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Evidence That Regulation of Thyrotropin Secretion by the Heterotopic Pituitary Is Independent of Endogenous Thyrotropin-Releasing Hormone from Any Source[1] (41168)

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Abstract. Equal quantities of rabbit antithyrotropin-releasing hormone serum (TRH-AS) or normal rabbit serum (NRS) were administered to rats made hypothyroid by chronic feeding of propylthiouracil. TRH-AS produced a 37% decrease in plasma thyrotropin (TSH) ($P < 0.01$) in intact rats, but caused no significant decrease in plasma TSH concentration in hypophysectomized rats bearing three heterotopic pituitary transplants under the kidney capsule. NRS had no significant effect on plasma TSH in either group. This supports other data indicating that secretion of TSH and prolactin by heterotopic pituitaries is not under significant control by TRH in the general circulation.

Thyrotropin (TSH) secretion is primarily under positive control by thyrotropin-releasing hormone (TRH) (1) from the hypothalamus. Pituitaries removed from direct contiguity with the hypothalamus by heterotopic transplantation secrete reduced quantities of TSH (2). However, TSH secretion by heterotopic pituitaries can be increased or decreased by appropriate experimental manipulation, such as altering the concentration of circulating thyroid hormones (3). TRH has an important influence on TSH synthesis and secretion and is believed to come primarily from the anterior hypothalamus. However, TRH is ubiquitous throughout the central nervous system (4–6) and has been detected in extraneural tissues (7) and perhaps in plasma (8–10). It is unclear what role TRH may play in the control of TSH secretion by heterotopic pituitaries.

Medial basal hypothalamic ablation (MBHA) in animals with eutopic pituitaries causes a profound drop in plasma TSH concentration (11). In contrast, MBHA had no significant effect on plasma TSH in hypophysectomized rats with heterotopic pituitaries under the kidney capsule (12). This indicates that the hypothalamus has no significant influence on TSH secretion by the heterotopic pituitaries. However, it does not exclude the possibility that TRH in the general circulation from some extra-hypothalamic source might be influencing the secretion of these two hormones by the heterotopic pituitary.

The present experiment was designed to complement the previous studies (12) by determining whether an injection of anti-TRH antiserum (TRH-AS) in a quantity which significantly depressed TSH secretion in rats with eutopic pituitaries (13) would affect TSH secretion in rats with heterotopic pituitaries. If no effect of TRH-AS was observed in the latter, this would indicate that physiologically significant quantities of TRH from any source do not reach the heterotopic pituitary through the general circulation.

Materials and Methods. Thirty-two male Sprague–Dawley rats (170–210 g BW) were obtained from Simonsen Labs, Gilroy, California, and kept in a temperature- (24 ± 1°) and light-controlled (light from 0600–1800 hr) environment. Three pituitaries from 21-day-old (40–60 g BW) Sprague–Dawley rats of either sex were transplanted beneath the left kidney capsule in 22 of the rats. All rats were then fed a pelleted low-iodine diet containing 0.15% propylthiouracil (obtained from

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Teklad Mills, Madison, Wisc.) and tap water *ad libitum* until the termination of the experiment. After 2 weeks the transplant-bearing rats were hypophysectomized. One week later, transplant-bearing (H-T) and intact control rats were briefly anesthetized with ether and injected (via a lateral tail vein) with 50 μl/100 g BW of either TRH-AS (13) or normal rabbit serum (NRS). Blood samples were collected percutaneously from the subclavian vein immediately before and 60 min after the injection of serum. The rats were under light ether anesthesia for both venipunctures. This experiment was repeated 3 weeks later with a cross-over design. All rats which previously received TRH-AS now received NRS, and vice versa.

Heparinized plasma samples were frozen and stored until assayed for TSH by a specific radioimmunoassay using a kit obtained from the NIAMDD Rat Pituitary Hormone Program through the National Pituitary Agency. Plasma TSH concentration was calculated as μU/ml based upon the biological potency of reference NIAMDD-Rat TSH-RP-1 (0.22 USP bovine TSH units/mg, McKenzie assay). All samples from each experiment were measured in the same assay.

Statistical evaluation of the data was made with Student's paired *t* test.

Results. In the first experiment, TRH-AS produced a 37% decrease in plasma TSH in the intact rats ($P < 0.01$) but had no significant effect in the rats with heterotopic pituitaries (Experiment 1, Fig. 1). In the cross-over experiment (Experiment 2, Table I), in which the injections to each group were reversed three weeks later, TRH-AS produced a 19% decrease in plasma TSH concentration in the intact group. An unexplained increase ($P < 0.01$) in plasma TSH followed TRH-AS injection in the rats with heterotopic pituitaries in the second experiment. NRS did not have a significant effect on plasma TSH concentration in the rats with eutopic or heterotopic pituitaries in either experiment ($P > 0.05$).

Discussion. These data indicate that insufficient TRH from any source is present in the general circulation to influence the secretion of TSH by heterotopic pituitaries. The administration of TRH-AS adequate to produce a significant decrease in plasma TSH concentration in intact rats did not reduce plasma TSH concentration in H-T rats. This confirms and extends the previous studies (12), in which it was found that MBHA produced a dramatic fall in plasma TSH in rats with eutopic, but not with heterotopic, pituitaries. It thus seems unlikely that extrahypothalamic TRH in the general circulation significantly influences secretion of TSH.

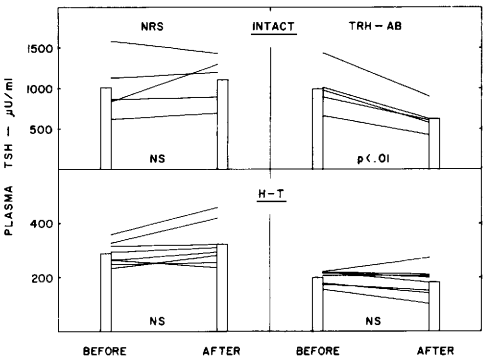

FIG. 1. Effect of TRH-AS (TRH-AB) on plasma TSH concentrations in PTU-fed hypophysectomized pituitary-transplanted (H-T) and intact control rats. Plasma TSH (μU/ml) is shown before and 60 min following injection of NRS (left panel) or TRH-AS (right panel). Bars represent the mean TSH value for the individual animals, which are indicated by the lines connecting the 0- and 60-min bars. Level of significance shown is that determined by the paired Student's *t* test.

It is possible that some depression of plasma TSH following TRH-AS might have been detected in the rats with heterotopic pituitaries if different time intervals were chosen for sampling. However, the previous studies with intact hypothyroid rats (13) indicated that a maximum fall in plasma TSH concentration was produced within 1 hr after the iv injection of antiserum. Due to the very limited supply of TRH-AS available, the present experiments could not be repeated. A single sampling 1 hr after injection was chosen to minimize the deleterious effect of blood loss, especially from the hypothyroid rats with heterotopic pituitaries.

In Experiment 2, there was a puzzling postinjection rise in plasma TSH in the rats with heterotopic pituitaries and a lesser decrease in plasma TSH in those with eutopic pituitaries after TRH-AS injection, in com-

TABLE I. EFFECT OF TRH-AS (EXPERIMENT 2) ON PLASMA TSH CONCENTRATION IN RATS WITH EUTOPIC OR HETEROTOPIC PITUITARIES

Pituitary location	(n)	Injection	Plasma TSH (μU/ml) 0 min	60 min
Eutopic	(5)	NRS	782 ± 113[a]	902 ± 158
	(5)	TRH-AS	1037 ± 175	822 ± 97*[b]
Heterotopic	(8)	NRS	224 ± 42	261 ± 43
	(8)	TRH-AS	261 ± 43	368 ± 57**

Note. Blood samples were collected immediately before and 60 min following administration of TRH-AS (antiserum to TRH) or NRS (normal rabbit serum).

[a] Mean ± SE.

[b] Difference from 0-min value (Student's paired t test). *$P < 0.05$. **$P < 0.01$.

parison to Experiment 1. In part, this may have been due to the development of antibodies to rabbit serum in the rats, since 3 weeks elapsed between the first and second studies.

The rats were fed a low-iodine, propylthiouracil diet before removal of the eutopic pituitary to insure maximum depletion of thyroid hormone stores and thus obtain a supranormal concentration of plasma TSH before TRH-AS was administered. Animals with heterotopic pituitaries fed a normal diet have plasma TSH concentrations just above the limits of detectability (12, 15). It would have been difficult to determine any significant fall in plasma TSH concentration without first making the animals severely hypothyroid. If propylthiouracil feeding had been started after removal of the eutopic pituitary, a much longer period would have been necessary to adequately exhaust preformed thyroid hormone stores to produce the elevation of plasma TSH seen under basal conditions in these experiments. Because of the sluggish rate of secretion of TSH compared to normal rats, depletion of stored thyroid hormone is much slower in animals in which the hypothalamic influence on pituitary TSH secretion is reduced or abolished (14, 15).

Finally, these studies, in combination with other data cited above, strongly suggest that secretion of TSH by heterotopic pituitaries can be directly affected by the concentration of circulating thyroid hormones independent of TRH. TRH injected iv induces prompt secretion of TSH from heterotopic pituitaries, indicating there is no significant loss of their ability to respond to TRH (15).

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1. Florsheim, W. H., Control of thyrotropin secretion. *in* "Handbook of Physiology" (E. Knobil and W. H. Sawyer, eds.), Section 7, Vol. 4, Part 2, p. 449. Amer. Physiol. Soc., Washington, D.C. (1974).
2. Greer, M. A., Scow, R. O., and Grobstein, C., Proc. Soc. Exp. Biol. Med. **82**, 28 (1953).
3. Scow, R. O., and Greer, M. A., Endocrinology **56**, 590 (1955).
4. Hökfelt, T., Fuxe, K., Johansson, D., Jeffcoate, S., and White, N., Eur. J. Pharmacol. **34**, 389 (1975).
5. Jackson, I. M. D., and Reichlin, Endocrinology **95**, 854 (1974).
6. Kardon, F. C., Winokur, A., and Utiger, R. D., Brain Res. **122**, 578 (1977).
7. Morley, J. E., Life Sci. **25**, 1539 (1979).
8. Jackson, I. M. D., and Reichlin, S., Life Sci. **14**, 2259 (1974).
9. Montoya, E., Seibel, M. J., and Wilber, J. F., Endocrinology **96**, 1413 (1975).
10. Emerson, C. H., and Utiger, R. D., J. Clin. Invest. **56**, 1564 (1975).
11. Tonooka, N., and Greer, M. A., Endocrinol. Exp. **13**, 75 (1979).
12. Greer, M. A., Tonooka, N., and Thompson, M. E., Prog. 53rd Ann. Mtg. Amer. Thyroid Assoc. Abstract T-23 (1977).
13. Harris, A. R. C., Christianson, D., Smith, M. S., Fang, S.-L., Braverman, L. E., and Vagenakis, A. G., J. Clin. Invest. **61**, 441 (1978).
14. Fukuda, H., and Greer, M. A., Endocrinology **100**, 911 (1977).
15. Greer, M. A., Tonooka, N., Ikeda, H., and Thompson, M. E., Endocrinology **108**, 382 (1981).

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