Vol. 96, No. 3, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 16, 1980 Pages 1143-1149

QUANTITATIVE MODULATION OF THYROID IODIDE PEROXIDASE BY THYROID STIMULATING HORMONE

AKIO NAGASAKA AND HIROYOSHI HIDAKA*

Department of Internal Medicine, Fujita-Gakuen University School of Medicine, Toyoake 470-11 and *Department of Pharmacology, Mie University School of Medicine, Tsu 514, Japan

Received August 26,1980

SUMMARY

The activity of rat thyroid iodide peroxidase fell to 8% of the normal value 48 hours after hypophysectomy. Rats given injections of thyroid stimulating hormone manifested an enzyme activity indistinguishable from that of the sham-operated animals. Cycloheximide prevented the thyroid stimulating hormone-induced restoration of the enzyme activity. The incorporation of ^{14}C-leucine into the thyroid gland decreased gradually and reached two thirds of the sham-operated group by 48 hours after hypophysectomy. Thyroid stimulating hormone administration prevented this decrease, as observed for iodide peroxidase activity. Thyroidal RNA contents decreased also in hypophysectomized rats, thyroid stimulating hormone treatment prevented the reduction of RNA contents and no significant change was observed in thyroidal DNA contents. These data are consistent with the idea that protein biosynthesis is involved in thyroid stimulating hormone regulation of thyroidal iodide peroxidase and that the life span of the peroxidase is less than 48 hours.

INTRODUCTION

Data have accumulated concerning the effect of TSH on specific chemical and enzymatic events in the thyroid (1-6). Thyroid hormone synthesizing enzyme, iodide peroxidase, is augmented by chronic administration of TSH regimens (7, 8). We found that TSH depletion by hypophysectomy induces a decrease in the enzyme activity and that this was reversed by TSH treatment; these changes in the enzyme activity required only a short interval (9). We now present evidence that the modulation of thyroidal iodide peroxidase by

---

The abbreviations used are: TSH - thyroid stimulating hormone; ACTH - Adrenocorticotropic hormone; TCA - trichloroacetic acid; DNA - deoxyribonucleic acid; RNA - ribonucleic acid.

0006-291X/80/191143-07$01.00/0

TSH is possibly produced by an effect on the biosynthesis of the enzyme protein, and that the life span of the enzyme in the rat thyroid is less than 2 days.

MATERIALS AND METHODS

All animals used were male inbred Wistar rats (110 - 150 g) fed a regular diet. They were either intact or sham-operated, or they had been subjected to surgical removal of the hypophysis. Individual thyroid lobes were carefully removed from rats killed by a sharp blow on the head, and were then weighed. Four lobes collected from two rats were combined and homogenized in 9 volumes of Krebs Ringer buffer (pH 7.4). The particulate fraction was prepared by centrifugation at 105,000 x g for one hour and used for measuring iodide peroxidase activity. The specific enzymatic activity in the particulate fraction is twice that in the homogenate and the recovery of the activity in the particulate fraction was 80 to 90%. Iodide peroxidase assay is based on iodotyrosine formation (10). The reaction mixture contained iodide, tyrosine and a H_2O_2 source, such as the glucose-glucose oxidase system. The activity was measured by ^{131}I incorporation into tyrosine, as reported by DeGroot and Davis (11). For the study of leucine incorporation into the thyroid protein, each rat was given 10 μCi of ^{14}C-leucine in saline into the tail vein 6 hours before sacrifice. The thyroid glands were homogenized in 5% TCA solution. The precipitates were centrifuged at 1,000 x g for 15 minutes and were washed 3 times by resuspension in 5% TCA and then dissolved in 10% NH_4OH and mixed with Bray's solution, to determine the radioactivity by liquid scintillation counting (Beckman model LS233). Thyroidal DNA and RNA contents were measured according to Schmidt-Thannhauser-Schneider's method (12). Protein concentration was determined by the method of Lowry et al.(13). Bovine TSH was purchased from Armour Co. Ltd. Synthetic ACTH (Cortrosyn Z) was from Organon Co. Ltd. Other chemicals were from commercial sources.

RESULTS

Iodide peroxidase activities in the thyroid gland of intact rats treated with 1.0 unit of bovine TSH every 12 hours for 3 days were elevated by 50% of the control: 6.2 ± 0.5 mμmol/mg protein (1.1 ± 0.08 mμmol/mg wet weight); TSH treatment: 10.2 ± 1.0* (1.7 ± 0.1*, statistical significance, $p < 0.05$, n=5), as compared to that without TSH. Therefore, in order to confirm the direct action of TSH on the enzyme activity, the time course of the effect of hypophysectomy was studied. Sham-operated rats or hypophysectomized rats injected with TSH or with saline were killed 0.5, 1, 3, 6, 24, 48 hours, 5 and 12 days after the operation. The activity decreased to 8% of the normal value 2 days after the surgical intervention and after that the activities were hardly measurable. Peroxidase activity remained constant over the

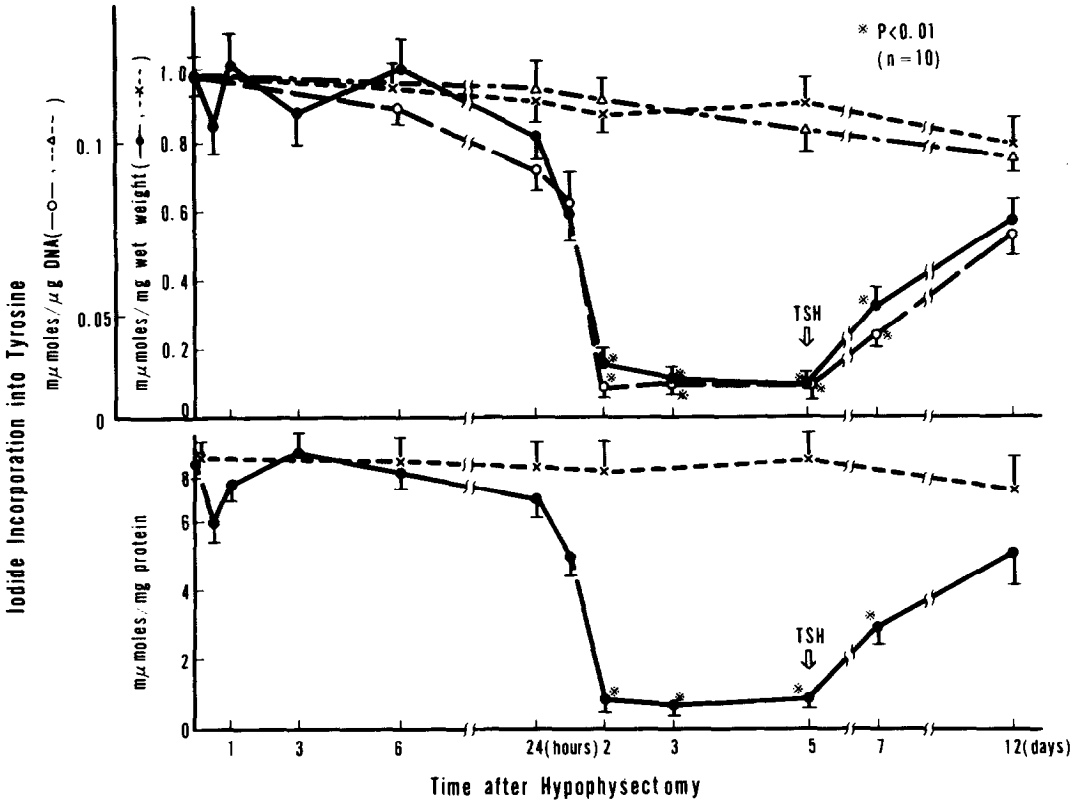

Fig. 1 Effect of hypophysectomy followed by TSH administration on thyroid iodide peroxidase. Rats were sacrified at the times indicated after hypophysectomy. Rats were treated with 1.0 unit of TSH dissolved in physiological saline within 10 minutes before injection twice daily starting immediately after hypophysectomy (X------X △---△) and without TSH (●——● o-----o). (⇩) From the fifth day after hypophysectomy, 1.0 unit of TSH was administered intraperitoneally twice daily for a week. Iodide peroxidase activity was measured as described in Methods. Each value represents mean ± S.E. of 10 experiments. *Statistically significant (Student's t-test).

12-day period in sham-operated rats and hypophysectomized rats in which TSH administration was begun immediately after hypophysectomy. When TSH administration was initiated on the fifth day, the activity increased slowly to approximately 70% of the normal value by the twelfth day (Fig. 1). Km of the enzyme did not differ between sham-operated and hypophysectomized rats, or these receiving TSH.

To determine whether a reduction in the enzyme activity in hypophysectomized rat is consistent with a quantitative decrease of protein synthesis,

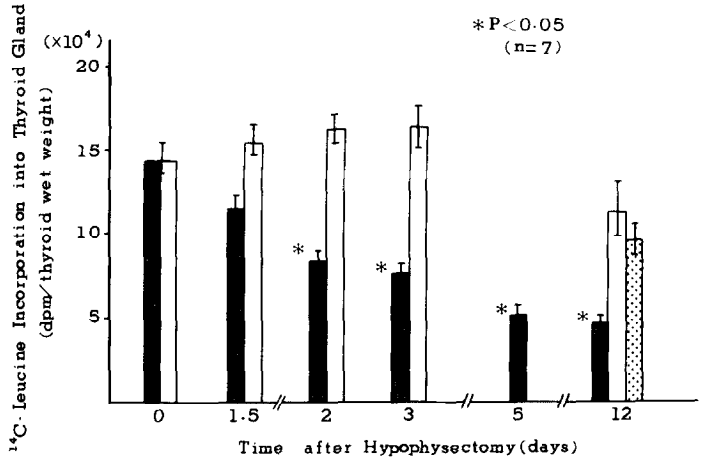

Fig. 2 Effect of hypophysectomy and TSH administration on ^{14}C-leucine incorporation into the rat thyroid gland. Details are in the text.

□ : TSH injection twice a day.
▨ : Saline injection.
⊞ : Saline injection for the first 5 days and TSH injection twice a day for the last 7 days.
Each value represents mean ± S.E. of 6 experiments.
*Statistically significant (Student's t-test).

^{14}C-leucine incorporation into total thyroid protein was determined. The ^{14}C-leucine incorporation found in the TCA precipitates of the thyroid gland was gradually reduced, reaching two thirds of the control values at 48 hours and one third at 96 hours after hypophysectomy. Repeated injection of 1.0 unit of TSH twice daily just after hypophysectomy prevented this decrease. TSH administration initiated on the fifth day after hypophysectomy restored the incorporation of ^{14}C-leucine toward control values and was concomitant with restoration of the enzyme activity (Fig. 2).

Alterations in thyroidal DNA and RNA content were checked in hypophysectomized rats treated with or without the TSH regimen just after or on the fifth day after hypophysectomy. DNA contents per thyroid wet weight were not affected by hypophysectomy or TSH treatment, but RNA contents per thyroid wet weight decreased significantly at 2 days after hypophysectomy. The levels then gradually fell to one half of the control at 12 days after hypophysectomy. The decreased RNA levels returned to near the control value with consecutive

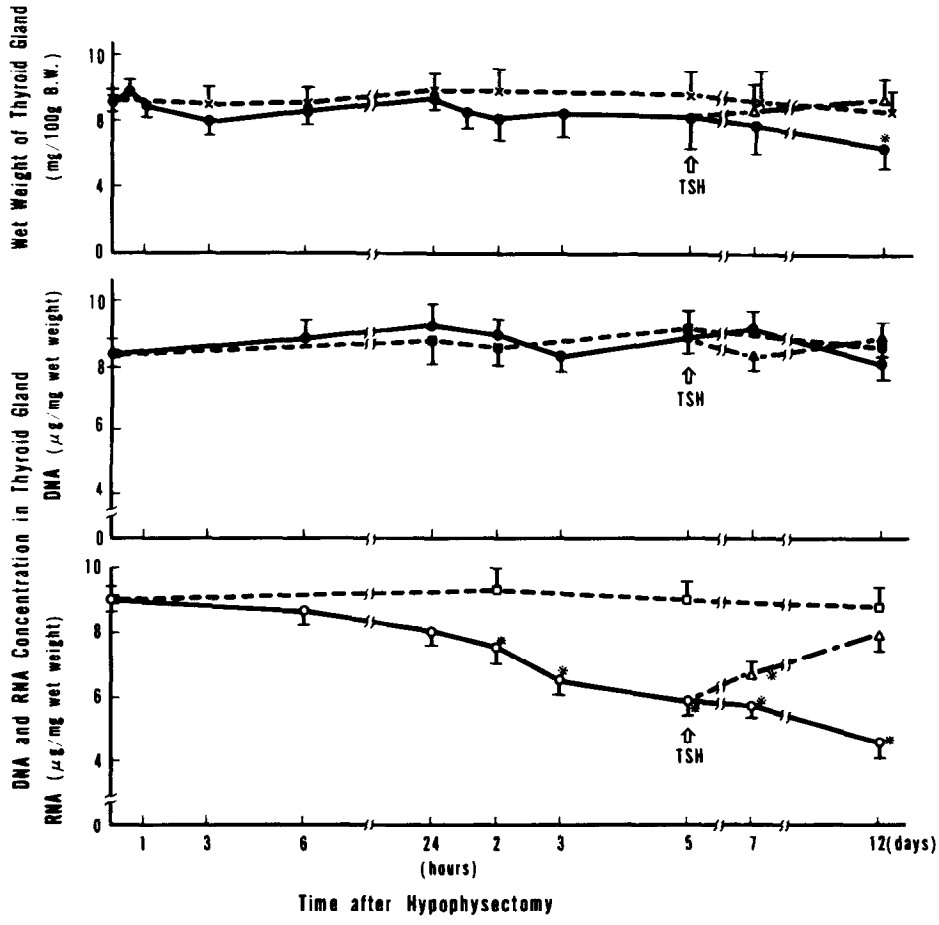

Fig. 3 Effect of hypophysectomy and TSH administration on thyroidal DNA and RNA contents. The condition is the same as in Fig. 1. Rats were treated with TSH (X----X ■----■ □--□) and without TSH (●—● ○—○) twice daily immediately after hypophysectomy. (⇩) TSH administration was initiated on the fifth day after hypophysectomy (△--△ ▲-·-▲). The values are mean ± S.E. of 5 experiments.
*Statistically significant ($p < 0.05$, Student's t-test).

TSH administration for 7 days. The alteration of thyroidal RNA contents by hypophysectomy and TSH treatment was consistent with changes in thyroidal iodide peroxidase activity and ^{14}C-leucine incorporation into thyroid proteins (Fig. 3).

As these data suggested that the modulation of iodide peroxidase by TSH may be via biosynthesis of the enzyme, cycloheximide and TSH were given intraperitoneally to hypophysectomized rats on the fifth day after hypophy-

TABLE 1

Effects of ACTH and cycloheximide on iodide peroxidase activity in hypophysectomized rat thyroid

	Thyroid Wet Weight (mg/100g BW)	Thyroidal Protein Concentration (mg/mg wet weight)	Iodide Incorporation into Tyrosine	
			(mμmol/mg w.w.)	(mμmol/mg protein)
Control	6.7 ± 0.3	0.14 ± 0.02	1.90 ± 0.08	11.0 ± 0.3
ACTH	7.8 ± 0.4	0.14 ± 0.03	0.16 ± 0.03*	1.3 ± 0.1*
Cycloheximide + TSH	8.1 ± 0.5	0.10 ± 0.05	0.08 ± 0.01*	0.6 ± 0.1*

ACTH(Cortrosyn-Z, 0.05 mg/day) was intraperitoneally administered just after hypophysectomy for 12 days.

Cycloheximide(300 μg/day) and TSH(1.0 unit/day) were intraperitoneally given to rats from the 5th day after hypophysectomy for 7 days.

*Statistically significant (Student's t-test) ($p < 0.01$, n=6).

sectomy. In contrast to the activity in hypophysectomized rats treated with TSH, the enzyme activity was not restored by TSH with the concomitant administration of cycloheximide (Table 1). Synthetic ACTH had no effect on the enzyme activity in hypophysectomized rats, thereby suggesting that changes in the enzyme activity seen with TSH are not due to the action of ACTH contaminated in commercial TSH preparations (Table 1).

DISCUSSION

Greer reported that the concentration of triiodothyronine and thyroxine in the thyroid gland decreased at 2 days after hypophysectomy (14). This may correspond to the disappearance time of iodide peroxidase activity by TSH depletion. These changes in the enzyme activity clearly corresponded to the changes in rate of leucine incorporation into the thyroid gland and to the changes in RNA contents in the thyroid gland, which did not show a time lag, and the TSH-induced restoration of peroxidase activity was prevented by the inhibitor of protein synthesis. It has been reported that TSH enhanced the incorporation of leucine into thyroid proteins measured in vitro within 2 days

of administration (15). Wägar reported that long-term regulation of thyroidal protein synthesis by TSH is mediated at the transcriptional level and TSH-induced stimulation of thyroid protein synthesis is partly dependent on synthesis of new RNA (16). Our results indicate that the life span of iodide peroxidase in the rat thyroid is less than 48 hours and that the rate of biosynthesis of thyroid peroxidase is regulated by TSH.

ACKNOWLEDGMENTS

We thank Dr. A. Tanaka, Shionogi Institute, for preparing the hypophysectomized rats, Dr. Leslie J. DeGroot (The University of Chicago) and M. Ohara for pertinent advice.

REFERENCES

1. Rosenberg, L.L. and Cavelieri, R.R. (1971) Endocrinology 89, 439 - 444.
2. DeGroot, L.J. and Dunn, A.D. (1966) Endocrinology 78, 1032 - 1036.
3. Greer, M.A. and Allen, C.F. (1972) Endocrinology 90, 915 - 923.
4. Rosenfeld, P.S. and Rosenberg, I.N. (1966) Endocrinology 78, 621 - 627.
5. Zimmerman, A.E. and Yip, C.C. (1968) Canadian J. Physiol. Pharmacol. 46, 449 - 452.
6. Taurog, A., Tong, W. and Chaikoff, I.J. (1958) Endocrinology 62, 664 - 676.
7. Yamamoto, K. and DeGroot, L.J. (1974) Endocrinology 95, 606 - 612.
8. Nagataki, S., Uchimura, H., Masuyama, Y. and Nakao, K. (1973) Endocrinology 92, 363 - 371.
9. Nagasaka, A. and Hidaka, H. (1972) Excerpta Medica International Congress Series 256, 317 (Abstract).
10. Alexander, N.M. (1959) J. Biol. Chem. 234, 1530 - 1533.
11. DeGroot, L.J. and Davis, A.M. (1962) Endocrinology 70, 492 - 504.
12. Schneider, W.C. (1946) J. Biol. Chem. 164, 747 - 751.
13. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.L. (1951) J. Biol. Chem. 193, 265 - 271.
14. Greer, M.A., Allen, C.F., Torresani, J., Roques, M. and Lissitzkey, S. (1974) Endocrinology 94, 1224 - 1231.
15. Lecocq, R.E. and Dumont, J.E. (1972) Biochim. Biophys. Acta 281, 434 - 441.
16. Wägar, G. (1974) Acta Endocrinol. 77, 64 - 70.