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Evidence That Thyroxine Inhibits either Basal or TRH-Induced TSH Secretion Only after Conversion to Triiodothyronine[1] (42679)

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Abstract. When TRH was administered every 15 min for 2 hr in euthyroid rats, equivalent modestly supraphysiologic doses of either T_4 or T_3 suppressed TRH-induced TSH secretion after 45 min. Pretreatment with iopanoic acid blocked the ability of T_4 but not of T_3 to suppress TRH-induced TSH secretion 2 hr after administration of the respective thyroid hormone. Pretreatment with iopanoic acid also blocked the ability of T_4, but not of T_3, to depress the elevated basal plasma TSH concentration of hypothyroid rats within 2 hr. Propylthiouracil did not significantly inhibit the ability of T_4 to depress TRH-induced TSH secretion and only slightly depressed the ability of T_4 to reduce the elevated plasma TSH of hypothyroid rats. Our data support the concept that although equivalent physiologic doses of T_4 or T_3 inhibit basal or TRH-induced TSH secretion equally rapidly, TSH inhibition produced by T_4 is probably dependent on its rapid conversion to T_3, either within the pituitary or peripherally. T_3 thus seems to be exerting almost all the negative feedback effects on TSH secretion under the conditions of our experiments.

Whether both T_4 and T_3 possess an innate ability to inhibit TSH secretion and exert hormonal action (2, 3) or whether T_4 must first be converted to T_3 to exert these effects has not been unequivocally settled (4–11). Although the nuclear binding affinity for T_3 is much greater than that for T_4 (12), it is not certain that all thyroid hormone action depends on nuclear binding (13–16). Both T_4 and T_3 depress basal TSH secretion and inhibit the ability of TRH to stimulate TSH secretion within 1 hr. The rapid effect of T_4 to inhibit TSH secretion has been postulated by Larsen and Silva to be due to intrapituitary conversion of T_4 to T_3 (6, 14). Iopanoic acid inhibits conversion of T_4 to T_3 (17–20) and significantly depresses the ability of T_4, but not of T_3, to inhibit basal TSH secretion several hours after administration of thyroid hormone. However, previous studies by Boado *et al.* (3) made less than 30 min after T_4 or T_3 administration have indicated that iopanoic acid does not significantly inhibit the ability of T_4 to depress TRH-induced TSH secretion. This suggests that T_4 may have a direct inhibitory effect on this phenomenon. We therefore designed the following experiments to explore this problem further.

Materials and Methods. Adult 350- to 400-g male Simonsen rats were used in all experiments. All drugs were given in a dose adjusted per 100 g body weight. TRH was given as a 1-μg iv bolus via atrial catheter every 15 min for 2 hr or under light ether anesthesia via jugular puncture. T_4 was given as 1 or 2.5 μg iv; T_3 was given as 0.25 or 0.625 μg iv. Some rats were made hypothyroid with 0.03% MMI in the drinking water for 2 weeks. Some rats were given 5 mg iopanoic acid or 2 mg PTU ip 24, 16, and 1.5 hr before beginning TRH injections. All blood samples were taken via an atrial catheter or by jugular puncture.

All experiments were performed between 0830 and 1600 hr. An indwelling intraatrial catheter was implanted through the jugular vein and fed out subcutaneously through the top of the head 18 hr before the experiment. Following implantation of the cannula, the rats were maintained in an isolated room in individual metal wastebaskets with sawdust in the bottom and with free access to food and water. The extracorporeal portion of the cannula was suspended above the rat's head

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to allow free mobility. Heparin in 0.9% saline (1 USP U/ml) was maintained in the cannula between sampling and injection, which were performed through the same cannula.

TRH administration and blood sampling. Bolus injections of TRH (Sigma) were given through the catheter every 15 min for 2 hr, using a 1-ml syringe. The TRH was diluted to give a concentration of 1 μg in 50 μl of saline. Blood samples of 0.25 ml were taken at each indicated time and replaced with an equal volume of 0.9% saline to minimize blood volume depletion. Samples were centrifuged at 4°C. Plasma was separated and frozen at −20°C until analyzed.

TSH assay and statistics. All hormone assays from any given experiment were performed at the same time. TSH was measured by radioimmunoassay of 0.1 ml of plasma or 1:1000 diluted pituitary homogenates, using rat TSH kits kindly supplied by the National Hormone and Pituitary Program, NIADDK. The stated potency of the reference standard supplied (NIAMDD-rTSH-RP-1, 0.22 USP U/mg) was used to calculate plasma hormone concentration. Our range of sensitivity was 15–1000 ng/tube (intraassay variation <7%, interassay variation <10%). Statistical analysis was performed by using Student's paired or unpaired *t* test and the Newman–Keuls multiple comparison test.

Results. *Dose and temporal characteristics of T_4 and T_3 inhibition of TRH-induced TSH secretion.* The first set of experiments was designed to determine for both T_4 and T_3 the dose and time parameters necessary to obtain a clear depression of TRH-induced TSH secretion. We had previously established (21, 22) that repetitive bolus doses of 1 μg TRH iv through an indwelling atrial catheter in freely moving unanesthetized euthyroid rats induced a biphasic rise in plasma TSH concentration with an initial sharp rise at 15 min, a nadir at 60 min, and a second rise with a slower rate of increase and lower but sustained amplitude which usually peaked at approximately 90 min, as shown in the top panel, Fig 1.

Intravenous injection of 1 μg T_4 or 0.25 μg T_3 at time 0 did not appreciably alter this pattern (lower panels, Fig 1.) However, increasing the dose to 2.5 μg T_4 or 0.625 μg T_3 caused a significant suppression of TRH-induced TSH secretion but only after 45 min (Fig 2).

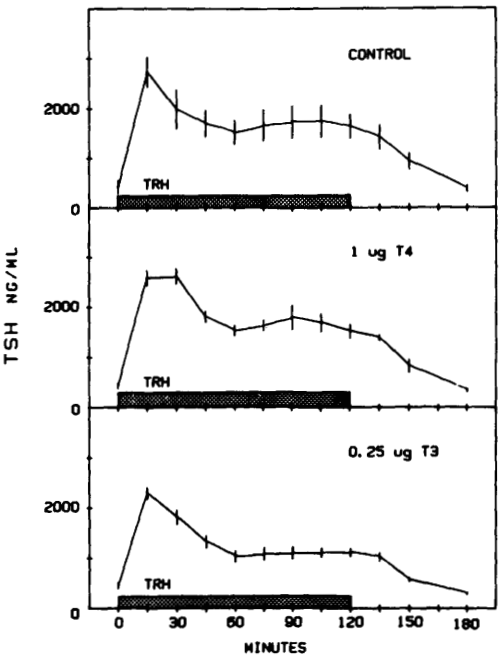

FIG. 1. Effect of 1 μg T_4 or 0.25 μg T_3 at 0 min on TRH-induced TSH secretion in euthyroid rats. There were four animals in each of the three groups identified by the legend in each panel. All animals had chronic indwelling cannulas. One microgram TRH per one hundred grams body weight was injected every 15 min for 2 hr as indicated by the horizontal stippled bar in each panel. The mean ± SE of plasma TSH concentration is indicated by each point and vertical line.

Effect of iopanoic acid on T_4- and T_3-induced inhibition of TRH-induced TSH secretion in euthyroid rats. The above experiments indicated that a reasonable time to test the early effects of blocking intrapituitary T_4 to T_3 conversion with iopanoic acid would be 2 hr after administration of T_4. Accordingly, plasma TSH was measured immediately before and 15 min after a bolus injection of 1μg TRH; 2.5 μg T_4 was given immediately after the second blood sampling. Two hours later, the TRH test was repeated. One group was pretreated with iopanoic acid, a drug which blocks conversion of T_4 to T_3 in both the pituitary and the peripheral tissues; one group was pretreated with propylthiouracil, a drug which blocks peripheral but not intrapituitary conversion of T_4 to T_3 (23–27), and

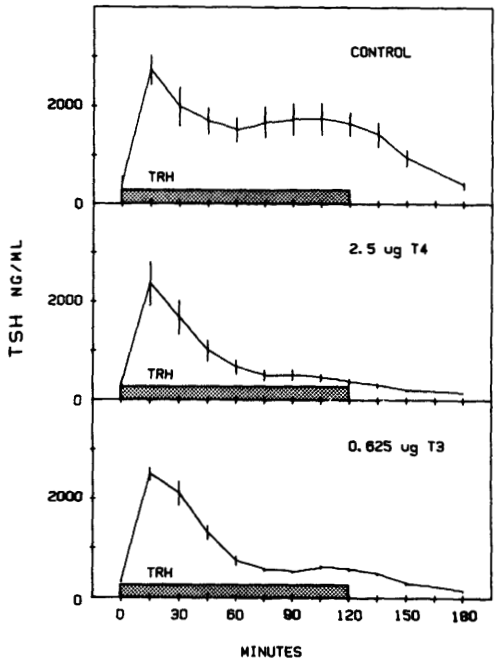

FIG. 2. Effect of 2.5 μg T_4 or 0.625 μg T_3 at 0 min on TRH-induced TSH secretion in euthyroid rats. The data are shown as in Fig. 1. There were four rats in each of the control and T_4 groups and three rats in the T_3 group.

a control group was not pretreated with drugs and was given saline instead of thyroid hormone at the end of the first TRH test.

There was no significant difference in the response to TRH between the first and second tests in the control rats injected with saline (Fig. 3). Treatment with 2.5 μg T_4 at the end of the first TRH test nearly completely blocked the rise in plasma TSH from the second TRH test 2 hr later. The blocking effect of T_4 was prevented by pretreatment with iopanoic acid but not by pretreatment with propylthiouracil.

The iopanoic acid study was repeated using 0.625 μg T_3 instead of the equivalent dose of 2.5 μg T_4 (Fig. 4). Iopanoic acid had no effect on the ability of T_3 to block TRH-induced TSH secretion.

Studies in hypothyroid rats. Similar studies were also made in hypothyroid rats. We have previously reported that the biphasic TSH response pattern seen with repetitive or continuous TRH administration in euthyroid rats is not seen in hypothyroid rats (22). Instead, there is only an initial stimulation of secretion, followed by a return of plasma TSH concentration to or slightly below the

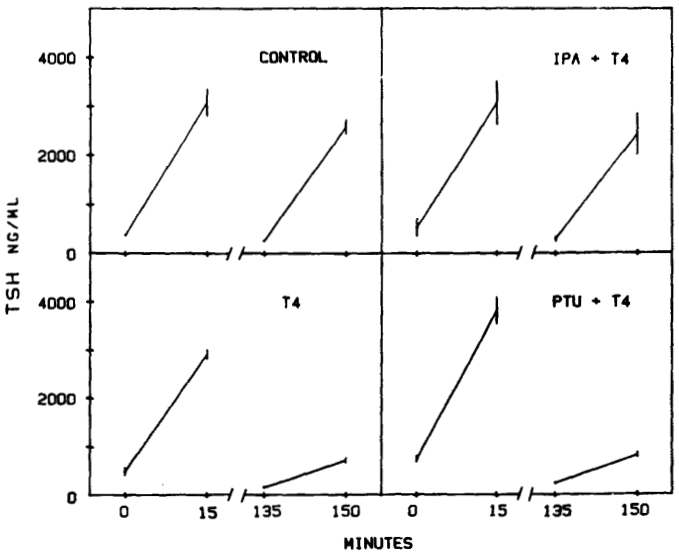

FIG. 3. Effect of iopanoic acid or propylthiouracil on 2.5 μg T_4 inhibition of TRH-induced TSH secretion. There were four of five rats in each group. Plasma TSH is shown as mean ± SE. An intrajugular bolus injection of 1 μg TRH was given at 0 and 135 min. Jugular blood was sampled just before and 15 min after TRH injections for plasma TSH concentration. T_4 (2.5 μg) was given in a bolus iv injection immediately after the 15 min blood sampling. Pretreatment with iopanoic acid (IPA) and propylthiouracil (PTU) was as indicated in the text.

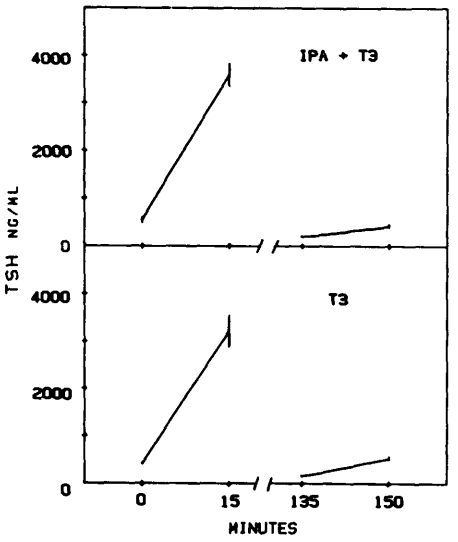

FIG. 4. Effect of iopanoic acid on 0.625 μg T_3 inhibition of TRH-induced TSH secretion. There were four animals in each group. The experiment was analogous and the data are plotted as in Fig. 3.

initial basal levels. There was no significant effect of either 2.5 μg T_4 or 0.625 μg T_3 given at 0 time on TRH-induced TSH secretion in hypothyroid rats (Fig. 5), in contrast to the clear effect of both thyroid hormones given in these concentrations in the euthyroid rats. However, plasma TSH was significantly lower in both the T_4- and T_3-treated groups than in the controls at 180 min ($P < 0.05$).

The spontaneous refractoriness of the hypothyroid rat to repetitive TRH precluded an exact repetition of the euthyroid studies in hypothyroid animals. Therefore, we examined the ability of iopanoic acid and propylthiouracil to block the respective abilities of T_4 or T_3 to depress basal plasma TSH secretion in hypothyroid rats 2 hr after their administration. Similar studies have been reported by Larsen examining the plasma TSH at the slightly later interval of 3 hr and using smaller doses (6).

Our data confirm the earlier report of Larsen (6) and are consistent with the TRH studies in the euthyroid rat presented above. Either 2.5 μg of T_4 (Fig. 6) or 0.625 μg of T_3 (Fig. 7) caused a significantly greater depression of basal plasma TSH in hypothyroid rats 2 hr later than did administration of saline. The effect of T_4, but not that of T_3, was blocked by iopanoic acid. Propylthiouracil partially blocked the effect of T_4 (Fig. 6), but to a much lesser extent than did iopanoic acid. Propylthiouracil was not tested with T_3.

Discussion. The primary object of these studies was to determine whether the early effect of T_4 on inhibiting TRH-induced TSH secretion was more likely due to a direct effect of T_4 on the thyrotroph or to the rapid conversion of T_4 to T_3 within the pituitary cell. The report of Boado *et al.* (3) indicated that there was no inhibition of the early T_4 suppression of TRH-induced TSH secretion in rats treated with iopanoic acid, a substance known to markedly depress intrapituitary conversion of T_4 to T_3, thus suggesting that T_4 itself might have some intrinsic action. Other evidence had suggested that the action of T_4 at the pituitary might be entirely dependent on its conversion to T_3 (6–9, 20, 25). Two potential problems in interpreting the data of Boado *et al.* are that quite large supraphysiologic doses of T_4 were given (10

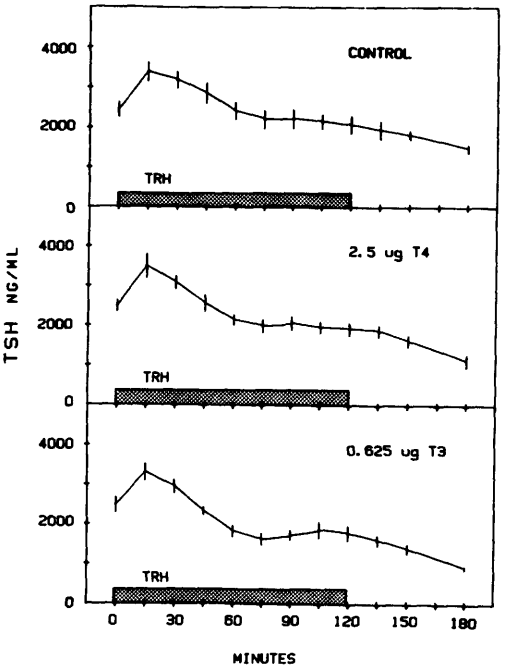

FIG. 5. Effect of 2.5 μg T_4 or 0.625 μg T_3 at 0 min on TRH-induced TSH secretion in hypothyroid rats. There were four rats in each group. The data are plotted as in Fig. 1.

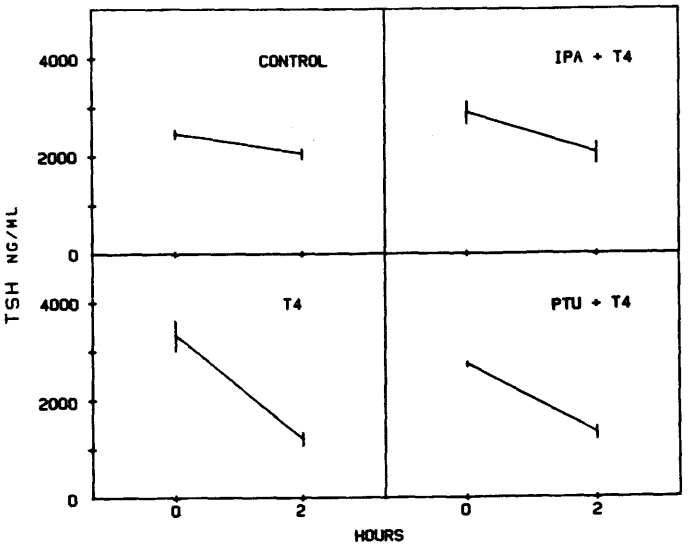

FIG. 6. Effect of iopanoic acid or PTU on 2.5 μg T_4 inhibition of basal TSH secretion in hypothyroid rats. There were four to five rats in each group. T_4 was given in a bolus intrajugular injection at 0 h. Jugular blood was sampled for TSH concentration just before and 2 hr after T_4 injection. There was a slight drop in plasma TSH in the vehicle-injected control group. However, there was a significantly greater drop in plasma TSH in the group given T_4 alone ($P < 0.01$). The group given IPA plus T_4 was not significantly different from the control ($P > 0.05$). However, that given PTU plus T_4 was statistically different from both the control ($P < 0.05$) and the T_4 only ($P < 0.05$) groups.

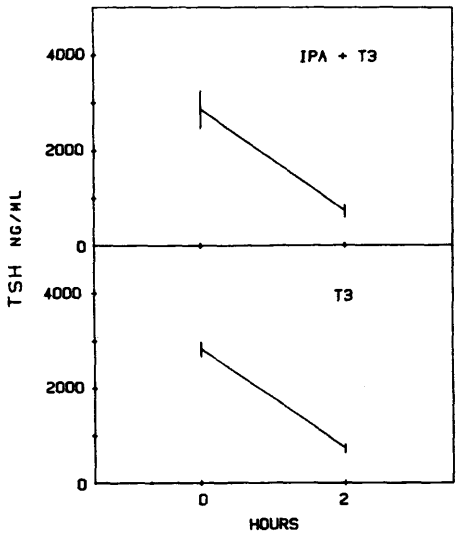

FIG. 7. Effect of iopanoic acid on 0.625 μg T_3 inhibition of basal TSH secretion in hypothyroid rats. The experiment was performed as in Fig. 6. There were five animals in the IPA plus T_3 group and four in the T_3 only group. There was no significant difference between the two groups in the depression of plasma TSH induced by T_3 ($P > 0.1$).

μg/100 g body wt) and the study was made within 30 min after administration of T_4 to minimize any possible conversion of T_4 to T_3.

Our initial experiments were designed to determine the earliest time at which a clear inhibition by either T_4 or T_3 of the TRH-induced TSH stimulation of TSH secretion could be detected and to ascertain the smallest dose of each hormone which could unequivocally produce this inhibition. We felt that such knowledge would permit us to evaluate more conclusively whether there might be a direct effect of T_4 on this phenomenon. T_3 was approximately four times more potent than T_4 in inhibiting TRH-induced TSH secretion under the conditions of our experiments, as would be expected from a vast number of earlier comparisons using a variety of parameters. Clear inhibition of TRH-induced TSH secretion could be seen only after 45 min from the time of administration of either thyroid hormone.

Our data indicate that the failure of Boado *et al.* to detect an inhibitory effect of iopan-

oic acid on the ability of T_4 to depress TRH-induced TSH secretion may be because they made their study less than 30 min after administering T_4, probably earlier than a significant effect of T_4 or T_3 could be detected. Also, Boado *et al.* used the rather high dose of 10 μg T_4/100 g body wt. Under these conditions there may have been some low-affinity binding of T_4 to nuclear or other receptors to induce an inhibition of TSH secretion which would not be seen at T_4 concentrations closer to normal.

In our experiments, pretreatment with iopanoic acid blocked the ability of T_4 but not of T_3 to suppress TRH-induced TSH secretion 2 hr after administration of the respective thyroid hormone. Pretreatment with iopanoic acid also blocked the ability of T_4, but not of T_3, to depress the elevated basal plasma TSH concentration of hypothyroid rats within 2 hr.

In conclusion, our data are consistent with the theory proposed by Silva and Larsen (6, 14) that T_4 inhibits TSH secretion only after conversion to T_3, whether this conversion is in peripheral tissues or within the pituitary itself. It thus seems likely that T_4 conversion to T_3 must occur very rapidly and the quantity of T_3 thus generated is sufficient to inhibit TSH secretion.

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