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Effects of Hypophysectomy and TSH on Thyroidal Fucokinase in Rats (40667)

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There is a marked heterogeneity in the carbohydrate moieties of thyroglobulin (1), but the role of these carbohydrates in the function of thyroglobulin as a substrate for thyroid hormone production and storage (2, 3) is currently unclear. One sugar of the thyroglobulin molecule, L-fucose, is unique because it is a 6-deoxy sugar found at the nonreducing termini of a number of oligosaccharide chains (4). Previous studies, which have shown that terminal sialyl (5) or fucosyl (6) groups on glycoproteins can control metabolic function, suggest that the terminal fucosyl residues of thyroglobulin may in part control the further utilization of this repository for thyroid hormones. We have begun an examination of the enzymes involved in fucose metabolism with a view to determining whether these enzymes exert control of thyroglobulin synthesis and degradation and hence of thyroid hormone storage and release. The first such enzyme is fucokinase, which was first studied in pig liver (7) and which we have detected and isolated from canine thyroid (8). This enzyme converts L-fucose to β-L-fucose 1-phosphate (7, 8), a first step in its ultimate incorporation into thyroglobulin (9). In this paper, we report the effects of hypophysectomy and TSH administration on fucokinase activity in rat thyroid tissue.

Materials and methods. Rat fucokinase was assayed by the disk assay used for canine thyroid fucokinase in which conversion of [^{14}C]fucose to [^{14}C]fucose-1-phosphate was followed by absorption of the phosphorylated sugar on an ion-exchange disk subsequently counted by liquid scintillation techniques (8). The assay solution contained the following reagents in a total volume of 0.3 ml: 6 m*M* $MgSO_4$, 5 m*M* ATP, 8.33 m*M* KF, 3.33 m*M* dithiothreitol, 90 m*M* morpholinopropane sulfonic acid (MOPS) buffer, pH 6.5, and 26.7 μ*M* L-[1-^{14}C]fucose containing 0.5 μCi of radioactivity per assay volume. The enzyme solutions were assayed for 0-, 30-, 60- and 90-min periods. One unit of fucokinase activity is defined as the amount which is required to catalyze the formation of 1 nmole of L-fucose-1-phosphate per 20 min at 37°.

In order to establish that concentrations of L-fucose and ATP-Mg were at appropriate levels, double-reciprocal plots (10) were constructed for the rat enzyme. K_m values of 4.8 × 10^{-3} *M* and 2.3 × 10^{-6} *M* were obtained for ATP and L-fucose, respectively, under the conditions of the assay with crude extracts (18,000 *g* supernatant solution) of enzyme or with enzyme passed through a Bio-Gel P-2 column (data not shown).

We report the results of experiments in which we used the following two protocols: I. For studies in which the thyroid fucokinase levels of intact, hypophysectomized, or hypophysectomized-TSH-treated rats were compared (Table I), 66 male Sprague–Dawley rats, of which 27 were intact (Sprague–Dawley Co., Madison, Wis.) and 39 had been hypophysectomized just before shipment (Hormone Assay Laboratories, Chicago, Ill.), were maintained on water and Purina chow, *ad libitum*, in an air-conditioned windowless room lighted from 0800 to 2000 hr. After maintenance for 18 days, the rats were divided into three groups, as follows: 27 intact rats ("intact") and 22 hypophysectomized rats ("hypophysectomized") were given injections (i.p.) of 0.5 ml 0.9% saline at 48 and 24 hr prior to sacrifice; the remaining 17 hypophysectomized rats ("hypophysectomized-TSH-treated") were given injections of 1.0 unit of TSH (Armour Thytropar, Armour Pharmaceutical Co., Phoenix, Arizona) in 0.9% saline at 48 and 24 hr prior to sacrifice. II. The protocol for determining the fucokinase response to TSH in intact rats is given in Table II. Rats were maintained as described above.

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TABLE I. THYROID FUCOKINASE ACTIVITY IN INTACT, HYPOPHYSECTOMIZED, AND HYPOPHYSECTOMIZED-TSH-TREATED RATS

Rat treatment[a]	Fucokinase activity[b] (units/mg DNA)		Percentage of activity in intact rats			
			Crude enzyme		P-2 enzyme	
	Crude enzyme	P-2 enzyme	%	P[c]	%	P[c]
Intact	0.37 ± 0.02	0.20 ± 0.04	100	—	100	—
Hypophysectomized	0.21 ± 0.05	0.09 ± 0.03	57	<0.025	45	<0.025
Hypophysectomized TSH treated	0.70 ± 0.28	0.18 ± 0.04	189	NS	90	NS

[a] Protocol I of materials and methods.
[b] For each treatment, this represents the mean ± SD for three groups of rats with five or more rats/group.
[c] Determined from Student's t test to assess difference from activity in intact rats. NS, not significant ($P > 0.05$).

TABLE II. FUCOKINASE RESPONSE TO TSH IN INTACT RATS

Expt No.[a]	Number of rats			Fucokinase activity[b] ratio intact TSH treated[c]
	Intact	Intact TSH treated	Sex	Intact
1	6	6	F	2.2
2	7	6	F	1.5
3	6	6	M	2.1

[a] In experiments 1 and 2, each rat of the "intact-TSH-treated" group was injected (i.p.) with 1.67 units of TSH in 0.9% saline at 25, 17, and 2 hr prior to sacrifice while each rat of the "intact" group was injected at these times with 0.9% saline only. In experiment 3, each rat of the "intact-TSH-treated" group was injected (i.p.) with 1.0 unit TSH in 0.9% saline at 48 and 24 hr prior to sacrifice while each rat of the "intact" group was injected at these times with 0.9% saline only.
[b] Thyroid fucokinase activity per animal was determined for homogenates treated with Bio-Gel P-2 (Materials and Methods). The fucokinase activity of intact rats is given in Table I.
[c] The differences between intact and TSH-treated rats were significant in all cases ($P < 0.025$).

All sacrifices were made between 0900 and 1100 hr to minimize possible effects of diurnal variation on fucokinase activity. The rats were anesthetized with sodium pentobarbital. The thyroid glands were removed from the rats while still attached to sections of the trachea and were immediately dropped into a homogenization buffer (0.02 M MOPS, 0.25 M sucrose, 0.005 M β-mercaptoethanol, pH 6.91). Tissues and tissue preparations were kept at a temperature of 2° unless noted otherwise. After the trachea sections had been collected from all animals, the thyroids were excised and cleaned free of fat, external blood vessels, membranes, and parathyroids on an iced watch glass under an illuminated magnifier. In order to provide sufficient enzyme activity (8) for assay, the thyroids from five or more rats of a given group were combined prior to homogenization in an ice-cooled Potter–Elvehjem homogenizer containing 0.4 ml of the homogenization buffer. Each homogenate was washed into a preweighed centrifuge tube so that the final volume was 1.0 ml (by weight). An aliquot (0.1 ml) was stored at −10° for later assay of DNA by a procedure, adapted especially for use in thyroid tissue (11), in which we used salmon sperm DNA as the standard. The remaining homogenate, 0.9 ml, was centrifuged for 15 min at 18,000 g, and the supernatant solution was decanted and retained. The pellet was resuspended in 0.2 ml of the homogenization buffer, and the suspension was centrifuged as before. The 18,000 g supernatants were combined, 0.1 ml was removed for immediate fucokinase assay, and the remainder was transferred to the top of a Bio-Gel P-2 column having a 27-ml bed volume. The original container and the transferring syringe were washed with 0.2 ml of the homogenization buffer and the wash was also transferred to the top of the gel column. The column, which had been preequilibrated with the homogenization buffer, was eluted with the same buffer, and 1-ml fractions were collected. After $A_{280\ nm}$ determination, the fractions containing protein were combined and concentrated to 0.2 ml in a Minicon B-15 Macrosolute Concentrator (Amicon Corporation, Lexington, Mass.). The retentate was transferred to a preweighed glass vial. The ultrafilter surface was washed with small aliquots of homogenization buffer, and the washes were combined with the retentate to

obtain a final volume of 0.45 ml (by weight). This enzyme preparation was later assayed for fucokinase activity. The rat thyroid fucokinase preparations were made on a standardized time schedule (min) such that each step was begun as follows: anesthetic injection, 0; trachea removal, 20; thyroid excision, 35; thyroid cleaning, 60; homogenization, 120; 18,000 *g* centrifugation, 150; assay of fucokinase in 18,000 *g* supernatant solution, 210; Bio-Gel P-2 gel filtration, 220; Minicon B-15 concentration, 250; assay of fucokinase in Minicon retentate, 450.

Results. It is essential in a comparison of enzyme activities between tissues from different animals that any potential effector substances that might modify enzyme activity be removed. Both positive and negative effectors of fucokinase activity have been identified among the nucleotides and nucleotide derivatives normally present in crude tissue extracts (12). Consequently it was not surprising that 18,000 *g* supernatant extracts of thyroid tissue showed a nonlinear response when assayed for fucokinase activity as a function of enzyme concentration (Fig. 1). It was necessary, therefore, to remove these or other effectors from the extracts in a rapid and consistent manner so that comparisons between tissue samples could still be drawn. This was accomplished by a molecular sieving procedure with a Bio-Gel P-2 column followed by solution concentration with an Amicon membrane concentrator (Materials and Methods). The result of this approach is seen in Fig. 1, where a linear response was observed with P-2-treated extracts when fucokinase activity was plotted as a function of enzyme concentration.

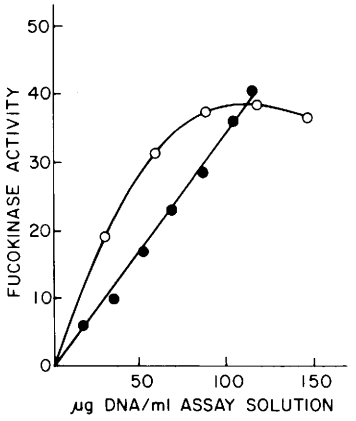

FIG. 1. Demonstration that the removal of small molecules from rat thyroid extracts provides a fucokinase preparation whose expressed activity (here as pmole of fucose-1-phosphate formed in 20 min) is a linear function of the quantity of extract used in the assay. (○) 18,000 *g* supernatant; (●) preparation treated with Bio-Gel P-2.

With these procedures, hypophysectomy appreciably reduced thyroid fucokinase activity whether the enzyme was measured directly in 18,000 *g* extracts or in preparations treated with Bio-Gel P-2 (Table I). Fucokinase levels appeared to be approximately 45–55% lower after removal of the hypophysis. Treatment of hypophysectomized animals with TSH restored thyroid fucokinase activity essentially to normal (Table I). In intact animals, treatment with TSH increased thyroid fucokinase activity significantly (Table II).

Discussion. These studies provide strong evidence that rat thyroid fucokinase is under hormonal control. The 45–55% decrease of thyroid fucokinase activity in hypophysectomized rats suggests that TSH may be responsible for maintaining appropriate fucokinase levels in the thyroid. The restoration of thyroid fucokinase activity under TSH replacement therapy in hypophysectomized rats and the response of thyroid fucokinase to TSH in intact rats pinpoint TSH as the responsible hormone. Our results parallel those of Pavlovic-Hournac *et al.* (13, 14), who observed that the absolute rate of thyroglobulin synthesis, but not of general thyroid protein synthesis, decreased by 50–60% after hypophysectomy and was restored by TSH to a nearly normal rate. Thus there is a marked correlation between changes in thyroid fucokinase activity and changes in thyroglobulin synthesis. This observation supports the hypothesis that TSH can modulate thyroglobulin synthesis by regulating the level of enzymes leading to its synthesis.

Only two pathways for the biosynthesis of GDP-fucose, the immediate precursor to the formation of fucosylated glycoproteins (15), have been described for mammalian tissues: a. GDP-D-mannose → GDP-4-keto-6-deoxy-D-mannose → GDP-L-fucose (16); b. L-fucose → β-L-fucose 1-phosphate → GDP-L-fucose (7, 17). Of the enzymes that catalyze these reactions, the only one detected and studied in thyroid tissue thus far is fucokinase

(8). It is probable that fucokinase exists in thyroid tissues in all species, where it serves to convert cellular fucose to fucose 1-phosphate. This cellular fucose might originate from exogenous sources (diet, activity of intestinal flora, release from other body tissues) or from the action of a fucosidase (18) on thyroid glycoproteins such as thyroglobulin, which contains from 13 to 26 fucose residues per molecule in all of the eight species examined (19, 20). Thus one role for thyroid fucokinase would be to act as a salvage enzyme for fucose endogenous to the thyroid. A system for the *de novo* synthesis of GDP-fucose, as found in some tissues (16), has not yet been detected in thyroid tissue, but work is under way to determine whether such a system is present and whether it contains responsive enzymatic steps.

Summary. The purpose of the present investigation was to determine whether rat thyroid fucokinase is responsive to hypophysectomy and to administration of thyroid stimulating hormone (TSH). Prior to measurements of fucokinase activity in thyroid extracts, any potential low-molecular-weight effectors were removed from the extracts by a molecular sieving procedure. With this procedure, thyroid fucokinase activity in hypophysectomized rats was found to be 55% lower than in intact rats. Injections (i.p.) of TSH into the hypophysectomized rats, however, restored the activity lost upon hypophysectomy to levels that were not significantly different from those of intact animals. Also, when intact rats were given injections (i.p.) of TSH, thyroid fucokinase activity was elevated significantly above that found in intact animals given injections of carrier only.

1. Arima, T., Spiro, M. J., and Spiro, R. G., J. Biol. Chem. **247,** 1825 (1972).
2. Tong, W., Pharmacol. Ther., Part B, **1,** 769 (1975).
3. Yamada, T., Pharmacol. Ther., Part C, **1,** 3 (1976).
4. Spiro, R. G., and Spiro, M. J., *in* "Current Topics in Thyroid Research" (C. Cassano, and M. Andreoli, eds.), p. 157. Academic Press, New York (1965).
5. Ashwell, G., and Morrell, A. G., *in* "Glycoproteins of Blood Cells and Plasma" (G. A. Jamieson, and T. Greenwalt, eds.), p. 173. Lippincott, Philadelphia (1971).
6. Remold, H. G., J. Exp. Med. **138,** 1065 (1973).
7. Ishihara, H., Massaro, D. J., and Heath, E. C., J. Biol. Chem. **243,** 1103 (1968).
8. Richards, W. L., and Serif, G. S., Biochim. Biophys. Acta **484,** 353 (1977).
9. Herscovics, A., Biochem. J. **117,** 411 (1970).
10. Lineweaver, H., and Burk, D., J. Amer. Chem. Soc. **56,** 658 (1934).
11. Wollman, S. H., and Breitman, T. R., Endocrinology **86,** 322 (1970).
12. Richards, W. L., Kilker, R. D., and Serif, G. S., J. Biol. Chem. **253,** 8359 (1978).
13. Pavlovic-Hournac, M., Rappaport, L., and Nunez, J., Endocrinology **89,** 1477 (1971).
14. Pavlovic-Hournac, M., Rappaport, L., and Nunez, J., *in* "Further Advances in Thyroid Research" (K. Fellinger and R. Hofer, eds.), p. 693. Verlag der Wiener Medizinischen Akademie, Vienna (1971).
15. Jabbal, I., and Schachter, H., J. Biol. Chem. **246,** 5154 (1971).
16. Foster, D. W., and Ginsberg, V., Biochim. Biophys. Acta **54,** 376 (1961).
17. Ishihara, H., and Heath, E. C., J. Biol. Chem. **243,** 1110 (1968).
18. Levvy, G. A., and McAllan, A., Biochem. J. **80,** 435 (1961).
19. Spiro, R. G., and Spiro, M. J., J. Biol. Chem. **240,** 997 (1965).
20. Ui, N., J. Biochem. (Tokyo) **74,** 593 (1973).

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