DISPLACEMENT OF THYROXINE FROM BINDING TO ADENOHYPOPHYSEAL PROTEINS BY THE THYREOTROPHIN-RELEASING HORMONE *in vitro*

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Extract of rat adenohypophyses in 0·1 M-NaCl was preincubated for 30 min with a solution of thyroxine-^{125}I and then for 30 min with a solution of synthetic thyreotrophin-releasing hormone (pyro-Glu-His-Pro-NH_2) or of two control tripeptides (H.Glu.Ala.Cys and H.Pro.Leu.Glu.NH_2). After precipitation with potassium ferrocyanide it was found that the radioactivity of the precipitate after incubation with TRH vas significantly lower.

Although it was found repeatedly in studies of the thyreotrophin-releasing activity of hypothalamic extracts[1,2] that the thyreotrophin-releasing hormone (TRH) affects both the release and the synthesis of thyreotrophin (TSH) most of the existing evidence indicates[3] that TRH acts mainly or solely on the release of TSH. Nothing is known about the participation of TRH in trophic and differentiation reactions of adenohypophysis on thyroidectomy. It is known that the adenohypophyseal reaction to TRH is blocked by thyroxine[4]. Recently, Pittman and coworkers[5] showed that TRH supports glucose oxidation by adenohypophysis *in vitro*, the reaction being also inhibited by thyroxine. Interaction with thyroxine at the adenohypophyseal level thus represents apparently a part of the mechanism of action of TRH. In a series of studies of adenohypophyseal hypertrophy following application of estrogens we estimated the association of thyroxine with adenohypophyseal proteins *in vitro*[6–8]. Now we decided to apply the method to determining the effect of TRH. In a previous communication we showed the inhibitory effect of TRH (0·5–4·0 μg/ml) on the association of thyroxine with adenohypophyseal protein *in vitro*[9]. The problem of specificity of this reaction remained open. The present communication contains results of tests in which TRH and two other tripeptides with different structure were investigated.

EXPERIMENTAL

In the experiments, male rats (experiment 1 and 2) and female rats (experiment 3 and 4) of the Wistar strain were used. Their initial body weight was 150–160 g and they were kept on a standard laboratory diet (Larsen) and received water *ad libitum*, at 22°C. In experiments 1 and 2, a total

of 24, in experiments 3 and 4, a total of 40 animals were used. The rats were decapitated, their adenohypophyses were excised, weighed and homogenized in 0·1M-NaCl (0·5 ml/mg tissue). The homogenates were filtered (Filtrak No 388) and stored at −18°C. After thawing and mixing, 0·6 ml aliquots were used for the estimation of proteins[10]. One ml of the extract was combined with 1 ml thyroxine containing 0·1 μg stable thyroxine (Thyroxine Roche) and 0·025 μg ^{125}I-labelled thyroxine (Radiochemical Centre, Amersham, England) and preincubated for 30 min at 21°C without shaking. Subsequently, TRH (Abbott; pyroGlu.His.Pro.NH_2) was added in experiments 1 and 2, or TRH Kabi (Kabi, Stockholm) in experiments 3 and 4, to a concentration of 0·5 μg/ml. Samples of other groups contained the same amounts of control tripeptides I and II (H.Glu.Ala.Cys; H.Pro.Leu.Glu.NH_2). After mixing, the samples were incubated for further 30 min under the same conditions and, after acidifying with acetic acid, proteins were precipitated with 2 ml 5% potassium ferrocyanide. The samples were centrifuged (4 000 r.p.m., 15 min), the sediment was washed with an equal volume of the precipitation solution, recentrifuged and its radioactivity measured in a well detector attached to the amplitude analyzer NK 108 (Gamma, Budapest). Association of thyroxine was expressed as percentage of radioactivity adsorbed by the proteins in 1 ml extract and as μg thyroxine per mg adenohypophyseal protein. Averages and 95% confidence limits were calculated and, for a statistical evaluation, variance analysis and Duncan's test were applied[11].

TABLE I

Effect of TRH and of Control Tripeptides on the Association of Thyroxine with Proteins of Rat Adenohypophysis *in Vitro*

Means ± confidence limits. Six (exp. 1,2) or ten (exp. 3,4) samples in each group. The numbers of groups with statistically different means are given in parentheses.

Experiment	Group	Thyroxine	
		%/ml	μg/mg protein
1	controls	28·77 ± 0·36 (2—4)	0·198 ± 0·000 (2—4)
	TRH	24·86 ± 0·43 (1,3,4)	0·170 ± 0·004 (1,3,4)
	peptide I	27·16 ± 1·28 (1,2)	0·186 ± 0·010 (1,2)
	peptide II	27·61 ± 0·84 (1,2)	0·190 ± 0·006 (1,2)
2	controls	29·02 ± 0·47 (2—4)	0·191 ± 0·003 (2—4)
	TRH	24·32 ± 0·48 (1,3,4)	0·161 ± 0·003 (1,3,4)
	peptide I	27·19 ± 0·35 (1,2)	0·180 ± 0·002 (1,2)
	peptide II	27·61 ± 0·74 (1,2)	0·182 ± 0·006 (1,2)
3	controls	29·10 ± 0·48 (2)	0·202 ± 0·004 (2)
	TRH	25·68 ± 0·33 (1,3,4)	0·178 ± 0·003 (1,3,4)
	peptide I	28·41 ± 0·60 (2)	0·197 ± 0·004 (2)
	peptide II	28·92 ± 0·21 (2)	0·197 ± 0·004 (2)
4	controls	28·29 ± 0·34 (2—4)	0·197 ± 0·003 (2—4)
	TRH	25·88 ± 0·36 (1,3,4)	0·180 ± 0·003 (1,3,4)
	peptide I	27·68 ± 0·28 (1,2)	0·192 ± 0·002 (1,2)
	peptide II	27·77 ± 0·26 (1,2)	0·193 ± 0·002 (1,2)

RESULTS AND DISCUSSION

The results of experiments are summarized in Table I. In all the four experiments, TRH displaced more radioactivity from the binding to proteins than did control tripeptides. An ideal result was obtained in experiment 3, only the TRH group being statistically different from the controls. The mechanism of the TRH effect is not understood. It may consist in displacing thyroxine from binding to adenohypophyseal protein or in association of thyroxine with a molecule of TRH. Since both control tripeptides were structurally different from TRH, one cannot draw conclusions on the specificity of this effect of TRH. It would be necessary to test TRH analogues with a similar structure as TRH but devoid of biological activity.

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REFERENCES

1. Schreiber V., Eckertová, A. Franc Z., Rybák M., Gregorová I., Kmentová V., Jirgl V.: Physiol. Bohemoslov. *12*, 1 (1963).
2. Sinha D. K., Meites J.: Endocrinology *78*, 1002 (1966).
3. Geschwind I. I. in the book: *Hypophysiotropic Hormones of the Hypothalamus* (J. Meites, Ed.). Williams & Wilkins, Baltimore 1970.
4. Guillemin R., Yamazaki E., Gard D. A., Jutisz M., Sakiz E.: Endocrinology *73*, 564 (1963).
5. Pittman J. A., Dubovsky E., Beschi R. J.: Biochem. Biophys. Res. Commun. *40*, 1246 (1970).
6. Schreiber V., Přibyl T., Roháčová J.: Physiol. Bohemoslov. *19*, 501 (1970).
7. Schreiber V., Přibyl T., Roháčová J.: Physiol. Bohemoslov. *19*, 511 (1970).
8. Schreiber V., Přibyl T., Roháčová J.: Sborník lékařský *73*, 49 (1971).
9. Schreiber V., Přibyl T., Roháčová J.: Sborník lékařský *74*, 26 (1972).
10. Lowry H. O., Rosebrough N. S., Farr A. L., Randall R. J.: J. Biol. Chem. *193*, 265 (1951).
11. Duncan D. B.: Biometrics *11*, 1 (1955).

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