. The kit was sensitive to a T_4 concentration of 0.3 μg/dl. The variation within assays was 6.1% and that between assays was 7.5%. The genotype of all pups was established by using body weight and circulating T_4 levels. The hypothalami of all pups of the same genotype within a given litter were pooled for extraction of CRF.

CRF was extracted from hypothalami by the method of Angwin and Barchas (8) as slightly modified (5). Recovery of rat CRF standard from the extraction procedure was greater than 70%. Hypothalamic CRF content was determined using an RIA kit commercially available from Peninsula Laboratories, Inc. (San Carlos, CA). Although the primary antibody for this kit is rabbit anti-rat CRF, serial dilutions of mouse hypothalamic extract of 1:20 gave results which paralleled the standard curve. Furthermore, the hypothalamic CRF content in euthyroid young mice was similar to that found in euthyroid young rats (4) using kits from the same source. In our hands, the lower limit of sensitivity for this kit was 14 pg CRF. The CRF content of all samples was determined with one kit in one assay, so variation between assays was not a consideration. Variation within the assay was 2.6%.

Comparison between means of a given parameter for euthyroid and hypothyroid pups of a given age was accomplished with the Student's *t* test, with significance ascribed to $P < 0.05$. In addition, the CRF data were subjected to two-way analysis of variance (age × genotype). Regression analysis was also performed to determine whether a linear relation existed between age and body weight for either euthyroid or hypothyroid littermates.

Results. At all ages tested, body weights of euthyroid (+/*hyt*) animals were greater than those of their hypothyroid (*hyt*/*hyt*) littermates (Table I). Body weights of euthyroid pups were highly linearly correlated with days of age ($r = 0.97$) by the relationship $Y = 0.36X + 1.97$. Body weight and age were not highly correlated in hypothyroid young ($r = 0.23$), largely as a result of a decline in weight between 20–25 and 25–30 days. The difference in thyroid status was clearly evidenced by circulating T_4 levels, with those of hypothyroid pups consistently below 1 μg/dl and those of euthyroid pups averaging 5.7–7.7 μg/dl (Table I).

Mean hypothalamic CRF levels of 15-, 20-, and 25-day-old hypothyroid mice were approximately 80% of those in euthyroid littermates, but the differences were not statistically significant at these ages (Fig. 1). However, at 30 days, hypothalami of hypothyroid pups contained significantly less CRF than did those of euthyroid young (169.5 vs 238.9 pg/hypothalamus). In addition, analysis of variance revealed a significant main effect of genotype ($P < 0.05$).

Discussion. The growth patterns of littermate euthyroid and hypothyroid mice in the present study are similar to those presented in the original paper reporting development of this animal model (6). Circulating T_4 levels corroborate that depressed body weight in *hyt*/*hyt* mice is the result of consequences attendant with the hypothyroid condition (Table I). Although studies using genetically hypothyroid mice have focused primarily on the pituitary–thyroid system (7), reports of normal ultrastructure of pituitary corticotropes (9) and of modified female adrenocortical X-Zone structure (10) have appeared. To our knowledge, this is the first report of measurement of

TABLE I. BODY WEIGHTS AND CIRCULATING THYROXINE LEVELS IN 15–30-DAY-OLD EUTHYROID (+/*hyt*) AND HYPOTHYROID (*hyt*/*hyt*) MICE[a]

	15 day		20 day		25 day		30 day	
	+/*hyt*	*hyt*/*hyt*	+/*hyt*	*hyt*/*hyt*	+/*hyt*	*hyt*/*hyt*	+/*hyt*	*hyt*/*hyt*
Body wt (g)	7.3 ± 0.4	5.8 ± 0.4[b]	9.6 ± 0.5	7.9 ± 0.3[b]	10.1 ± 0.5	7.5 ± 0.5[b]	13.1 ± 0.7	6.5 ± 0.8[b]
T_4 (μg/dl)	6.0 ± 0.4	0.7 ± 0.1[b]	5.7 ± 0.3	0.8 ± 0.1[b]	7.7 ± 0.4	0.9 ± 0.1[b]	6.6 ± 0.1	0.9 ± 0.1[b]

[a] Means ± SEM for six to eight litters of pups.
[b] Significantly different from +/*hyt* by Student's *t* tests ($P < 0.05$).

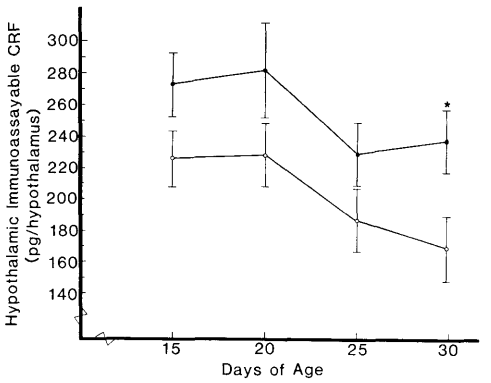

FIG. 1. Hypothalamic immunoassayable corticotropin-releasing factor in hypothalamic extracts from phenotypically euthyroid (+/*hyt*, ●) and hypothyroid mice (*hyt/hyt*, ○) at 15, 20, 25, and 30 days of age. Data points represent means of determination from six to eight litters of mice, and bars represent the standard error of the mean. *Significantly different from the hypothyroid value ($P < 0.05$).

the functional potential of the hypothalamic component of the HPA axis in genetically hypothyroid mice.

In the present study, mean hypothalamic content of immunoreactive CRF is less in *hyt/hyt* mice than in euthyroid littermates at all ages studied, but is not significantly subnormal until 30 days of age (Fig. 1). On the other hand, immunoassayable hypothalamic CRF content was more severely depressed in 15-day-old thiouracil-fed rats, being approximately 40% of normal (5). This discrepancy may be partially explained by the difference in the thyroid status of the mothers of young in the two studies. Since thiouracil was supplied to the young rats by way of the maternal diet, the thyroid status of the mother, as well as of the pups, was depressed from early in pregnancy. In the present study, all mother mice received a thyroid-supplemented diet coincident with genetically hypothyroid males in the mating cage and were genotypically heterozygous and phenotypically euthyroid as well. Placental passage of thiouracil and its direct effect on the fetus have been established (11), whereas transplacental influence of thyroid hormones continues to be debatable (12), rendering equivocal the supposition of a direct influence of circulating maternal T_4 levels on fetuses *in utero*. However, the supply of thyroid hormones present in maternal milk (13) would be less available or unavailable to young rats born to thiouracil-fed mothers as compared to young mice born to euthyroid mothers. In light of this suggestion, it is interesting to note that mean hypothalamic CRF content in the present study appeared to decline in both euthyroid and hypothyroid mice at the time of weaning by the mother (20–25 days), with the decline continuing in hypothyroid pups to 30 days of age (Fig. 1). While these results may be caused by previously unknown pleiotropic effects of the *hyt* mutation, they are suggestive of a direct effect of hypothyroidism.

As is the case with any secretory product, the tissue content of CRF at any given instant is directly tied to its rate of synthesis and rate of release. Thus, data from the present study speak only indirectly to the functional capability of the hypothalamus of *hyt/hyt* mice. It would be of interest to determine whether genetic hypothyroidism decreases the ability of the hypothalamus to release immunoreactive CRF *in vitro*. However, attempts to measure immunoreactive CRF in hypothalamic incubates have met with little success (14). It would also be of interest to determine the influence of hypothyroidism upon CRF levels in hypothalamo–hypophyseal portal blood, but the small size of young mice in general and *hyt/hyt* mice in particular makes collection of such blood samples technically unfeasible. Additionally, there is evidence that hypothyroidism modifies metabolism (15) and condensation (16) of brain tubulin into microtubules, a defect which would impair the secretory ability of neurosecretory cells. In view of the above technical difficulties, the reported modification of tubulin status, and the previously determined correlation between thiouracil-induced depression of HPA axis function (1–3), hypothalamic CRF bioactivity (4) and immunoreactivity (5), the present study relates genetic hypothyroidism to hypothalamic CRF content. Studies in progress will determine the development of overall responsivity of the HPA axis in the *hyt/hyt* mouse, since investigations in the hyporesponsive young rat (17) have suggested that hypothalamic CRF levels similar to those in the present study are sufficient to activate the pituitary component of the axis.

In conclusion, genetic hypothyroidism depresses the hypothalamic CRF content of

young mice born to euthyroid mothers, with the depression becoming more severe after weaning. This effect appears to be the result of hypothyroidism, but nonthyroidal effects of the *hyt* mutation have not been ruled out. CRF reduction resulting from genetic hypothyroidism appears less pronounced than that caused by thiouracil feeding. The influence of genetic hypothyroidism on the development of overall HPA axis function remains to be determined.

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