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Research Article

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Altered Thyroidal Responsivity to Thyrotropin Induced by Circulating Thyroid Hormones

A "SHORT-LOOP" REGULATORY MECHANISM?

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Abstract We studied the effect of thyroid hormone administration on responsivity of murine thyroid to exogenous thyrotropin (TSH) in order to explore the possibility that the thyroid gland might be directly inhibited by its own hormones. In the rat both L-thyroxine (T_4) and 3,5,3′-L-triiodothyronine (T_3) pretreatment inhibited TSH-induced thyroidal ornithine decarboxylase (ODC) activity in vivo in a dose-related manner (half-maximal inhibition, 1.7 μg/rat and 0.6 μg/rat, respectively). Other structurally related compounds exhibited the following inhibitory potencies compared to T_4: T_3, 283%; triiodothyroacetic acid, 40%; D-T_4, 18%; 3,5-L-diiodothyronine, 9%. Monoiodotyrosine, diiodotyrosine, and iodide were not inhibitory. The full inhibitory effect of T_4 or T_3 was observed when thyroid hormone was administered from 96 to 12 h before TSH and was also seen in hypophysectomized animals. Pretreatment with T_4 or T_3 in divided doses over 2½ days inhibited TSH-induced increase in [1-^{14}C]glucose oxidation to $^{14}CO_2$ and [^{3}H]leucine incorporation into protein in rat thyroid.

In the mouse T_4 or T_3 pretreatment (0.25–25 μg daily) caused dose-related inhibition of both thyroidal ODC activity and ^{131}I release induced by TSH in vivo. In mice on a low-iodine diet (LID) but not in animals on a regular diet (RD) NaI pretreatment also blunted TSH-induced thyroidal ODC activation and ^{131}I release. When LID or RD mice were pretreated with 12.5–125 μg of T_4 or T_3 over 2½ days, TSH-induced in vitro stimulation of thyroid cyclic 3′,5′-adenosine monophosphate formation was inhibited in a dose-related manner; NaI pretreatment was inhibitory in the LID mouse only.

Prior administration of exogenous TSH blunted the activation of thyroid ODC and thyroid hormone release induced by subsequent TSH administration in rat and mouse. These studies indicate altered thyroid responsivity to TSH under the influence of circulating thyroid hormones and suggest the existence of a "short-loop" negative feedback regulating thyroid function.

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INTRODUCTION

The results of recent studies (1, 2) suggest that an increase in circulating thyroid hormone levels may impair thyroidal responsivity to exogenous thyrotropin (TSH).[1] It was therefore felt to be of interest to explore this possibility further by studying the effect of thyroid hormone administration on TSH-augmented thyroid function in the rat and mouse. The results indicate that such treatment does, in fact, occasion decreased thyroid gland response to exogenous TSH and suggest the existence of a "short-loop" negative feedback regulating thyroid function.

METHODS

The rat

Male Holtzman rats, weighing 180–200 g, were maintained on standard laboratory chow and tap water. Five animals were usually assigned to each treatment group. At the time of sacrifice the rats were anesthetized with ether and killed by a blow on the head. The thyroid gland was removed immediately thereafter, trimmed, and weighed. The average pooled weight of the five glands used in these studies was between 50 and 60 mg.

[1] *Abbreviations used in this paper:* cyclic AMP, cyclic adenosine 3′,5′-monophosphate; LID, low-iodine diet; ODC, ornithine decarboxylase; RD, regular diet; T_3, 3,5,3′-L-triiodothyronine; T_4, L-thyroxine; TCA, trichloroacetic acid; TSH, thyrotropin.

Hypophysectomized rats were obtained from Hormone Assay Laboratories, Chicago, Ill. These animals were also maintained on standard laboratory chow and tap water ad lib. supplemented only by orange slices. Animals were used for ornithine decarboxylase assay 2–4 days after hypophysectomy. At the end of the experiments, the animals were killed, and the hypophysial fossa was examined with a 10 × dissecting microscope to ascertain the completeness of hypophysectomy. No pituitary remnants (other than the proximal end of the stalk) were found in any animal. Additionally, circulating TSH levels in these animals were measured by radioimmunoassay (3) before experimentation (using reagents supplied by the National Institutes of Arthritis, Metabolism, and Digestive Diseases Rat Pituitary Hormone Distribution Program) and uniformly found to be < 6 μU/ml (3).

Ornithine decarboxylase (ODC) assay. Animals were killed 4 h after i.p. administration of TSH; timing of thyroid hormone pretreatment was generally 20 h before sacrifice unless otherwise indicated.

Tissue preparation. For each treatment group, thyroid glands from five rats were pooled and weighed. They were homogenized with a Polytron homogenizer (Brinkmann Instruments, Inc., Westbury, N. Y.) at a concentration of 48 mg/ml in 0.05 M sodium potassium phosphate buffer, pH 7.2, containing 10 mM tetrasodium EDTA and 5 mM dithiotreitol, at 4°C. The supernatant fractions were immediately used for enzyme assay.

Enzyme assay. ODC was assayed by a semi-micromodification of the method of Russell and Snyder (4). The reaction mixture contained 0.3 μCi of DL-[1-^{14}C]ornithine hydrochloride, 58 or 61 mCi/mmol (Amersham/Searle Corp., Arlington Heights, Ill.), 0.1 μmol pyridoxal phosphate, 0.3 ml of the 20,000-*g* thyroid supernatant fraction, and buffer (described above) to make a final volume of 0.5 ml. Activity was assayed (usually in triplicate) as recently described elsewhere (5). Results are expressed as picomoles of $^{14}CO_2$ liberated from DL-[1-^{14}C]ornithine per gram wet weight tissue per 30 min incubation.

Glucose oxidation. Rats were injected subcutaneously with 5 μg L-thyroxine (T_4), 3,5,3′-L-triiodothyronine (T_3), or NaI (5 μg in 0.2 ml normal saline), or 0.2 ml normal saline twice daily (9:00 a.m. and 4:00 p.m.) for 2 days. On the 3rd day, the hormone, iodide, or saline was administered i.p. 1 h before death. Six rats were assigned to each experimental group; the 12 thyroid lobes so obtained were selectively distributed to three tubes so that no paired lobes were placed in the same tube. Glucose oxidation was measured by the method of Tong (6) with minor modifications. Thyroid tissue was incubated with 1 ml Eagle's basal medium (Earle's salts) containing 20% (vol/vol) fetal calf serum and 1.0 μCi of [1-^{14}C]glucose (sp act, 5–10 mCi/mmol; New England Nuclear Corp., Boston, Mass.) with or without 200 mU/ml TSH, in a 12-ml round-bottom plastic centrifuge tube sealed with a rubber stopper containing a center well for $^{14}CO_2$ collection. The incubation was carried out in a Dubnoff metabolic shaking incubator for 90 min at 37°C. The reaction was stopped by injecting 0.2 ml 7 N H_2SO_4 into the incubation medium. The $^{14}CO_2$ liberated from [1-^{14}C]glucose was collected in the Hyamine-filled center well as described for ODC assay (5). Results are expressed as cpm $^{14}CO_2$ released per milligram tissue.

Protein synthesis. Rats were pretreated with T_4, T_3, or saline as described for glucose oxidation studies for 2 days. On the following day, at 7 a.m., the animals were given yet another (i.p.) injection of thyroid hormone immediately before i.p. administration of 4 U TSH and, again, just before [^{3}H]leucine administration. 10 μCi [^{3}H]leucine (sp act, 50 Ci/mmol; Amersham/Searle Corp.) was administrated i.p. 7 h after TSH administration and 2 h before death. Four animals were used per experimental group; their thyroids were pooled and homogenized in 1 ml of 10% (wt/vol) trichloroacetic acid (TCA) containing 0.1% "cold" leucine. The resulting precipitate was then washed three times with 1 ml 10% TCA, incubated with 1 ml 10% TCA at 90°C for 15 min, and centrifuged at 1,000 *g* for 15 min. The resulting precipitate was washed with 1 ml absolute ethanol and 1 ml of ethanol-ether mixture (1:1, vol/vol) and solubilized by incubation in 1 ml 0.3 N NaOH at 37°C for 1 h. An aliquot of the resulting solution was used for protein determination (7). 0.5 ml of the solution was used for radioactivity determination using 10 ml Instagel (Packard Instrument Co., Downers Grove, Ill.) as the scintillation counting medium. Results are expressed as cpm per milligram protein.

The mouse

Female weanling mice of the Swiss-Webster strain were used throughout. For all experiments to be described one group of mice was placed on a low-iodine diet (LID) 2 wk before and during the experiment, while a second group was fed regular Purina laboratory chow (RD).

ODC assay. Animals were killed by a blow on the head 4 h after the i.p. administration of TSH and, where indicated, at varying times after the administration of thyroid hormone. The thyroid gland and underlying trachea were removed en bloc and trimmed of excess connective tissue. 15–20 animals were used for each variable tested, and ODC measurements were made on the pooled thyroid-trachea preparations.

For assay of ODC activity in mouse thyroid, the procedure utilized in the rat was modified slightly as follows: 0.1 ml of mouse thyroid enzyme preparation was incubated with 0.3 μCi [1-^{14}C]ornithine and 0.1 μmol pyridoxal phosphate in a final volume of 0.2 ml; the reaction was terminated by injecting 0.2 ml 2 M acetic acid into the incubation medium.

In LID mice, simultaneous thyroid ODC assay (15 animals per group) and McKenzie bioassay (6 animals per group, method of Shishiba et al. [2]) were carried out as follows: on the 14th day of the LID, the six McKenzie bioassay animals received 4 μCi of ^{131}I i.p. An hour later and on the 15th and 16th days, they received the stated amount of T_3, T_4, or iodide s.c. On the 17th day, 24 h after the last injection of thyroid hormone, 0.5–10 mU TSH was injected i.p. In 6 of the 15 animals in each group blood specimens were obtained from the orbital veins before and 3 h after TSH injection. All animals were killed 4 h after TSH administration for thyroid ODC measurement. In some experiments (see Results), LID mice were pretreated with thyroid hormone or iodide administered s.c. only 24 and 12 h before TSH administration.

Cyclic AMP measurements. For cyclic AMP studies, LID and RD mice were pretreated with T_4, T_3, NaI, or saline for 2½ days; thus, two s.c. injections were given at 9:00 a.m. and 4:00 p.m. on each of the 2 days before death; on the following day, one i.p. injection of thyroid hormone or iodide was given at 9:00 a.m., and the animals were killed 1 h later. The thyroid gland was removed, carefully trimmed, and weighed on a Sartorius balance sensitive to 0.01 mg (Brinkmann Instruments, Inc.); average gland weight was 1.5 mg. The gland was then incubated with varying amounts of TSH in 1 ml Krebs-Ringer bi-

carbonate buffer containing 0.1% glucose, 0.2% bovine serum albumin, and 10 mM theophylline for 30 min at 37°C, after which the gland was homogenized in 6% TCA (with trace amounts of [^{3}H]cyclic AMP for recovery) in a VirTis glass homogenizer (VirTis Co., Inc., Gardiner, N. Y.). The TCA supernatant fraction was extracted with ether, and the cyclic AMP content was measured by radioimmunoassay (8) using a kit supplied by Schwarz Bioresearch, Inc., Orangeburg, N. Y.

In an additional series of cyclic AMP experiments, thyroid glands were removed from untreated RD mice and preincubated under an atmosphere of 95% O_2–5% CO_2 with T_4, T_3, or NaI in Krebs-Ringer bicarbonate buffer (containing glucose and albumin as described above but without theophylline) for 3 h at 37°C; thereafter, the glands were removed from the incubation medium, washed twice with buffer, blotted, and incubated with TSH as described above.

All data were analyzed for statistical significance by Student's *t* test (two-tailed) unless otherwise stated.

Chemicals and Reagents. TSH (Thytropar) was purchased from Armour Pharmaceutical Co., Kankakee, Ill. T_4, T_3, D-T_4, diiodothyronine, diiodotyrosine, monoiodotyrosine, tyrosine, and dithiotreitol were purchased from Sigma Chemical Co., St. Louis, Mo. NCS tissue solubilizer was purchased from Amersham/Searle Corp. Fetal calf serum and Eagle's Basal medium (Earle's salts) were purchased from Grand Island Biological Co., Grand Island, N. Y.

T_3, T_4, and their analogues were prepared for in vivo or in vitro use by solubilization in the minimum quantity required of 0.025 N NaOH; further dilution was made with normal saline to the final volume of 0.2 ml used for administration to rat or mouse. For the control or NaI-treated groups, a similar minimal quantity of 0.025 N NaOH was added to the normal saline or NaI to make the final injection solutions of similar alkalinity to the solutions of iodothyronine hormones and their analogues.

RESULTS

The rat

Effects of T_4 and T_3 pretreatment on TSH-induced thyroid ODC activity

We have previously shown (5) that TSH in doses from 1 to 8 U increases rat thyroid ODC activity in a concentration-related manner. As can be seen from Fig. 1, i.p. administration of 4 U TSH increases rat thyroid ODC activity 30- to 40-fold over saline control; however, this TSH-induced ODC activity is virtually abolished when the rat is pretreated with 5 μg T_4 or T_3 administered s.c. 16 h before TSH administration. Basal ODC activity is unaffected by such pretreatment.

The inhibitory effect of thyroid hormone pretreatment on TSH-induced ODC activity is dose related (Fig. 2); the half-maximal inhibitory dose of T_4 is 1.7 μg/rat, and that of T_3 is 0.6 μg/rat. Rats pretreated with large doses of NaI show no alteration in TSH-induced thyroid ODC activity.

As can be seen from Table I, the maximal increase in circulating T_4 and T_3 levels associated with pretreatment of the rats with 1.7 μg T_4 or 0.6 μg T_3 is comparable to that seen after administration of 2 U TSH and represents only a twofold (T_4) to threefold (T_3) increase over basal levels. (Serum T_4 and T_3 levels were also measured at shorter and longer time intervals after treatment, but only the basal and maximal values are shown here.)

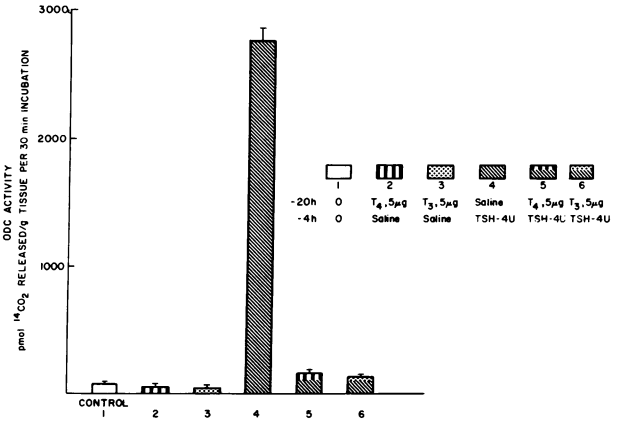

FIGURE 1 Effect of thyroid hormone pretreatment on TSH-induced rat thyroid ODC activity. Rats (five animals per experimental group) were pretreated with saline, 5 μg T_4, or 5 μg T_3 injected s.c. 16 h before i.p. administration of 4 U TSH (−20 h). The rats were killed 4 h after TSH injection (−4 h) and thyroid ODC activity assayed. Each vertical bar represents the mean±SE of three experiments.

To examine further the specificity of T_4 and T_3 inhibition of TSH-induced rat thyroid ODC, the effects of a variety of iodotyrosines, iodothyronines, and related compounds were studied. The half-maximal inhibitory doses of these compounds (obtained, in the

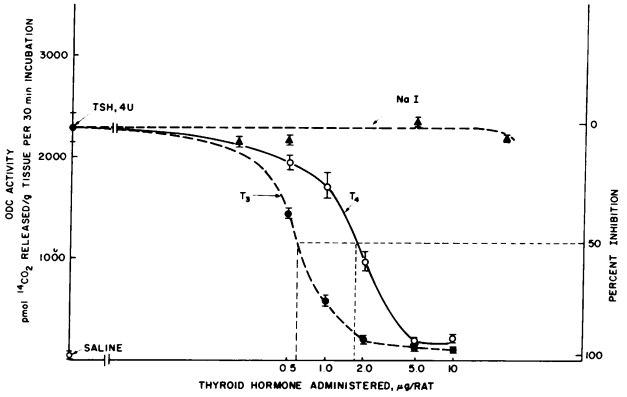

FIGURE 2 Concentration-related inhibitory effect of exogenous thyroid hormone on TSH-stimulated rat thyroid ODC activity. Increasing quantities of T_4, T_3, or NaI were administered s.c. to groups of rats (five animals per experimental group) 16 h before i.p. injection of 4 U TSH. 50% reduction in TSH-induced ODC activity is indicated by horizontal broken line; half-maximal inhibitory doses of T_4 and T_3 are 1.7 μg per rat and 0.6 μg per rat, respectively. Each point represents the mean±SE of three experiments.

TABLE I

Serum T_4 and T_3 Levels in T_4-, T_3-, and TSH-Treated Rats

Sampling time*	Treatment‡	Serum thyroid hormone level§ Control	TSH, 2 U	T_4, 1.7 μg	T_3, 0.6 μg
h					
0	T_4‖	2.3±0.4	—	—	—
	T_3¶	55±5	—	—	—
+2	T_4	—	4.7±0.3	4.6±0.9	—
	T_3	—	120±18	—	156±15
+4	T_4	—	4.9±0.5	4.1±0.6	—
	T_3	—	122±12	—	133±15

* After hormone administration.
‡ TSH administered i.p., T_4 and T_3 given s.c.
§ Each value is the mean ±SE of five determinations in five rats.
‖ Micrograms per deciliter.
¶ Nanograms per deciliter.

first five instances listed, from sigmoid inhibitory dose-response curves) are listed in Table II. Of the compounds tested, only triiodothyroacetic acid, D-T_4, and diiodothyronine exhibit any significant inhibitory activity.

To determine whether an increase in the dose of exogenous TSH could overcome the inhibitory effect of thyroid hormone on (stimulated) thyroid ODC activity, graded doses of TSH ranging from 1 to 8 U were given to rats pretreated with 0.5 μg T_3. As shown in Fig. 3, the inhibitory effect of T_3 is maintained despite increasing doses of exogenous TSH.

The time-course of T_4 and T_3 inhibition of TSH-induced ODC activity is depicted in Fig. 4. 95–97% inhibition of ODC activation is demonstrable when rats are pretreated with 10 μg T_4 or 5 μg T_3 between 12 and 96 h before TSH administration. Statistically significant ($P < 0.01$) inhibition of stimulated ODC activity is demonstrable in animals pretreated as late as

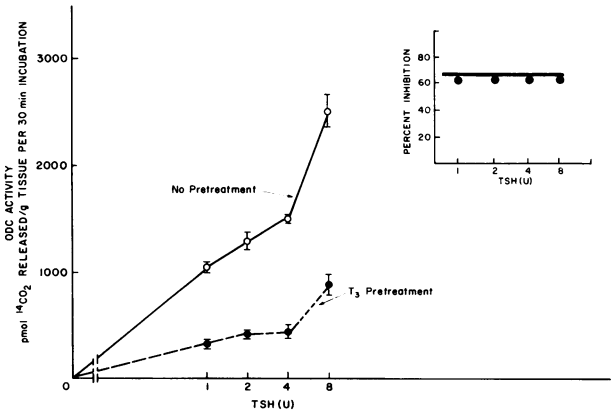

FIGURE 3 Inhibitory effect of fixed-dose (0.5 μg) T_3 pretreatment on rat thyroid ODC activity induced by increasing concentrations of TSH. Each point represents the mean ±SE of three experiments (five animals per experimental group).

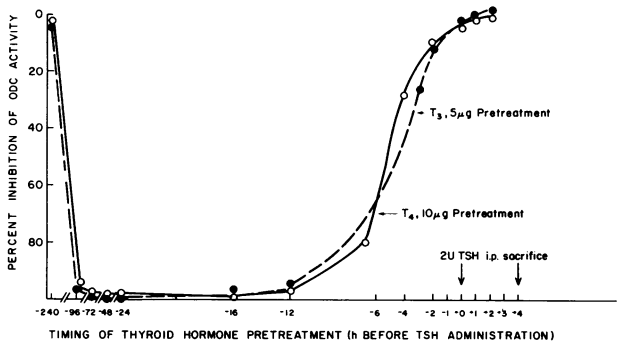

FIGURE 4 Time-course of T_4 or T_3 inhibition of TSH-induced rat thyroid ODC activity. Rats were pretreated with 10 μg T_4 or 5 μg T_3 administered s.c. at various intervals before or after TSH administration. TSH-induced ODC activity (2,050±72 pmol $^{14}CO_2$ released/g tissue per 30 min incubation) is regarded as 0% inhibition and the basal level of ODC activity (78±30 pmol $^{14}CO_2$ released/g tissue per 30 min incubation) is regarded as 100% inhibition. Each point represents the mean of closely agreeing triplicate determinations.

(but no later than) 4 h (T_4) or 3 h (T_3) before TSH administration.

To determine whether an intrathyroidal inhibitor of thyroid ODC is synthesized or activated as a result of thyroid hormone pretreatment, the 20,000 *g* supernatant fractions prepared from glands obtained from T_4 only- and TSH only-treated rats were combined and tested for ODC activity. In addition, 0.02 μg T_4 was added

TABLE II

*Relative Inhibitory Potency of T_4, T_3, and Thyroid Hormone Analogues on TSH-Induced Thyroid ODC Activity**

Compounds	Half-maximal inhibitory quantity	Relative inhibitory potency‡
	μg/rat	%
T_4	1.7	100
T_3	0.6	283
Triiodothyroacetic acid	4.3	40
D-T_4	9.3	18
Diiodothyronine	18	9
Thyronine§	≫125	<1
Diiodotyrosine§	≫125	<1
Monoiodotyrosine§	≫125	<1
Tyrosine§	≫125	<1

* TSH, 4 U, was administered i.p. 4 h before sacrifice and the various compounds tested were given s.c. 16 h before TSH administration.
‡ Calculation of the relative inhibitory potency of the compounds tested is based on the half-maximal inhibitory quantity of each compound compared to that of T_4.
§ No significant inhibitory effect on TSH-induced thyroid ODC activity was obtained when rats were treated with 125 μg or more of each of these compounds.

TABLE III

Effect of TSH Pretreatment on TSH-Induced Rat Thyroid ODC Activity

Treatment				
First TSH administration		Second TSH administration		
Time interval*	Dose/rat	Time interval*	Dose/rat	ODC activity‡
h		*h*	*U*	*pmol $^{14}CO_2$/g*
−16	Saline	−4	2	1,872±66
−16	2 U	−4	2	986±71§
−25	Saline	−4	2	1,979±101
−25	2 U	−4	2	923±117§
−45	Saline	−4	2	1,810±88
−45	2 U	−4	2	561±40§
−74	Saline	−4	2	1,749±76
−74	2 U	−4	2	508±66§

* Time interval is that between i.p. administration of TSH or saline and sacrifice.

‡ Each value is the mean±SE of triplicate determinations (five animals per experimental group).

§ Significantly ($P < 0.01$) less than corresponding saline/TSH-treated group.

TABLE IV

Effect of Thyroid Hormone Pretreatment on TSH-Induced Thyroid ODC Activity in Hypophysectomized Rats

Pretreatment*	Treatment‡	ODC activity§
		pmol $^{14}CO_2$/g
Saline	Saline	27±16
Saline	TSH, 2 U	1,918±183
T_4, 1 μg	TSH, 2 U	1,178±167‖
T_4, 2 μg	TSH, 2 U	890±132‖
T_4, 5 μg	TSH, 2 U	178±96‖
T_3, 1 μg	TSH, 2 U	908±31‖
T_3, 2 μg	TSH, 2 U	318±59‖
T_3, 5 μg	TSH, 2 U	104±76‖

* Administered s.c. 16 h before TSH injection.

‡ Administered i.p. 4 h before sacrifice.

§ Animals tested 3 days after hypophysectomy. Each value is the mean±SE of triplicate determinations (five animals per experimental group).

‖ Significantly ($P < 0.01$) less than saline/TSH-treated group.

directly to 0.05 ml of 20,000 *g* supernatant fraction prepared from TSH-stimulated glands. In neither instance is the resulting enzyme activity of the mixture any different than that found in the TSH-stimulated glands alone (data not shown here). These findings make unlikely the possibility of formation of a substance inactivating or inhibiting preformed enzyme consequent to thyroid hormone pretreatment.

Since an increase in circulating thyroid hormone levels is shown to reduce thyroid gland response to exogenous TSH, it was of interest to determine whether prior treatment with TSH reduces thyroidal response to subsequent TSH administration. Pretreatment of rats with 2–8 U TSH results in significant reduction in the magnitude of ODC activation induced by a second injection of 2 U TSH compared to that observed in animals pretreated with saline only (Table III). (A total of six such experiments was carried out; data from one representative experiment, detailing results of 2 U TSH pretreatment only are shown in the Table.) Such inhibition is demonstrable when the first injection of TSH is given anywhere from 21 to 70 h before the second injection of TSH (i.e., 25–74 h before sacrifice); under these conditions, no residual TSH effect on thyroid ODC activity was present at the time the second TSH injection was administered (data not shown here). The reduction in response to the second dose of TSH appears not to be related to the size of the first TSH dose (4-U and 8-U TSH pretreatment data not shown) or its timing (within the limits outlined above). (The inhibition observed with a 12-h interval between TSH injections was not entirely reproducible [three of six experiments].) Basal serum T_4 levels in these experiments averaged 2.6±0.5 μg/dl and averaged (for all TSH doses) 4.7±0.6, 5.7±0.7, 6.0±0.6, and 5.8±0.8 μg/dl at 3, 6, 9, and 12 h, respectively, after the first injection of TSH; the increase in serum T_4 after the first injection of TSH was statistically the same at all sampling periods whether the dose administered was 2, 4, or 8 U.

TABLE V

Effect of T_4, T_3, or NaI Pretreatment on In Vitro TSH-Induced Glucose Oxidation in Rat Thyroid

Experimental conditions		
In vivo pretreatment*	In vitro incubation	Glucose oxidation‡
		cpm $^{14}CO_2$ released/mg wet wt
Saline	—	61.8±7.3
Saline	TSH, 200 mU/ml	127.5±6.2§
T_4, 25 μg	—	56.4±4.9
T_4, 25 μg	TSH, 200 mU/ml	88.6±7.2‖
T_3, 25 μg	—	56.3±8.1
T_3, 25 μg	TSH, 200 mU/ml	79.0±9.6‖
NaI, 25 μg	—	65.2±5.5
NaI, 25 μg	TSH, 200 mU/ml	131.7±10.3§

* Pretreatment was given in five divided doses over a 3-day period as described in text.

‡ Results are the means±SE of closely agreeing triplicate determinations.

§ Significantly ($P < 0.01$) greater than saline control.

‖ Significantly ($P < 0.01$) less than TSH alone.

TABLE VI

Effect of Thyroid Hormone Pretreatment on In Vivo TSH-Induced Protein Synthesis in Rat Thyroid

Pretreatment*	Treatment	[^{3}H]Leucine incorporation‡
		cpm/mg protein
Saline	Saline	259±21
Saline	TSH, 4 U§	618±30‖
T_4, 30 μg	Saline	265±13
T_4, 30 μg	TSH, 4 U	406±10¶
T_3, 30 μg	Saline	251±11
T_3, 30 μg	TSH, 4 U	399±32¶

* Pretreatment was given in six divided doses over 3-day period as described in text.

‡ Each value is the mean±SE of triplicate determinations (four animals per experimental group).

§ Administered i.p. 7 h before i.p. injection of 10 μCi [^{3}H]-leucine and 9 h before sacrifice.

‖ Significantly ($P < 0.01$) greater than control.

¶ Significantly ($P < 0.01$) less than TSH alone.

To exclude the possibility that T_4 or T_3 pretreatment acts by suppressing endogenous TSH secretion, thus altering the sensitivity of the thyroid gland to subsequent exogenous TSH stimulation, ODC experiments were also carried out in hypophysectomized rats. As can be seen from Table IV, a dose-related inhibitory effect of T_4 or T_3 pretreatment on TSH-induced thyroid ODC activity is also seen in these animals. Similar pretreatment with iodide or thyroid hormone analogues was ineffective (data not shown here).

Effects of T_4 and T_3 pretreatment on TSH-induced glucose oxidation in thyroid

As shown in Table V, pretreatment of rats with T_4 or T_3 in divided doses over a 2½-day period (total dose, 25 μg) results in significant inhibition of the increase in [1-^{14}C]glucose oxidation to $^{14}CO_2$ induced by 200 mU TSH. Basal glucose oxidation was unaffected under these conditions. NaI pretreatment did not influence basal or TSH-stimulated glucose oxidation. Qualitatively identical results were obtained in three additional experiments.

Effects of T_4 and T_3 pretreatment on TSH-induced protein synthesis in thyroid

As shown in Table VI, pretreatment of rats with T_4 or T_3 as for glucose oxidation studies results in significant inhibition of the increase in [^{3}H]leucine incorporation into protein induced by in vivo administration of TSH. Basal protein synthesis is unaffected by thyroid hormone pretreatment. Qualitatively identical results were obtained in three additional experiments.

The mouse

ODC activity and hormone release

Characterization of TSH-induced ODC activation. As shown in Fig. 5, TSH exerts a dose-related stimulatory effect on mouse thyroid ODC activity in animals killed 4 h after i.p. administration of the hormone. Although the maximal stimulatory effect on both thyroid ODC activity and ^{131}I release is observed with the same dose of TSH (10 mU), significant augmentation of mouse thyroid hormone release is observed with doses of TSH (e.g., 1 mU, Fig. 5) that do not influence ODC activity in the gland.

The time-course of TSH-induced ODC activation and ^{131}I release after the i.p. administration of 10 mU TSH is depicted in Fig. 6. Although a modest elevation of thyroid ODC activity is observed as early as 2 h after TSH administration, clear-cut stimulation of ^{131}I release is more readily discernible at this time; the peak increase in both thyroid ODC activity and ^{131}I release is observed at 4 h.

(Although there is considerable variability in the magnitude of TSH-induced ODC activation in mouse thyroid over the extended period of time required for completion of these studies [e.g., ODC responses in Figs. 5 and 6], the dose-related stimulatory effects of TSH and the inhibition thereof by T_4 and/or T_3 (*vide infra*) is entirely reproducible.)

Effect of thyroid hormone or iodide pretreatment on TSH-induced ODC activity and hormone release. As indicated in Methods, both LID and RD mice were used for the ODC experiments whereas LID mice only were used in the McKenzie bioassay. It is thus appropriate to note that neither basal nor TSH-induced thyroid ODC activity is modified by iodine lack (data not shown here).

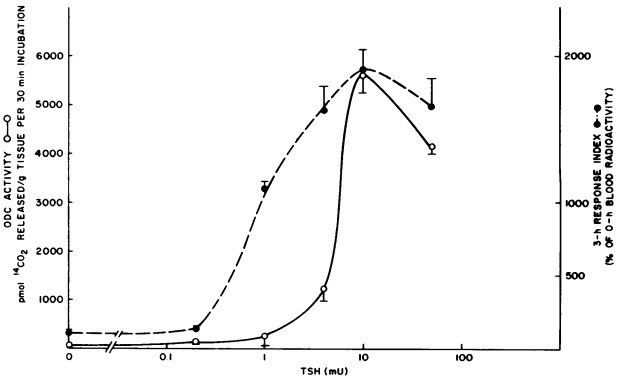

FIGURE 5 Dose-related TSH stimulation of mouse thyroid ODC activity (○) and thyroid hormone release (●). Each point represents the mean±SE of three experiments (15 animals per experimental group in ODC study; 6 animals per experimental group in ^{131}I release bioassay).

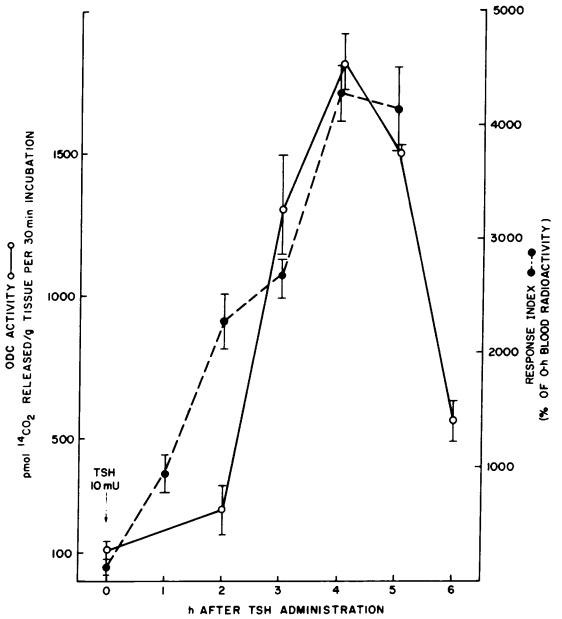

FIGURE 6 Time-course of TSH-induced mouse thyroid ODC activation (○) and thyroid hormone release (●). 10 mU TSH was administered i.p. at 0 time and the mice sacrificed at the times indicated for measurement of thyroid ODC activity. For measurement of thyroid ^{131}I release, the mice were given 4 μCi ^{131}I i.p. and 0.25 μg T_3 s.c. 24 h before TSH administration; a second s.c. injection of 0.25 μg T_3 was given 8 h later. Blood was obtained by orbital sinus puncture at 0 h and at varying times after TSH injection. Each point represents the mean±SE of three experiments (15 animals per experimental group in ODC study; 6 animals per experimental group in ^{131}I release bioassay).

When LID mice are pretreated with up to 1.5 μg T_4 or T_3 for only 1 day (i.e., at −24 and −12 h) before TSH administration, no inhibition of thyroid ODC activity is observed (Table VII). Under these conditions, however, pretreatment with larger doses of T_4 or T_3 (10 μg) significantly reduces TSH stimulation of thyroid ODC activity.

In contrast to the lack of inhibitory effect of short-term low-dose thyroid hormone pretreatment, more prolonged (i.e., 2½ days) pretreatment with as little as 0.25 μg daily doses of T_4 or T_3 results in significant inhibition of TSH-induced mouse thyroid ODC activity (Fig. 7). In contrast to our findings in the rat (*vide supra*), NaI pretreatment of the LID mouse also markedly blunts TSH-induced ODC activation. Although larger doses of thyroid hormone or iodide are required than those necessary for ODC inhibition, 2½-day thyroid hormone or NaI pretreatment of LID mice inhibits TSH-induced thyroid ^{131}I release (Fig. 8).

As can be seen from the data depicted in Fig. 9, the RD mouse pretreated with NaI responds exactly as does the rat, i.e. there is no effect on TSH-induced

TABLE VII

Effect of Short-Term Thyroid Hormone Pretreatment on Thyroid ODC Activity in the LID Mouse

In vivo pretreatment*	ODC activity‡
	pmol $^{14}CO_2$/g
Control (normal saline)	54±17
4 mU TSH	2,906±143§
1.5 μg T_4	65±55
1.5 μg T_3	85±9
1.5 μg T_4 + 4 mU TSH	2,864±60§
10 μg T_4 + 4 mU TSH	2,046±87‖
0.25 μg T_3 + 4 mU TSH	2,574±49§
1.5 μg T_3 + 4 mU TSH	3,127±105§
10 μg T_3 + 4 mU TSH	1,878±138‖

* Mice on LID for 14 days were given the stated amount of T_4 or T_3 s.c. 24 h and 12 h before i.p. TSH administration. The mice were killed 4 h after TSH or saline administration.
‡ Each value is the mean±SE of triplicate determinations.
§ Significantly ($P < 0.01$) greater than control.
‖ Significantly ($P < 0.01$) less than TSH alone.

mouse thyroid ODC activity, whereas the inhibitory effect of T_4 or T_3 pretreatment on TSH-activated thyroid ODC is qualitatively the same in both the LID and RD mouse.

As in the rat, pretreatment with TSH significantly reduces mouse thyroid ODC response to a subsequent dose of TSH (Table VIII). Thus, 1-, 2-, or 3-day pretreatment with 20 mU TSH results in approximately half the increase in ODC activation in response to a subsequent 20-mU dose of TSH compared to that observed in mice pretreated with saline only. The results

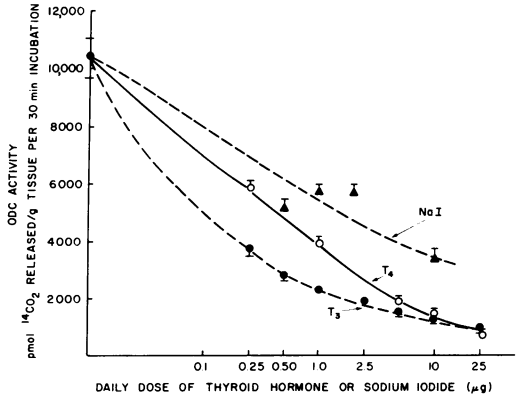

FIGURE 7 Inhibitory effect of exogenous thyroid hormone or sodium iodide on TSH-stimulated thyroid ODC activity in the LID mouse. The mice were pretreated with varying amounts of T_4, T_3, or NaI for 3 consecutive days. On the 4th day, the animals were sacrificed 4 h after i.p. administration of 10 mU TSH. Each point represents the mean ±SE of three experiments (15 animals per experimental group).

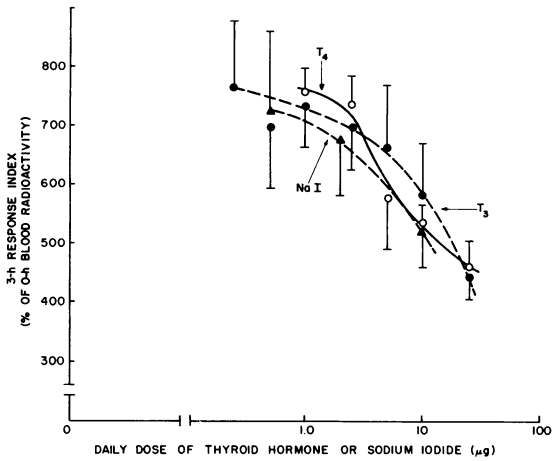

FIGURE 8 Inhibitory effect of exogenous thyroid hormone or sodium iodide on TSH-stimulated thyroid hormone release in the LID mouse. The mice were pretreated with varying amounts of T_4, T_3, or NaI for 3 consecutive days. 4 μCi ^{131}I had been given i.p. on the 1st day 1 h before the first s.c. injection of T_4, T_3, or NaI. On the 4th day, the animals were bled at 0 h, then given 0.5 mU TSH i.p. and bled again 3 h later. The animals pretreated with NaI were also given 0.25 μg T_3 s.c. daily to suppress endogenous TSH before exogenous TSH administration.

with 1-day pretreatment only are shown here. If more than 24 h separate the TSH "pretreatment" and the "test" dose of TSH, no inhibition of thyroid ODC activation is observed (data not shown). As was also the case in the rat, no residual "pretreatment" TSH effect on thyroid ODC or serum T_4 levels was observed at the time the second or "test" dose of TSH was administered. Although less marked than the reduction in ODC activation, TSH pretreatment does result in

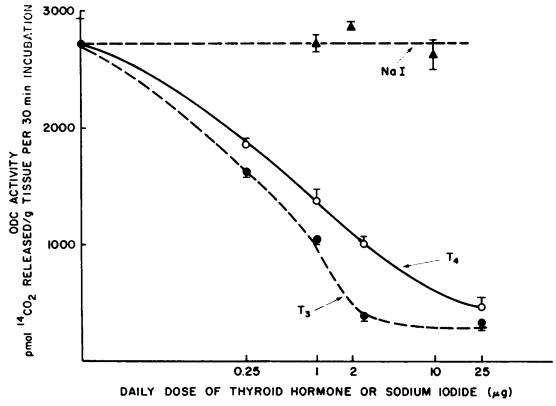

FIGURE 9 Effect of exogenous thyroid hormone or sodium iodide pretreatment on TSH-stimulated thyroid ODC activity in the RD mouse. The mice were pretreated with varying amounts of T_4, T_3, or NaI for 3 consecutive days. On the 4th day, the animals were killed 4 h after i.p. administration of 10 mU TSH. Each point represents the mean±SE of three experiments (15 animals per experimental group).

TABLE VIII

Effect of TSH Pretreatment on TSH-Induced Mouse Thyroid ODC Activity and Thyroid Hormone Release

Group	TSH administration* Day 1	Day 2	ODC activity‡	Serum T_4‡
			pmol $^{14}CO_2$/g	*μg/dl*
1	—	20 mU	9,852±588	6.9±0.3
2	—	Saline	44±5	2.5±0.5
3	20 mU	20 mU	4,951±342§	5.3±0.4§
4	20 mU	Saline	64±6	3.3±0.6

* TSH was given i.p. at 11 a.m. on day 1; on day 2, TSH or saline was given i.p. at 7 a.m., 4 h before orbital sinus puncture for serum T_4 sampling and sacrifice.

‡ Each value is the mean±SE of triplicate determinations (six animals per experimental group).

§ Significantly ($P < 0.05$ to $P < 0.01$) less than (day 2, TSH only-treated) group 1.

a lesser (albeit statistically [$P < 0.05$] significant) rise in serum T_4 levels in response to the "test" (day 4) dose of 20 mU TSH (group 3) compared to that observed in nonpretreated animals (group 1).

CYCLIC AMP FORMATION

When LID mice are pretreated with varying amounts of T_4 or T_3 for 2½ days as described in Methods, TSH-induced in vitro stimulation of thyroid cyclic AMP formation is inhibited in a concentration-related manner (Table IX).

Although qualitatively similar findings with T_3 and T_4 pretreatment are observed in RD mice, as in the case for ODC activity, NaI pretreatment inhibits TSH-induced thyroid cyclic AMP formation in LID mice only (data not shown here).

As can be seen from Table X, in vitro preincubation of mouse thyroid glands for 3 h with as little as 1 μg/ml T_4 markedly inhibited cyclic AMP accumulation induced by a subsequent 30-min incubation with 5 mU TSH. (Addition of the 0.025 N NaOH diluent only to the preincubation was without effect.) Although similar preincubation with NaI at a concentration of 50 μM (6.3 μg/ml) also effected a more modest reduction in TSH-induced cyclic AMP accumulation, addition of 2 mM methimazole to the preincubation medium abolished the inhibitory iodide effect. The inhibitory effect of T_4 could not be reproduced by up to 20 μg/ml triiodothyroacetic acid or D-T_4 nor was it affected by addition of methimazole to the preincubation medium (data not shown here).

DISCUSSION

The thesis that the thyroid gland might be ". . . directly inhibited by its own hormone . . ." was first advanced by Galli-Mainini in 1941 (9). He reported that the in

vitro stimulatory effect of TSH on oxygen consumption by guinea pig thyroid slices was inhibited by the addition of thyroid hormone to the incubation medium. In 1944, Cortell and Rawson (10) reported that the administration of T_4 to the immature female guinea pig depresses thyroidal response to exogenous TSH as determined by the mean cell height of the thyroid epithelium. These authors also noted that the administration of T_4 to hypophysectomized male rats depressed thyroidal response to exogenous TSH as determined both by mean thyroid epithelial cell height as well as by thyroidal radioiodine uptake (10). The possible implications of these findings were apparently ignored during the next two decades until Florsheim and co-workers (1) and subsequently Shishiba et al. (2) noted that the response of the McKenzie bioassay mouse to TSH or long-acting thyroid stimulator was blunted by prior exposure to increasing doses of exogenous thyroid hormone. The latter authors concluded that ". . . this study suggests a possible alteration of thyroidal responsiveness to stimulators under the influence of circulating thyroid hormone and warrants further investigation . . ." (2).

TABLE IX

Effect of Thyroid Hormone Pretreatment on In Vitro TSH-Induced Thyroid Cyclic AMP Formation in the LID Mouse

In vivo pretreatment*	In vitro incubation‡	Cyclic AMP content§
		pmol/mg tissue
Exp. 1		
Saline	—	4.03±1.00
Saline	1 mU TSH	39.03±3.60‖
Saline	5 mU TSH	158.97±11.56‖
0.25 μg T_4	—	2.98±0.75
0.25 μg T_4	1 mU TSH	44.38±5.28‖
0.25 μg T_4	5 mU TSH	119.60±18.50‖
2.5 μg T_4	—	4.23±0.99
2.5 μg T_4	1 mU TSH	27.23±2.96¶
2.5 μg T_4	5 mU TSH	43.53±5.17¶
25 μg T_4	—	2.73±0.30
25 μg T_4	1 mU TSH	13.98±1.32¶
25 μg T_4	5 mU TSH	33.32±11.32¶
Exp. 2		
Saline	—	3.63±0.42
Saline	1 mU TSH	31.60±4.06‖
Saline	5 mU TSH	93.81±6.79‖
0.25 μg T_3	—	3.00±0.52
0.25 μg T_3	1 mU TSH	21.53±3.53‖
0.25 μg T_3	5 mU TSH	90.37±2.65‖
2.5 μg T_3	—	2.30±0.87
2.5 μg T_3	1 mU TSH	18.55±1.66¶
2.5 μg T_3	5 mU TSH	42.90±3.89¶
25 μg T_3	—	4.16±1.14
25 μg T_3	1 mU TSH	15.17±2.27¶
25 μg T_3	5 mU TSH	38.33±5.56¶

* Mice placed on a low-iodine diet for 14 days were then given thyroid hormone or saline s.c. at 9 a.m. and 4 p.m. on each of the 2 days before sacrifice; on the following day an i.p. injection of thyroid hormone or saline was given 1 h before sacrifice.

‡ 30 min at 37°C.

§ Each value is the mean±SE of three experimental determinations, each assayed in triplicate.

‖ Significantly ($P < 0.01$) greater than control.

¶ Significantly ($P < 0.01$) less than corresponding concentration of TSH alone.

TABLE X

Effect of T_4 Preincubation on TSH-Induced Mouse Thyroid Cyclic AMP Formation

Preincubation (3 h)*	Final incubation (30 min)‡	Cyclic AMP content§
		pmol/mg tissue
Buffer[a]	Buffer[b]	1.38±0.21
Buffer	TSH, 5 mU/ml	65.01±0.88‖
T_4, 1 μg/ml	Buffer	0.97±0.30
T_4, 1 μg/ml	TSH, 5 mU/ml	11.94±1.12¶
NaI, 50 μM	Buffer	1.38±0.30
NaI, 50 μM	TSH, 5 mU/ml	48.16±3.20¶
Methimazole, 2 mM	Buffer	1.54±0.29
NaI, 50 μM, +methimazole, 2 mM	TSH, 5 mU/ml	69.37±2.88‖

* Preincubation was carried out at 37°C in Krebs-Ringer bicarbonate buffer[a], pH 7.4, containing 1% glucose and 1% bovine serum albumin under an atmosphere of 95% O_2-5% CO_2.

‡ Final incubation was carried out at 37°C in Krebs-Ringer bicarbonate buffer[b], pH 7.4, containing 1% glucose, 1% bovine serum albumin, and 10 mM theophylline.

§ Each value is the mean±SE of three experimental determinations, each assayed in triplicate.

‖ Significantly ($P < 0.01$) greater than buffer alone.

¶ Significantly ($P < 0.01$) less than glands incubated with buffer/TSH.

The results of the present study confirm and extend these observations. Thus, a number of exogenous TSH effects on murine thyroid, including stimulation of cyclic AMP formation and hormone release, are inhibited by prior administration of thyroid hormone to the experimental animals. The inhibitory effect is concentration-related and achieved at a dose (and resultant circulating concentration) of T_4 and/or T_3 comparable to that required for inhibition of goitrogenesis in the rat (11) and/or that resulting in inhibition of thyrotropin-releasing hormone-TSH release response in the mouse (12). The inhibitory effect in the rat appears to be reasonably specific for iodothyronines. In contrast, NaI is also inhibitory in the LID but not in the RD mouse. We believe the inhibitory effect of NaI in the LID mouse merely reflects avid uptake of iodide by the iodine-depleted thyroid gland and its rapid incorporation into intrathyroidal thyroid hormone. Results of

preliminary studies wherein concomitant perchlorate or propylthiouracil administration abolish the inhibitory NaI effect (Yu, S., Y. Friedman, and G. Burke, unpublished observations) as well as a similar block by methimazole of NaI inhibition in vitro of TSH-induced cyclic AMP formation in mouse thyroid (Table X) support this premise.

Van Sande et al. (13) have presented data compatible with the thesis that iodide trapped by the thyroid is oxidized and then bound to an intracellular organic compound X, which then exerts a negative feedback on thyroid cell function; these findings and conclusions are entirely consistent with the postulate advanced herein. Rapoport and Ingbar (14) have reported similar in vitro findings with iodide and rat thyroid; in their studies, direct measurement of organic iodine content and cyclic AMP production in paired thyroid lobes incubated in graded concentrations of stable iodide demonstrated proportionality between organic iodine content and the extent of inhibition of adenylate cyclase responsiveness to TSH. Thus, the inhibitory effect of NaI in the LID mouse may derive solely from its conversion to thyroid hormone and does not vitiate the apparent specificity of the inhibitory iodothyronine effect on TSH stimulation.

While one cannot entirely exclude the possibility that thyroid hormone pretreatment accelerates the clearance of exogenous TSH, this appears rather unlikely since the inhibitory effect of in vivo T_4/T_3 pretreatment is also demonstrable when in vitro TSH effects are studied (e.g., rat, [1-^{14}C]glucose oxidation; mouse, cyclic AMP formation). These findings are in keeping with the observations of Shishiba et al. (2) that a reduction in the colloid droplet response was also observed in thyroid glands of mice pretreated with large doses of T_3 in vivo and incubated with TSH or LATS in vitro. Additionally, an inhibitory effect of T_4 and/or T_3 on the in vitro stimulation of mouse thyroid cyclic AMP formation by TSH is demonstrable when the extirpated mouse thyroid gland is preincubated with thyroid hormone (Table X).

Since exposure of the thyroid gland to increased circulating thyroid hormone levels blunts the stimulatory effect of exogenous TSH, one would anticipate that "pretreatment" of the experimental animal with TSH and the consequent increase in circulating T_4 and T_3 levels should result in decreased thyroidal response to further TSH administration. Such was found to be the case in both rat and mouse when TSH effects on ODC activation and thyroid hormone release were tested under these conditions. These observations, as well as the inhibitory effect of T_4/T_3 pretreatment on TSH-induced thyroid ODC activity in hypophysectomized rats and the demonstration of an in vitro inhibitory effect of thyroid hormone on TSH-stimulated mouse thyroid cyclic AMP formation, do not support the recent suggestion of Gafni et al. (15) that reduced thyroidal response to TSH following pretreatment of mice with T_3 is a consequence of (endogenous) TSH "withdrawal."

Although the inhibitory effect of T_4 and/or T_3 pretreatment on TSH-induced ODC activation persists for up to 4 days, the $t_{\frac{1}{2}}$ of exchangeable tissue T_3 in the rat varies between only 4 and 6 h (16). In this regard, the inhibition by circulating thyroid hormones of thyroidal response to TSH parallels a variety of tissue effects of injected T_3 in thyroidectomized rats wherein all of the observed effects of the hormone appear to decline with similar $t_{\frac{1}{2}}$'s in the order of 4–6 days (17–19). Thus, our findings merely represent yet another instance where the tissue effect of the hormone appears to persist long after all of the administered hormone has disappeared from the circulation.

The precise relationship of ODC activation in thyroid to hormonogenesis or hormone secretion is uncertain. We (5), and others (20) have recently shown that the activity of the latter enzyme, which catalyzes the initial step in polyamine synthesis from ornithine, is regulated by endogenous circulating TSH. To the extent that ODC activity may serve as a biological marker for the growth-promoting effects of TSH, the inhibitory effect of thyroid hormone on TSH-induced ODC activation suggests that both hormone secretion and gland growth can be directly inhibited by circulating thyroid hormones. Clearly, the precise locus and nature of this inhibition remains to be defined. While there is currently some disagreement as to whether such inhibition occurs before (15) or beyond (21) cyclic AMP formation, the inhibition by thyroid hormone of TSH-induced cyclic AMP formation[2] in the gland as reported here may well be of pivotal importance. (Since thyroid hormones have been shown to inhibit rather than augment thyroid cyclic AMP phosphodiesterase activity [23, 24], the inhibition of TSH-induced cyclic AMP formation cannot be attributed to thyroid hormone-induced enhancement of the activity of the degradative enzyme.)

In this regard, the recent observation of Takasu et al. (25) suggest that the findings reported here may be relevant to the pathogenesis of Graves' hyperthyroidism in man. These workers reported that addition of T_4 or T_3 in vitro decreased TSH stimulation of adenylate cyclase and cyclic AMP formation in plasma membrane fractions and slices prepared from thyroid glands surgi-

[2] Although we have been unable to demonstrate a dibutyryl cyclic AMP effect on rat thyroid ODC (5), Zussman and Burrow (22) recently reported that dibutyryl cyclic AMP and aminophylline caused a 12-fold increase in rat thyroid ODC activity 5 h after administration.

cally removed from euthyroid subjects. Since thyroid tissue from hyperthyroid patients was less susceptible to T_4- and T_3-induced suppression of TSH-augmented adenylate cyclase activation and cyclic AMP formation, Takasu et al. suggested that thyroid hormones play an important role in the control of human thyroid function through depression of the thyroidal adenylate cyclase-cyclic AMP system activated by TSH (25).

Thus, from the results of studies of other workers as well as our own, it appears reasonable to conclude that thyroidal responsivity to TSH is altered under the influence of circulating thyroid hormones and, further, to suggest the existence of a thyroid-hormone-mediated "short-loop" negative feedback important in the regulation of thyroid function.

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