Effects of Exposure of Pregnant Rats to TRH on Development of the Pituitary-Thyroid Axis in their Progeny

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Abstract

TRH (10 and 1000 $\mu\text{g}/\text{kg}$ body weight (BW)) was injected ip into pregnant rats daily from day 0 to 20 of pregnancy, and the pituitary-thyroid axis of their pups (Mat-TRH rats) was examined on days 0, 4, 10, 21 and 90 after birth. The pituitary TSH content of male Mat-TRH rats was significantly lower on day 4, and higher on day 10 than that of control rats. The serum TSH was significantly higher on day 10 (except female 10 $\mu\text{g}/\text{kg}$ group).

An exaggerated TSH response to exogenous TRH (10 $\mu\text{g}/\text{kg}$ BW; ip) was observed on day 10 (males, 1000 $\mu\text{g}/\text{kg}$ group). The serum T_4 level of female Mat-TRH rats was low on day 4 (1000 $\mu\text{g}/\text{kg}$ group), and higher on day 10. On days 21 and 90, the levels of pituitary TSH, serum TSH and T_4 in Mat-TRH rats were similar to those in controls, but the TSH response to TRH was still exaggerated (1000 $\mu\text{g}/\text{kg}$ group).

No significant difference between control and TRH-treated mothers was seen on days 10 and 90 postpartum except for a decreased pituitary TSH content on day 10 in the 1000 $\mu\text{g}/\text{kg}$ group. It is concluded that repeated administration of TRH to pregnant rats shows an effect on the pituitary-thyroid axis function of their progeny in later life.

The pituitary-thyroid axis in fetal rats is thought to be immature: in fetuses the concentration of circulating thyroid hormones and TSH are very low (Fukuda and Greer 1978), and TSH secretion from the pituitary shows little dependency on hypothalamic control (Tonooka and Greer 1978; Theodoropoulos *et al.*, 1979).

Injection of TRH into pregnant animals has been found to increase both the maternal and fetal serum TSH level in rats (D'Angelo *et al.*, 1972; Kojima and Hershman 1974) and in rhesus monkeys (Azukizawa *et al.*, 1976), but the effect of TRH on postnatal development of the pituitary-thyroid axis is unknown. Recently, we found that neonatal exposure to TRH suppressed the TSH response to exogenous TRH in later life of developing rats (Azukizawa *et al.*, 1981; Kawai *et al.*, 1983), and we have concluded

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that this impaired TSH response to TRH was induced by persistent abnormality of pituitary cells. In the present study we examined the effects of repeated administration of TRH to pregnant rats on the pituitary-thyroid axis of their progeny in later life.

Materials and Methods

Animals: Male and virgin female Wistar rats were purchased at 5 weeks of age from Kitayama Labes Co., Kyoto, Japan. They were maintained in an air-conditioned animal room with lighting between 8:00 and 20:00, at a temperature of $22 \pm 1^\circ\text{C}$ and relative humidity of 50–60%. Water and food (CA-1, containing $42 \mu\text{g}$ of iodine per 100 g of diet, Nippon Clea, Tokyo, Japan) were given *ad libitum*. After a 3-week acclimatization period, females were mated overnight with males. The day when spermatozoa were found in vaginal smears was taken as day 0 of pregnancy. After delivery, newborn rats were taken at random from several litters to avoid maternal influences, and the litter size was adjusted to 8 pups (4 of each sex) per mother. Pups were kept with the mothers until weaning on day 21 of postnatal life. After weaning, the animals were maintained until day 90. The mothers were also kept until 90 days postpartum.

Experimental procedure: Synthetic TRH (Tanabe Seiyaku Co., Osaka, Japan) was dissolved in saline and injected ip into pregnant rats in a volume of 1 ml/kg body weight (BW) at doses of 10 and $1000 \mu\text{g/kg}$ BW once a day from day 0 to 20 of pregnancy, then their pups (Mat-TRH rats) were examined. Control pregnant rats received saline instead of TRH.

During the suckling period, the external differentiation and reflexologic functions of pups were examined by the method described previously (Kawai *et al.*, 1983).

Pups were killed on days 0, 4, 10, 21 and 90 after birth. Blood was taken by thoracic incision on days 0, 4 and 10 after birth and blood from 2 to 4 pups of each group was pooled. On days 21 and 90 after birth, the animals were bled by decapitation under anesthesia with sodium pentobarbital between 13:00–15:00. The serum was separated and stored at -20°C until assayed. The pituitaries were obtained on days 4, 10 and

90 after birth and stored at -20°C . They were homogenized in 1 ml of a saline solution buffered at pH 7.4 with 0.5 M phosphate salts and the supernatant were used for measurement of TSH.

The mothers were killed on days 10 and 90 postpartum. Blood was taken from the abdominal aorta of animals under anesthesia with sodium pentobarbital and then the pituitary was excised.

Fig. 1. Pituitary TSH contents (mean $\pm$ SE) of Mat-TRH rats on postnatal days 4, 10 and 90. Asterisks indicate significant differences between Mat-TRH rats and saline-treated control (* $p < 0.05$; ** $p < 0.01$). Figures above columns indicate numbers of animals used.

TRH-test on pups and mothers: Blood samples were withdrawn after ip injection of TRH at 10 $\mu\text{g}/\text{kg}$ BW to animals. Blood was collected from pups by thoracic incision without anesthesia on day 10 or via an indwelling needle in the right jugular vein under anesthesia with sodium pentobarbital on days 21 and 90 after birth. The TRH test on mothers was done in the same way on days 10 and 90 postpartum. Sera were stored at -20°C until assayed.

Radioimmunoassay (RIA): RIA of TSH in serum and pituitary gland was done by a double antibody method described previously (Rookh *et al.*, 1979; Kawai *et al.*, 1983). The reagents for rat TSH RIA were kindly provided under the Rat Pituitary Hormone Distribution Program of the NIH. The sensitivity of the assay was 5 $\mu\text{U}/\text{ml}$. Intraassay and interassay coefficients of variation were 4.3 and 5.4%, respectively.

Thyroxine (T_4) in serum was measured by RIA using polyethylen glycol separation of the bound and free forms (Rookh *et al.*, 1979; Kawai *et al.*, 1983). The sensitivity of the assay was 0.3 $\mu\text{g}/\text{dl}$. Intra- and interassay coefficients of

variation were 4.4 and 13.3%, respectively.

Statistical analysis: The significances of differences between values were evaluated by the "t" and " χ^2 " test.

Results

Effects of the TRH exposure of mothers during pregnancy on development of their pups.

The growth, external differentiation and reflexes of Mat-TRH rats did not differ from those of controls.

Pituitary-thyroid axis during the neonatal period: The pituitary TSH content was significantly reduced on postnatal day 4 and significantly increased on day 10 in male Mat-TRH rats (Fig. 1). The serum TSH level was significantly raised on day 10, except in females of the 10 $\mu\text{g}/\text{kg}$ group (Fig. 2). An exaggerated response on TRH

Fig. 2. Basal serum TSH levels of Mat-TRH rats on postnatal days 4, 10, 21 and 90. Vertical bars indicate SE. Asterisks indicate significant differences between Mat-TRH rats and controls (* $p < 0.05$). Figures above columns indicate numbers of pooled samples (days 4 and 10) or animals (day 21 and 90) used.

Fig. 3. Serum TSH response 15 min after TRH administration (10 µg/kg; ip) in Mat-TRH rats on postnatal days 10, 21 and 90. Vertical bars indicate SE. Asterisks indicate significant differences between Mat-TRH rats and controls (* $p < 0.05$; ** $p < 0.01$). Figures above columns indicate numbers of animals used.

test was observed on day 10 in males in the 1000 µg/kg group (Fig. 3).

The serum level of T_4 in Mat-TRH and control rats at birth was very low [≤ 0.8 µg/dl or below the limit of detection ($0.3 < \mu\text{g/dl}$)]. On day 4, the serum T_4 level was significantly lower in females of the 1000 µg/kg group. In contrast, on day 10, the serum T_4 level was significantly raised in Mat-TRH female rats (Fig. 4).

Pituitary-thyroid axis in adulthood: The pituitary TSH content and serum TSH and T_4 levels in Mat-TRH rats were comparable to those in controls on days 21 and 90 (Figs. 1, 2 and 4). An exaggerated response on TRH test was, however, observed on days 21 and 90 in Mat-TRH rats of 1000 µg/kg group (Fig. 3).

Effects of TRH exposure of mothers during pregnancy on their pituitary-thyroid axis postpartum.

On day 10 postpartum, the pituitary TSH content of mothers treated with TRH at 1000 µg/kg during pregnancy (331 ± 41 mU/pituitary, $n=5$) was significantly lower ($P < 0.05$) than that of controls (545 ± 72 mU/pituitary, $n=10$). No significant difference was seen in the serum TSH, T_4 or the response on TRH test between TRH-treated and control mothers. On day 90 postpartum, the pituitary TSH content, serum TSH- and T_4 -levels and the response on TRH test in the TRH-treated mothers were similar to those in controls.

Discussion

Table 1 shows a schematic representation of our results. The present study indicates that maternal TRH-treatment of pregnant rats produced effects on the pituitary-thyroid function of their pups.

The serum thyroid hormone levels have been found to be increased in human subjects (Snyder and Utiger 1973; Ahuja *et al.*,

1980) and animals (Chopra and Solomon 1973) after repeated treatment with TRH. These data are comparable with our data on day 10. The TSH in their reports, however, was found to be blunted. Therefore, the TSH response to TRH in Mat-TRH rats is different from these observations.

Nemeroff *et al.* (1980) reported that repetitive administration of TRH to rats and mice lowered the pituitary TSH content and serum TSH-, and T_4 - and T_3 levels. Pre-

viously, we (Kawai *et al.*, 1983) found that neonatal exposure to TRH resulted in decreases in pituitary TSH content and serum TSH and T_4 levels. These observations are comparable with the slight suppression of pituitary-thyroid function in Mat-TRH rats on day 4 and in TRH-treated mothers on day 10.

The absence of a late effect on pituitary TSH secretion in TRH-treated mothers postpartum suggests that the cause of this

Fig. 4. Basal serum T_4 levels of Mat-TRH rats on postnatal days 4, 10, 21 and 90. Vertical bars indicate SE. Asterisks indicate significant differences (* $P < 0.05$; ** $P < 0.01$). Figures above columns indicate numbers of pooled samples (days 4 and 10) or animals (days 21 and 90) used.

Table 1. Summary of results

Days after delivery	Mat-TRH rat		TRH-treated mothers			
	neonatal period		adult life			
	4	10	21	90	10	90
pituitary TSH	↓ m	↑ m		→	↓ *	→
Serum TSH	→	↑ m, f *	→	→	→	→
TRH test		↑ m *	↑ *	↑ *	→	→
Serum T_4	↓ f *	↑ f	→	→	→	→

Comparison with TRH, ↑: increase ↓: decrease →: not different m: male f: female
*: TRH 1000 µg/kg group

late effect in Mat-TRH rats may be altered pituitary-thyroid function during the early neonatal period.

Rats with a brief period of perinatal hypothyroidism showed increased pituitary and serum TSH and serum TSH and T₄ levels in their later life, and the TSH response to TRH was reduced (Bakke *et al.*, 1976). On the other hand, neonatal hyperthyroidism resulted in a reduced TSH response to TRH and lower levels of TSH, T₄ and T₃ in adult life (Azizi *et al.*, 1974; Bakke *et al.*, 1974; Dussault *et al.*, 1980). Neither consequence due to hypo- or hyperthyroidism is accounted for the changes observed in Mat-TRH rats, because the TSH response to TRH was enhanced and thyroid hormone levels were normal in adult life.

As has been shown in fetal monkeys (Melmend *et al.*, 1979), fetal lambs (Klein and Fisher 1980) and neonatal rats (Walker *et al.*, 1980), the pituitary TSH response to TRH is greater during the early period of development than after maturity. In neonatal rats, this augmented TSH response to TRH progressively declines with age in parallel with the increase in thyroid hormones (Walker *et al.*, 1980). Recently, Banerji and Prasad (1982) showed that the amount of TRH receptor of pituitary cells was greater in neonatal rats than in adults. Thus, it can be postulated that the enhanced TSH secretion in Mat-TRH rats is due to increasing sensitivity of thyrotrophs caused by the increase in the amount of TRH receptor.

The role of hypothalamic TRH in the development of the pituitary-thyroid axis is unknown. Tonooka and Greer (1978) reported that neither maternal nor fetal TRH played any role in controlling pituitary TSH secretion in the rat fetus. Walker and Dussault (1979) reported that intermittent administration of pharmacological doses of TRH to rats throughout the fetal and neonatal period did not induce any effects on the hypothalamic-pituitary-thyroid axis in

adult life. However, the present and previous findings (Kawai *et al.*, 1983) indicate that repetitive administration of TRH in fetal or neonatal life has a late effect on development of the pituitary-thyroid axis that persists into adult life. The discrepancy of the results of Walker and Dussault and the present study remains unexplained, but the differences in period of administration and dose of TRH might be related to this discrepancy. In addition, it is noticeable that there are differences in TSH responses to TRH between adult rats exposed to repetitive TRH during fetal and neonatal period. It is possible that the pituitary-thyroid function is altered by the perinatal treatment, but the mechanisms remains unclear.

Although TRH was administered to pregnant and puerperal women (Ylikorkala *et al.*, 1980; Roti *et al.*, 1981), the effects of maternal exposure of TRH on fetuses and neonates remain unclear. In this connection, the present and previous studies demonstrate the effects of maternal or neonatal injection of TRH on the pituitary-thyroid function in developing rats.

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