EFFECT OF GRADED DOSES OF THYROXINE ON THE PLASMA TSH LEVELS IN MICE

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Summary : The well known effect of T4 administration is suppression of TSH level in plasma ; with small doses of T4 has been shown to potentiate goitrogenesis induced by goitrogenic drugs. The effects of short term treatment with small doses of T4 on plasma and pituitary TSH levels were studied in mice. Very small doses of T4 caused an increase in circulating TSH while larger doses produced the usual decrease. Pituitary TSH levels decreased with the smallest dose employed; taken along with the larger plasma TSH level, this would indicate a greater release of TSH by pituitary, probably mediated though the hypothalamus. Increasing the dose of T4 caused an increase in pituitary TSH content, with a lowering of plasma TSH, probably inhibiting release while synthesis is going on. With larger doses, T4 probably inhibited both synthesis and release of TSH as shown by the reduction of TSH levels in pituitary and plasma.

Key words : T4 potentiation of TSH release synthesis and release of TSH plasma TSH pituitary TSH

Hypothyroidism, brought about by thyroidectomy or goitrogenic drugs, induces an increase of the thyrotropin (TSH) level in the blood (1-3). Sellers and Schonbaum (4) have shown that long term treatment with small doses of thyroxine potentiate goitrogenesis in the rat. They also concluded that the potentiation seemed to be mediated via the adenohypophysis or higher centres (5). The effect is possibly by an increased secretion of TSH by the pituitary gland. Clinically, treatment with thyroxine sometimes resulted in an initial increase in TSH level before the well-known suppression effect is seen. Short duration experiments with small doses of T4 were likely to show the better changes in TSH level than long term ones which tend to produce cumulative effects and reach quilibrium states. It was therefore, decided to test the effect of single doses of varying quantities of 1-thyroxine (T4) on TSH secretion, in intact mice.

MATERIALS AND METHODS

Female mice weighing between 18 and 20 gm. on arrival in the laboratory, were divided into 6 groups of 10 - 12 animals each and were given graded doses of thyroxine ranging from 0.1 μg to 10.0 μg subcutaneously. Control animals received saline. At the end of 48 hours, the animals were bled through the ophthalmic sinus using heparinized micropipettes; equal volumes of blood were collected from each animal. The blood was pooled together for each

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group, plasma separated by centrifuging at 3600 R.P.M. for 10 minutes and kept frozen till required for assay.

The pituitary glands were collected immediately after bleeding the animal and were pooled together in groups. They were homogenized in a glass homogenizer with the addition of isotonic saline and stored frozen till required for assay.

The samples of plasma were assayed by the Kirkham method of assay for TSH, as described by Desbarate-Schonbaum *et al.* (6).

RESULTS

Table I gives the results of administering varying doses of T4 to intact mice on the plasma TSH concentration. With the smallest dose of T4 used (0.1 μg), there was a significant increase in TSH concentration. Thereafter, there was a progressive diminution of TSH with increasing doses, but the TSH did not become undetectable even with the largest (10.0 μg) dose of T4 used.

Table I: Effect of varying doses of T4 on plasma TSH in mice

	No. of animals	*Plasma TSH in uU/ml*
Saline	12	163.42 (125.00—225.99)
0.1 μg T4	12	244.26 (181.15—357.80)
0.3 μg T4	12	181.42 (137.81—254.39)
1.0 μg T4	12	137.75 (106.36—186.58)
3.0 μg T4	10	98.43 (76.76—129.09)
10.0 μg T4	11	87.97 (68.63—114.47)

The pituitary content of TSH is given in Table II. The content was small in the intact control animals. It became less (reduced to undetectable amounts at the dilution employed) with 0.1 μg T4. With the next higher dose, the pituitary TSH content was about half that of the control value and thereafter, it increased with higher amounts of T4 except for the largest dose of T4 (10.0 μg), where the TSH content was about the same as the control and only one-third of that in the group receiving 3.0 μg T4, where the peak value had been observed.

DISCUSSION

In the intact mice, very small dose of T4 caused an increase in the circulating TSH instead of the well-known effect of T4 suppressing TSH secretion and decreasing the level of TSH in circulation. The effective dose was 0.1 μg/mouse/day; on a weight basis, this was comparable to the dose employed by Sellers and Schonbaum (5), viz. 5 μg/rat/day (allowing

Table II : Effect of varying doses of T4 on the pituitary TSH content in mice.

	No. of pituitaries	*TSH content/pituitary in μU*
Saline	12	2.15
0.1 μg T4	12	undetectable at the dilution employed
0.3 μg T4	12	1.07
1.0 μg T4	12	3.66
3.0 μg T4	10	7.24
10.0 μg T4	11	2.40

*The homogenates were diluted with saline such that 1 *ml* was equal to 1/4 of a pituitary for the first three samples and 1/8 of a pituitary for the last three samples.

also for the different routes of administration), which failed to prevent goitres and in some cases produced goitres of larger size than those produced by the goitrogen treatment alone.

The pituitary TSH content in the control, saline-treated mice was low. The smallest dose of T4 decreased the TSH level below the control level making it undetectable at the dilution employed. This might indicate a greater release, mediated probably by a greater activity of thyrotropin-releasing factor of the hypothalamus, and accounting for the increased concentration of TSH in the circulation. The decrease in pituitary TSH was not observed by Van Rees (7). The explanation for the failure to observe the decrease might be that a sufficiently small dose had not been used and the experiment was of relatively longer duration than the present series. If a still smaller dose was employed or the experiment was of a shorter duration, probably the depleting effect on pituitary TSH might have been observed. The difference could also be possibly accounted for by differences in species. Increasing dose of T4, caused an increase in the pituitary TSH content. With 0.3 μg T4, the pituitary TSH level was less than the control, while the blood TSH level was higher than the control value. This effect is probably by inhibiting TSH release (borne out by the reduced blood levels), while synthesis goes on. With the largest dose employed, there was a reduction in pituitary TSH, T4 probably inhibiting synthesis as well as release. Sinha and Meites (8) found that treatment with T4 resulted in a significant fall in TSH content. The highest dose used by them viz. 25 μg/100 *g* body weight/day in the rat is comparable to the larger dose in the mice but they observed an equal fall (to about one-fourth of that in the control rats) with 5.0 μg/100 *g*/day. Probably the longer treatment with T4 (2 weeks instead of 2 days) produced the suppression of pituitary TSH in the rat.

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