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Peptide inhibitors of pulmonary angiotensin I converting enzyme

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SUMMARY

A number of peptides have been found to inhibit a particulate angiotensin I converting enzyme preparation from rabbit lung; bradykinin-potentiating peptide C, bradykinin, and methionyl-lysyl-bradykinin inhibit competitively. The latter two peptides are hydrolyzed by the converting enzyme preparation; bradykinin-potentiating peptide C is not. Thus, it is possible that although bradykinin and methionyl-lysyl-bradykinin do compete with angiotensin I for the active site of the converting enzyme, they may not be hydrolyzed while bound to this enzyme. The converting enzyme activity is Cl^- dependent; bradykininase is independent of the presence of Cl^-; therefore, there does seem to be some distinction between converting enzyme activity and bradykininase activity.

Studies on the angiotensin I converting enzyme in the lung have revealed a high level of activity for this enzyme[1-3]. It has also been suggested that this enzyme in lung may play a role in bradykinin metabolism[2]. Yang and co-workers[4, 5] have reported the isolation of an enzyme preparation from hog lung, plasma, and kidney cortex (kininase II or dipeptide hydrolase) which not only inactivates bradykinin, ostensibly by releasing the C-terminal dipeptide phenylalanyl-arginine, but converts angiotensin I to angiotensin II by removing the dipeptide histidyl-leucine. They have suggested that these two activities are due to one enzyme. In an effort to clarify the interrelationship between converting enzyme activity and bradykininase activity, we have studied the effects of bradykinin, certain bradykinin analogues, bradykinin-potentiating peptide C (ref. 6), certain synthetic substrates, and several polypeptide hormones on a membrane-bound angiotensin I converting enzyme preparation from rabbit lung[7].

Converting enzyme was isolated from rabbit lung using the methodology previously described from this laboratory[7]. The 25 000 $\times g$ pellet was resuspended in 0.25 M sucrose-1 mM $MgSO_4$ and this fraction was used for converting enzyme and bradykininase assays. Angiotensin I converting enzyme was assayed on the isolated rabbit

aorta[8] and bradykininase on the isolated guinea pig ileum[9]. Angiotensins I and II, bradykinin, methionyl-lysyl-bradykinin, hippuryl-glycyl-glycine, and bradykinin-potentiating factor were obtained from Schwarz-Mann, Orangeburg, N.Y. Histidyl-leucine (His-Leu) and phenylalanine-arginine (Phe-Arg) were products of Cyclo Chemicals, Los Angeles, Calif. All hormones used were purchased from Sigma Chemical Co., St. Louis, Mo. All-D-bradykinin was furnished by Dr. John Stewart, University of Colorado School of Medicine, Boulder, Colo. Hippuryl-histidyl-leucine was synthesized in our laboratory; it showed one spot on paper chromatography. Bradykinin-potentiating peptide C was obtained from the Institute for Protein Research, Osaka, Japan. Bradykinin-potentiating peptide C is an undecapeptide (PCA-Gly-Leu-Pro-Pro-Gly-Pro-Pro-Ile-Pro-Pro), isolated from the venom of the Japanese poisonous snake *Agkistrodon halys blomhoffii*, which has been shown to inhibit the converting enzyme from dog lung[6]. An additional sample of hippuryl-glycyl-glycine was obtained by courtesy of Dr. F. Dorer, VA Hospital, Cleveland, Ohio.

Cushman and Cheung[10] have reported that hippuryl-histidyl-leucine serves as a substrate for the angiotensin I converting enzyme. Yang and co-workers[4, 5] have used hippuryl-glycyl-glycine to assay kininase II; Dorer has used this latter tripeptide to assay

TABLE I

INHIBITORS OF ANGIOTENSIN I CONVERTING ENZYME

Incubation mixtures contained 6 nmoles of angiotensin I, 0.1 ml enzyme solution, the indicated volume of inhibitor, and 0.2 M phosphate buffer (1% NaCl), pH 7.4, to a final volume of 1 ml. Enzyme solution and buffer were preincubated for 3 min; angiotensin I was added and allowed to react for 6 min (at 37°). At this time an aliquot was removed and assayed on the isolated rabbit aorta. Inhibitor was included in the preincubation mixture except in those cases indicated by an asterisk, in which the inhibitor and angiotensin I were added simultaneously. This was done where destruction of the inhibitor during the preincubation period was considered as possibly significant.

Inhibitor	*Concentration (M)*	*Inhibition (%)*
His-Leu	$1 \cdot 10^{-3}$	30
	$5 \cdot 10^{-3}$	72
Phe-Arg	$1 \cdot 10^{-3}$	43
	$5 \cdot 10^{-3}$	93
Hippuryl-histidyl-leucine	$1 \cdot 10^{-3}$	63
	$5 \cdot 10^{-3}$	100
Hippuryl-glycyl-glycine	$1 \cdot 10^{-3}$	0
	$5 \cdot 10^{-3}$	18
Bradykinin	$7.9 \cdot 10^{-6}$	53*
	$1.6 \cdot 10^{-5}$	74*
Methionyl-lysyl-bradykinin	$1.7 \cdot 10^{-5}$	41*
	$3.8 \cdot 10^{-5}$	68*
Bradykinin-potentiating peptide C	$1.9 \cdot 10^{-5}$	47
	$3.8 \cdot 10^{-5}$	61

converting enzyme (personal communication). If these synthetic substrates are true converting enzyme substrates, then, when added to a converting enzyme incubation mixture containing angiotensin I, they should inhibit the conversion of angiotensin I to angiotensin II. Yang and co-workers[4, 5] have shown that preincubation of kininase II with hippuryl-histidyl-leucine produces inhibition of both the bradykininase and converting enzyme activity of that enzyme.

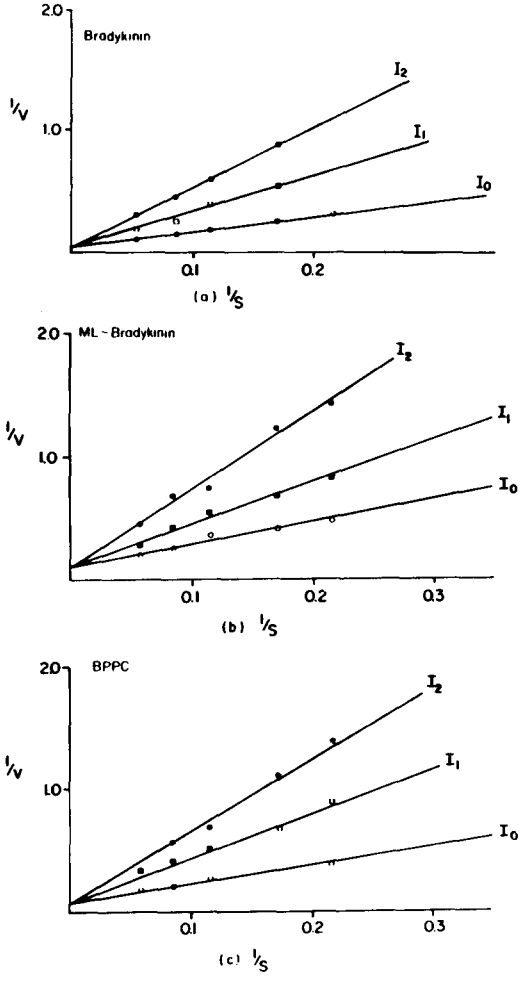

Fig. 1. The effects of various concentrations of bradykinin, methionyl-lysyl-bradykinin and bradykinin-potentiating peptide C on angiotensin I converting enzyme. Lineweaver-Burk representations of the effects of varying substrate (S) and inhibitor (I) concentrations on the rate of angiotensin II formation (v), using (a) bradykinin: I_1 = 8 nmoles, I_2 = 16 nmoles; (b) methionyl-lysyl-bradykinin (ML-bradykinin): I_1 = 17 nmoles, I_2 = 38 nmoles; (c) bradykinin-potentiating peptide C (BPPC): I_1 = 19 nmoles, I_2 = 38 nmoles. The incubation conditions are as described in Table I. v is expressed as μg angiotensin II formed/ml incubation mixture and S as nmoles angiotensin I added/ml incubation mixture. In all three instances the common intersection occurs on the $1/v$ axis, indicating competitive inhibition.

As can be seen in Table I, both hippuryl-histidyl-leucine and hippuryl-glycyl-glycine show inhibition of the converting enzyme, but at concentrations far in excess of those of angiotensin I. Hippuryl-histidyl-leucine is the stronger inhibitor, showing inhibition at $5 \cdot 10^{-4}$ M, and completely inhibiting at $5 \cdot 10^{-3}$ M. Hippuryl-glycyl-glycine concentrations of $5 \cdot 10^{-3}$ M are required before any inhibition can be demonstrated. The greater activity of hippuryl-histidyl-leucine over hippuryl-glycyl-glycine may be explained in terms of the greater structural similarity of hippuryl-histidyl-leucine to angiotensin I, since both of the latter molecules end with the dipeptide His-Leu.

The cleavage products of the converting enzyme and bradykininase (dipeptide hydrolase), His-Leu and Phe-Arg, respectively, at concentrations 500 times greater than that of angiotensin I were shown to inhibit the converting enzyme (Table I); Phe-Arg was the more potent inhibitor. Both of these peptides have also been shown to inhibit the dipeptide hydrolase activity at similar concentrations[4, 5]. Bradykinin, methionyl-lysyl-bradykinin, and bradykinin-potentiating peptide C all strongly inhibited the lung converting enzyme at concentrations slightly in excess of that of angiotensin I. The Lineweaver-Burk plots for converting enzyme activity in the presence of bradykinin (Fig. 1a), methionyl-lysyl-bradykinin (Fig. 1b), and bradykinin-potentiating peptide C (Fig. 1c) are indicative of competitive inhibition, suggesting that these peptides do compete with angiotensin I for the active site of the converting enzyme. Bakhle[11] and Freer and Stewart[12] have reported that the pentapeptide bradykinin-potentiating factor also competitively inhibits the lung enzyme both *in vivo* and *in vitro*. Although these data suggest a strong similarity between the converting enzyme activity and bradykininase activity, the two enzymes show a dissimilarity in Cl^- requirement and response to insulin, suggesting that there does exist some distinction between them. Table II illustrates the effects of Cl^- on converting enzyme and bradykininase activity from typical experiments. The converting enzyme shows an absolute Cl^- dependency whereas bradykininase activity, using either methionyl-lysyl-bradykinin or bradykinin as substrate, is Cl^- independent. Yang and co-workers[4, 5] have reported that porcine insulin ($1 \cdot 10^{-6}$ M) greatly inhibited angiotensin I conversion; we have been unable to show inhibition of converting enzyme with bovine insulin concentrations 50-fold greater than that reported above.

Several polypeptide hormones were also tested as possible converting enzyme inhibitors. ACTH, oxytocin, STH, FSH, and LTH (all at $2.4 \cdot 10^{-5}$ M) and glucagon ($8.7 \cdot 10^{-5}$ M) failed to show any inhibitory activity. All-D-bradykinin[13] ($2.7 \cdot 10^{-5}$ M) did not show inhibition, indicating that the active site of the converting enzyme is apparently specific for the L-amino acid configuration.

Since bradykinin-potentiating peptide C, although structurally quite different from bradykinin and methionyl-lysyl-bradykinin, displays the same type of inhibition pattern, it is possible that the converting enzyme has a single active site. Many peptides (His-Leu, Phe-Arg, hippuryl-histidyl-leucine, hippuryl-glycyl-glycine, angiotensin I, bradykinin, methionyl-lysyl-bradykinin, bradykinin-potentiating factor and bradykinin-potentiating peptide C) appear capable of binding at this site; however, it has not yet been clearly demonstrated which of these are hydrolyzed while bound by the enzyme. Bradykinin and methionyl-lysyl-bradykinin are hydrolyzed by the enzyme preparation (Table II), but this does not necessarily indicate that the Cl^--independent bradykininase

TABLE II

THE EFFECT OF Cl^- ON THE ACTIVITIES OF ANGIOTENSIN I CONVERTING ENZYME AND BRADYKININASE IN RABBIT LUNG

Data are reported as specific activity: Converting enzyme, nmoles angiotensin II formed/mg protein per 6 min incubation time; bradykininase, nmoles bradykinin or methionyl-lysyl-bradykinin destroyed/mg protein per 6 min incubation. Incubation conditions are as indicated in Table I for converting enzyme assays. Bradykininase reaction mixtures contained 40 nmoles bradykinin (21 nmoles methionyl-lysyl-bradykinin), 0.25 ml enzyme solution, and 0.2 M phosphate buffer (pH 7.4) to a final volume of 1 ml. The enzyme and buffer were preincubated for 3 min; bradykinin was added and the reaction run for 6 min (at 37°). An aliquot was then removed and assayed on the isolated guinea pig ileum.

	No NaCl	*1% NaCl*
Bradykininase	65.2* 92.7	65.9* 91.7
Converting enzyme	0	2.9

*Methionyl-lysyl-bradykinin used as substrate.

activity responsible for this cleavage is identical to the Cl^--dependent converting enzyme activity. The degree of inhibition caused by preincubation with His-Leu and Phe-Arg is not altered by simultaneous addition of the dipeptide and angiotensin I; this suggests that no destruction of either His-Leu or Phe-Arg occurs. In addition, when bradykinin-potentiating factor and bradykinin-potentiating peptide C are applied to the isolated guinea pig ileum together with bradykinin, the same degree of potentiation is obtained whether or not the potentiating factor or peptide have been incubated with the enzyme preparation. The ability of the potentiating factor to inhibit the conversion of angiotensin I to angiotensin II is also unchanged by preincubation with the enzyme preparation. It is conceivable that just as His-Leu, Phe-Arg, bradykinin-potentiating peptide C and bradykinin-potentiating factor apparently bind to the active site of converting enzyme without being hydrolyzed, bradykinin could likewise be bound to this site without hydrolysis, and subsequently cleaved by the bradykininase active site once released by the converting enzyme.

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