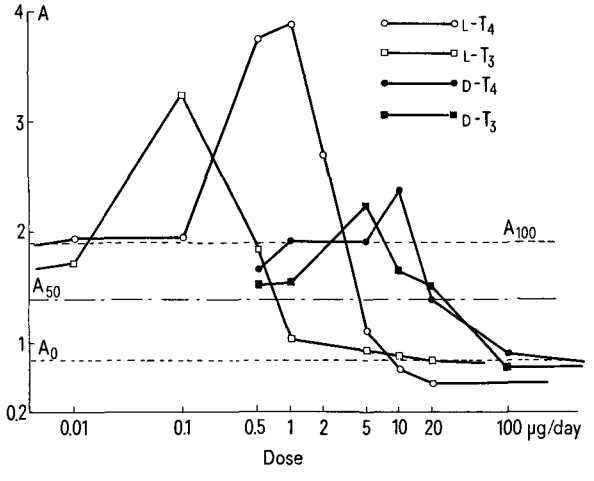
Inhibition of TRH-Induced TSH Release by L-Thyroxine, L-Triiodothyronine, and their D-Isomers in Mice

The hormones L-thyroxine (L-T4) and L-triiodothyronine (L-T3) are secreted by the thyroid under the control of thyrotropic hormone (TSH), the release of which from the pituitary is caused by the hypothalamic thyrotropic releasing hormone (TRH) and in turn is inhibited by L-T4 and L-T3[1,2]. This negative feedback system is used to find out the daily amount of L-T4 and L-T3 necessary to suppress in the mouse the TRH-induced TSH release from the pituitary. Further the suppressive activity of the isomers D-thyroxine (D-T4)[3] and D-triiodothyronine (D-T3)[3] is compared with that of the natural hormones L-T4 and L-T3.

As test model we used a modified McKenzie-bioassay[4] for TSH with female mice (20 g) not on a low-iodine diet. In the morning of the 1st day each mouse received 10 μCi tracer-free $Na^{125}I$ i.p. On the 4th day 0.1 ml blood was withdrawn from the retroorbital venous plexus. The counted radioactivity of the sample served as blank (J_0). Thereafter i.p. injection of 500 ng TRH, synthesized by an automated procedure[5], resulted in a reproducible maximal increase of blood radioactivity (J_3) 3 h after injection. Further increase of the amount of TRH beyond 500 ng did not enhance the response, whereas amounts smaller than 500 ng did not always give consistent results.



Relative change of blood iodine-125 radioactivity (A) 3 h after aTRH bolus in mice pretreated with various doses of L-thyroxine (L-T4), L-triiodothyronine (L-T3), D-thyroxine (D-T4), and D-triiodothyronine (D-T3) as shown on the abscissa.

Table I.

Thyroid hormone (x)	Regression function (x in μg/d)	Range of x (number of groups)	Number of value pairs (A:x)
L-T3	A = 2.02–2.15 (lg$x$ + 0.42)	0.1– 1.0; 3	27
L-T4	A = 2.23–3.38 (lg$x$ — 0.45)	1.0– 10.0; 4	42
D-T3	A = 1.48–1.01 (lg$x$ — 1.31)	5.0–100.0; 4	36
D-T4	A = 1.80–1.46 (lg$x$ — 1.33)	10.0–100.0; 3	38

Table II.

Thyroid hormone	ED_{50} (nmol/d)		Relative suppressive activity (L-T4 = 1.00)
	Mean	Confidence limits for 1–2 P = 0.95	
L-T3	1.39	0.71– 2.75	4.78
L-T4	6.67	5.14– 8.65	1.00
D-T3	38.65	30.43–46.09	0.17
D-T4	54.56	37.89–78.56	0.12

The ratio A = J_3/J_0 was used as a measure of response to TRH and TSH injections; *500 ng TRH in 0.5 ml* 154 m*M* NaCl with 0.5% bovine serum albumin injected i.p. resulted in a 3-hour response of $A_{100} = 1.93$ (SD ± 0.26; n = 43), which was nearly equivalent to the 3-hour response of 3.0 mU highly purified bovine TSH (A = 1.85 ± 0.24; n = 20). Control injections of 0.5 ml saline with 0.5% bovine serum albumin gave a response of $A_0 = 0.86 \pm 0.16$ (n = 39).

On the 2nd, 3rd and 4th day after the $Na^{125}I$ application, each animal was injected i.p. daily doses of L-T4, L-T3, D-T4, or D-T3 ranging from 0.001 to 1000 μg. Thereafter the 3-hour response A to an i.p. injection of 500 ng TRH was measured. The results are shown in the respective log-dose-response curves in the Figure. With increasing dose of thyroid hormones, the TRH responsiveness first enhances to values significantly above A_{100} and then falls asymptotically to a minimum around A_0. The enhanced responsiveness is most conspicuous after treatment with L-T4 and L-T3 and also discernible after D-T4 and D-T3. From the data obtained it cannot be decided whether the enhancement is produced by the pituitary or thyroid or at both sites.

Regression functions (Table I) were calculated of the individual A-values of the nearly linear, descending slope of the 4 TRH response curves. These functions enabled us to evaluate the half maximal effective dose (ED_{50}) at $A_{50} = (A_{100} + A_0)/2 = 1.40$ with respect to suppression of TSH release by the thyroid hormones and their D-isomers. The relative suppressive activity (Table II) of L-T4 and L-T3 is fairly well equal to their metabolic effect on the activity of the mitochondrial L-α-glycerophosphate dehydrogenase in rat liver[6]. The suppressive activity of the D-isomers is much lower than that of the L-hormones, but cannot solely be attributed to the impurities of 0.3% L-isomers. On the contrary, a genuine suppressive activity must be ascribed to the D-isomers or they are partly isomerized to the L-form in the organism.

Zusammenfassung. Bei Mäusen lässt sich die TRH-induzierte Freisetzung von TSH durch L-Trijodthyronin, L-Thyroxin, D-Trijodthyronin und D-Thyroxin hemmen. Für 20 g schwere Tiere ergibt sich eine entsprechende ED_{50} von 1,39; 6,67; 38,65 und 54,56 nmol/die.

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