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Effect of Thyroid Hormone Excess and Deficiency on Serum Thyrotropin in Rats Immunized Passively with Antiserum to Somatostatin[1] (39537)

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It is now known that somatostatin, in addition to inhibiting the release of growth hormone (GH) (1-5), also inhibits the secretion of several other hormones, among them, thyrotropin (TSH), insulin, glucagon, and gastrin, in various animal species, both *in vivo* and *in vitro* (6-12). However, the physiological role of somatostatin in regulating the secretion of these hormones is still not clear.

Recently, a specific antiserum to somatostatin was generated in sheep in our laboratory (13). This antiserum has been used to neutralize the effect of endogenous somatostatin in order to clarify the possible physiological role of that hormone in the regulation of the secretion of GH and TSH. It has thus been found that passive immunization of rats with the antiserum to somatostatin (anti-SS) prevented a stress-induced decrease in serum GH (13), augmented the TSH release after exposure to cold (14), and enhanced the TSH response to thyrotropin-releasing hormone (TRH) (15). These findings suggested that somatostatin plays a physiologically important role in the regulatory mechanism of the secretion of GH and TSH.

To examine the interaction between somatostatin, TRH, and thyroid hormones in the regulation of TSH secretion we studied the effect of triiodothyronine (T_3) on the basal and TRH-stimulated TSH concentrations in sera of rats passively immunized with anti-SS.

Materials and methods. Male Charles River CD-strain rats were used throughout the experiments. They were maintained in animal quarters with controlled temperature (24°) and illumination (0500-1900 hr) and were given free access to tap water and Purina laboratory chow.

Experiment 1. Rats weighing 300-360 g were divided into three groups of 16 rats each. The first group was injected with 0.1 ml of 0.9% saline, the second with 100 ng of T_3, and the third with 200 ng of T_3/100 g BW sc. These three groups were divided into two subgroups of eight animals each. Each subgroup was then injected iv under ether anesthesia with either 2 ml of anti-SS or 2 ml of normal sheep serum (NSS) at the same time as T_3 was given. Two hours later all animals were injected iv with 200 ng of TRH/rat. Blood was drawn from the jugular vein before and 5 min after TRH.

Experiment 2. Rats weighing 300-350 g were divided into three groups of 10 rats each. The first group was injected with 0.1 ml of 0.9% saline, the second with 1 μg of T_3, and the third with 10 μg of T_3/100 g BW sc. These three groups were divided into two subgroups of five animals each. These subgroups were then given iv either 2 ml of anti-SS or 2 ml of NSS at the same time as T_3. Two hours later all rats were injected iv with 200 ng of TRH/rat. Blood was drawn from the jugular vein before and 5 min after TRH.

Experiment 3. Thirty immature rats weighing 70-80 g were thyroidectomized under Surital anesthesia (3.5 mg/100 g BW) and then divided into three groups of 10 animals each. These groups were then divided into two subgroups of five animals each. These subgroups were injected iv with 1 ml of anti-SS or 1 ml of NSS 1, 3, or 7 days after the thyroidectomy. Two hours later the animals were killed by decapitation, and blood was collected from the trunk.

Another 30 rats were sham-operated and divided into groups in the same manner as

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the thyroidectomized animals. Each subgroup was injected iv with either 1 ml of anti-SS or 1 ml of NSS 1, 3, or 7 days after the operation. Two hours after the injection, the blood was collected. In addition, 10 intact animals were divided into two groups, one of which was injected with anti-SS and the other with NSS, and blood was collected 2 hr after injection. TSH was determined by radioimmunoassay (RIA) as described by Kiefer *et al.* (16), with the use of NIAMDD rat pituitary kit for rat TSH.

The antiserum to somatostatin was generated in sheep as described elsewhere (17). The antiserum used in the present study (No. 774) bound 85% of ^{125}I-labeled-Tyr^1-somatostatin (sp act, 330 μCi/μg) at 1:70 dilution. At 1:14,000 dilution of antiserum, the tracer–antibody binding was inhibited by unlabeled cyclic somatostatin in a dose-related manner in a range from 32 to 4096 pg/tube. There were no cross-reactions with TRH, luteinizing hormone-releasing hormone (LHRH), rat luteinizing hormone (LH), follicle-stimulating hormone (FSH), TSH, GH, or prolactin. It was shown earlier that the administration of this antiserum did not interfere with the determination of rat TSH (15).

The means of serum TSH levels for each group were compared by using the Student's *t* test or Duncan's new multiple range test (18).

Results. Experiment 1 (Fig. 1). In the first group of rats, injected with saline and not with T_3, the basal serum TSH level was significantly higher in the animals pretreated with anti-SS than in those given NSS (1.25 ± 0.23 vs 0.54 ± 0.05 μg/ml, $P < 0.01$). There was a significant increase in serum TSH 5 min after TRH in both anti-SS- and NSS-treated animals, and the mean TSH level after TRH was significantly higher in the anti-SS-treated animals than in those pretreated with NSS (3.14 ± 0.23 vs 2.02 ± 0.21 μg/ml, $P < 0.01$).

In the second group injected with 100 ng of T_3/100 g BW both the basal and post-TRH serum TSH levels were suppressed to about half of those in respective control animals. The basal as well as the TRH-stimulated TSH levels were significantly higher in the anti-SS-treated rats than in the NSS-treated rats (0.50 ± 0.07 vs 0.22 ± 0.04 μg/ml, $P < 0.01$, and 1.73 ± 0.15 vs 0.91 ± 0.04 μg/ml, $P < 0.01$, respectively).

In the third group of rats treated with 200 ng of T_3/100 g BW the basal serum TSH

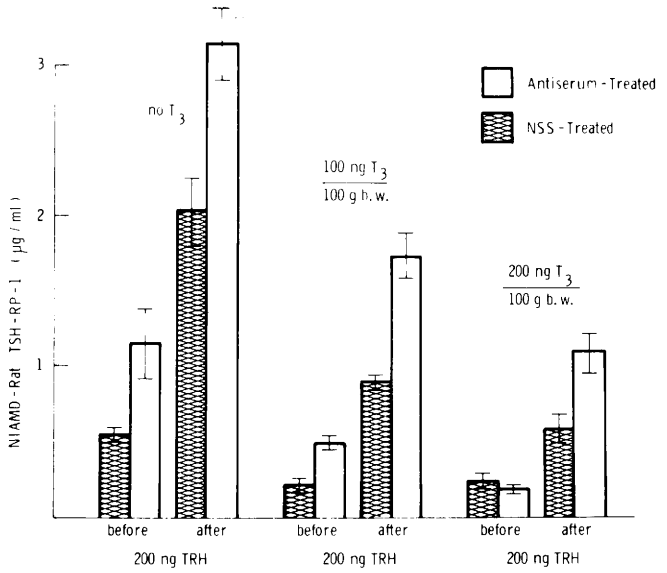

FIG. 1. Effect of T_3 on serum TSH levels before and 5 min after TRH in rats pretreated with sheep antiserum to somatostatin or normal sheep serum (NSS). Both T_3 and serum were administered 2 hr prior to the injection of TRH. The significance of differences between the corresponding TSH values in the antiserum- and NSS-treated groups was calculated using Student's *t* test (** $P < 0.01$).

level did not differ between the subgroup pretreated with antiserum and that pretreated with NSS (0.19 ± 0.02 vs 0.24 ± 0.04 μg/ml). In both subgroups, the TSH level rose significantly after TRH. The increase in serum TSH level in response to TRH was significantly greater in anti-SS than in NSS-pretreated rats (1.09 ± 0.13 vs 0.59 ± 0.10 μg/ml, $P < 0.05$).

Experiment 2. As in the previous experiment, in the first group of animals which had not received T_3, both the basal and the TRH-stimulated TSH levels were significantly higher after pretreatment with anti-SS than with NSS (1.00 ± 0.11 vs 0.58 ± 0.05 μg/ml, $P < 0.01$, and 3.97 ± 0.31 vs 2.69 ± 0.41 μg/ml, $P < 0.01$, respectively).

Administration of 1 μg of T_3/100 g BW significantly suppressed the basal serum TSH and blocked the TSH response to TRH. There was no difference in either the basal or the post-TRH TSH levels between groups pretreated with anti-SS or NSS (0.25 ± 0.04 vs 0.25 ± 0.02 μg/ml and 0.28 ± 0.04 vs 0.27 ± 0.03 μg/ml). Similar results were obtained in the animals treated with 10 μg of T_3/100 g BW (0.22 ± 0.02 vs 0.22 ± 0.02 μg/ml and 0.27 ± 0.03 vs 0.25 ± 0.04 μg/ml).

Experiment 3 (Fig. 2). In the unoperated rats, the basal serum TSH concentration was significantly higher after pretreatment with anti-SS than with NSS (1.15 ± 0.19 vs 0.29 ± 0.05 μg/ml, $P < 0.01$). There was a significant rise in serum TSH on the day after thyroidectomy, with a further increase 3 and 7 days after the operation. The TSH level was significantly higher in the anti-SS- than in the NSS-treated animals both on Day 1 and Day 3 after thyroidectomy (2.32 ± 0.27 vs 1.01 ± 0.07 μg/ml, $P < 0.01$, and 6.18 ± 0.88 vs 3.33 ± 0.70 μg/ml, $P < 0.01$, correspondingly). However, on Day 7 after thyroidectomy there was no difference in serum TSH between the anti-SS- and NSS-treated groups (6.99 ± 0.36 vs 6.88 ± 0.58 μg/ml).

In sham-operated rats the TSH levels were significantly lower on Days 1, 3, and 7 after operation than in the corresponding groups of thyroidectomized animals treated with anti-SS and NSS, respectively. There was no difference in the corresponding anti-SS- and NSS-treated groups between nonoperated and sham-operated animals, and the serum TSH levels were significantly higher in the anti-SS- than in the NSS-treated rats on Days 1, 3, and 7 after the operation (results not shown). Thus, the stress of the operation did not cause any nonspecific differences between the groups.

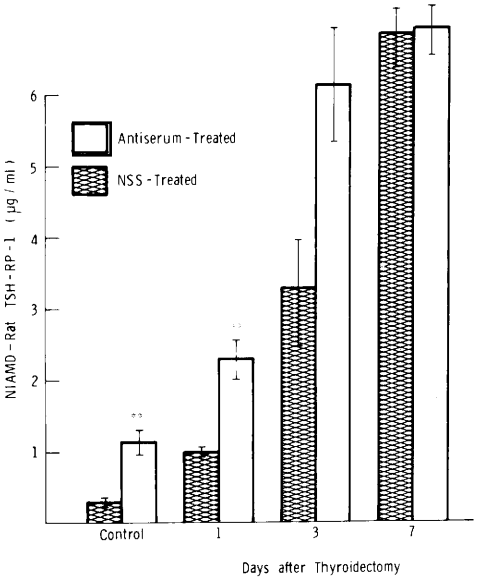

FIG. 2. Serum TSH levels in rats pretreated with antiserum to somatostatin or normal sheep serum (NSS) 1, 3, or 7 days after thyroidectomy and in nonoperated control rats. The significance of differences between the corresponding TSH values in the antiserum- and NSS-treated groups was calculated using Student's t test (*$P < 0.05$, **$P < 0.01$).

Discussion. The present study confirms and extends our previous observation that passive immunization by specific antiserum to somatostatin increases the basal as well as the TRH-stimulated TSH secretion in rats (15). This phenomenon is most probably due to the nullification of the action of endogenous somatostatin by passive immunization.

TRH stimulates TSH secretion presumably by binding to specific receptor sites on the plasma membrane of thyrotrophs. This binding causes an activation of adenylate cyclase resulting in an increase of intracellular cyclic adenosine monophosphate (cAMP) accumulation, which ultimately leads to alteration of specific subcellular

functions and synthesis and release of TSH (19). TRH seems to have a modulating rather than a dynamic effect on the TSH secretion. There is now evidence that somatostatin and TRH do not act in a competitive manner at the receptor site of the thyrotroph, but that somatostatin suppresses the TRH action at a later stage (19). It has been shown that somatostatin inhibits cAMP accumulation *in vitro* in the pituitary, parallel to suppressing GH and TSH release (19). When the action of endogenous somatostatin is neutralized by passive immunization, its tonic suppression of cAMP accumulation in the thyrotrophs as well as in the somatotrophs may be eliminated, resulting in enhancement of the TSH response to TRH. Thyroid hormones exert their negative feedback effect on the TSH secretion at the pituitary level, without affecting to any major degree the TRH secretion (20). The action of thyroid hormones on the thyrotrophs has not been clarified yet, although there is some recent evidence that thyroid hormones reduce the number of TRH binding sites at the plasma membrane of the thyrotroph (19). The site of action may also be beyond the generation of cAMP in the cell nucleus (21, 22).

Administration of T_3 to rats naturally suppressed the basal TSH level and the TSH response to TRH in a dose-dependent manner. T_3 given at 100 ng/100 g BW suppressed the basal TSH level and blunted the response to TRH to about half the control level. Administration of the antiserum to somatostatin significantly increased the basal as well as post-TRH TSH levels as compared to the NSS-treated group. When 200 ng of T_3/100 g BW was administered, the antiserum to somatostatin did not alter the blocking effect of T_3 on the basal serum TSH, but still augmented the TRH-stimulated TSH secretion, as compared to the NSS-treatment. When greater doses of T_3 were given, the antiserum could not overcome the blocking effect of T_3 even after TRH. Vale *et al.* (6) have shown that somatostatin and thyroid hormones exhibit a summation of effects in inhibiting the TSH secretion both *in vivo* and *in vitro*. Our results are in good agreement with theirs, showing an interaction between somatostatin and thyroid hormones under physiological conditions.

The rise of serum TSH after thyroidectomy was significantly increased by pretreatment with antiserum to somatostatin. However, on the seventh day after thyroidectomy, while the serum TSH level had possibly reached its maximum, it could not be further increased by passive immunization with somatostatin.

It has been shown that somatostatin only slightly reduces the basal TSH concentration in serum in both animals and man, but suppresses the response of TSH to TRH. The higher the TSH levels, the greater is the absolute reduction of TSH concentration caused by somatostatin (6–9, 23). There is also a positive correlation between the basal TSH levels and the net increment in serum TSH after TRH. Our results show a positive correlation between the TSH levels in the corresponding NSS-treated control groups and the net increase in TSH in anti-SS-treated groups.

Previous findings (14, 15) as well as those reported in this paper suggest that endogenous somatostatin may play an important role in regulating the TSH secretion, unless the circulating thyroid hormone concentration is extremely low or high. Thus, under normal conditions, somatostatin could be a physiological regulator of the TSH secretion, in addition to TRH and thyroid hormones. The role of somatostatin in regulation of the TSH secretion can, however, be completely evaluated only when we are able to measure fluctuations of endogenous somatostatin under various conditions.

Summary. Passive immunization of rats with antiserum to somatostatin (anti-SS) caused about a 2-fold increase in the basal serum TSH levels and a 1.5-fold rise in serum TSH after TRH, as compared to rats treated with normal sheep serum (NSS). When the basal and the TRH-stimulated TSH levels were suppressed to about half the control levels with 100 ng of T_3/100 g BW, the neutralization of the endogenous somatostatin secretion with anti-SS significantly increased the basal as well as the post-TRH TSH levels. After administration of 200 ng of T_3/100 g BW, the basal TSH levels in the anti-SS- and NSS-treated ani-

mals were the same, but, in response to TRH, the TSH levels rose significantly higher in the anti-SS-treated group. When higher doses of T_3 (1 and 10 μg/100 g BW) were given, anti-SS failed to affect the basal or the post-TRH TSH levels. The elevated TSH levels 1 and 3 days after thyroidectomy were further increased by treatment with anti-SS as compared to NSS. The highly elevated level of TSH on the seventh day after thyroidectomy was, however, not affected by anti-SS. These findings indicate that somatostatin modulates the TSH secretion rate under physiological conditions and can be considered with TRH and thyroid hormones as another regulator of TSH secretion.

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