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Novel activity of human angiotensin I converting enzyme: Release of the NH_2- and COOH-terminal tripeptides from the luteinizing hormone-releasing hormone

(kininase II/chloride activation/tripeptidyl aminopeptidase/tripeptidyl carboxypeptidase)

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ABSTRACT **Angiotensin I converting enzyme (ACE; kininase II; peptidyldipeptide hydrolase, EC 3.4.15.1) cleaves COOH-terminal dipeptides from active peptides containing a free COOH terminus. We investigated the hydrolysis of luteinizing hormone-releasing hormone (LH-RH) by homogeneous human ACE. Although this decapeptide is blocked at both the NH_2 and COOH termini, it was metabolized to several peptides, which were separated by HPLC and identified by amino acid analysis. A major product was the NH_2-terminal tripeptide, $\overset{1}{\text{<Glu}}\text{-}\overset{2}{\text{His}}\text{-}\overset{3}{\text{Trp}}$, and another was LH-RH-(4–10) heptapeptide, indicating that the $\overset{3}{\text{Trp}}\text{-}\overset{4}{\text{Ser}}$ bond is cleaved to release the NH_2-terminal tripeptide. ACE also released the COOH-terminal tripeptide, $\overset{8}{\text{Arg}}\text{-}\overset{9}{\text{Pro}}\text{-}\overset{10}{\text{Gly}}\text{-}NH_2$, and then sequentially the dipeptides $\overset{6}{\text{Gly}}\text{-}\overset{7}{\text{Leu}}$ and $\overset{4}{\text{Ser}}\text{-}\overset{5}{\text{Tyr}}$, leaving <Glu-His-Trp intact. Thus, <Glu-His-Trp was formed by both NH_2- and COOH-terminal hydrolysis. The cleavage of LH-RH was inhibited by specific ACE inhibitors and by antibody to ACE but not by inhibitors of other enzymes, showing that the hydrolysis was indeed due to ACE. In the absence of chloride, the hydrolysis proceeded at only 16% of the maximal rate (in 500 mM NaCl), but in 10 mM NaCl it increased to 64%. In 500 mM NaCl solution, 86% of the hydrolysis was accounted for by the release of the NH_2-terminal tripeptide, whereas in 10 mM NaCl, the COOH-terminal and NH_2-terminal cleavage occurred about equally. The K_m of LH-RH in 500 mM NaCl was 167 μM and the catalytic constant k_{cat} was 210 min^{-1}. When the NH_2-terminal pyroglutamic acid was replaced with glutamic acid ([Glu^1]LH-RH), ACE liberated almost exclusively the COOH-terminal tripeptide in 10 mM NaCl. Thus, human ACE, although it is named peptidyl dipeptidase or dipeptidyl carboxypeptidase, can cleave a protected peptide at the NH_2 or COOH terminus. The enzyme could be involved in the *in vivo* metabolism of LH-RH and possibly other blocked peptides.**

Angiotensin converting enzyme (ACE) or kininase II is distributed throughout the body, including the central nervous system (1–4). The enzyme was originally named for its ability to convert angiotensin I to angiotensin II (5) or to inactivate bradykinin (6, 7). After extensive studies with a variety of peptide substrates, it was concluded that ACE cleaves mainly COOH-terminal dipeptides from oligopeptides with a free COOH terminus (8, 9). Thus, it was named dipeptidyl carboxypeptidase, or, more correctly, peptidyldipeptide hydrolase (EC 3.4.15.1). However, the results of some recent studies do not entirely support this thesis. For instance, we found that human ACE cleaves the tridecapeptide neurotensin (10, 11), which is longer than kinins or angiotensin I, the naturally occurring substrates. ACE also cleaves the penultimate peptide bond of substrates in which the last amino acid is replaced by nitrobenzylamine (12). It releases the COOH-terminal tripeptide from substrates with a penultimate proline and an antepenultimate serine or alanine—e.g., des-Arg^9-bradykinin (13). We reported recently that human ACE cleaves the COOH-terminal tripeptide from substance P (10, 11), a peptide with a blocked COOH-terminal amino acid. Similar results were observed by others with rat brain (14) or rabbit lung (15) ACE.

Because the decapeptide luteinizing hormone-releasing hormone (LH-RH) also has a blocked COOH-terminal amino acid (and a blocked NH_2 terminus as well) and is in tissues known to contain ACE (2, 16), we wondered if it is a substrate for human ACE. We found that ACE released the protected NH_2-terminal tripeptide of LH-RH, in addition to the COOH-terminal tripeptide. In high (0.5 M) NaCl solution, the NH_2-terminal hydrolysis was the main route of metabolism of LH-RH *in vitro* by human ACE.

MATERIALS AND METHODS

Chemicals. LH-RH, [Glu^1]LH-RH, the NH_2-terminal tripeptide $\overset{1}{\text{<Glu}}\text{-}\overset{2}{\text{His}}\text{-}\overset{3}{\text{Trp}}$, and LH-RH-(4–10) heptapeptide were purchased from Vega Biochemicals, Tucson, AZ. (*S*)-[*N*-(1-Carboxy-3-phenylpropyl)alanyl]proline (designated MK-422) was a gift from R. Mumford of Merck. Trifluoroacetic acid and premixed HCl/propionic acid, 50:50 (vol/vol), were "sequenal grade" from Pierce. *N*-(α-rhamnopyranosyloxyhydroxyphosphinyl)leucyltryptophan (phosphoramidon) was from Peninsula Laboratories, Belmont, CA. (*S*)-*N*-(3-Mercapto-2-D-methylpropanoyl)proline (designated captopril) was donated by Z. Horovitz, of Squibb.

Enzyme Purification. ACE was solubilized from a membrane fraction of human kidney with trypsin and purified by DEAE-cellulose and hydroxylapatite chromatography, followed by reverse immunoadsorption chromatography (17). The preparation was homogeneous as determined by NaDodSO$_4$/polyacrylamide gel electrophoresis with Coomassie blue staining; however, when the more sensitive silver staining technique (18) was used, minor contaminant bands became evident in the stored preparation. Thus, an additional step of purification was included. Highly purified fractions of ACE were obtained by gel filtration on a 0.75 × 30 cm TSK-G3000SW column (Varian) in an HPLC system previously described (19). The column was run at room temperature with 0.07 M sodium phosphate buffer (pH 7.2) at a flow rate of 0.3 ml/min, and fractions (0.15 ml) were collected. ACE was eluted at 19.7 min, and the first three fractions containing pure enzyme (as determined by NaDodSO$_4$/poly-

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Abbreviations: LH-RH, luteinizing hormone-releasing hormone; ACE, angiotensin I converting enzyme.

acrylamide gel electrophoresis with silver staining) were stored in 50% glycerol at −20°C.

Enzyme Assays. Hydrolysis of LH-RH and [Glu[1]]LH-RH by human ACE was measured by HPLC. The optimum pH for hydrolysis of LH-RH was found to be 8.0. For routine assays, ACE (25–100 ng) was incubated with substrate (0.1 mM) and 0.05 M Hepes (pH 8.0) containing either 10 or 500 mM NaCl in a final volume of 0.1 ml. Reactions were run at 37°C for 20–300 min and were stopped with 20 μl of 5% trifluoroacetic acid. Inhibitors, if any, were preincubated with enzyme for 30 min at 4°C. Antisera to human ACE, raised in rabbits (17), and preimmune serum were heated at 56°C for 30 min and preincubated with ACE at a 1:1000 dilution for 24–62 hr at 4°C. For kinetic studies, ACE (150 ng) was incubated with LH-RH at 10 different substrate concentrations (ranging from 1 to 200 μM) in the buffer containing 500 mM NaCl.

Aliquots (40 μl) of each reaction were analyzed in a Waters automated gradient HPLC system with a μBondapak C_{18} reverse-phase column (19). The products were separated with a gradient of increasing concentrations of CH_3CN/0.035% trifluoroacetic acid (solvent B) in H_2O/0.035% trifluoroacetic acid (solvent A). The concentrations changed linearly from 98% A and 2% B to 64% A and 36% B in 26 min. Peptide products were detected by absorbance at 214 nm. Product formation or substrate degradation was quantitated by comparing the integrated peak area to the area of a known amount of authentic standard. For determination of kinetic constants, data were plotted according to Hanes (as described in ref. 20) and fit to the best straight line by linear regression. Correlation coefficients of $r = 0.98$ or better were obtained.

Amino Acid Analysis. Products of LH-RH hydrolysis were collected after separation by HPLC and lyophilized. Peptides were hydrolyzed at 160°C for 15 min in HCl/propionic acid, 50:50 (vol/vol) (21). Acid was removed by lyophilization, and the amino acids were resuspended in 200 μl of saturated sodium borate (pH 9.5). Amino acids were analyzed by HPLC after derivatization with *o*-phthaldialdehyde as reported (22).

UV Spectroscopy. The UV spectrum of <Glu-His-Trp was determined in a Varian DMS 100 spectrophotometer equipped with a computer (Varian DS-15 data station).

RESULTS

Hydrolysis of LH-RH by Human ACE. Incubation of homogeneous human ACE with LH-RH produced several peptide products as determined by HPLC (Fig. 1). The released peptides were separated, collected from the column, and identified by amino acid analysis (Table 1). The major product (peak 5 in Fig. 1) was the NH_2-terminal tripeptide, <Glu-His-Trp. Amino acid analysis showed only the presence of glutamic acid and histidine in this peptide because tryptophan was destroyed by the acid hydrolysis (21). The identity of peak 5 as <Glu-His-Trp was confirmed by two other methods. Synthetic <Glu-His-Trp used as the standard was coeluted with peak 5 from the HPLC column (not shown). In addition, the absorption of peak 5 in the UV range (Fig. 2) indicated the presence of tryptophan, and the spectrum was identical with that of synthetic <Glu-His-Trp (Fig. 2). Another prominent product of LH-RH hydrolysis was peak 4, which was identified as LH-RH-(4–10) heptapeptide (Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2). This was proved by both amino acid analysis (Table 1) and by coelution of peak 4 with authentic LH-RH-(4–10) (not shown). Therefore, LH-RH-(4–10) arose from the hydrolysis of the peptide bond between Trp-3 and Ser-4 to release the NH_2-terminal tripeptide and the COOH-terminal heptapeptide.

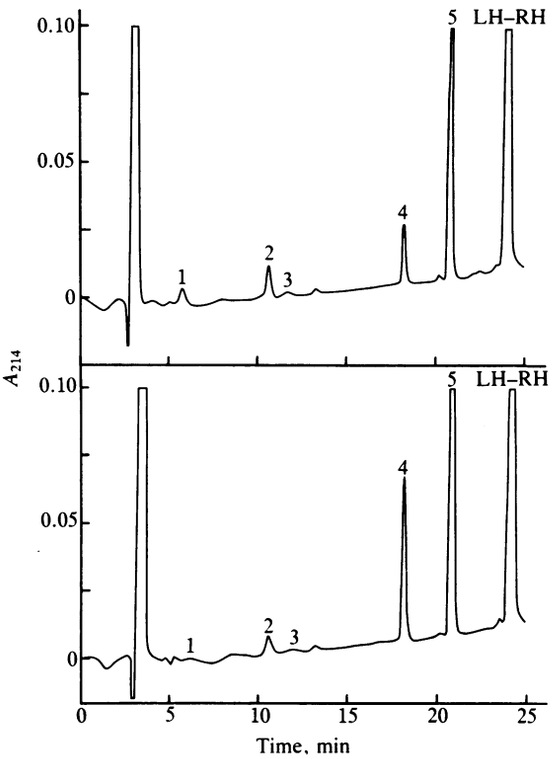

FIG. 1. HPLC analysis of the products of cleavage of LH-RH by homogeneous human ACE. ACE (24.5 ng) was incubated at 37°C with 0.1 mM LH-RH in the presence of 10 mM (*Upper*) or 500 mM (*Lower*) NaCl. Because the overall hydrolysis rate is 1.56 times faster in 500 mM NaCl than in 10 mM NaCl (see Table 3), the reaction in 10 mM NaCl (*Upper*) was run 1.56 times longer (282 min) than the reaction in 500 mM NaCl (180 min) to achieve equal degradation of LH-RH for comparison of the products. Products were separated by HPLC as described. Numbered peaks are products that were identified by amino acid analysis (see Table 1).

Identification of the other peptide products revealed a sec-

Table 1. Amino acid analysis of the products of LH-RH hydrolysis by human ACE

Peak no.	Relative quantities of amino acids found*							Peptide sequence identified
	Glu	His	Ser	Tyr	Gly	Leu	Arg	
1	—	—	—	—	1.00	—	1.00	Arg-Pro-Gly-NH_2†
2	—	—	0.82	1.00	—	—	—	Ser-Tyr
3	—	—	—	—	1.07	1.00	—	Gly-Leu
4	—	—	0.74	0.89	1.88	1.00	1.00	Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2†
5	1.00	0.92	—	—	—	—	—	<Glu-His-Trp‡

*Results are the average of two to five separate experiments consisting of hydrolysis of LH-RH by ACE, collection of the peaks from the HPLC, and amino acid analysis.
†Proline is not detected by the *o*-phthaldialdehyde derivatization used (22).
‡Tryptophan was detected prior to acid hydrolysis spectrophotometrically (see Fig. 2).

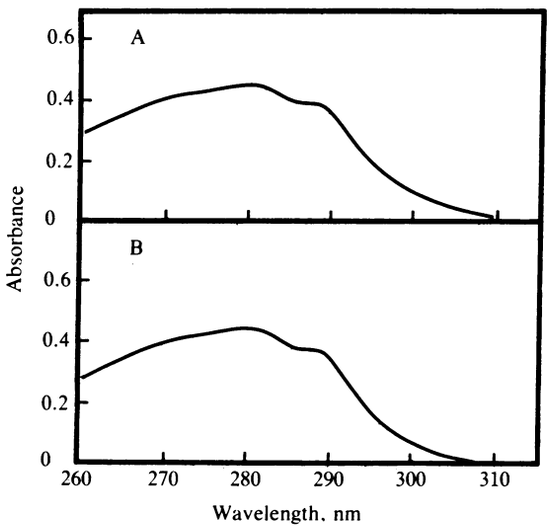

FIG. 2. Ultraviolet spectra of authentic <Glu-His-Trp (A) and peak 5 collected from the HPLC column (B). For details, see text.

ond hydrolysis pathway that also can result in the appearance of NH_2-terminal tripeptide (Fig. 3). Peak 1 was identified as $\overset{8}{\text{Arg}}\text{-}\overset{9}{\text{Pro}}\text{-}\overset{10}{\text{Gly}}\text{-}NH_2$, peak 2 as $\overset{4}{\text{Ser}}\text{-}\overset{5}{\text{Tyr}}$, and peak 3 as $\overset{6}{\text{Gly}}\text{-}\overset{7}{\text{Leu}}$ (Table 1). These results indicate that ACE first cleaves at the bond between Leu-7 and Arg-8 to release the COOH-terminal tripeptide and then sequentially removes the dipeptides Gly-Leu at positions 6 and 7 and Ser-Tyr at positions 4 and 5, leaving the NH_2-terminal tripeptide, <Glu-His-Trp. This is similar to the pattern of cleavage of substance P by ACE (11), another peptide with an amidated COOH-terminal amino acid.

While hydrolysis at the N-terminal end was totally unexpected, it was not due to a trace contaminant in the preparation, as shown by the effects of NaCl and specific inhibitors on the hydrolysis (Table 2). In the absence of chloride ion, a known activator of ACE (23), the rate of total LH-RH degradation was only 16% of that seen in the presence of 0.5 M NaCl (Table 2). The release of all products from LH-RH was inhibited 97% by MK-422 (10 nM) and 98% by captopril (100 nM), specific inhibitors of ACE (24). Inhibitors of neutral endopeptidase 24.11 (25–27), aminopeptidases, catheptic enzymes, and serine proteases (i.e., phosphoramidon, bestatin, *p*-chloromercuriphenylsulfonate, and diisopropyl fluorophosphate, respectively) were ineffective (Table 2). Antiserum to ACE [1:1000 (vol/vol) dilution] also inhibited the reaction (67%), whereas preimmune serum did not.

Effect of Sodium Chloride on LH-RH Hydrolysis. The rate of hydrolysis of LH-RH in the absence of chloride ion was slow (Table 2). When NaCl was added to 10 mM, it increased to 64% of the optimal rate established in 500 mM NaCl (Table 3). However, the concentration of salt altered the cleavage pattern as proven by analysis of the products obtained in the presence of 10 mM and 500 mM NaCl (Fig. 1 and Table 3). In 10 mM NaCl, peaks 1, 2, and 3 were higher and peak 4 was lower than in 500 mM NaCl (Fig. 1). Because peaks 1, 2, and 3 were derived from the COOH-terminal cleavage and peak 4 was derived from the NH_2-terminal cleavage (Fig. 3), high salt concentrations obviously favor the liberation of the NH_2-terminal tripeptide. The amount of LH-RH-(4–10) (peak 4) produced was used to quantitate the NH_2-terminal hydrolysis because in control experiments authentic LH-RH-(4–10) was not cleaved by ACE in 0, 10, or 500 mM NaCl (not shown). In 10 mM NaCl, the amount of LH-RH-(4–10) formed was 43% of the total substrate hydrolyzed, and this increased to 86% in the presence of 500 mM NaCl (Table 3). Thus, at low salt concentration, the cleavage of the COOH-terminal tripeptide (Arg-Pro-Gly-NH_2) is preferred, while in high salt concentration the hydrolysis is due almost exclusively to cleavage of the NH_2-terminal tripeptide (<Glu-His-Trp). The importance of the protected NH_2 terminus for the NH_2-terminal hydrolysis was shown in the following experiments. When the LH-RH derivative containing a free NH_2-terminal glutamic acid ([Glu^1]LH-RH) was the substrate, the rate of cleavage at the NH_2-terminal end was very low. In 10 mM NaCl, the rate of NH_2-terminal tripeptide release was only 1.4% of the overall rate of [Glu^1]LH-RH hydrolysis, and this increased only to 11% in 500 mM NaCl (Table 3).

Kinetics of LH-RH Hydrolysis by ACE. To determine the kinetic constants of the NH_2-terminal tripeptide hydrolysis of LH-RH by ACE, studies were carried out in 500 mM NaCl. The turnover number (k_{cat}) for LH-RH with ACE was 210 min^{-1}, comparing favorably with that of substance P (11)

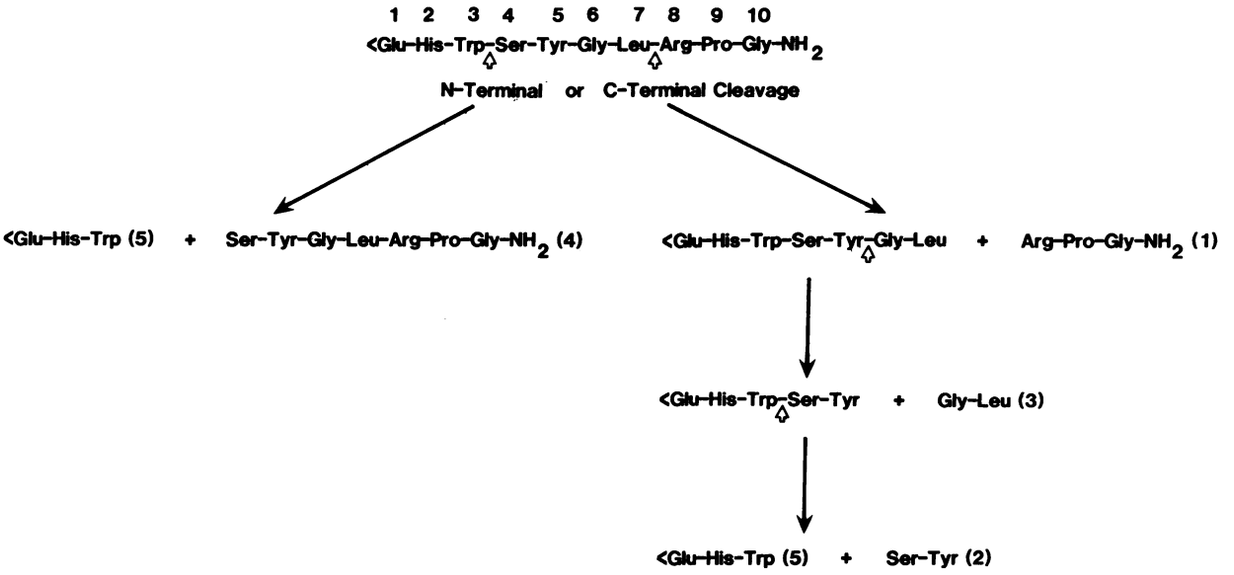

FIG. 3. Hydrolysis of LH-RH by human ACE. ⇩, Sites of cleavage by ACE. Numbers in parentheses refer to the peak number on the HPLC tracing (see Fig. 1).

Table 2. Inhibition of hydrolysis of LH-RH

Inhibitor*	Molarity	NaCl†	Activity, %
None	—	+	100
None	—	−	16
MK-422	1×10^{-8}	+	3
Captopril‡	1×10^{-7}	+	2
Phosphoramidon	1×10^{-6}	+	95
Bestatin	1×10^{-5}	+	95
PCMS	1×10^{-4}	+	98
iPr_2P-F	1×10^{-3}	+	87

*Inhibitors were preincubated with enzyme for 30 min at 4°C then substrate was added to initiate the reaction. PCMS, *p*-chloromercuriphenylsulfonate; iPr_2P-F, diisopropyl fluorophosphate.
†Reactions were conducted in the presence (+) or absence (−) of 0.5 M NaCl.
‡NaCl = 0.24 M.

(Table 4). The K_m was 167 μM, higher than that of substance P, but much lower than the K_m of [Leu^5]enkephalin or hippurylhistidylleucine (28) (Table 4).

DISCUSSION

ACE of human kidney (10, 11), rabbit lung (15), and rat brain (14) inactivates substance P, a peptide with an amidated COOH-terminal amino acid, by releasing the COOH-terminal tripeptide, $\overset{9}{\text{Gly}}$-$\overset{10}{\text{Leu}}$-$\overset{11}{\text{Met}}$-NH_2. In the present study we showed that LH-RH, which also contains a blocked COOH-terminal amino acid, was cleaved by human ACE at the bond between Leu-7 and Arg-8 to release the COOH-terminal tripeptide, $\overset{8}{\text{Arg}}$-$\overset{9}{\text{Pro}}$-$\overset{10}{\text{Gly}}$-NH_2, similar to the results found with substance P. ACE also hydrolyzed the bond between Trp-3 and Ser-4 to release the NH_2-terminal tripeptide, <Glu-His-Trp. This finding was quite unexpected as ACE usually cleaves dipeptides from the COOH terminus of peptide substrates with free COOH-terminal amino acids (8, 9) and has not been shown before to cleave at the NH_2 terminus. Peptide substrates with free NH_2-terminal amino acids are not cleaved at the NH_2 terminus by ACE to any significant degree probably because of the positively charged free amino group, which would be repelled by the positively charged arginine in the active center (11, 29). The presence of the NH_2-terminal pyroglutamic acid in LH-RH must allow the NH_2 terminus to more readily interact with the active center. Indeed, when the LH-RH derivative containing a free NH_2-terminal glutamic acid ([Glu^1]LH-RH) was the substrate, the NH_2-terminal hydrolysis was greatly reduced. However, since 11% of the enzymatic hydrolysis of [Glu^1]-LH-RH still proceeded at the NH_2-terminal end in high-salt solution (0.5 M), other molecular interactions can also be important.

ACE requires chloride ion for optimal activity with the

Table 3. Effect of NaCl on LH-RH hydrolysis by human ACE

Substrate	NaCl, mM	Products formed, nmol/min per mg of enzyme: $\overset{1}{\text{<Glu}}$-$\overset{2}{\text{His}}$-$\overset{3}{\text{Trp}}$	LH-RH-(4–10)	Total LH-RH cleaved
LH-RH	10	290 ± 29	158 ± 13	369 ± 69
	500	537 ± 26	498 ± 21	580 ± 57
[Glu^1]LH-RH	10	—	2.5 ± 0.3	173 ± 8
	500	—	16 ± 2	141 ± 15

ACE was incubated with substrate (0.1 mM) in the presence of either 10 or 500 mM NaCl, and the products were analyzed by HPLC. Results are the average ± SEM of three to seven determinations.

Table 4. Hydrolysis of LH-RH and other peptide substrates by ACE

Substrate	K_m, μM	k_{cat}, min^{-1}	k_{cat}/K_m ($\mu M^{-1} \cdot min^{-1}$)
LH-RH*	167 ± 14	210 ± 6	1.3 ± 0.1
Substance P†	25	225	9.0
[Leu^5]Enkephalin‡	1000	700	0.7
Hip-His-Leu‡	1300	6800	3.6

*Results are the average ± SEM of three separate determinations.
†From Skidgel *et al.* (11).
‡From Stewart *et al.* (28); Hip, hippuryl.

majority of peptide substrates (23). However, this phenomenon depends on the peptide substrate being cleaved. For example, ACE hydrolyzes bradykinin in the absence of additional chloride ions (23), and kinin hydrolysis is maximally enhanced by a low (20 mM) concentration of chloride ion (30). The other major natural substrate, angiotensin I, is not cleaved in the absence of chloride (23) and requires 200 mM NaCl for optimal activity (30). Because the primary hydrolysis of LH-RH took place at two different peptide bonds, it was of interest to determine the effect of chloride ions on these actions. In 10 mM NaCl, the overall LH-RH hydrolysis rate was 64% of the maximum achieved (in 0.5 M NaCl), and the substrate was hydrolyzed about equally at the two termini. At high salt concentration (0.5 M NaCl), however, the hydrolysis pattern was shifted and favored NH_2-terminal cleavage, as 86% of the hydrolysis was due to the release of the NH_2-terminal tripeptide. Thus, the COOH-terminal tripeptide cleavage was optimal at low chloride ion concentrations, as found with substance P and human ACE, where liberation of the COOH-terminal tripeptide was near maximal in 10 mM NaCl (11).

The mechanism for ACE activation by chloride ion has not been elucidated completely, but it has been hypothesized to involve anion binding to one or two essential lysine residues (31, 32), which could alter the ionic character of the active site and/or the conformation of ACE (33). These ionic or conformational alterations could enhance binding of the NH_2-terminus of LH-RH to the active site of ACE.

The hydrolysis of LH-RH by ACE probably involves only one active site as indicated by the following. First, when the NH_2-terminal hydrolysis was stimulated with high salt, the hydrolysis at the COOH-terminal end decreased. Second, MK-422 and captopril, active site-directed inhibitors of ACE (24), inhibited both the COOH-terminal and NH_2-terminal hydrolysis of LH-RH. Finally, at high chloride ion concentration, LH-RH inhibited the hydrolysis of a short synthetic substrate of ACE, furylacryloyl-Ala-Phe-Gly-Gly (unpublished data).

LH-RH acts on the pituitary to promote the release of luteinizing hormone and follicle-stimulating hormone (16). Cell bodies containing LH-RH project axons to the median eminence, which allows LH-RH to be released into the hypophyseal portal capillaries (16). Enzymes that could inactivate LH-RH (and thereby regulate its levels) are presumably localized at the site of release or action of LH-RH (i.e., median eminence and pituitary). The pituitary has been shown to contain at least three enzymes that can hydrolyze LH-RH: a pyroglutamate aminopeptidase, the post-proline-cleaving enzyme, and an "LH-RH endopeptidase" that cleaves at the bond between Tyr-5 and Gly-6 (34–38). The major products of LH-RH degradation by extracts of the rat median eminence were shown to be LH-RH-(1–5), LH-RH-(6–10), and <Glu-His-Trp (38). The first two products resulted from cleavage of the bond between Tyr-5 and Gly-6 by the "LH-RH endopeptidase," but the source of the <Glu-His-Trp NH_2-terminal tripeptide was not identified (38). Because <Glu-His-Trp is the major product of LH-RH hydrolysis by

ACE and the enzyme is known to be present in the median eminence (2), it is possible that ACE is involved in the metabolism of LH-RH *in vivo*. Although the K_m for LH-RH (167 μM) is higher than that of other substrates of ACE and, therefore, might be considered unfavorable for significant *in vivo* hydrolysis, it is similar to the value of 190 μM obtained with the "LH-RH endopeptidase" (35–38). In fact, the K_m for overall LH-RH degradation in tissue extracts of the median eminence was 177 μM (38). In addition, kinetic constants established with the soluble enzyme do not necessarily reflect those of the membrane-bound enzyme *in vivo* (2).

While ACE could function centrally to inactivate LH-RH, it also may metabolize LH-RH at peripheral sites. LH-RH and LH-RH-like peptides have been found in seminal plasma (39), testis (40), and placenta (41–44)—sites that contain high levels of ACE (45, 46). Renal ACE, which is localized in the proximal tubular brush border (47–49), may inactivate circulating LH-RH. Previous studies have shown kidney homogenate (50) and renal proximal tubules *in vitro* (51) or *in vivo* (52) to readily metabolize LH-RH. A major metabolite of LH-RH in the above experiments was the NH_2-terminal <Glu-His-Trp (50–52), which is also the major product of LH-RH degradation by ACE found in the present study.

The physiological significance of these findings remains to be explored. However, it is possible that administration of ACE inhibitors, used clinically as antihypertensive drugs (53), can affect the degradation of LH-RH as well as other biologically active peptides. Thus, some of the actions of ACE inhibitors *in vivo* could be due to inhibition of the hydrolysis of peptides other than angiotensin I or bradykinin.

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