64. Inherited Functional Alterations in the Hypothalamo-Pituitary-Thyroid System in Rats raised from Thyroidectomized Mothers

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We have reported that maternal parathyroidectomy in the rat during pregnancy affects the functional development of calcium regulatory system in the offspring over several generations. Less marked hypocalcemic response to the removal of parathyroid glands was observed in the 3rd or 4th generation offspring as well as in the first generation offspring when mother rats were parathyroidectomized on the 5th day of gestation.⁷⁾ A delay of reproductive aging in the descendants of the parathyroidectomized mother was noted for several generations.¹⁰⁾ When mother rats were parathyroidectomized on the 15th day of gestation, the offspring rats showed a decreased threshold to the lethal dose of calcium chloride over 10 generations.⁹⁾ These results provide evidence showing considerably large influence of maternal environment on the phenotypic expression in fetuses.

Thyroid hormones are well known to play a crucial role in the development of human and animals. However, because of that the available information from human and animal studies have indicated a limited transfer of thyroid hormones either from mother to fetus or from fetus to mother,⁴⁾ the effect of maternal thyroid disorders on the functional development of fetuses have been inadequately studied. We report here that maternal thyroidectomy during pregnancy resulted in alterations in the hypothalamo-pituitary-thyroid function in the sequential offspring.

Materials and methods. Wistar-Imamichi male and female rats were mated and the day on which sperm was present in the vaginal smear was designated as day 0 of pregnancy. The pregnant rats were surgically thyroidectomized (Tx) under ether anesthesia on the 5th day of gestation and 1% calcium chloride solution was given as drinking water until the time of weaning. Control pregnant rats were sham-operated. Tx mothers delivered normally on the 22nd day of gestation. Litter-size of the Tx rats did not differ from that of controls. All pups were weaned at 21 days of age. The subsequent generation offspring rats were developed by sister-brother mating. The second (F₂) and third (F₃) generation rats of Tx or sham-operated groups were thyroidectomized at 3 months of age. They were killed by decapitation 1, 2, 3 or 7 days after the operation and the trunk blood was collected. In another series of experiments, the sham- and Tx-F₄ rats were tested for pituitary thyrotropin (TSH) response to synthetic thyrotropin-releasing hormone (TRH) administration. Blood samples of the sham- and Tx-F₄ rats were obtained from the jugular vein by venipuncture with 26-G needle for determination of the basal TSH level. These animals were then divided into two groups; one group was injected intravenously with 5 μ g/kg

of synthetic TRH (Peptide Institute Inc., Osaka) dissolved in saline to give an injection volume of 1 ml/kg and the other group with saline alone to serve as controls. The rats were killed by decapitation 5, 15 or 30 min later and the trunk blood was collected. Isolated serum was kept frozen at -20°C . Serum TSH was measured by the double antibody radioimmunoassay²⁰⁾ using the rat pituitary hormone kit provided by the National Pituitary Agency, NIAMDD. The standard used was NIAMD-Rat TSH-RP1. Serum thyroxine (T_4) was measured using a radioimmunoassay (SpacTM, Dai-ichi Radioisotope Lab., Tokyo). The rats were kept in a temperature-controlled room ($22 \pm 3^{\circ}\text{C}$) with a lighting schedule of 14 hr light (06:00–20:00) and 10 hr darkness and supplied with a stock diet (Oriental Yeast Co., Ltd.) and water *ad libitum*. Statistical analyses of data were performed using Student's *t*-test.

Results. There was no significant difference in the body growth, basal serum T_4 and TSH levels between the sham- and Tx-offspring rats. Serum TSH levels in both male and female rats of the sham- F_2 groups showed a significant increase from the basal level ($P < 0.05$) 24 hr after thyroidectomy and the level continued to increase until the 7th day of the operation examined. In the Tx- F_2 male rats, serum TSH levels showed a significant increase 24 hr after thyroidectomy, but it reached a plateau on the 2nd to 3rd day of the operation and no further increase was observed on the 7th day, i.e., the TSH level on the 7th day was significantly lower than that in the sham- F_2 males ($P < 0.01$) (Fig. 1).

Fig. 1. Serum TSH levels following thyroidectomy in the second (F_2) or third (F_3) generation rats raised from sham-operated (N) or thyroidectomized (Tx) mothers. ** $P < 0.01$ vs. N males.

Serum TSH levels in the Tx- F_2 female rats did not increase 24 hr after thyroidectomy but showed a significant progressive increase to the same level as in the sham- F_2 females on the 2nd, 3rd and 7th day of thyroidectomy. Thyroidectomy in the sham- F_3 male and female rats also resulted in a progressive increase in serum TSH levels. Its changing pattern was similar to that shown in the sham- F_2 rats. As shown in the Tx- F_2 male rats the Tx- F_3 male rats also showed a less marked increase in serum TSH levels 7 days after thyroidectomy; the

level was significantly lower than that in the sham-F₃ males ($P < 0.01$) (Fig. 1). The Tx-F₃ female rats responded to thyroidectomy with no increase in serum TSH levels at 24 hr and then with a progressive increase toward the same level in the sham-F₃ control females on the 7th day of the operation. This changing pattern in the serum TSH level was quite similar to that in the Tx-F₂ females.

Table I. Serum TSH levels ($\mu\text{g/ml}$ serum) following an intravenous injection of synthetic TRH ($1 \mu\text{g/kg}$) in the 4th generation rats raised from sham-operated or thyroidectomized (Tx) mothers

Time after TRH (Min)	Male		Female	
	Control	Tx	Control	Tx
0	$0.153 \pm 0.040^*$ (4)	0.125 ± 0.015 (4)	0.132 ± 0.010 (5)	0.126 ± 0.024 (4)
5	1.087 ± 0.191 (4)	0.740 ± 0.115 (4)	0.944 ± 0.185 (5)	0.902 ± 0.056 (4)
15	2.081 ± 0.135 (3)	$1.385 \pm 0.193^{**}$ (4)	1.319 ± 0.258 (5)	1.467 ± 0.475 (4)
30	1.224 ± 0.096 (3)	$0.896 \pm 0.138^*$ (4)	0.598 ± 0.067 (5)	$0.913 \pm 0.152^*$ (4)

* Mean $\pm$ SEM. Number in parentheses indicate the number of rats examined.

A single intravenous injection of $5 \mu\text{g/kg}$ TRH to the sham-F₄ and Tx-F₄ offsprings induced a marked increase in serum TSH levels. The maximal increase was shown 15 min after the injection of TRH in male and female rats of both groups. However, the magnitude of the increase in serum TSH levels in the Tx-F₄ male rats was significantly lower than that in sham-F₄ male rats (Table I).

Discussion. The present results clearly indicated that the offspring rats, particularly male offspring, raised from mothers thyroidectomized on the 5th day of gestation possess heritable deficits in the hypothalamo-pituitary-thyroid function. In the Tx-F₂, F₃ and F₄ generation male rats, the responsiveness of the hypothalamo-pituitary axis to the deprivation of thyroid hormones or to exogenous TRH administration showed a marked decline. It has been observed in human and animals that thyroidectomy results in an increase in the release of TRH from the hypothalamus and of TSH from the pituitary gland via negative feedback mechanism. The decreased release of TSH in response to thyroidectomy or to TRH administration observed in the present experiments might suggest a decrease in (1) pituitary TRH receptors, (2) TRH concentrations released into the portal circulation, or (3) TSH synthesis in thyrotrophs. Although mechanisms involved in the present findings including sex difference in the effect of maternal thyroidectomy are unknown, the decreased TSH response to exogenous TRH stimulation which is demonstrated in the Tx-F₄ male rats may suggest alterations of thyrotrophs. TRH crosses the placenta in rats¹¹⁾ and presumably in human. But the placenta is essentially impermeable to TSH and thyroxine and triiodothyronine.^{4,15)} Moreover, it has been indicated that the fetal hypothalamo-pituitary-thyroid system develops and functions largely independent of maternal system.⁵⁾ However, maternal hypothyroidism has been known to induce a certain change in fetal development or in fetal pituitary-thyroid function in human¹⁴⁾ or in rats.^{17),18)} The present results also indicated that maternal thyroid function is of an extreme importance for the functional

development of fetal hypothalamo-pituitary-thyroid system. Animal studies have shown increased responsiveness of the pituitary-thyroid system to TRH or to thyroxine during pregnancy.¹²⁾ This suggests that thyroidectomy during pregnancy would result in an expanded alterations in the hypothalamo-pituitary-thyroid system.

There are still a few reports on the vertical transmission of altered phenotypic expression in the progeny affected by maternal environment. As mentioned above, maternal parathyroid ablation induces alterations in the regulatory system of serum calcium or in the threshold to calcium itself in the offspring for several generations in the rat.^{7),9),10)} When neonatal female rats were made hypothyroid with large doses of thyroxine and mated to normal males, their F₁ and F₂ offspring showed a variety of abnormal endocrine functions.¹⁾ Maternal under-nutrition results in cerebral deficiency²¹⁾ or in immune-deficiency³⁾ for several generations. Maternal zinc deprivation in mice has been found to induce deficiency of antibody formation in the F₂ and F₃ generation offspring.²⁾ Effects of parental exposure to drugs also results in heritable functional alterations in the offspring over several generations. Behavioral changes in the F₂ or F₃ generation offspring of mice or rats treated with morphine,⁶⁾ 6-mercaptopurine,¹⁹⁾ phenytoin⁸⁾ or tricyclic antidepressants¹³⁾ have been reported. Transmission of tumor incidence into the 3rd generation offspring of mice exposed to urethan or radiation has been observed by Nomura.¹⁶⁾ The present results would extend a new sight into maternal hypothyroidism as a factor which modifies phenotypic expression in offspring, though the mechanism remains unsolved.

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