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PITUITARY–THYROID ACTIVITY AND LONGEVITY IN NEONATALLY THYROXINE-TREATED RATS

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SUMMARY

Wistar rats were made hypothyroidic by intraperitoneal thyroxine (T4) injection during the first 10 days of neonatal life. Levels of T4, 3,3',5-triiodothyronine (T3), thyroid-stimulating hormone (TSH) and prolactin in the blood of these rats were measured by radioimmunoassay. The T4 levels are about two-thirds of control values up to 20 months of age. T3 level is low only at a young age. TSH level shows no significant difference from control, but is about half that of control after the stimulation of secretion by 6-propyl-2-thiouracil. The level of prolactin is much higher in the T4-treated group than in controls. In male rats, life duration of hypothyroid rats was longer than control by about 4 months. The life extension effect of hypothyroidism was observed also in females, although the difference was smaller than that in males. The concentration of T4 in the blood of male rats is higher than females, and the decrease in T4 level by neonatal T4 treatment is also more marked in males.

Key words: Rat; Hypothyroidism; Lifespan; Pituitary hormones; Radioimmunoassay

INTRODUCTION

Food restriction is one of the most successful procedures to elongate the lifespan of experimental animals [1,2]. However, the mechanism of the life-expanding effect of food restriction has not been elucidated yet. In poikilothermic animals, the length of the life has been postulated to be determined by "the rate of living" [3], and the lifespan of these animals is longer if they live at low temperature [4]. In the case of mammals, it has been reported that the lifespan correlates with metabolic rate both among various species [5] and among various strains of the mouse [6]. According to these theories, thyroid activity possibly

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affects the lifespan of mammals. Addition of thyroid gland to the food reduces the lifespan of mice [7]. Thyroxine increases the aging of tail tendon collagen fibers [8,9], and induces a decrease in O_2 consumption with age [10]. Rats which were kept at 9°C did not live as long as those kept at 28°C, and the life-shortening effect of prolonged exposure to cold was postulated to be due to an increase in thyroxine level [11]. Tryptophan deficiency retards aging of the rat, and pituitary–thyroid activity is suppressed in these rats [12].

It has been frequently reported that the neonatal administration of thyroid hormones (T3 or T4) induces a chronic hypothyroidic state in the rat [13–16]. Since active thyroid-stimulating hormone (TSH) secretion is also inhibited in neonatally T4-treated rats, a disturbance in the normal development of the brain–pituitary system is postulated [17,18]. In the present study, moderate hypothyroidism was induced by neonatal T4 treatment, and the effect of hypothyroidism on the lifespan of the rat was examined.

MATERIALS AND METHODS

To make rats hypothyroidic we adopted neonatal hormone treatment instead of surgical thyroidectomy or treatment with antithyroid drug to avoid drastic hormone deficiency and possible harmful effects. About 200 male and female Wistar rats were injected intraperitoneally with 0.5, 1, and 2 μg of T4 per g body weight on days 2 (the day after the day of birth), 4, 6, 8, and 10 of neonatal life. Half of the rats in the control group received alkaline saline, the vehicle. The rats were kept on commercial chow (F2, Funabashi Co.) and tap water.

Body weight and food consumption of control and T4-treated rats were measured for the first 2 months after weaning. Pair-fed control rats were provided with the same amount of food as consumed by T4-treated rats every day.

One group of rats was treated with the antithyroid drug 6-propyl-2-thiouracil (PTU). PTU, 0.05%, was administered in the drinking water over a 2-week period, prior to sacrifice. The amount of drinking was reduced in the PTU-treated rats to about two-thirds of control. Neonatally T4-treated rats and pair-fed rats consumed almost the same amount of PTU solution as normal PTU-treated rats.

Blood was obtained by cardiac puncture (utilizing a heparinized syringe) from ether-anesthetized rats. Blood plasma was stored at −20°C after centrifugation to remove red blood cells.

The concentrations of T4 and T3 in blood were measured by a commercial radioimmunoassay kit (Amerlex T4 and Amerlex T3, Amersham). TSH and prolactin (PRL) levels in the blood were determined by double-antibody radioimmunoassay with standard rat pituitary hormones and antibodies provided by the NIAMDD Rat Pituitary Hormone Distribution Program. Five or six rats per group were used for all assays, and radioimmunoassay tubes were duplicated for each rat. Statistical analyses were done with Student's *t*-test.

RESULTS

Changes in body weight of normal and neonatally T4-treated rats are shown in Fig. 1. The body weight of neonatally T4-treated rats is 80–85% that of the controls during young and middle age. The average body weight of male Wistar rats reaches a maximum at 15 months of age. Neonatally T4-treated male rats maintain their maximum body weight longer and show almost no weight loss until 22 months of age. The average body weight of rats older than 22 months is not shown because of the sudden increase in the death rate at this age in males.

Figure 2 shows changes in food efficiency (the ratio of weight gain per day to food consumed per day) of control and neonatally T4-treated rats from 30 to 60 days' old. Food efficiency of T4-treated rats was significantly higher than controls during the periods around 34 days and older than 55 days.

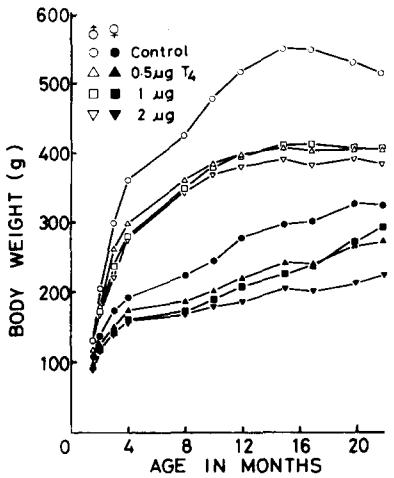

Fig. 1. Changes in body weight of rats treated with 0.5, 1, or 2 μg of T4 per g body weight during the first 10 days of life.

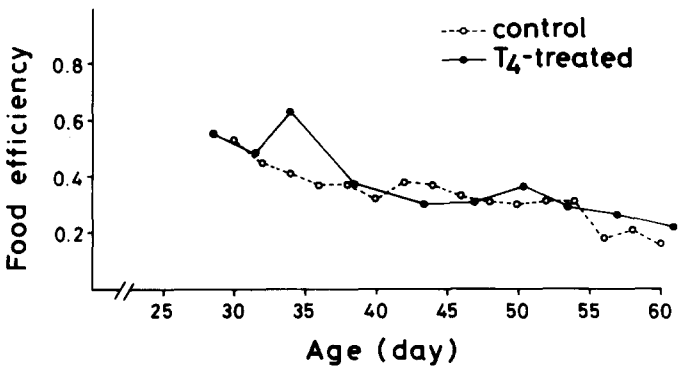

Fig. 2. Food efficiencies of control and neonatally T4-treated male rats from 30 to 60 days' old.

The T4 level in blood of neonatally T4-treated rats is about two-thirds of control at 20 months of age (Fig. 3). The T3 level is also lower than controls in 25-day-old neonatally T4-treated rats, but the difference is not significant in 20-month-old rats (Fig. 3). The T4 and T3 levels of pair-fed control rats at 2 months were 6.3 ± 0.85 μg per 100 ml and 1.2 ± 0.22 ng/ml, respectively, and showed no significant difference from the levels in the intact rats.

As shown in Fig. 4, the level of TSH is slightly lower (but is not statistically significant) in neonatally T4-treated rats than in controls. The difference between the normal and the T4-treated group is more remarkable after the administration

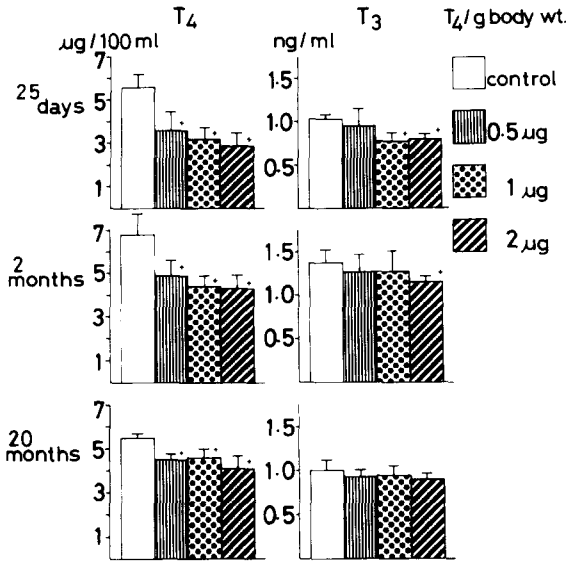

Fig. 3. Concentrations ±S.D. of T4 and T3 in the blood of control and neonatally T4-treated male rats at various ages. + = $p < 0.01$ (control *vs.* experiment).

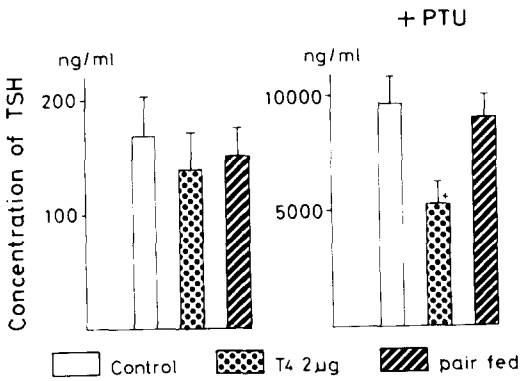

Fig. 4. Concentrations ±S.D. of TSH in the blood of control, neonatally T4-treated and pair-fed male rats (40 days' old). +PTU: the levels after the administration of 6-propyl-2-thiouracil for 2 weeks. + = $p < 0.01$ (control *vs.* experiment).

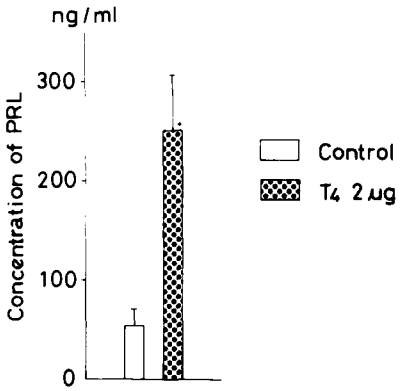

Fig. 5. Concentrations ±S.D. of PRL in the blood of control and neonatally T4-treated male rats (40 days' old). + = $p < 0.01$ (control *vs.* experiment).

of PTU for two weeks. In normal rats the TSH level is elevated about 60 times after PTU; however, after PTU treatment the TSH level of the T4-treated rats is only about half that of the control PTU-treated rats, although it is, nevertheless, higher than before PTU treatment. The change in TSH level of pair-fed rats by PTU treatment did not differ from that of normal rats. These results indicate that the maximal activity of TSH cells of neonatally T4-treated rats is less than that of normal rats, and suggest that neonatal T4 administration results in permanent damage of the TSH cells.

The concentration of PRL in blood (Fig. 5) significantly increases in T4-treated rats. Since TRH stimulates PRL secretion, this result suggests increased hypothalamic TRH secretion in these rats, and that the suppression of TSH cell activity in T4-treated rats is probably not due to the inhibition of TRH secretion as a consequence of hypothalamic injury.

The survival curves in Fig. 6 show that the life duration of male hypothyroid rats is longer than that of control. The mortality rate of hypothyroid animals is almost the same as controls until they are 24 months' old, but it is remarkably lower after 24 months. The period of 50% survival increased to 28 months, which is significantly greater than that of control rats, 24 months. The maximum life duration was 35 months for hypothyroid rats and 31 months for control rats. In the females, the life extension effect of neonatal T4 treatment was also observed, although the decrease in the mortality rate after 24 months was not so marked as in male rats. The period of 50% survival lengthened from 28 to 31 months, and maximum life duration was 36 months for control and 38 months for hypothyroid rats. Table I shows the mean lifespan for various doses of neonatal T4 treatment. In male rats, all three doses of T4 are effective in elongating lifespan, but 0.5 μg of T4 per g body weight had no effect in females. In another group with 3 μg of T4 per g body weight the death rate in sucklings was high, and these rats were eliminated from the lifespan experiment. These results indicate that the best dose for life extension is 1–2 μg of T4 per g body weight.

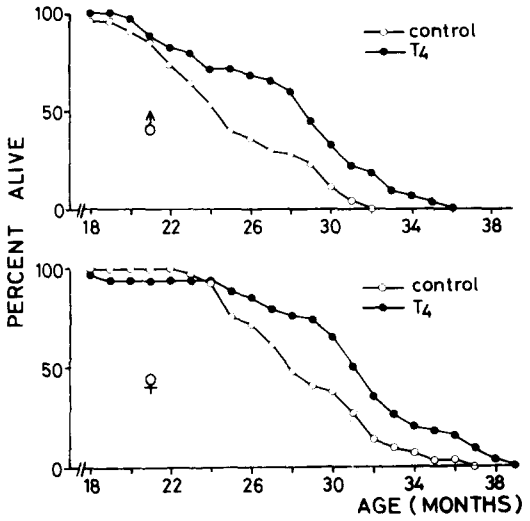

Fig. 6. The survival curves of control and T4-treated (1 and 2 μg of T4 per g body weight) male and female Wistar rats. Numbers of rats per group were 47 (male, control), 34 (male, experimental), 29 (female, control) and 34 (female, experimental). In control groups, the vehicle-injected rats were added to the intact rats because no effect of vehicle injection was detected.

TABLE I
LIFESPAN OF CONTROL AND NEONATALLY T4-TREATED RATS
Control groups include both intact and the vehicle-injected rats because no statistical difference was detected between them.

	Male	*Female*
Control	24.8 ± 0.545[a] (47)[b]	28.5 ± 0.660 (29)
T4 (μg/g body weight)		
0.5	26.5 ± 0.954 (22)	27.5 ± 0.989 (18)
1	28.8 ± 1.13 (16)	30.9 ± 1.41 (14)
2	27.6 ± 0.943 (18)	31.8 ± 0.902 (20)

[a]Mean ±S.E.M.
[b]Number of rats used.

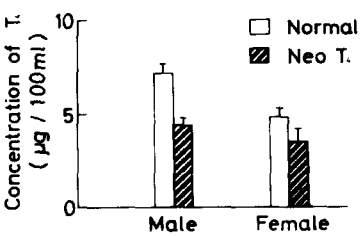

Fig. 7. Concentrations ±S.D. of T4 in the blood of control and neonatally T4-treated 2-month-old male and female rats. $p < 0.01$ (control male *vs.* control female, control *vs.* experimental for both male and female).

Figure 7 shows the sex difference in concentration of T4 in the blood of control and neonatally T4-treated rats. Male Wistar rats have a very high T4 level in the blood compared with females and the data on rats of other strains (approx. 5 μg per 100 ml) [19], and the decrease in T4 concentration by neonatal T4 treatment is greater in male rats than in female rats.

DISCUSSION

There is a discrepancy in the earlier reports regarding the levels of TSH in the neonatally T4-treated rat: Azizi *et al.* [17] reported a decrease in TSH levels, whereas Bakke *et al.* [18] observed no significant difference in TSH levels between control and T4-treated animals, although a marked difference is apparent after activation of TSH secretion by thyroidectomy. The present study also shows that the difference is not significant unless the pituitary is stimulated as a consequence of PTU administration.

Results of the present study indicate that moderate hypothyroidism elongates the lifespan of the rat. Everitt *et al.* [20] have reported that hypophysectomy in early life of the male Wistar rat retards aging, and extends mean lifespan. The characteristics of the survival curve in hypophysectomized rats are similar to the survival curve of hypothyroidic male rats in the present study, and it is possible that the effect of hypophysectomy on the lifespan is mainly due to the deficiency of TSH, although long-term growth-hormone therapy has been reported to accelerate renal aging [20]. The life-expanding effect of hypothyroidism is more conspicuous in male rats than in females. It may be related to the fact that the blood concentration of T4 in males is higher than in females or in rats of other strains, and that the lifespan of male Wistar rat is extraordinarily short among rats of various strains.

The relationship between aging retardation by hypothyroidism and by food restriction is not clear. The body weight of our hypothyroidic rats is about 80% that of controls, and the inhibition of growth may act as an anti-aging factor. Previous investigations have suggested that the increase in spontaneous exercise in food-restricted rats is the decisive factor in prolonging lifespan [21]. However, the present study is contradictory to this hypothesis because it indicates that the rate of aging is suppressed by the endogenous growth retardation which is not accompanied by an increase in voluntary activity.

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