Studies on the hydrolysis of bradykinin by angiotensin-converting enzyme (kininase II)[1]

F.E. Dorer, J.M. Stewart and J.W. Ryan

Hypertension Research Laboratory, Veterans Administration Hospital and Department of Biochemistry, Case Western Reserve University, Cleveland (Ohio 44106, USA), Department of Biochemistry, University of Colorado Medical Center, Denver (Colorado 80220, USA) and Department of Medicine, University of Miami School of Medicine, Miami (Florida 33152, USA), 14 March 1978

Summary. Arg-Pro-Pro-Gly-Phe, the N-terminal pentapeptide of bradykinin, is not an inhibitor of angiotensin-converting enzyme and is not hydrolyzed by the enzyme. Arg-Pro-Pro, the N-terminal tripeptide is a relatively potent (IC_{50}= 2.3×10^{6} M) inhibitor but its higher homolog, Gly-Arg-Met-Lys-Arg-Pro-Pro is not an inhibitor of angiotensin-converting enzyme.

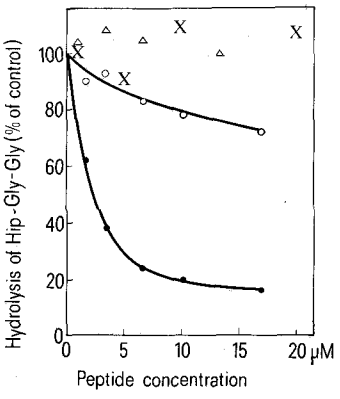
Using highly purified preparations of lung angiotensin-converting enzyme (also known as kininase II, EC 3.4.15.1), a number of investigators have shown that bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) is cleaved to yield the 2 C-terminal dipeptides Phe-Arg and Ser-Pro[2,3]. Although the enzyme might be expected to hydrolyze the remaining pentapeptide (Arg-Pro-Pro-Gly-Phe), no evidence has been found to indicate that angiotensin-converting enzyme yields Gly-Phe. Recently, Borges et al.[4] have reported that isolated, blood-free liver preparations (perfused with μM concentrations of bradykinin) yield Gly-Phe as well as Ser-Pro. Phe-Arg and other metabolites which may have originated from bradykinin. From their results, it was suggested that angiotensin-converting enzyme can hydrolyze the Pro-Gly bond of bradykinin. Further interest in this possibility arises from the finding that the N-terminal tripeptide, Arg-Pro-Pro, is a moderately potent inhibitor of bradykinin[5]. The degree of inhibition is pH dependent, the tripeptide being more potent at pH 8.0 than at 6.0[6]. Nonetheless, the intriguing possibility is suggested that if angiotensin-converting enzyme hydrolyzes the Pro-Gly bond of bradykinin, it will have formed an inhibitory metabolite. Thus, the purpose of the present study was to examine the ability of angiotensin-converting enzyme to hydrolyze Arg-Pro-Pro-Gly-Phe, or alternatively, to determine whether the pentapeptide is capable of inhibiting the enzyme.

Materials and methods. Angiotensin-converting enzyme was purified from porcine lung as described previously[7]. The preparation used throughout was equivalent to Fraction G (purity of approximately 35%). Arg-Pro-Pro-Gly-Phe, Arg-Pro-Pro, BPP_{9a} (< Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro), and Gly-Arg-Met-Lys-Arg-Pro-Pro were prepared by the Merrifield solid phase method[8]. The B-chain of insulin (bovine) was obtained from Armour Pharmaceutical Co., Phoenix, AZ, USA. Hippurylglycylglycine (Hip-Gly-Gly) was synthesized as described previously[7].

Assays of angiotensin-converting enzyme were performed as follows: Each 5-ml reaction mixture contained 0.05 M Hepes buffer at pH 6.0 or 8.0, 0.1 M NaCl, 1 mM Hip-Gly-Gly, and 0.38 μg of enzyme. Hydrolysis was measured by a quantitative ninhydrin technique[6]. Except where noted, inhibitory peptides were added at concentrations in the range 1–20 μM. In experiments where Arg-Pro-Pro-Gly-Phe was tested as a substrate for angiotensin-converting enzyme, Hip-Gly-Gly was omitted from the reaction mixture and the pentapeptide was added at concentrations of 10 and 50 μM. The quantity of enzyme used (3.8 μg) was 10 times the amount needed to detect hydrolysis of bradykinin.

Results and discussion. Confirming results of previous studies[6,9], we found that BPP_{9a} (SQ 20881), at low concentrations, inhibited the hydrolysis of Hip-Gly-Gly by angiotensin-converting enzyme ($IC_{50}=1.8\times10^{-8}$ M). Arg-Pro-Pro also inhibited the reaction at pH 8 ($IC_{50}=2.3\times10^{-6}$ M) but was relatively ineffective at pH 6 (figure). Arg-Pro-Pro-Gly-Phe did not inhibit angiotensin-converting enzyme and was not a substrate, even when the enzyme concentration was increased 10fold. These results are in agreement with our previous finding that Gly-Phe was not a product of the hydrolysis of bradykinin by angiotensin-converting enzyme[2] and suggest that the pentapeptide does not bind to the enzyme. The formation of the dipeptide Gly-Phe from bradykinin in isolated liver preparations[4] cannot be accounted for by the action of angiotensin-converting enzyme. Although Arg-Pro-Pro clearly inhibits angiotensin-converting enzyme, our data show that this tripeptide is not formed.

In view of the potency of BPP_{9a} and the structural similarity of Arg-Pro-Pro (both peptides contain the C-terminal Pro-Pro sequence), we investigated the possibility of using an N-terminal higher homolog of Arg-Pro-Pro as an enzyme inhibitor. Gly-Arg-Met-Lys-Arg-Pro-Pro was tested in the concentration range of 1–30 μM. No significant inhibition was detected. Similar results were obtained with the B-chain of insulin; a polypeptide reported to be a substrate for angiotensin-converting enzyme[9].



Inhibition of angiotensin-converting enzyme catalyzed hydrolysis of Hip-Gly-Gly by peptides structurally related to bradykinin. Peptides were tested for inhibitory activity at either pH 6 or 8. Added peptides were: Arg-Pro-Pro (pH 6), ○-○; Arg-Pro-Pro (pH 8), ●-●; Arg-Pro-Pro-Gly-Phe (pH 8), X; Gly-Arg-Met-Lys-Arg-Pro-Pro (pH 8), △.

1 This work was supported in part by grants from the US Public Health Service (HL 18415, HL 15691, HL 19764) and the John A. Hartford Foundation, Inc., and by the Medical Research Service of the Veterans Administration.

2 F.E. Dorer, J.R. Kahn, K.E. Lentz, M. Levine and L.T. Skeggs, Circulation Res. *34*, 824 (1974).
3 R.L. Soffer, R. Reza and P.R.B. Caldwell, Proc. nat. Acad. Sci. USA *71*, 1720 (1974).
4 D.R. Borges, E.A. Limaos, J.L. Prado and A.C.M. Camargo, Naunyn-Schmiedeberg's Arch. Pharmac. *295*, 33 (1976).
5 G. Oshima and E.G. Erdos, Experientia *30*, 733 (1974).
6 F.E. Dorer, J.R. Kahn, K.E. Lentz, M. Levine and L.T. Skeggs, Biochim. biophys. Acta *429*, 220 (1976).
7 F.E. Dorer, J.R. Kahn, K.E. Lentz, M. Levine and L.T. Skeggs, Circulation Res. *31*, 356 (1972).
8 J.M. Stewart and J.D. Young, Solid Phase Peptide Synthesis. W.H. Freeman, San Francisco 1969.
9 R. Igic, E.G. Erdos, H.S.J. Yeh, K. Sorrells and T. Nakajima, Circulation Res. *31* suppl. 2, 51 (1972).