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METABOLITES OF THYROTROPIN RELEASING HORMONE INHIBIT ANGIOTENSIN CONVERTING ENZYME IN VITRO

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SUMMARY: Pyroglutamylhistidylproline and histidylproline, reported metabolites of thyrotropin releasing hormone, were found to competitively inhibit purified rabbit lung angiotensin converting enzyme with K_I values of 0.76 μM and 1.7mM, respectively. Native thyrotropin releasing hormone and histidylprolinediketopiperazine at concentrations of 10 mM and 5 mM, respectively, had no effect on angiotensin converting enzyme activity. Neither the native hormone nor its deamidated derivative served as substrate for angiotensin converting enzyme.

Angiotensin converting enzyme (peptidyl dipeptide hydrolase, EC 3.4.15.1) catalyzes two key reactions in the process of blood pressure regulation; the conversion of angiotensin I to the powerful vasoconstrictor angiotensin II, and the inactivation of bradykinin, a vasodilator (for a review see ref. 1). The combination of these two reactions produces a strong vasopressor response (1). The most important physiological site for these reactions is thought to be the luminal surface of pulmonary vascular endothelial cells (1). Angiotensin converting enzyme is inhibited by a wide variety of peptides and peptide derivatives (for a review see ref. 2). Oligopeptides with proline at the COOH-terminus, a hydrophobic or basic residue in the penultimate position, and a pyroglutamyl residue at the NH_2-terminus were found

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Abbreviations: TRH, thyrotropin releasing hormone; TRH-FA, pyroglutamylhistidylproline (deamidated TRH); Hispro, histidylproline; Cyclohispro, histidylprolinediketopiperazine; Hiphisleu, hippurylhistidylleucine; HPLC, high pressure liquid chromatography; K_I, equilibrium constant for binding of enzyme to inhibitor.

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to be especially potent inhibitors (2). Several peptide-derived angiotensin converting enzyme inhibitors are used clinically in the treatment of human hypertension (3).

Thyrotropin releasing hormone, a tripeptide produced in the hypothalamus which stimulates the release of thyroid-stimulating hormone from the anterior pituitary, is used diagnostically to evaluate patients with disorders of the hypothalamo-pituitary system (4). TRH has been shown to increase mean arterial blood pressure in normotensive rats and in rats with experimentally induced hypovolemic or endotoxic shock (5). These effects were attributed to the action of TRH as an antagonist of certain opiate-mediated events (5). In contrast, diastolic blood pressure was found to decrease or remain unchanged in hyperthyroid human patients following injections of TRH (6). Hypothyroid and euthyroid patients, however showed increased diastolic blood pressures after receiving injections of TRH (6). At least two alternative pathways exist for the catabolism of TRH: One produces TRH-FA by deamidation of the COOH-terminal proline of TRH (7,8), the other involves hydrolysis of the NH_2-terminal glutamyl residue with subsequent cyclization of the histidylprolineamide to form histidylprolinediketopiperazine (9). It is possible that the different blood pressure responses observed following the injection of TRH into hyperthyroid as compared to euthyroid and hypothyroid patients (6) may be due to the employment of different catabolic pathways for TRH as a function of thyroid status (10,11). In view of the observed cardiovascular effects produced by TRH injections in rats and humans, and because of the close structural similarity between metabolites of TRH and inhibitors of angiotensin converting enzyme we have tested TRH and several of its known metabolites to determine their activity as inhibitors of angiotensin converting enzyme. In the present communication we report that the deamidated TRH derivative, pyroglutamylhistidylproline, is a potent converting enzyme inhibitor.

MATERIALS AND METHODS

Purified angiotensin converting enzyme from rabbit lung, prepared by the method of Das and Soffer (12), was generously provided by Dr. R.L. Soffer of this department. Enzyme activity was assayed by spectrophotometric measurement of hippuric acid liberated from the synthetic substrate hippurylhistidylleucine (Research Plus, Bayonne, New Jersey) as described by Cushman and Cheung (13). One unit of enzyme activity is defined as the amount required to catalyze the release of

one μmole of hippuric acid per minute at 37°C. Enzyme used in these studies had a specific activity of 92 units/mg of protein. Protein concentrations were determined using the procedure described by Lowry et al. (14) standardized with bovine serum albumin. Plots of kinetic data were constructed according to the method described by Segel (15). High pressure liquid chromatography was performed using a Waters model 6000A solvent delivery system and a model 660 solvent programmer. An LKB 2138 Uvicord S equipped with a 206 nm filter was used for detection of peptides and amino acids. Specific conditions for HPLC experiments are described in the figure legend. TRH and TRH-FA were from Beckman Instruments Inc., Palo Alto, Calif. Cyclohispro and Hispro were from Chemical Dynamics Corp., South Plainfield, New Jersey.

RESULTS AND DISCUSSION

Pyroglutamylhistidylproline, a deamidated metabolite of TRH (16,17), was found to be a potent inhibitor of angiotensin converting enzyme, with a K_I of 0.76 μM (Table I, and Fig. 1C and 1D). This is comparable to the inhibition produced by Teprotide (SQ 20,881) a nonapeptide used in the diagnosis and treatment of some forms of hypertension in humans (18,19). The strong inhibition produced with TRH-FA is consistent with its structure: An oligopeptide with a cyclized NH_2-terminal glutamyl residue, a basic histidyl residue in the penultimate position, and a proline at the COOH-terminus. Histidylproline was about 2000-fold less effective as an inhibitor than TRH-FA, with a K_I of 1.7 mM (Table I, Fig. 1A and 1B). The graphical analysis presented in Fig. 1 demonstrates that the inhibition produced by TRH-FA and Hispro is competitive in nature. Native TRH which contains an amide group on the COOH-terminal proline, and the TRH metabolite Cyclohispro (9,16) had no effect on converting enzyme activity at concentrations of 10 mM and 5 mM, respectively (Table I).

β-lipotropin has been reported to inhibit angiotensin converting enzyme with a K_I of 0.78 μM (20) a potency comparable to that of TRH-FA. Competitive inhibition of converting enzyme by other endogenous peptides, including methionine enkephalin, leucine enkephalin and β-endorphin, has also been reported (21) but the K_I values for

TABLE I

INHIBITION OF ANGIOTENSIN CONVERTING ENZYME BY METABOLITES OF THYROTROPIN RELEASING HORMONE

Peptide	K_I
Thyrotropin releasing hormone	No inhibition at 10 mM
Deamidated TRH	0.76 μM
Hispro	1700 μM
Cyclohispro	No inhibition at 5 mM

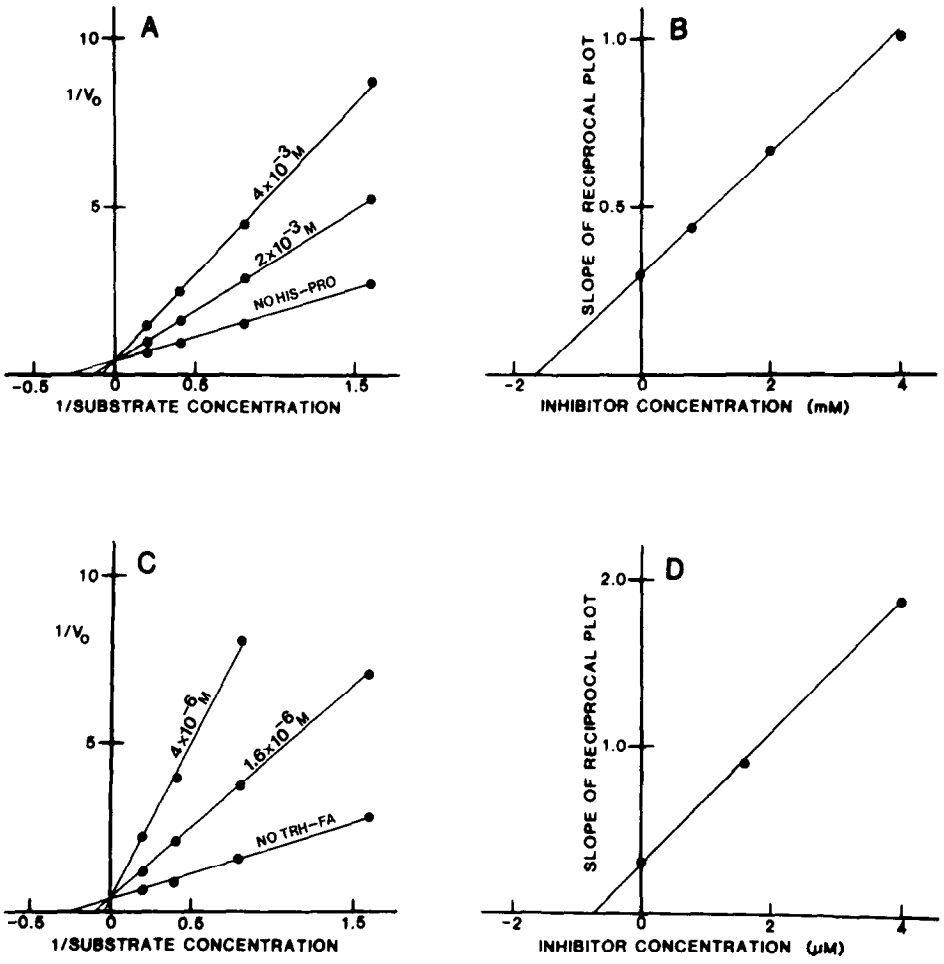

Fig. 1: Inhibition of rabbit lung angiotensin converting enzyme by histidylproline (A and B) and TRH-FA (C and D). A. Enzyme activity was assayed in the presence of 1 mM (not shown), 2 mM and 4 mM Hispro, and in the absence of Hispro, as indicated in the figure. Substrate (Hiphisleu) concentrations were: 5.0, 2.5, 1.25, and 0.63 mM. The double-reciprocal plot was constructed as described by Segel (15). B. The data in A are replotted as indicated. The K_I may be read as the absolute value of the intercept of the abscissa (1.7 mM). C. As in A except Hispro is replaced by 1.6 μM and 4 μM TRH-FA. D. The data in C are replotted as indicated. The K_I (determined as in B) is 0.76 μM.

these peptides were found to be 40 to 200-fold greater than for TRH-FA. Recently Verma et al. have reported that peptides derived from ACTH act as potent non-competitive inhibitors of angiotensin converting enzyme in partially purified preparations from dog lung (22).

Mixtures of TRH, TRH-FA, Hispro and Cyclohispro could be separated rapidly by HPLC (Fig. 2). This provided a convenient assay to test whether TRH or TRH-FA could serve as a substrate for angiotensin converting enzyme. In assays conducted at 37°C

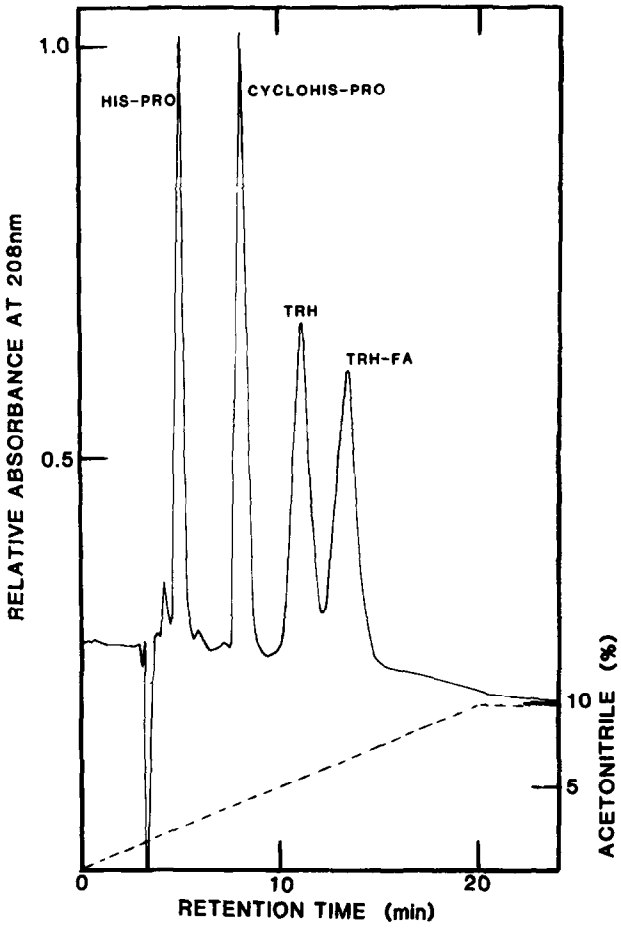

Fig. 2: Separation of Hispro, Cyclohispro, TRH and TRH-FA by high pressure liquid chromatography. A total of 8 nanomoles of each of the four peptides was injected in a volume of 20 µl onto a C-18 column (Waters Associates, Milford, Mass., µBondapak C-18, 3.9 x 300 mm). Elution was accomplished at a flow rate of 60 ml/hr using a CH_3CN gradient formed by mixing 0.1% trifluoroacetic acid with increasing proportions of 100% CH_3CN, to a final CH_3CN concentration of 10%. The gradient is shown as a broken line.

in 0.1 M potassium phosphate buffer, pH 8.3 or pH 7.5, containing 30 mM NaCl, no TRH or TRH-FA was consumed and no new products were detected after incubation of each peptide with converting enzyme.

Although the clinical significance of inhibition of angiotensin converting enzyme by TRH-FA requires further investigation, it is important to note that an amidase in serum capable of converting TRH to TRH-FA has been identified (7,8). Furthermore, we have observed a decrease in serum angiotensin converting enzyme activity following injection of TRH into human patients (23). The results presented in this communication provide a possible explanation for this observed decrease in activity, namely that angiotensin converting enzyme is inhibited by TRH-FA.

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