13TH ANNUAL ASPEN CONFERENCE ON RESEARCH IN EMPHYSEMA

Bradykinin Potentiating Factor

Lewis J. Greene, Ph.D.; Sergio H. Ferreira, Ph.D.; and John M. Stewart, Ph.D.

The venom of the Brazilian snake Bothrops jararaca contains a mixture of polypeptides, bradykinin potentiating factor, which potentiates the pharmacologic actions of bradykinin in vitro as well as in vivo. We have isolated nine biologically active peptides from the venom by ultrafiltration and column chromatographic procedures. The peptides contain 5-13 amino acid residues per molecule and have molecular weights in the range 600-1400. They have a high proline content (20-40 mole percent) and each has the cyclized form of glutamic acid (pyrrolidone carboxylic acid) as the amino terminus.

The structure of a biologically active pentapeptide was shown to be pyrrolidone-carboxyl-Lys-Trp-Ala-Pro. This peptide was synthesized by the solid phase method as proof of structure and several analogues were prepared in order to examine the relationship between structure and biologic activity on the isolated guinea pig ileum. The synthetic pentapeptide potentiates the activity of bradykinin two-fold at the same concentration (1 x 10^{-7} M) as the natural pentapeptide. The related peptides Glu-Lys-Trp-Ala-Pro, Trp-Ala-Pro and pyrrolidinecarboxyl-Lys-Trp-Pro, had 1/20, 1/200 and <1/200 of the activity of the pentapeptide.

Synthetic pyrrolidinecarboxyl-Lys-Trp-Ala-Pro like bradykinin potentiating factor increased the activity of bradykinin on smooth muscle contraction, capillary permeability and blood pressure. The available evidence indicates that the potentiation of bradykinin activity is due to the inhibition of enzymes that normally inactivate bradykinin.

CHEST, VOL. 59, NO. 5, MAY 1971 SUPPLEMENT
bradykinin.\textsuperscript{4}

Vane and coworkers\textsuperscript{5} have demonstrated that the pulmonary vascular bed plays the major role in the disappearance of circulating bradykinin and conversion of angiotensin I to angiotensin II. Bradykinin potentiating factor inhibits the pulmonary disappearance of bradykinin\textsuperscript{\ast} and the pulmonary conversion of angiotensin I to II in vivo.\textsuperscript{7} Bradykinin potentiating factor\textsuperscript{\ast} and the purified peptides isolated from Bothrops jararaca venom\textsuperscript{\ast} inhibit angiotensin converting enzyme in fractions derived from homogenized dog lung. We have used the blood pressure systemic response method\textsuperscript{10} to evaluate the effect of infusion of synthetic pyrrolidonecarboxyl-Lys-Trp-Ala-Pro on these pulmonary processes in the rat. Infusion of the peptide at 100-200 \textmu g/min inhibited the pulmonary conversion of angiotensin I to II and inhibited the pulmonary disappearance of bradykinin by more than 70 percent. However, the inhibitory effect of the peptide disappeared when the infusion was stopped. These infusion experiments illustrate the use of enzyme inhibitors to control the metabolism of these hormones in the pulmonary vascular bed. By using specific inhibitors of one or more of these enzymes it should be possible to demonstrate the participation of these hormones in various physiologic situations and perhaps to use these inhibitors as drugs to selectively control the flux of these peptides through certain pathways.

References

2. Ferreira SH, Bartelt DC, Greene LJ: Biochemistry 9:2583, 1970
6. Roblero J, Stewart JM: unpublished data

Properties of the Angiotensin-Converting Enzyme of Lung

D. W. Cushman, Ph.D., H. S. Cheung, Ph.D., and A. E. Peterson, Ph.D.

Angiotensin-converting enzyme catalyzes the formation of the pressor octapeptide, angiotensin II, from an inactive precursor, the decapeptide, angiotensin I. First isolated by Skeggs et al\textsuperscript{1} from horse plasma, the enzyme was later shown to be present at much higher and physiologically more significant concentration in the vascular beds of the lung.\textsuperscript{2} The rapid conversion of angiotensin I in the pulmonary circulation and the observed particulate nature of the isolated enzyme, suggested a localization in or on the membrane of the capillary endothelial cell,\textsuperscript{3} perhaps associated with specialized structures such as the pinocytotic vesicles discussed by Dr. Smith. The probable location of the angiotensin-converting enzyme on the vast area of membrane in direct contact with capillary blood, and the role of this enzyme in the metabolism of vasoactive peptides make it worthy of consideration for a possible physiologic or pathologic role in the lung, as well as in the cardiovascular system.

Purification and characterization of this important enzyme require the development of a simpler and more quantitative assay than the biologic assays which have been employed by most workers. A protected tripeptide, hippuryl-L-histidyl-L-leucine (HHL), was found to be cleaved by the angiotensin-converting enzyme to yield histidylleucine and hippuric acid, the latter of which can be extracted and quantitated by UV spectrophotometry at 228 nm. The specific activities of angiotensin-converting enzyme in various rat tissues assayed by this method were found to be in good agreement with the results obtained by Roth and coworkers,\textsuperscript{4} activity in lung was 30-fold greater than that of serum, and at least 5-fold greater than the activity in any other tissue.

Substrate specificity studies employing a 70-fold purified angiotensin-converting enzyme from dog lung indicate that the enzyme is a carboxypeptidase that cleaves dipeptides rather than amino acids from the carboxyl terminus of polypeptides. The enzyme does not hydrolyze the methyl ester of HHL, hippuryl-L-histidine, or benzoyloxybenzyl-L-histidyl-L-leucine. In addition to catalyzing the formation of angiotensin II, the enzyme can inactivate another important vasoactive peptide, bradykinin, by cleavage of the C-terminal phenylalanylarginine residue. It is uncertain what role, if any, this reaction might play in the pulmonary destruction of kinins, which was discussed by Drs. Ryan and Stewart.

Optimal activity of the enzyme in vitro is obtained at pH 8.3 in the presence of 300 mM NaCl. The apparent Michaelis constant for HHL was 2.6 mM, indicating that this tripeptide has a much lower affinity for the enzyme than angiotensin I. The angiotensin-converting enzyme is a metalloprotein which is activated 60 percent by addition of excess divalent cobalt ion, but not by other divalent metal ions. After removal of the naturally occurring metal ion by treatment with a high concentration of EDTA, followed by exhaustive dialysis, activity can be restored 160 percent by cobalt ion, 100 percent by zinc ion, or 40 percent by manganese ion. It seems probable that the enzyme is a zinc metalloprotein. It is inhibited by a great variety of metal-chelating or binding reagents and by heavy metal ions such as cadmium and mercury which probably exchange with the natural metal ion to

CHEST, VOL. 59, NO. 5, MAY 1971 SUPPLEMENT