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Research Article

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Rapid Alteration in Circulating Free Thyroxine Modulates Pituitary Type II 5′ Deiodinase and Basal Thyrotropin Secretion in the Rat

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Abstract

TSH secretion is decreased by both T_4 and T_3. This negative feedback control of TSH secretion has been correlated with an increase in pituitary nuclear T_3 content, and it is not clear whether T_4 exerts its effect directly on the thyrotroph or after its deiodination to T_3. However, levels of the pituitary enzyme catalyzing T_4 to T_3 conversion, 5′D-II, are decreased in the presence of an increased amount of T_4. Thus, it is unclear why the thyrotroph would have a mechanism for modulating the production of T_3, if T_3 is, in fact, the sole bioactive signal providing negative feedback inhibition.

To examine this apparent paradox, we administered EMD 21388, a compound which inhibits the binding of T_4 to transthyretin resulting in a rapid increase in circulating free T_4 levels, to rats pretreated with radiolabeled T_4 and T_3. We observed increases in pituitary and liver T_4 content of >150%, without increases in the respective tissue T_3 contents. The EMD 21388-treated rats also exhibited a 25% decrease in pituitary 5′D-II activity (103.8±15.8 fmol ^{125}I released · mg protein^{-1} · h^{-1}, vs. control, 137.4±15.9, mean±SE), as did rats treated with sodium salicylate, another compound that inhibits T_4-TTR binding (100.8±7.1). TSH levels significantly decreased 2 h after the administration of EMD 21388. These data demonstrate that despite a T_4-mediated decrease in pituitary 5′D-II activity, an increase in T_4 independently decreases TSH secretion. (*J. Clin. Invest.* 1991. 88:898–903.) Key words: thyrotropin • flavonoid • deiodinase • transthyretin • thyroxine

Introduction

Thyrotropin (TSH)[1] regulates the synthesis and secretion of thyroxine (T_4) and triiodothyronine (T_3) from the thyroid gland. Pituitary TSH secretion is stimulated by thyrotropin releasing hormone (TRH), and decreased by other hypothalamic factors, including dopamine and somatostatin (1). Thyroid hormones play a major role in regulating TSH secretion. In thyroidectomized rats, the administration of physiologic replacement doses of L-T_3 decreases serum TSH by ~ 50% in 2 h, and a similar decrease is seen after the administration of L-T_4 (2, 3). Silva and Larsen reported that, following the intravenous administration of 70 ng L-T_3/100 g body weight or a 10-fold higher amount of T_4, the fall in serum TSH was associated with a rise in the percentage of T_3 bound to pituitary nuclear receptors (3). This led to the suggestion that T_4 exerts its inhibitory effect on TSH secretion primarily after its deiodination to T_3.

This idea was further supported by a number of subsequent findings. First, pituitary TSH suppression was dependent upon nuclear T_3 content and serum T_3 concentration (4). Second, the simultaneous administration of replacement doses of L-T_3 and L-T_4 doubled the pituitary nuclear T_3 content and augmented the decrease in serum TSH beyond that of L-T_3 administration alone (4). Furthermore, the administration of iopanoic acid, a potent inhibitor of T_4 to T_3 conversion, prevented the T_4 induced decrease in TSH secretion in thyroidectomized rats (5, 6). Iopanoic acid was also found to block the effect of T_4 on TRH-induced TSH secretion in euthyroid rats (7). In intact rats, Emerson et al. reported that serum T_3 appears to more closely correlate with serum TSH levels than serum T_4 (8). It became clear, then, that both circulating T_3 and the intrapituitary conversion of T_4 to T_3 play key roles in the regulation of TSH secretion. The physiological importance of pituitary iodothyronine 5′-deiodination was underscored by the finding that 50% of pituitary T_3 is generated locally from T_4 in euthyroid rats (9).

If T_3 is the major signal inhibiting TSH secretion, then under conditions in which serum T_4 is increased and serum T_3 remains unchanged, any decrease in TSH levels should be dependent upon local pituitary T_3 production via type II 5′ deiodinase (5′D-II), the pituitary enzyme catalyzing T_4 to T_3 conversion (10). Interestingly, however, numerous studies have provided in vivo and in vitro evidence that an increase in the T_4 concentration rapidly decreases 5′D-II activity (11–16). This would appear paradoxical in a system thought to respond in a negative-feedback fashion, since the intracellular concentration of T_3, the bioactive signal, would be expected to remain constant over a wide range of T_4 concentrations.

To examine this paradox, we pretreated rats with [^{125}I]-T_4 and [^{131}I]-T_3 and studied the intrapituitary thyroid hormone content and 5′D-II activity after administration of EMD 21388 (3-methyl-4′,6-dihydroxy-3′,5′-dibromo-flavone). This synthetic flavonoid blocks the binding of T_4 to transthyretin (TTR), the major thyroid hormone binding protein in the rat. Intraperitoneal administration of EMD 21388 increases the serum free T_4 concentration and decreases the serum total T_4 within 3 min, without affecting serum free T_3 levels (17). Basal serum TSH levels decrease within 1–2 h after EMD 21388 administration, and remain depressed for at least 4 h (17, 18).

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1. *Abbreviations used in this paper:* 5′D-II, type II 5′ deiodinase; T_3, triiodothyronine; T_4, thyroxine; TRH, thyrotropin-releasing hormone; TSH, thyrotropin; TTR, transthyretin.

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This model allowed us to examine the negative-feedback control of basal TSH secretion under steady-state conditions, without requiring the administration of pharmacologic amounts of thyroid hormones.

We observed a > 150% increase in the pituitary T_4 content in the EMD 21388-treated animals, without an increase in pituitary T_3 content derived from either local deiodination or from the circulation. Pituitary 5'D-II activity was decreased. These findings were associated with a decrease in serum TSH concentration. Thus it appears likely that T_4 can act independently to suppress basal TSH secretion in euthyroid rats, and may, therefore, be an additional bioactive signal to the thyrotroph.

Methods

Animals and reagents. Male Sprague-Dawley rats, 200–250 g, were obtained from Charles River Laboratories (Kingston, RI). They were maintained under standard conditions with a 12-h light cycle, and were allowed *ad libitum* access to Purina Standard Chow (Ralston-Purina, St. Louis, MO) and water before all studies.

EMD 21388 was kindly supplied by E. Merck (Darmstat, FRG) and was dissolved in 40 mM NaOH and given intraperitoneally. Sodium salicylate was dissolved in H_2O and given by gavage. [^{125}I]-T_4 and [^{131}I]-T_3 were freshly prepared from T_3 and T_2 (Henning, Berlin, FRG) by methods previously described (19). ^{125}I was obtained from Amersham (Arlington Heights, IL), and ^{131}I from New England Nuclear (Boston, MA). The purity of each radiolabeled iodothyronine was > 95%, as determined by HPLC analysis. Both tracers were mixed in normal rat serum and administered intravenously, or used in the determination of 5'D-II activity.

Experimental procedures. Tissue iodothyronine levels were examined in rats given 250 μCi of [^{125}I]-T_4 (2.2 Ci/μmol) 16 h, and 25 μCi [^{131}I]-T_3 (~ 2.5 Ci/μmol) 1 h before the study, using a modification of the method of Silva et al. (9). At time 0, group I rats were given i.p. ketamine anaesthesia and sacrificed by aortic exsanguination. Group II rats were given 40 mM NaOH vehicle, and group III rats were given EMD 21388 (2 μmol/100 g i.p.). These two groups were sacrificed 1 h later. Rats were perfused with 60–90 ml of saline. Pituitaries and 0.5 g liver were harvested and homogenized in buffer containing 10 mM Hepes and 10 mM iopanoic acid. Radioactivities in aliquots of serum, liver, and pituitary homogenates were measured in a gamma well counter (Autogamma 5000; Packard Instrument Co., Inc., Arlington Heights, IL). Corrections for decay, energy cross-over, and background were made for both isotopes. Tissue T_4 and T_3 content were analyzed using descending paper chromatography in a solvent system containing 1:5:6 hexane, *t*-amyl alcohol, and 2N NH_4OH (20). Bands of T_4 and T_3 were identified by staining with deazotized sulfanilic acid (Pauly's reagent) and iodine was identified with palladium chloride stain. Each T_3 and T_4 band was cut, counted, and the percentage of total counts was determined by dividing the counts for each isotope in both the T_3 and T_4 bands by the total counts present on each paper strip. The percentage of total counts was then multiplied by the counts present in a milligram of tissue, and expressed as a percent of the total counts of each isotope administered.

In the second set of experiments, group I rats were treated with 40 mM NaOH vehicle i.p. Group II rats received EMD 21388 (2 μmol/100 g i.p.), and group III animals received sodium salicylate (30 mg/100 g p.o.). All rats were sacrificed 1 h later by decapitation and trunk blood and pituitaries were collected. Serum was assayed for TSH, total T_4, total T_3, and free T_4. Pituitaries were immediately placed in liquid nitrogen, and later homogenized in 800 μl of a buffer containing 250 mM sucrose, 20 mM Hepes, 1 mM EDTA, and 1 mM DTT, pH 7.0, just before measuring 5'D-II activity.

In the third experiment, rats were treated with either 40 mM NaOH vehicle or EMD 21388 (2 μmol/100 g i.p.) and sacrificed 1 or 2 h later. Trunk blood was collected, and serum was assayed for TSH.

Assays. Serum T_4 and T_3 were measured by RIA as previously described (21). Serum TSH concentrations were measured by RIA using materials kindly supplied by the National Institutes of Diabetes, Digestive and Kidney Diseases National Hormone and Pituitary Program, National Institutes of Health, Bethesda, MD. All three hormones were measured in duplicate, in random order, and in the same assay for each experiment. The intraassay coefficients of variation were: T4, < 1%; T3, < 5%; and TSH, < 1%.

Salicylate levels were determined by a modification of Trinder's method (22) in the serum of salicylate-treated rats, and in equilibrium dialysis buffer after an 18-h incubation with serum containing 2.1 mM sodium salicylate. Percent free T_4 was measured in duplicate by equilibrium dialysis of undiluted serum at 37°C for 18 h as described previously (21), and the free T_4 concentration was calculated as the product of total T_4 and percent free T_4.

To assess whether a significant concentration of "free" EMD 21388 could be present in vivo and available to cells, we used the ability of EMD 21388 to displace T_4 from serum as an index of "free" flavonoid. In the first experiment, increasing quantities of EMD 21388 were added to serum aliquots, creating concentrations of 0–5 mM. In the second experiment, rats were treated with either 2 μmol/100 g or 4 μmol/100 g EMD 21388 intravenously, sacrificed in 3 min, and trunk blood was obtained. Each serum sample was dialyzed against an equal volume of phosphate buffer for 18 h at 37°. After dialysis, the dialysate was added to an equal volume of fresh normal rat serum, and this mixture was incubated with tracer [^{125}I]-T_4 for 30 min. 50 μl aliquots of each incubation mixture were loaded onto nondenaturing PAGE gels and run for 5 h at 140 V in recirculating 0.02 M phosphate buffer (23). PAGE-gels were dried and exposed for radioautography for 24 h, and the quantity of radiolabeled T4 bound to TTR was determined by counting the labeled TTR spot and expressing it as a percentage of the total counts in that aliquot.

5'D-II activity was measured by the release of ^{125}I from [^{125}I]-T_4 in the presence of 1 mM propylthiouracil, as previously described (12), using 20-μl aliquots of pituitary homogenate and 100 or 200 pM [^{125}I]-T_4 as substrate. Protein determinations were performed using the Bio-Rad Protein Assay (Bio-Rad Laboratories, Inc., Richmond, CA).

Statistical analysis. All data are presented as the mean±SE. Data were analyzed using one way analysis of variance (ANOVA) and Student-Neumann-Keuls multiple comparisons test (SNK), or analysis of covariance, where appropriate.

Results

Effect of EMD 21388 on the kinetics of radioiodine-labeled T_3 and T_4. The ratio of pituitary to serum ^{125}I counts in the control group at time zero (0.23±0.02) was not different from that in the NaOH-treated group at 60 min (0.20±0.02), indicating that the rats were in equilibrium at the time of the experiment. The ^{125}I pituitary to serum ratio in the EMD 21388–treated group, however, was approximately double that of either control group (0.47±0.06, $P < 0.05$). Table I illustrates the pituitary content of [^{125}I]-T_4, [^{125}I]-T_3, and [^{131}I]-T_3. These results indicate that the differences in pituitary ^{125}I counts can be directly attributed to an increase in the pituitary T_4 content alone, and that this increase is not accompanied by a corresponding change in [^{125}I]-T_3 levels. There was also no increase in the systemic contribution to pituitary T_3 content, as demonstrated by the pituitary [^{131}I]-T_3 levels (Table I).

The content of hepatic radioiodine-labeled iodothyronines displayed a similar pattern (Table I). Again, the liver to serum ratio of ^{125}I was markedly higher in the EMD 21388 treatment group than in either of the control groups. (1.59 + 0.11 in the EMD 21388–treated rats, vs. 0.50 + 0.04 in the group I control rats, and 0.58 + 0.03 in the group II control rats; $P < 0.05$ by ANOVA). The liver content of [^{125}I]-T_4 was elevated in the

Table I. Pituitary and Liver Fractionated Radioiodothyronine Content in EMD 21388-treated Rats and Controls

	Pituitary			Liver		
Group	I Control (0 h)	II Control (1 h)	III EMD	I Control (0 h)	II Control (1 h)	III EMD
% dose/mg tissue						
[^{125}I]-$T_4 \times 10^{-7}$	3.27±0.46	3.64±0.49	5.70±0.52*	10.79±0.98	9.11±1.20	14.36±1.33*
[^{125}I]-$T_3 \times 10^{-7}$	2.25±0.33	2.51±0.16	2.97±0.49	1.65±0.21	1.98±0.10	1.78±0.20
[^{131}I]-$T_3 \times 10^{-5}$	1.80±0.24	1.74±0.13	1.63±0.14	0.76±0.05	0.95±0.12	0.60±0.04

Rats were treated with 250 μCi of [^{125}I]-T_4 16 h before the study, and 25 μCi [^{131}I]-T_3 1 h before the study. One control group was sacrificed at time 0, and one control group was given 40 mM NaOH vehicle and sacrificed at 1 h. EMD 21388-treated rats received 2 μmol/100 g and were sacrificed at 1 h. The data include the results of two separate experiments, each with three to five rats per group, and are expressed as the mean±SE. * $P < 0.05$ by ANOVA.

flavonoid-treated rats compared to rats in groups I and II, whereas the level of [^{125}I]-T_3 was not different. The liver content of [^{131}I]-T_3 (circulating T_3) was slightly lower in the EMD 21388–treated rats.

Effect of EMD 21388 and salicylate administration on pituitary 5'D-II activity. To determine why an increase in the pituitary content of T_4 was not associated with a corresponding increase in pituitary T_3 content, we studied the 5'D-II activity in pituitary homogenates of animals treated with either vehicle, EMD 21388, or sodium salicylate, another compound previously shown to partially inhibit T_4 binding to TTR in the rat (24).

To ensure an optimal estimation of pituitary 5'D-II activity, we measured the rate of 5'D-II activity under reaction conditions that showed linear production of T_3 at T_4 concentrations approximately two- and fourfold greater than those estimated to be present in the pituitary. T_3 production was linear for up to 40 min (data not shown) and the 5'D-II activity carried out at 100 and 200 pM [^{125}I]-T_4 is shown in Fig. 1. EMD 21388 and salicylate administration significantly decreased pituitary 5'D-II activity at both substrate concentrations, and greater enzyme activity was present in all groups using 200 pM T_4. This suggests that the similar levels of pituitary T_3 content seen in all groups in the first set of experiments were due to decreased enzyme activity, and not to a saturation of enzyme capacity.

Determination of free EMD 21388 activity in serum. When EMD 21388 was added to serum in increasing concentrations and then subject to equilibrium dialysis in duplicate, the percentage of [^{125}I]-T_4 bound to TTR in normal serum mixed with dialysate is shown in Fig. 2. In rats given 2 or 4 μmol EMD 21388/100 g body wt intravenously, sacrificed 3 min later, there was no evidence of "free" EMD 21388 activity. (57.5±1.6% in rats treated with 4 μmol EMD 21388/100 g body wt; 58.0±1.1% at 2 μmol/100 g, vs. 55.7±1.5% in controls; 4 rats per group, P = NS).

Effect of salicylate added in vitro on pituitary 5'D-II. To determine whether sodium salicylate was a direct inhibitor of 5'D-II, we assayed aliquots of normal pituitary homogenate for 5'D-II activity in the presence of 0, 2, and 10 mM sodium salicylate. The activities in triplicate samples were 47.0, 65.6, and 57.1 fmol ^{125}I released · mg protein^{-1} · h^{-1}, respectively (NS). Because the serum salicylate concentration in animals treated with 30 mg salicylate/100 g body wt was 34.2 mg/dl±1.0 (2.1 mM), it is clear that this administered dose of salicylate does not directly inhibit enzyme activity.

Effects of EMD 21388 and salicylate on serum TSH, total T_4, free T_4, and total T_3 concentrations. Table II illustrates the serum TSH and thyroid hormone levels in the three groups. The decrease in TSH at 1 h in the EMD 21388–treated group, although not statistically significant, was similar to that previously reported (18). The serum TSH concentration was also slightly lower in the salicylate-treated group. In both treatment groups there was a significant decrease in the serum total T_4 concentration compared to the control group. There was a significant increase in the percent free T_4 in the EMD 21388-

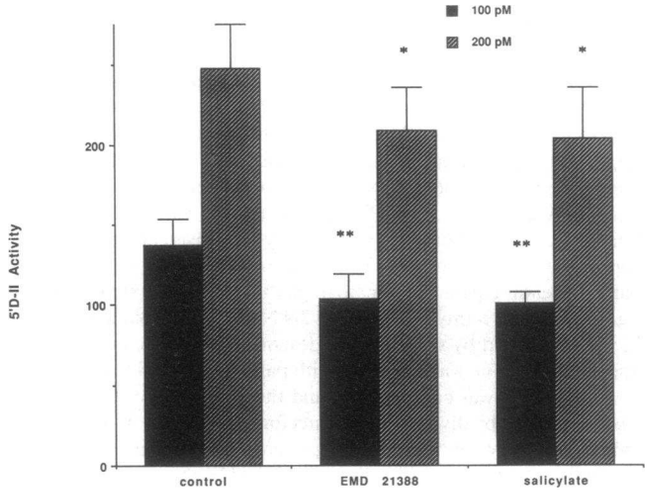

Figure 1. The effects of EMD 21388 and sodium salicylate administration on pituitary 5'D-II activity using 100 pM and 200 pM [^{125}I]-T_4 as substrates. Rats were treated with either 40 mM NaOH vehicle, 2 μmol EMD 21388/100 g body wt, or 30 mg sodium salicylate/100 g body wt and sacrificed 1 h later. Pituitaries were homogenized, and aliquots used to determine the fmol ^{125}I released · mg protein^{-1} · h^{-1} at each substrate concentration, after 10, 20, and 40 min of incubation. Activities were calculated by least squares regression analysis for each animal, and the means in each treatment group are shown above. Data are reported as the mean±SE of three separate experiments, with four to eight rats per group per experiment. Error bars indicate standard error. *$P < 0.05$ vs. control (analysis of covariance). **$P < 0.05$ vs. control (analysis of covariance).

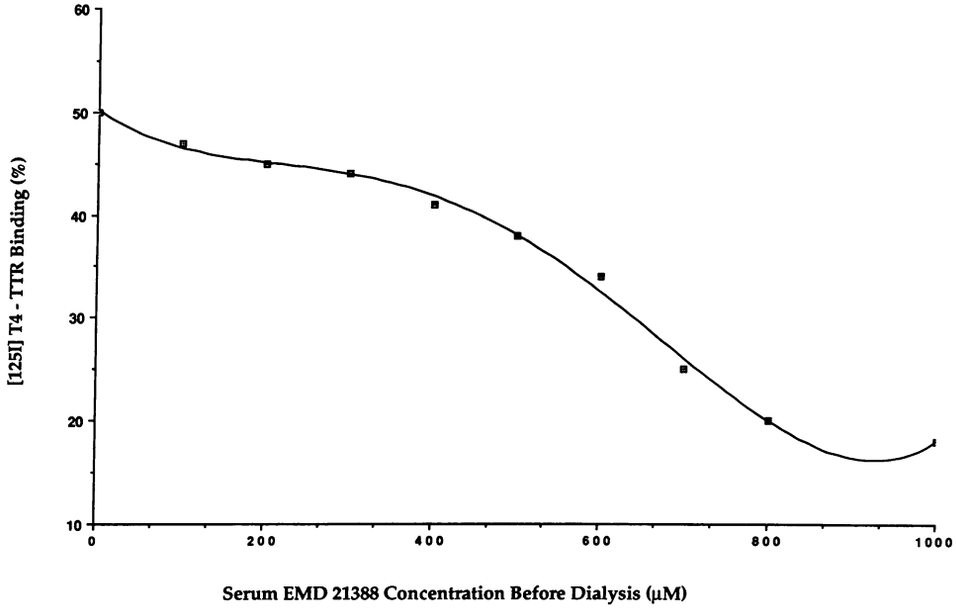

Figure 2. Estimation of serum free EMD 21388 activity. 0–1,000 μM EMD 21388 was added to normal rat serum in duplicate, and 1 ml of each was subject to equilibrium dialysis overnight. Each dialysate was added to normal rat serum containing [125I]-T_4, and the mixture was subject to SDS-PAGE. The radiolabeled band corresponding to TTR was counted and expressed as a percentage of total radioactivity for that lane.

treated group due to the inhibition of T_4 binding by TTR, and thus, a less pronounced but still highly significant increase in the serum free T_4 concentration. Salicylate administration has been reported to increase the percent free T_4 measured by equilibrium dialysis (24), and the percent free T_4 was significantly higher using this technique (salicylate-treated, 0.051±0.003%, vs. control, 0.032±0.002%, $P < 0.05$). However, we established in a separate experiment, that ~ 70% of the serum salicylate is present in the dialysate at equilibrium, and, therefore, equilibrium dialysis cannot accurately reflect the percent free T_4 present in vivo. Thus, the free T_4 concentration cannot be calculated. It is likely, however, that the serum free T_4 concentration in these rats is increased because Larsen has reported that salicylate, when added to human serum at a concentration of 30 mg/dl, doubles the free T_4 concentration as measured by an ultrafiltration technique (25).

Serum TSH concentrations 1 and 2 h after administration of EMD 21388. To demonstrate that the decrease in serum TSH becomes more pronounced over time after EMD 21388 administration, rats were treated with EMD 21388 and serum TSH concentrations measured 1 and 2 h later. The results are shown in Fig. 3 and demonstrate a significantly lower serum TSH concentration in the rats treated with EMD 21388 compared with control values at 2 h. These results are similar to those previously reported (18).

Table II. Effects of EMD 21388 and Sodium Salicylate on Serum TSH, T_4, Percent Free T_4, and T_3 1 h after Administration

	Control	EMD 21388	Salicylate
TSH (*μU/ml*)	63.7±6.8	55.8±4.6	49.4±3.3
T_4 (*μg/dl*)	5.68±0.02	3.00±0.16*	2.93±0.22*
% Free T_4	0.028±0.001	0.115±0.005*	—
Free T_4 (*ng/dl*)	1.57±0.09	3.42±0.23*	—
T_3 (*ng/dl*)	81.37±4.31	65.75±4.18*	55.38±3.67*

Serum values were measured from trunk blood obtained 1 h after treatment with either 40 mM NaOH vehicle, 2 μmol EMD 21388/100 g body wt, or 30 mg sodium salicylate/100 g body wt. Data are reported as the mean±SE of three separate experiments, with four to eight rats per group per experiment. * $P < 0.05$ vs. controls by ANOVA and SNK.

Discussion

These data demonstrate that an acute elevation in serum free T_4, due to inhibition of T_4 binding to TTR by EMD 21388, rapidly increases the pituitary and liver content of T_4. In the pituitary, this increased amount of T_4 did not result in a greater T_3 content, because the enzyme catalyzing the conversion of T_4 to T_3, 5'D-II, was decreased. The data further indicate that this decrease in enzyme activity is directly related to the increased concentration of substrate T_4, because salicylate administra-

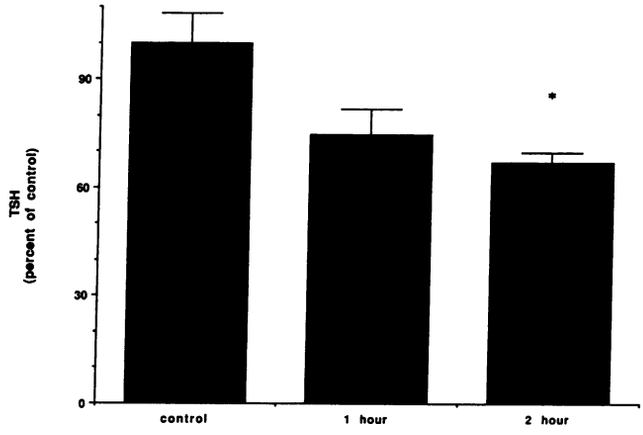

Figure 3. Serum TSH concentrations 1 and 2 h after EMD 21388 administration. Rats were treated with either 40 mM NaOH vehicle, or 2 μmol EMD 21388/100 g body wt. There were five rats per group. Error bars indicate standard error. *$P < 0.05$ vs. controls by ANOVA and SNK.

tion produced a similar decrease in 5'D-II activity in vivo, but had no effect on 5'D-II activity when added in vitro. Although EMD 21388 added in vitro has been reported to competitively inhibit 5'D-II, this effect occurs only in concentrations > 1 μM (26). It is highly unlikely that an administered intraperitoneal dose of 2 μmol/100 g would provide an intrapituitary concentration of 1 μM. Assuming that the entire injected dose is absorbed and retained in the circulation, in a 250-g rat the maximum EMD 21388 serum concentration would be 500 μM (5 μmol/10 ml serum). We have observed that the apparent "free" EMD 21388 at this serum concentration causes ~ 24% displacement of [125I]-T_4 from TTR. Assuming that TTR is the only serum protein that binds EMD 21388, and assuming a 1:1 stoichiometry, the concentration of free EMD 21388 would be 0.24 [TTR], or 0.24 [5.5 μM] (27), or 1.3 μM. The total amount of free EMD 21388 in the 10 ml of rat serum would therefore be 13 nmol. If that free EMD 21388 is equally distributed throughout the body, the pituitary concentration would be 13 nmol/250 ml, or 52 nM. The effect of EMD 21388 on the activity of 5'D-II in this system is, therefore, likely to be indirect, and caused by its ability to increase the pituitary content of T_4. While in earlier work (18) this effect on pituitary 5'D-II activity was not observed, subsequent in vivo experiments have demonstrated that the potency of EMD 21388 to inhibit T_4 binding to TTR is increased (17) perhaps due to the use of a more alkaline vehicle in the more recent experiments (17). This might result in a greater amount of free T_4 available to the pituitary to decrease 5'D-II activity.

The ability of T_4 to regulate intrapituitary T_3 production has been verified by numerous investigators. Incubation of pituitary fragments (11) or GH_4C_1 cells (12) with increasing concentrations of T_4 resulted in a decrease in deiodination of T_4 and T_3 production. Others have shown that rats treated with T_4 exhibit decreased pituitary deiodinase activity (13–15). Leonard et al. have reported that this effect is not inhibited by cycloheximide and is, therefore, not dependent on protein synthesis (16). Finally, Lum and co-workers have suggested that 5'-deiodinase activity is autoregulated in man, being reduced when serum T_4 concentration is elevated, and increased in the presence of a lower T_4 value (28). The present study demonstrates that in euthyroid rats, the basal activity of 5'D-II can be decreased by ~ 25% in 1 h by an increase in pituitary T_4 content without the administration of pharmacologic doses of L-T_4.

We have also demonstrated that, in the absence of an increase in intrapituitary T_3 levels, TSH begins to decrease at 1 h and decreases even further 2 h after EMD 21388 administration. Because previous data indicate that EMD 21388 has a low affinity for the T_3 nuclear receptor (29), and the intracellular T_3 that we measured is in dynamic equilibrium with nuclear T_3, there is no reason to expect a difference in T_3 nuclear receptor occupancy between EMD 21388-treated and control rats. Therefore, basal TSH secretion may decrease independently of the T_3 nuclear content.

The importance of T_3 in inhibiting TSH release is clear and has been reported to be more important than T_4. Hypothyroid rats treated with iopanoic acid (which blocks both intrapituitary and systemic T_4 to T_3 conversion) have a lower pituitary nuclear T_3 content than control rats, and TSH secretion is not decreased in response to an intravenous bolus of T_4 (5). Additionally, T_4 does not inhibit the TRH-induced increase in TSH secretion after pretreatment with iopanoic acid (7). Furthermore, Emerson et al. have recently reported that serum T_3 concentrations are more positively correlated with TSH secretion than serum T_4 concentrations under physiological conditions (8). Therefore, it is possible that a critical percentage of nuclear T_3 receptors must be occupied to suppress TSH secretion and maintain a basal secretory rate in the euthyroid rat. However, we have now demonstrated that elevations in pituitary T_4 content may decrease basal TSH secretion without an associated increase in pituitary T3 content.

It is important to note, however, that thyrotrophs constitute only ~ 10% of the euthyroid pituitary (30), and we may not be observing an accurate reflection of 5'D-II activity and iodothyronine content in these cells. Studies of enriched pituitary cell pools have shown that, while under hypothyroid conditions, thyrotrophs have less 5'-D activity than other anterior pituitary cell types, T_3 replacement induces a similar, lower level of activity in all cell pools (31). This may indicate that the 5'D-II may be less substrate-regulated in the thyrotroph than in other cell types, and it is possible that proportionately more thyrotroph-derived T_3 is available to its nucleus when pituitary T_4 content is elevated. Alternatively, the thyrotroph may be dependent, in a paracrine fashion, on T_3 generated by other pituitary cell types. When T_3 availability decreases within the pituitary, TSH secretion is stimulated. However, when T_4 content increases, T_3 production is blunted in other cell types such as lactotrophs and somatotrophs, and these cells are protected from increased amounts of T_3 generated by unchecked local conversion. Under these conditions the total pool of T_3 available to the thyrotroph is not different from the euthyroid state, but an additional signal for suppressing TSH secretion, T_4, remains intact.

In summary, we have demonstrated that an acute elevation in serum free T_4 concentration can increase pituitary (and liver) T_4 content, and can rapidly decrease the activity of pituitary 5'D-II. Pituitary T_3 content does not change and, despite this, TSH secretion decreases. These results suggest that T_4 may have an effect on TSH secretion independent of its conversion to T_3, and may explain how TSH secretion can decrease in the presence of a decrease in 5'D-II activity.

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