RESISTANCE OF VASOACTIVE PEPTIDES FROM VENOM OF Vespa orientalis TO ACTION OF PROTEOLYTIC ENZYMES

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The kinetics of the hydrolysis by proteolytic enzymes of vasoactive peptides isolated from the venom of the hornet Vespa orientalis has been investigated. It has been found that the half-period of decomposition of the peptides exceeds that of bradykinin. These facts are in harmony with in vivo results on the prolonged hypotensive action of the peptides.

Vasoactive peptides, especially kinins, are characterized by a broad spectrum of biological action which is manifested in very low concentrations [1]. They lower the blood pressure, exhibit a myotropic action, and are mediators of pain in the organism. However, the action of vasoactive peptides is extremely brief because of their rapid inactivation in the organism by various proteolytic enzymes, which substantially limits the use of vasoactive peptides.

The inactivation of kinins is performed by specialized enzymes: carboxypeptidase B (splitting out of the C-terminal arginine residue from bradykinin), kininase II (dipeptidyldipeptidase, splitting out the C-terminal dipeptide), and chymotrypsin [2]. Numerous attempts have been made at the directed modification of the molecules of kinins with the aim of obtaining analogs with a prolonged action [2, 3]. However, as a rule, chemical modification substantially lowers the differentiated activity of the peptide. It is obvious that to create new peptide bioregulators a structural-functional investigation of natural analogs of the kinins possessing increased resistance to the action of proteolytic enzymes is necessary.

Two vasoactive peptides possessing a hypotensive action prolonged in comparison with that of bradykinin have previously been isolated from the venom of the hornet Vespa orientalis [4]. As has been established, one of them (peptide II) is a close structural analog of bradykinin: Val-Pro-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg. The other – peptide I – has the following amino acid sequence: Phe-Leu-Arg-Ile-Ala-Gly-Leu-Leu-Leu-Lys-Ala.

In model experiments to study the comparative stability of the vasoactive peptides, we used chymotrypsins, carboxypeptidase B, and kininase II. The rate of inactivation of the peptides was determined by a biological method based on measuring the residual myotropic activity of the peptides in the early periods after incubation with the enzyme.

Figure 1, a, shows the results of a study of the kinetics of the hydrolysis of peptides I and II and bradykinin by kininase II. The half-period of decomposition of bradykinin amounted to 10 min, while for peptide II it was approximately 60 min and for peptide I it was considerably longer – at the 60th minute the residual myotropic activity amounted to 80%. It is obvious that the relatively higher resistance of peptide I to the action of kininase II as compared with bradykinin is connected with the different reactivities of the C-terminal dipeptides, Lys^{10}–Ala^{11} (in peptide I) and Phe^{8}-Arg^{9} (in bradykinin). On the other hand, the splitting out of the dipeptide Phe^{8}-Arg^{9} in peptide II and bradykinin took place at different rates. It may be assumed that the additional amino acid sequence at the N-end of peptide II lowered the efficacy of the action of the enzyme somewhat in comparison with bradykinin. A similar observation has been made in a study of the kinetics of the hydrolysis of bradykinin and its natural analogs by kininase II [5]. The best substrate for kininase II was bradykinin. Lys-bradykinin and, particularly, Met-Lys-bradykinin were less sensitive to degradation by this enzyme.

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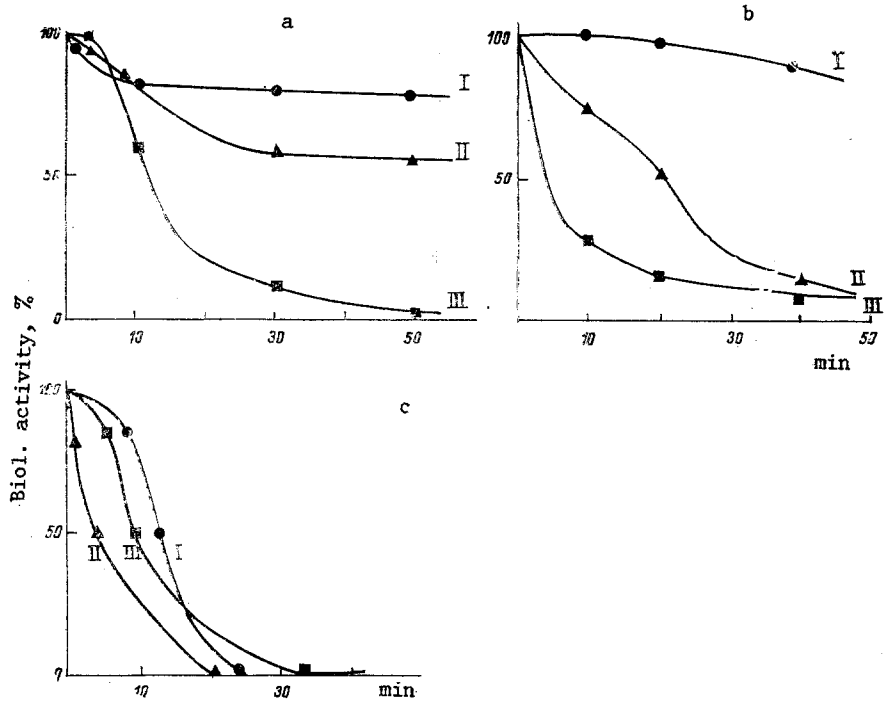

Fig. 1. Kinetic curves of the cleavage of peptides by kininase II (a), carboxypeptidase B (b), chymotrypsin (c): a) 0.05 M Tris-HCl, pH 4.5 37°C; enzyme:substrate ratio = 1:5; b) 0.025 M Tris-HCl, pH 7.6; 37°C; enzyme:substrate ratio = 1:100; c) 0.05 M Tris-HCl, pH 7.8; 37°C; enzyme:substrate ratio = 1:100. I) Peptide I; II) peptide II; III) bradykinin.

The splitting out of the C-terminal arginine residue from peptide II by carboxypeptidase B lowered its activity to a smaller degree than in the case of bradykinin. For example, after incubation for 5 min, the activity of bradykinin was 40%, while that of peptide II was 80%, of its initial level (Fig. 1, b). The time of half-decomposition of bradykinin on hydrolysis by carboxypeptidase B amounted to 5 min, while for peptide II it was 20 min. Peptide I was scarcely hydrolyzed by carboxypeptidase B, as could have been assumed on the basis of an analysis of its primary structure.

The peptides were hydrolyzed most intensively by chymotrypsin (Fig. 1, c). The times of half-decomposition of bradykinin, peptide I and peptide II were 10, 5, and 15 min, respectively. As had been established previously [6], chymotrypsin splits out the C-terminal arginine residue from bradykinin more rapidly than it hydrolyses the Phe^5-Ser^6 bond, but the relative contribution of chymotrypsin to the general metabolism of the kinins has so far been studied inadequately.

On generalizing the results given on the resistance of the peptides of the venom of V. orientalis to hydrolysis by proteolytic enzymes, it can be observed that, as was to be expected on the basis of an analysis of the primary structures of peptides I and II, peptide I was the most resistant to the action of the proteolytic enzymes, and this was reflected in in vivo experiment on the hypotensive action of the peptides from the hornet venom. On the other hand, it must be emphasized that in structural analogs of the bradykinin the following correlation is shown fairly clearly: with a lengthening of the polypeptide chain from the N-end resistance to the action of proteolytic enzymes increases, and this finds its reflection in in vivo experiments.

EXPERIMENTAL

The isolation of the vasoactive peptides from the venom of V. orientalis and their physiochemical and functional analyses were carried out as we have described previously [4].

The amino acid sequences of the peptides were determined by the degradation of the peptides from the N-end by Edman's method with identification of the amino acids in the form of

their dansyl derivatives on polyamide plates [7], and also in the form of phenylthiohydantoin derivatives [8].

The C-terminal amino acid sequences were established in the following way. The peptide (0.1 μmole) in 100 μl of 0.1 M triethanolamine acetate buffer, pH 5.5, was hydrolyzed with carboxypeptidase Y (Serva) at a molar ratio of substrate to enzyme of 10,000:1. After various intervals of time, aliquots of the hydrolysate were taken and the amino acids that had been split out were analyzed in the form of their dansyl derivatives.

Hydrolysis of the peptides by chymotrypsin (Worthington) was carried out at an enzyme: substrate ratio of 1:100 in 0.05 M Tris-HCl buffer, pH 7.8, at 37°C for 0-60 min. After various intervals of time, 100-μl samples were fixed in 0.4 ml of a mixture of 4% TCA and 4 mM oxalic acid. After neutralization of the solution with 1 N NaOH, the residual myotropic activity was analyzed on an isolated neck of a rat uterus [9]. Bradykinin triacetate was used as standard.

The hydrolysis of the peptides by kininase II was performed in 0.05 M Tris-HCl buffer, pH 4.5, containing 50 μg of the peptide and 10 μg of kininase II. The kininase II (carboxycathepsin) was obtained by the method of [10]. Samples were taken after predetermined intervals of time from the beginning of the reaction and were analyzed as described for chymotrypsin.

The hydrolysis of the peptide by carboxypeptidase B was performed in an incubation buffer of 0.025 M Tris-HCl, pH 7.6, at an enzyme:substrate ratio of 1:100 (the carboxypeptidase B was a preparation from porcine pancreas (Worthington)). After predetermined intervals of time 100-μ samples were taken and were analyzed as described above.

SUMMARY

1. The increase in the duration of the hypotensive action of peptide II in comparison with bradykinin is connected with the presence of the additional amino acids (-Val-Pro) at the N-end and correlates with the kinetics of its hydrolysis by proteolytic enzymes.

2. The hypotensive effect of peptide I, prolonged in comparison with that of bradykinin, is a consequence of a fall in the specificity of kininases in relation to the C-terminal grouping -Leu^9-Lys^{10}-Ala^{11} as compared with the -Pro^7-Phe^8-Arg^9 of bradykinin.

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