PHYSIOLOGICAL MODULATION OF THYROTROPIN SECRETION BY SOMATOSTATIN AND THYROLIBERIN

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SUMMARY: When injected into rats together with a rabbit antiserum to Somatostatin, Thyroliberin released significantly more Thyrotropin than when injected together with normal rabbit serum. This was true in normal, thyroidectomized, and even in hyperthyroxinemic rats, and in each case the maximum effect was seen soon after injection. The data are interpreted to indicate a rapidly-acting feedback control over Thyrotropin secretion involving Somatostatin as the agonist. Such a mechanism can explain a number of situations where the traditional model of the hypothalamo-pituitary-thyroid axis does not account for the experimental data.

Most endocrinologists believe that the secretion of Thyrotropin is stimulated primarily by the hypothalamic neurohormone, Thyroliberin. Inhibition of TSH secretion is supposed to be accomplished by thyroid hormone acting on the pituitary to induce an inhibitory protein (1, 2, 3) which prevents the pituitary response to TRF. The evidence for this inhibitory factor has been questioned (4) because supraphysiological levels of thyroid hormone are needed to induce its formation *in vitro*. Thyroid hormone appears to stimulate hypothalamic TRF synthesis, at least *in vitro* (5). This generally accepted control system appears inefficient, since it calls for the enhanced production of a stimulator while blocking its physiological action, and it does not explain satisfactorily how the lowered TRF production found in hypothyroidism could result in greatly enhanced TSH production and release.

For several years evidence has pointed to a dual hypothalamic control over TSH secretion. Peter (6) has shown that separation of the pituitary from hypothalamic influences in fish resulted in thyroidal hyperfunction.

*Abbreviations:
Thyroliberin or TRF; Thyrotropin Releasing Factor
Somatostatin or SRIF; Growth Hormone Release Inhibitory Factor
NRS; Normal Rabbit Serum
TSH; Thyrotropin

Joseph and Knigge (7) demonstrated a similar effect in kittens after surgical isolation of the medial basal hypothalamus. This evidence pointed toward the possibility of hypothalamic inhibition of pituitary TSH secretion which could become dominant under special circumstances. The discovery of Somatostatin by Brazeau et al. (8) and the demonstration that this neurohormone inhibited TRF-stimulated TSH secretion without affecting basal TSH levels, has pointed to a likely agonist for the inhibitory arc in the hypothalamo-pituitary-thyroid system (9). The experiments here described show that an antiserum to Somatostatin, when administered together with TRF, enhances the pituitary response to TRF stimulation. This work confirms and extends the recent report of Ferland et al. (10) that anti-SRIF serum elevated circulating TSH levels in rats without altering the diurnal TSH cycle.

Materials and Methods

Synthetic somatostatin was obtained through the kindness of Dr. R. Deghenghi of Ayerst Laboratories, Montreal and of Dr. S. Makineni of Bachem Fine Chemicals Co., (Marina del Rey, Calif.) who also donated the Tyr^1-SRIF used in evaluating SRIF binding by antisera. Somatostatin was coupled to Bovine Serum Albumin (Pentex, crystallized) with glutaraldehyde, using the procedure of Arimura et al. (11). Five rabbits were immunized at monthly intervals with 300 µg of the conjugate in complete Freund's adjuvant (Difco) at multiple subcutaneous sites. After 10 inoculations, 3 of the animals' sera, when diluted 1:3000, bound 24 - 30% of ^{125}I-Tyr^1-SRIF when 1 ng of the tracer was present. The present experiments were carried out with a pool of these 3 sera.

Adult female rats (Hilltop Farms, Philadelphia, PA) weighing 300 - 375 g were anesthetized with 20 ml/kg of 20% ethanol in saline in experiments continuing for more than 30 minutes, or with 40 mg/kg pentobarbital (Nembutal Sodium, Abbott) for shorter protocols. All animals received, by rapid intrafemoral injection 1.0 ml of saline solution of TRF (Bachem) together with 1.0 ml of either anti-SRIF serum or normal rabbit serum. Because of the reported rapid degradation of TRF by blood, the serum and TRF were drawn into the syringe immediately prior to injection.

Blood was obtained by cardiac puncture just prior to the administration of TRF plus serum and at 3 to 5 time periods after injection. Thyrotropin levels in the sera were determined using the radioimmunoassay reagents supplied by the N.I.A.M.D.D.'s Hormone Distribution Program.

Rats were made hypothyroid by surgical thyroidectomy 4 weeks prior to TRF administration. Hyperthyroxinemic rats were similarly thyroidectomized, but received daily subcutaneous injections of 50 µg L-Thyroxine for the last 21 days of the 4 week period prior to use.

Thyroliberin was dissolved in saline prior to injection in concentra-

tions ranging from 75 ng/ml to 2.5 µg/ml. No attempt was made to preclude adsorption to glassware by means of protein.

Results

When 6 rats were injected with 1.0 ml of our anti-SRIF serum alone, their serum TSH level slowly declined from a pre-injection value of 119 $\pm$ 27 ng/ml of NIAMDD rat RP-1 standard to 93 $\pm$ 12 ng/ml 10 minutes after injection and 74 $\pm$ 7 ng/ml after 20 minutes.

The maximal TSH response was found early after TRF injection. The elevation of TSH was significantly greater 6 minutes after TRF injection than after 3 minutes, but there was no further increase by 10 minutes and a marked fall by 20 minutes. Similar findings were obtained when injecting TRF together with anti-SRIF serum.

In the experiment described in Figure 1, the rats did not respond to the injection of TRF alone. These animals were older (11 months, compared

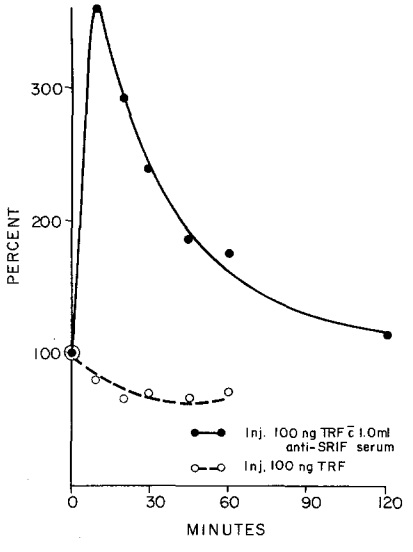

Figure 1:

Changes in serum thyrotropin levels in mature female rats after the intravenous injection of 100 ng of thyroliberin alone or of the same dose of thyroliberin together with 1.0 ml of anti-SRIF serum. Changes are presented as percent of pre-injection TSH levels. Rats were anesthetized with i.p. ethanol.

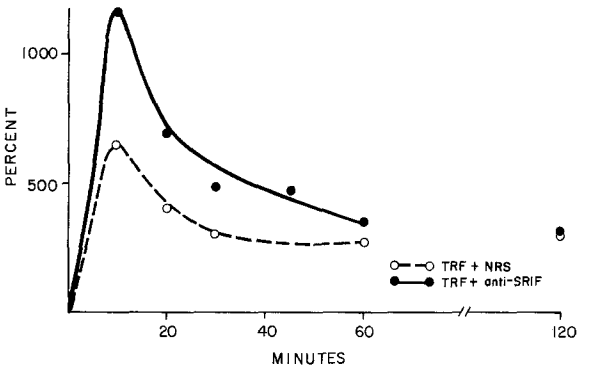

Figure 2:

Course of serum thyrotropin levels in young adult female rats after the injection of 100 ng thyroliberin either together with normal rabbit serum or anti-SRIF rabbit serum. Ethanol anesthesia was used.

to 5 months for the animals used in the remaining experiments). The controls in this experiment received half the volume of injectate given to the rats receiving both TRF and anti-SRIF. Initial TSH levels in the TRF controls also were significantly higher (512 $\pm$ 50 ng/ml as against 250 $\pm$ 10 ng/ml for the TRF plus anti-SRIF group). However, the two curves were so very different that we repeated the experiment in younger rats (Figure 2). This time the animals responded normally to 100 ng TRF, and, again, the response was significantly enhanced ($p < 0.02$) by simultaneous administration of anti-SRIF rabbit serum rather than NRS. In another experiment, the TSH level rose to 647 $\pm$ 66% of pre-injection levels 10 mins. after 100 ng of TRF given with NRS and to 1040 $\pm$ 55% when the same dose of TRF was given with 1.0 ml of anti-SRIF serum ($p < .001$).

Thyroidectomized rats showed a marked response to 75 ng of TRF when administered together with anti-SRIF, while the response to the same dose of TRF with normal rabbit serum was not significant (Figure 3). The difference between the two curves was significant at all sampling times, p being $< .001$ at 5 and 10 minutes after injection of TRF and $< .01$ at 15 and 20 minutes. Twelve rats were used per group.

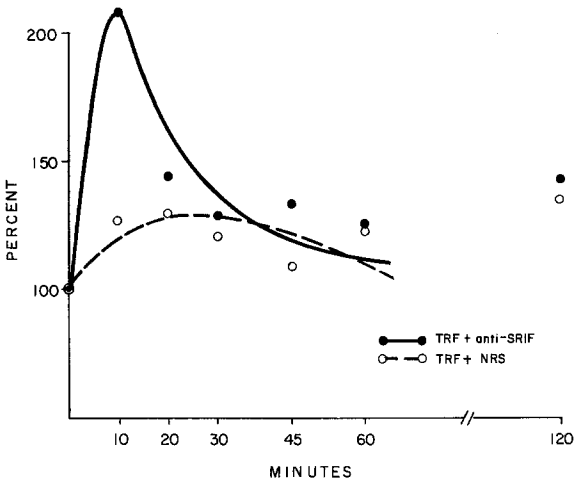

Figure 3:

Serum thyrotropin levels in young adult female rats surgically thyroidectomized 4 weeks prior to the experiment after injection of 75 ng TRF in normal or anti-SRIF rabbit serum. Ethanol anesthesia was used.

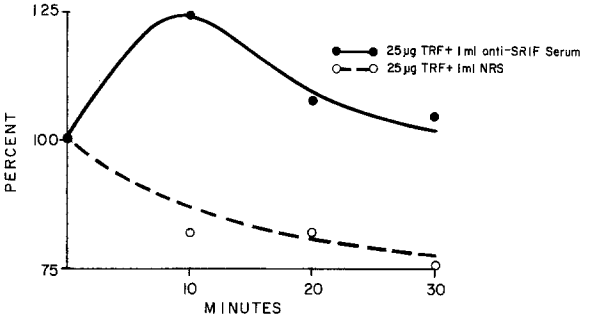

Figure 4:

Serum TSH in young adult female rats anesthetized with Pentobarbital after the injection of 2.5 µg of thyroliberin together with 1.0 ml of normal rabbit serum or anti-SRIF rabbit serum. Rats were thyroidectomized 4 weeks prior to the experiments and treated with 50 µg of L-Thyroxine for the last 21 days.

Hyperthyroxinemic rats failed to respond to a very large dose (2.5 µg) of TRF given with NRS (Figure 4), but the same dose of TRF given together with anti-SRIF did cause significant secretion of TSH. Even though only 4 rats were used to establish each curve, the difference between the two responses was significant ($p < 0.05$) 10 minutes after the injection.

Discussion:

In all our experiments, addition of potent antiserum against SRIF enhanced the secretion of TSH above that seen in rats receiving the same dose of Thyroliberin together with normal rabbit serum. The anesthetic used (ethanol in 3 experiments and nembutal in 2) appeared to make no difference.

The antiserum alone had no effect on basal TSH levels. Only a gradual decrease in serum TSH levels was produced, which did not attain statistical significance during the first 20 minutes after injection and which was similar to the gradual declines seen in the control groups in Figures 1 and 4. We ascribe these decreases to the effect to the diurnal TSH cycle (13) (since all our experiments were carried out between 1 and 3 p.m.) and to the hemodilution due to the blood loss caused by sampling. Our failure to detect any elevation of serum TSH after anti-SRIF serum alone agrees with the reported failure of Somatostatin to lower basal TSH levels, but it conflicts with the effect of anti-Somatostatin serum recently reported by Ferland et al. (10). Ferland's observations were made at least an hour after the administration of anti-SRIF, at which time we find a rapid decline of the anti-SRIF effect.

The slight, but significant, enhancement of TSH levels subsequent to the injection of anti-SRIF serum together with a large dose of TRF into hyperthyroxinemic rats indicates that the blocking effect of high doses of thyroxine (8 times the normal replacement dose in such animals) for 3 weeks was not exerted exclusively through the formation of a protein inhibitor of TRF action, as has been described *in vitro* (1, 2, 3).

We interpret our results to indicate that the control of TSH secretion may be modulated by both positive (Thyroliberin-mediated) and negative (Somatostatin-mediated) mechanisms in addition to the slow inhibition process mediated by thyroid hormone. Such a control system permits the very rapid changes in serum TSH levels reported in man by Odell (12) and in the rat by Greer (14) for which no satisfactory explanation has previously been offered. Our hypothesis could also explain the paradoxical finding of decreased TRF

synthesis in myxedema as well as the elevated TRF production and excretion in hyperthyroidism which has been repeatedly reported. Finally, such an expanded control system would also accomodate the findings of Peter (6) in fish and of Joseph and Knigge (7) in kittens where hypothalamic control over pituitary TSH secretion has been demonstrated to be predominantly inhibitory.

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