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Demonstration of Pyroglutamylpeptidase and Amidase Activities toward Thyrotropin-releasing Hormone in Hamster Hypothalamus Extracts

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Using a radioimmunoassay method for thyrotropin-releasing hormone, the presence of thyrotropin-releasing hormone-metabolizing activity in various hamster tissues was demonstrated. While there was substantial activity degrading thyrotropin-releasing hormone in hypothalamus, there was a notable absence of such activity in pituitary. The enzymatic activity in the hypothalamus was shown to be soluble and separable into two fractions. Analysis of the metabolic products formed by the two enzymes indicated that one possessed an amidase activity (<Glu-His-Pro-NH_2→<Glu-His-Pro) and the other possessed pyroglutamylpeptidase activity (<Glu-His-Pro-NH_2→<Glu+His-Pro-NH_2). Other peptides containing NH_2-terminal pyroglutamic acid or COOH-terminal amide groups did not block the hydrolysis of thyrotropin-releasing hormone, suggesting that the enzymes were specific. Some inhibitors preferentially blocked the activity of one or the other enzymes. Of possible biological significance is the observation that thyroid-stimulating hormone inhibited the amidase activity while hydrocortisone inhibited the pyroglutamylpeptidase activity.

Thyrotropin-releasing hormone (pyroglutamylhistidyl-prolineamide, TRH)[1] has been shown to stimulate the secretion of TSH (1, 2), prolactin (3, 4), and growth hormone (5, 6) from the pituitary gland. It has also been shown to have significant effects on behavior and on mood state (7–11). For example, TRH potentiates the behavioral excitation induced by L-dopa in mice (7) and also has mood-elevating effects in normal and depressed women (8–11). In recent years numerous reports have been published showing enzymatic hydrolysis of peptide hormones such as MIF (12, 13), the kinins (14–16), angiotensins I and II (17–20), LRH (21), and oxytocin (22–24) by neural tissues. It has been suggested that such studies may help in determining the role of peptide metabolism in neural tissues (14). However, only limited data are available concerning the synthesis and the breakdown of TRH by neural tissues (25–31). In the present study we have determined the pathway of TRH metabolism in the hypothalamus. Hamster hypothalamus possesses two separable soluble enzymes which break down TRH. One of the enzymes is an amidase and the other is a pyroglutamylpeptidase.

[1] The abbreviations used are: TRH, thyrotropin-releasing hormone, <Glu-His-Pro-NH_2; TSH, thyrotropin; acid TRH, <Glu-His-Pro; substance P, Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2; MIF, melanocyte-stimulating hormone release-inhibiting factor, Pro-Leu-Gly-NH_2; LRH, luteinizing hormone-releasing hormone, <Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2; <Glu, pyrrolidone carboxylic acid; $PhCh_2SF$, phenylmethane sulfonyl fluoride; *p*Cl-HgBzO, *p*-hydroxymercuribenzoate; Tos-LysCH_2Cl, Nα-tosyl-L-lysyl chloromethane; Tos-PheCH_2Cl, Nα-tosyl-L-phenylalanyl chloromethane; BSA, bovine serum albumin; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; *L*-dopa, 3,4-dihydroxy-L-phenylalanine.

METHODS

Source of Tissue and Preparation of Crude Extract—Randomly bred adult (female, 4 to 6 weeks) Golden Syrian hamsters were decapitated and various tissues were excised. The tissues were placed in chilled 50 mM sodium citrate buffer, pH 6.6, containing 8 mM β-mercaptoethanol (Cm-buffer). The tissues were minced, then homogenized (five strokes, 3,000 rpm) with a loose fitting, motor-driven, serrated Teflon pestle in a Pyrex glass vessel (10 ml volume). The crude homogenate was centrifuged at 27,000 × *g* for 30 min and the supernatant was used for enzyme assay unless indicated otherwise.

Preparation of Antibody—Rabbit anti-BSA-TRH was produced as described elsewhere (32) except that the antigen was injected subcutaneously. The antiserum was found to be specific for TRH; a 100-fold excess of LRH, acid TRH, His-Pro, His-Pro-NH_2·2HBr, pyrrolidone carboxylic acid, His-Pro, MIF, substance P, tetragastrin, Pro-NH_2, histidine, proline, or glutamic acid did not reduce the binding of TRH to antibody when tested at approximately the equivalence point for TRH·antibody complex formation.

Assay for TRH Metabolism—The assay mixture (25 μl) for TRH-metabolizing activity contained: sodium citrate buffer, pH 6.6, 50 mM; 2-mercaptoethanol, 7 mM; [*Pro*-^{3}H]TRH (40 mCi/μmol), 25 nM; and 5 to 15 μl of enzyme. The reaction was run at 37° in a tube (10 × 75 mm) and stopped by immersing the tube in a boiling water bath for 5 min. The amount of [*Pro*-^{3}H]TRH remaining was determined by radioimmunoassay. The tubes were cooled and then 122.5 μl of chilled phosphate-buffered saline (0.1 M potassium phosphate, pH 7.2 and 0.14 M NaCl) and 2.5 μl of antiserum was added. The reaction mixtures were incubated for 30 min at 0°, then filtered through Millipore filters (type HA, 0.45 μ, Millipore Corp., Mass.). The filters were washed with 10 ml of chilled phosphate-buffered saline, then soaked in 1 ml of 3%

ammonia. The eluted radioactivity was determined by scintillation counting in 10 ml of Triton X-100/toluene/Liquifluor (6/12/1). The amount of antibody used in this assay was sufficient to bind all the antigen. The specific activity is defined as units per mg of protein. One unit is that amount of enzyme which will catalyze the degradation of 1 fmol of TRH/min.

Thin Layer Chromatography—TRH and its metabolites were separated by thin layer chromatography on silica gel (Eastman Kodak No 6061) plates in two solvent systems: Solvent 1, $CHCl_3/CH_3OH$/99.7% CH_3COOH (3/2/1); and Solvent 4, $CHCl_3/CH_3OH$ (1/1).

Materials—the bacterial <Glu-peptidase was purified from *Aerobacter cloacae* up to the Sephadex G-100 stage as described by Doolittle and Armentrout (33). Substance P, TRH, MIF, and LRH were purchased from Beckman Instruments, Inc. The acid TRH was a product of Bachem, Calif. His-Pro-NH_2·2HBr and His-Pro were kindly provided by A. O. Geiszler of Abbott Laboratories. All other chemicals, hormones, and trypsin inhibitors were purchased from Sigma Chemical Co. The preparation of bovine TSH (Sigma) used had a biological activity of 1 IU/mg of dry material.

RESULTS

TRH-metabolizing Activity in Various Tissue Extracts—The results of a tissue survey of TRH-metabolizing activity are shown in Table I. In all tissues tested, the metabolizing activity showed a linear response to time and enzyme concentration. The highest specific activities were observed in heart, kidney, hypothalamus, and brain. Somewhat lower specific activities were seen in lung, diaphragm, eye, and skeletal muscle. The serum activity was quite low (2 to 10% of the tissue level). However, since the amount of total protein in serum is much greater than in any of the tissues tested, the total TRH-degrading activity in serum is relatively substantial.

While hypothalamus is a major source of TRH, the hypothalamo-hypophyseal portal system transports TRH to the pituitary, a site of TRH action. In contrast to the substantial level of TRH-degrading activity in hypothalamus, pituitary contains no detectable activity (Fig. 1). Since pituitary extract did not inhibit the hypothalamic degrading activity (Fig. 1), the inability to detect TRH-degrading activity in pituitary was probably not due to the presence of an inhibitor.

In order to examine the products of TRH metabolism, [^{3}H]TRH was incubated for 30 min with the supernatant fraction from various tissues. The different products were separated by thin layer chromatography as described in the legend to Fig. 2. The chromatograms suggested that lung extracts and serum converted TRH to acid TRH (Fig. 2, *top panel*). The data also indicated that proline was the major product of TRH metabolism by diaphragm, skeletal muscle, kidney, liver, brain, and heart (Fig. 2, *last three panels*). However, hypothalamic extracts produced proline and acid TRH in almost equal proportions and, in addition, some His-Pro or His-Pro-NH_2[2] (Fig. 4*A*) suggesting the presence of multiple TRH-metabolizing enzymes. His-Pro or His-Pro-NH_2 appeared to be only a minor fraction of the metabolic products in all other tissues and serum.

Fractionation of Hypothalamic TRH-metabolizing Activity by Differential Centrifugation—Table II shows the subcellular distribution of TRH-metabolizing activity in hypothalamic extract. About 75% of the activity in the crude extract was associated with the 27,000 × *g* supernatant. The activity associated with the membrane and mitochondrial fraction was less than 15%. Therefore in subsequent experiments the 27,000 × *g* supernatant from hypothalamus was used as the enzyme source.

Separation of Two TRH-metabolizing Activities—The analysis of the products of TRH metabolism by hypothalamic extract (Fig. 4*A*) showed the formation of proline, acid TRH, and His-Pro or His-Pro-NH_2. The multiplicity of products suggested the presence of more than one TRH-metabolizing enzyme in hypothalamus.

Sephadex G-100 chromatography of a 27,000 × *g* supernatant from hypothalamic extract revealed the presence of two

TABLE I

TRH degradation by extracts of various organs of Golden Syrian hamster

TRH-degrading activity was determined as described under "Methods." The results (average three to five determinations) are expressed as mean ±S.E.

Organ	Specific activity
	fmol TRH degraded/min/mg protein
Eye	151
Heart	325 ± 47
Lung	182 ± 26
Kidney	481 ± 25
Diaphragm	162 ± 63
Liver	261 ± 5
Skeletal muscle	90 ± 32
Hypothalamus	503 ± 55
Whole brain minus hypothalamus	524 ± 81
Serum	9.6 ± 4.5

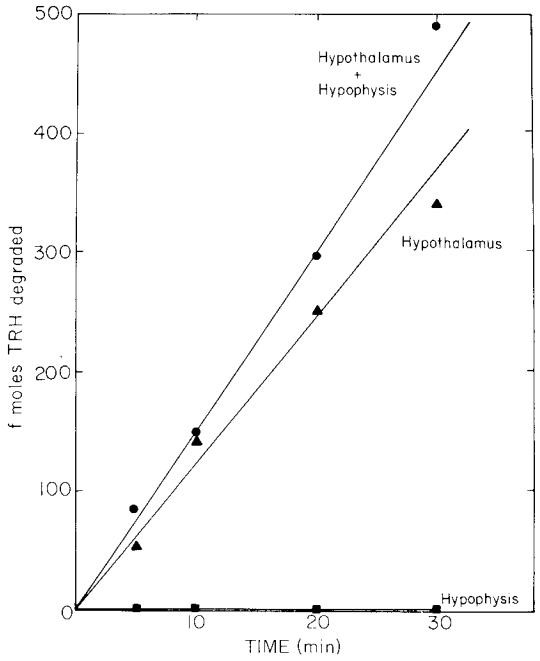

FIG. 1. Absence of TRH-degrading activity in hypophysis. TRH degradation was assayed as described under "Methods." Twenty-five micrograms of crude extract protein (hypothalamus or hypophysis) was used in each assay. The assay containing a mixture of hypothalamus and hypophysis contained 25 μg of each preparation.

[2] His-Pro-NH_2 nonenzymatically cyclizes to give His-Pro and ammonia (A. O. Geiszler, Abbott Laboratories, and R. Makineni, Bachem, Inc.; personal communication). The thin layer chromatography (silica gel, see "Methods") of authentic His-Pro, His-Pro-NH_2·2HBr and the product of TRH degradation by *Aerobacter cloacae* <Glu-peptidase in Solvents 1 and 4 gave R_F values of 0.66, 0.47, 0.67, and 0.77, 0.48, 0.76, respectively. Therefore, all the chromatographic data indicated as His-Pro-NH_2 actually are the properties of the diketopiperazine derived from His-Pro-NH_2 (His-Pro).

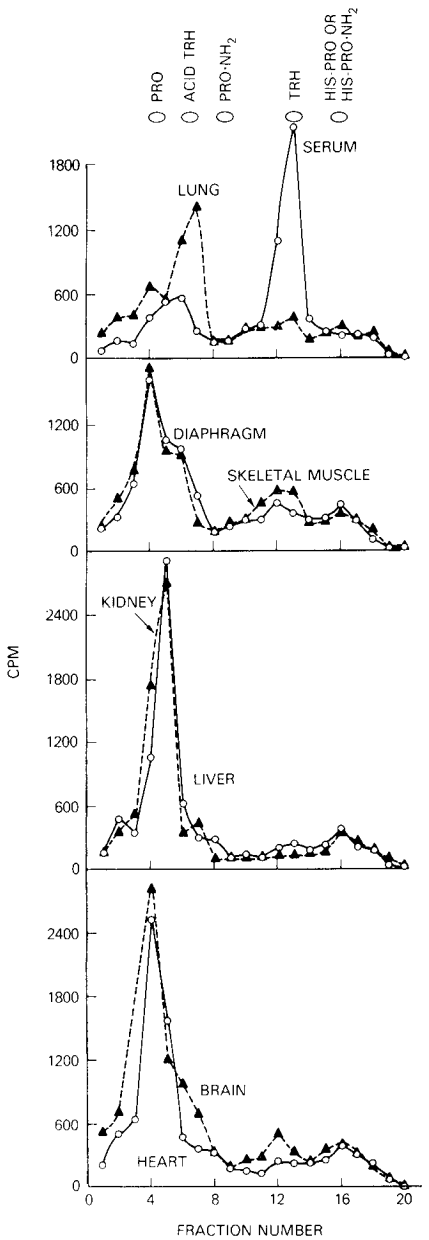

FIG. 2. Thin layer chromatographic analysis of the products of TRH inactivation. The extracts from different tissues were made as described under "Methods." Radioactive TRH was incubated with 5 μl of tissue extract (micrograms of protein: heart, 100; lung, 150; kidney, 100; diaphragm, 55; liver, 175; muscle, 60; hypothalamus, 10; and brain, 60), in a total volume of 25 μl, for 30 min at 37°. The reaction was terminated by boiling for 5 min. The reaction products were separated by thin layer chromatography in Solvent 4 (see "Methods"). The distribution of radioactivity on thin layer chromatography plates was determined by depositing 0.25-inch segments into scintillation vials, incubating with 1 ml of 90% MeOH for 15 min at room temperature and then counting by scintillation counting. The chromatographic profile of different standard compounds is shown at the *top* of the figure.

separable peaks of activity responsible for TRH metabolism (Fig. 3). To avoid cross-contamination, fractions from the ascending limb of Peak I and the descending limb of Peak II were pooled and used to determine the nature of the Peak I and II activities.

TABLE II

Subcellular localization of TRH-degrading activity in Golden Syrian hamster hypothalamus

Freshly excised hypothalami were homogenized as described under "Methods," centrifuged at 600 × *g* for 10 min, and the supernatant (crude extract) was collected. The crude extract was centrifuged at 10,000 × *g* for 10 min and the pellet was collected. The 10,000 × *g* supernatant was centrifuged at 27,000 × *g* for 30 min and the pellet was collected. Each pellet fraction was washed once before suspending in a small volume of extraction buffer (50 mM sodium citrate, pH 6.6 + 7 mM 2-mercaptoethanol) and the designated fractions were assayed for TRH-degrading activity as described under "Methods."

Cell fraction	Volume	Total protein	Specific activity	Total activity
	ml	*mg*	*fmol TRH inactivated/min/mg protein*	*units*
Crude extract	4.3	37.41	330	12,350
10,000 × *g* pellet	1.4	9.66	90	870
27,000 × *g* pellet	0.5	3.45	160	550
27,000 × *g* supernatant	2.9	16.24	540	8,770
% recovery		78.4		82.5

The products of TRH metabolism by Fraction I and II are shown in Fig. 4. The chromatogram of the Fraction I product (Fig. 4*B*) in Solvent 4 showed the presence of acid TRH as the sole reaction product. Therefore, Fraction I contains an enzyme that deamidates TRH (TRH-amidase). The chromatogram of the Fraction II product (Fig. 4*B*) in Solvent 4 showed a single new peak of radioactivity which co-migrated with His-Pro or His-Pro-NH_2. However, in Solvent 1 (Fig. 4*C*), this new peak of radioactivity co-chromatographed with the reaction product of TRH degradation by *Aerobacter aerogenes* <Glu-peptidase (His-Pro-NH_2). Therefore, Fraction II contains an enzyme that degrades TRH to pyroglutamic acid and His-Pro-NH_2 (<Glu-peptidase).

Effect of pH and Various Buffers on TRH-amidase and TRH-<Glu-peptidase Activity—Fig. 5 shows the results of studies on the effect of various buffers and pH values on both enzyme activities. The data do not allow a complete description of the pH optimum for either enzyme. However, it is clear that the two enzymes can be differentiated on the basis of the ion effects seen here. For TRH-amidase, assay at pH 7 in maleate buffer results in higher activity than in either Tris, Hepes, or phosphate buffers. In contrast, for TRH-<Glu-peptidase activity, assay at pH 7 in maleate buffer results in lower activity than either Tris, Hepes, or phosphate buffer. However, assay in citrate buffer (pH 6.6) results in almost maximum activity for both enzymes and was therefore adopted as standard.

Inhibitors of TRH-amidase and TRH-<Glu-peptidase Activities—A number of possible competitive inhibitors (substrate analogs) of TRH metabolism were examined for their effects on [^{3}H]TRH degradation (Table IIIA). The presence of high concentrations of a number of peptides containing COOH-terminal amide (LRH, Substance P, tetragastrin, etc.) or NH_2-terminal <Glu (LRH, acid TRH, <Glu-Ala) resulted in inhibition of the metabolism of [^{3}H]TRH. Since the concentration required for significant inhibition of TRH metabolism by these peptides was several hundredfold higher than the substrate concentration, the enzymes appear to be relatively specific for TRH. However, since not all the known

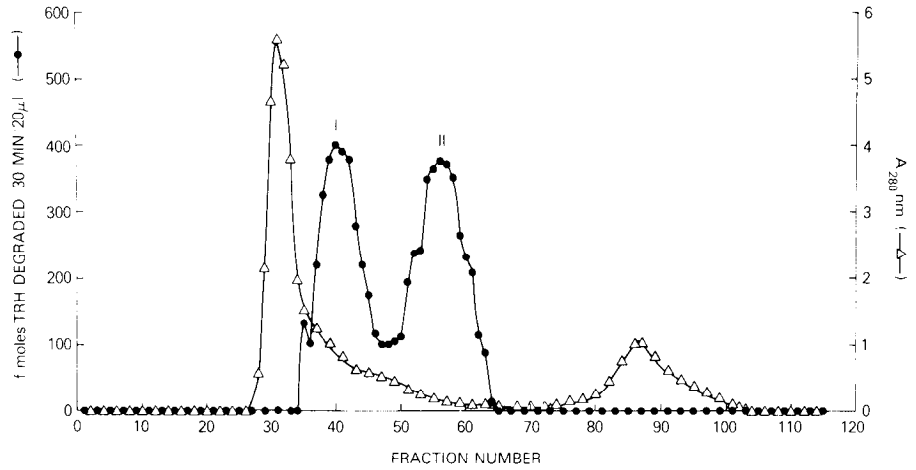

FIG. 3. The elution from Sephadex G-100 of TRH-degrading activity in crude hypothalamic extract. The crude extract was prepared from 16 freshly excised hamster hypothalami as described under "Methods." Five milliliters (50.5 mg of protein) of this extract was loaded on a Sephadex G-100 column (69 × 1.6 cm) equilibrated with Cm-buffer. The column was eluted with the same buffer at 4° and 1.4-ml fractions were collected. The protein concentration was measured by the absorbance at 280 nm and the TRH-degrading activity was assayed as described under "Methods."

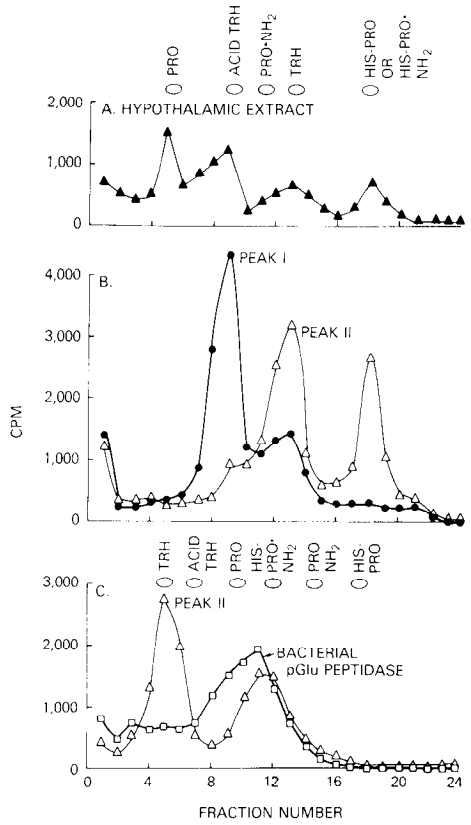

FIG. 4. Analysis by thin layer chromatography of the products of TRH degradation by Sephadex Fractions I and II. The incubation mixtures for degradation of TRH was prepared as described in Fig. 2 and contained 20, 10, 8, and 50 μg of protein for crude hypothalamic extract, Fraction I, Fraction II, and bacterial <Glu-peptidase, respectively. Chromatography was carried out in Solvent 4 for *Panels A* and *B* and in Solvent 1 for *Panel C* (see "Methods").

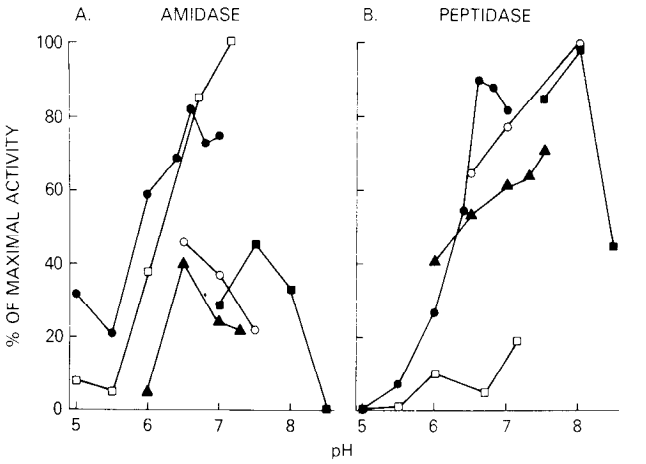

FIG. 5. pH activity curves for TRH degradation by TRH-amidase and TRH-<Glu-peptidase. TRH degradation reaction mixtures were prepared as described under "Methods" except that various buffers were used. For radioimmunoassay, 0.5 M potassium phosphate buffer, pH 7.3, was substituted for buffered saline in the standard assay. Sodium-citrate, ●——●; sodium-phosphate, ▲——▲; Tris-HCl, ■——■; sodium-maleate, □——□; and Hepes, ○——○.

brain peptides were tested, it is possible that one of them or some yet undiscovered peptide is a substrate for either enzyme. <Glu-His-Pro, the product of TRH-amidase action on TRH was tested as an inhibitor. Since a 1500-fold excess of this compound showed no inhibition of TRH amidase, it appears that there is no product inhibition of this enzyme. Several compounds previously shown to be inhibitors of proteolytic enzymes or peptidases were tested for their effects on TRH-amidase and TRH-<Glu-peptidase (Table IIIB). The trypsin inhibitors from soy bean and ovomucoid as well as pancreatic trypsin inhibitor and Tos-LysCH_2Cl, showed preferential inhibition of the TRH-amidase, whereas Tos-PheCH_2Cl, benzamidine, and $CoCl_2$ preferentially inhibited the TRH-<Glu-peptidase. A variety of heavy metals, tested at 10 mM ($NiCl_2$, $FeCl_2$, $CuCl_2$, $ZnCl_2$, $CdCl_2$, $CrCl_2$, and $Al(NO_3)_3$) inhibited both activities. None of the metals tested activated either enzyme and EDTA (10 mM) did not affect the activity. Iodoacetamide (1 mM) substantially inhibited both activities.

Some hormones and antimetabolites were also tested as potential inhibitors of the two activities. Thyroxin (1 mM) and reserpine (0.1 mM) showed no effect on either enzyme. Fig. 6 shows the concentration dependence for inhibition of TRH-amidase and TRH-<Glu-peptidase by bovine TSH and hydrocortisone. TSH inhibited TRH-amidase but not TRH-<Glu-peptidase; the dose-response curve was hyperbolic. However, hydrocortisone inhibited only the TRH-<Glu-peptidase activity; in this case, the dose-response curve was sigmoidal.

TABLE III

Effect of various agents on TRH-amidase and TRH-<Glu-peptidase activities

TRH-amidase and TRH-<Glu-peptidase activities were assayed as described under "Methods." In Part A, substrate and inhibitor were added together and the reaction was initiated by the addition of enzyme. In Part B, enzyme and inhibitor were incubated together for 10 min at 37° and the reaction was initiated by addition of substrate.

A. Amino acids and peptides

Inhibitor	Concentration	Inhibitor/substrate ratio	Per cent inhibition	
			TRH-amidase[a]	TRH<Glu-peptidase[b]
	μM			
LRH	24	900	55.0	15.5
Substance P	16	600	95.0	—[c]
Tetragastrin	40	1,500	37.0	—
<Glu-His-Pro	40	1,500	0	61.0
<Glu-Ala	24	900	—	44.0
Pyrrolidone carboxylic acid	40	1,500	—	38.0
Histidine	40	1,500	0	52.5
Glutamic acid	2,750	11×10^6	47.5	—

B. Peptidase inhibitors, metals, and metal chelators

Inhibitor	Concentration	Per cent inhibition	
		TRH-amidase[d]	TRH-<Glu-peptidase
	μM		
Ovomucoid	1.0[e]	90.0	0
Soy bean trypsin inhibitor	0.2[e]	100.0	0
Pancreatic trypsin inhibitor	400[f]	100.0	15.5
Tos-LysCH_2Cl	0.01	55.5	—
Tos-LysCH_2Cl	1.0	—	53.5
Tos-PheCH_2Cl	0.001	—	60.0
Tos-PheCH_2Cl	0.1	58.0	—
Benzamidine	5,000	0	98.0
EDTA	10,000	0	0
$CoCl_2$	10,000	0	52.0

[a] Concentrations of 40 μM MIF, Pro-Phe-NH_2, Phe-Leu-NH_2, Pro-NH_2, His-Pro, and proline showed no inhibition.

[b] Concentrations of 40 μM Pro-NH_2, His-Pro, and proline gave no inhibition.

[c] — signifies inhibition not tested.

[d] Inhibition was also observed with the trypsin inhibitors from beef pancreas and lima bean (0.2 mg/ml) as well as with 8-hydroxyquinoline (5 mM), 1,10-phenanthroline (0.1 mM), $PhCH_2SF$ (1 mM), and $HgCl_2$ (0.8 mM).

[e] Milligrams per ml.

[f] Kallikrein inactivating units per ml.

DISCUSSION

Previous studies indicated that incubation of TRH with rat, porcine, bovine, human, and hamster serum (34) as well as porcine hypothalamus (31) abolished biological and immunological activity. Digestion of samples of TRH with pepsin, subtilisin, Nagarse, trypsin, chymotrypsin, carboxypeptidase B, and aminopeptidase did not affect biological activity (35, 36).

Various workers have studied the effect of thyroid status of animals on TRH-metabolizing activity in plasma. While

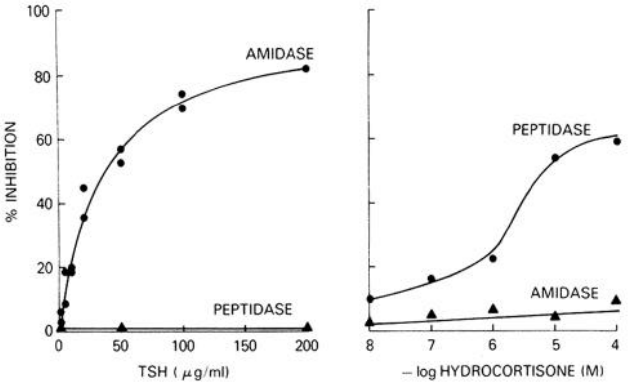

FIG. 6. Differential inhibition by thyrotropic hormone and hydrocortisone of TRH degradation by TRH-amidase and TRH-<Glu-peptidase. Standard reaction mixtures for TRH degradation (see "Methods") contained amidase (10 μg of protein) or peptidase (8 μg of protein) and the designated concentrations of TSH or hydrocortisone. Replicate reaction mixtures were terminated at 0, 5, 10, and 15 min by boiling. Rates of TRH degradation were calculated from these kinetic data. In the absence of added inhibitor the rates of TRH degradation by amidase and peptidase were 30 and 24 fmol/min, respectively.

Redding and Schally (37) reported that the TRH-inactivating activity of rat plasma was dependent on the thyroid status of the donor animal, these observations were not confirmed by Vale *et al.* (38). In a recent study, Bassiri *et al.* (39) have shown that there was no significant difference in serum TRH-inactivating activity between normal individuals and patients suffering from hyperthyroidism or hypothyroidism and hypopituitarism. Also, there was no effect of triiodothyronine or thyroxin *in vivo* or *in vitro* on TRH inactivation by human serum. Our studies indicate that thyroxin does not inhibit either TRH-amidase or TRH-<Glu-peptidase.

There are a number of studies in the literature that are difficult to rationalize with the concept that TRH serves as a common regulator of the release of TSH, growth hormone, and prolactin. Administration of apomorphine (40), 2-Br-ergocryptin (41), steroid hormones (42) or drugs modifying metabolism of biogenic amines (43), and chronic L-dopa therapy in Parkinson's disease (44) can lead to differential relative changes in the levels of one or more of these three pituitary hormones. Exposure of rat pituitary cells (GH_3) in culture to TRH leads to increased prolactin and decreased growth hormone secretion (45).

We propose that these results might be explained by a model in which the regulation of different pituitary hormones is controlled by different metabolites of TRH, such as acid TRH and His-Pro-NH_2. Such a model would attribute some regulatory significance to the TRH-metabolizing enzymes described in this report. The inhibition seen by TSH of TRH-amidase (catalyzing the synthesis of acid TRH) and by hydrocortisone of TRH-<Glu-peptidase (catalyzing the synthesis of His-Pro-NH_2) (Fig. 6) provides some basis for a serious consideration of a regulatory role of these enzymes. In this context, it should be pointed out that the organ distribution of the enzymes is widespread (Fig. 2), but that the regulatory action of either enzyme may be tissue-localized. Typical plasma levels of hydrocortisone are approximately 10 times lower than necessary to see inhibition of TRH-<Glu-peptidase; however, there may be a tissue that contains higher than plasma levels of hydrocortisone and a hormone-sensitive TRH-<Glu-peptidase

activity. The inhibition of TRH-amidase by TSH is especially noteworthy. While the pituitary may contain the highest levels of TSH, since it is the site of hormone synthesis, enzymes for TRH degradation are absent from pituitary (Fig. 1). Further, plasma levels of TSH are at least 100-fold lower than necessary to produce substantial inhibition of hypothalamic TRH-amidase. Although a substantial concentration of the TRH synthesized in hypothalamus may be transported to the pituitary via the hypothalamo-hypophyseal portal system, it is clear that the pituitary is not a site for TRH degradation and the portal blood is not subject to a hormonal regulation of TRH degradation. The significance of the hormone effects on these enzymes therefore remains to be evaluated.

Acknowledgments—We thank Dr. Jonas Galper for his valuable advice during the course of preparation of this manuscript and Dr. A. O. Geiszler (Abbott Laboratories) for the supply of authentic His-Pro-$NH_2 \cdot 2HBr$, and His-Pro. Ms. Peggy U. Veazey provided excellent assistance in preparation of the manuscript.

Note Added in Proof—Recently, a paper (46) has appeared describing experiments in which the administration of 3,3',5-triiodothyronine to rats led to an increase in serum TRH-inactivating activity. In view of the observations presented here that TSH inhibits TRH-amidase and the established fact that thyroid hormone inhibits the synthesis and release of thyrotropin (47), the possibility should be considered that the thyroid hormone effects on TRH degradation reported by Bauer may be mediated via TSH.

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