VASOACTIVE PEPTIDES FROM VENOM OF THE HORNET *Vespa orientalis*

PHYSICOCHEMICAL AND FUNCTIONAL CHARACTERIZATION

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Two peptides having kinin activity have been isolated from hornet venom. The peptides isolated exhibit hypotensive action and a myotropic effect, and they also liberate histamine from mast cells. One of the peptides is a close structural analog of bradykinin while the other differs considerably from it.

Among potentially possible cardiovascular drugs, an important place is assigned to vasoactive peptides, especially kinins, which exhibit a physiological action in extremely low concentrations. Normal physiological concentrations of kinins in the organism are of the order of 10^{-11} mole/g of tissue [1], but their direct use in medical practice is being held back by their extreme instability in vivo in relation to specialized enzyme systems. To solve this problem it is necessary to make a wide search for and a structural-functional investigation of vasoactive peptides from natural sources, the most suitable of which are hymenoptera venoms [2-4]. In the present paper we give information on the isolation and a physicochemical and functional study of vasoactive peptides from the venom of the hornet *V. orientalis*.

As can be seen from Fig. 1, kinin myotropic activity was detected in fractions I and II. In order to free them from biogenic amines the active fractions were subjected to gel filtration in a column of Sephadex G-10, which gave two peptides exhibiting a pronounced contractile effect. The isolated peptides migrated in the form of individual spots under TLC conditions on plates of silica gel with R_f 0.325 and 0.319, which distinguishes them from the histamine-liberating peptides obtained by A. I. Miroshnikov et al. [5], from which they also differ in molecular weight and amino acid composition. From the results of gradient electrophoresis under denaturing conditions, the peptides also consisted of homogeneous substances having molecular weights of 1100 ± 100 and 1500 ± 100 Da. The isoelectric points of the kinins of hornet venom were 10.65 and 10.50, and their N-terminal amino acids phenylalanine and valine. The amino acid compositions of peptides I and II are given below:

Amino acid	Peptide I	Peptide II
Alanine	1.88 (2)	0.14 (0)
Glycine	1.44 (1)	0.86 (1)
Valine	0.18 (0)	1.17 (1)
Isoleucine	0.69 (1)	0.13 (0)
Leucine	3.55 (4)	–
Phenylalanine	0.65 (1)	2.30 (2)
Glycine	1.25 (1)	–
Arginine	1.36 (1)	1.86 (2)
Serine	–	0.20 (1)
Proline	–	4.12 (4)
Total amino acids	11	11

The specific myotropic activity of peptide I under the conditions of biological testing on the neck of the rat uterus corresponded to 1/10 of the activity of bradykinin, while that of kinin II was approximately two orders of magnitude lower, but the resistance of the isolated peptides to the action of enzymes (chymotrypsin, kininase II) was substantially higher than that of bradykinin, as was reflected on their functional properties in vivo experiments.

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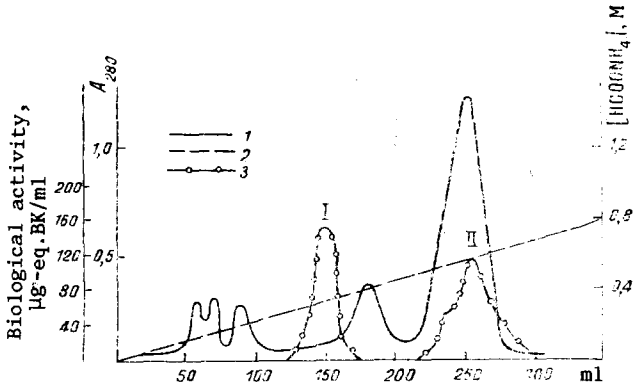

Fig. 1. Chromatographic separation of the whole hornet venom on a column (1.0 × 60 cm) of SP-Sephadex C-25 in a concentration gradient of ammonium formate; 0.01 M, pH 3.6-1.0 M, pH 6.6. 1) Optical density at 280 nm; 2) molarity gradient of the elution buffer; 3) biological activity, BK equivalent, μg/ml.

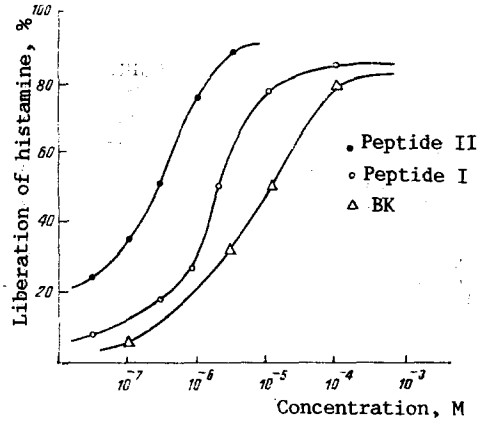

Fig. 2. Liberation of histamine from rat mast cells under the action of vasoactive peptides. The histamine-liberating activity of a peptide was evaluated from the magnitude of ED_{50}, i.e., the concentration of peptide at which 50% of histamine is liberated.

It was characteristic that preparative incubation with antibodies to bradykinin (BK) in the equivalence zone completely prevented the myotropic effect of the peptides.

On the intravenous injection into cats of kinin II and BK an equidepressor effect was observed at concentrations of 40 and 25 μg/kg, respectively, the threshold concentration of peptide II being approximately 1.5 times lower than that of BK. A lowering of the blood pressure under the action of the peptides set in twice as fast as that due to BK. The restoration of the pressure to the initial level was observed after 45 h for BK and peptide II. The hypotensive effect of kinin I required concentrations three times higher than those of BK but it had a distinctly prolonged nature.

One of the important properties of vasoactive peptides is the liberation of histamine from mast cells stimulated by them. The kinins of the hornet venom liberated histamine intensively from rat mast cells (Fig. 2). Peptide 2 exhibited the greatest activity (ED_{50} = $5 \cdot 10^{-7}$), which was somewhat higher than that of the classical histamine-liberating substance 48/80 (ED_{50} = $8.5 \cdot 10^{-7}$ M). The liberating activity of peptide (I) was also considerable (ED_{50} = $2 \cdot 10^{-6}$ M; ED_{50} for BK = $1.5 \cdot 10^{-5}$) and was comparable with the reaction of polistes-kinin (ED_{50} = $3 \cdot 10^{-6}$ M) [6].

In comparative experiments on the competitive action of ^{125}I-labeled BK and the peptides with antibodies to BK it was shown that a five-fold molar excess of kinin II, like unlabeled

BK, completely displaced [^{125}I]BK from its complex with antibodies, while the radioactivity detected in the precipitate when using peptide I amounted to 1/2 of the radioactivity of the supernatant solution. Apparently, kinin II is a close structural analog of bradikinin, while the structure of peptide I differs substantially from that of BK, and this is of additional interest for a structural-functional investigation.

EXPERIMENTAL

Lyophilized hornet venom obtained by electrostimulation was investigated.

Fractionation of the Venom. A column of SP-Sephadex C-25 was used for separation under the conditions described by Yoshida [7], fractions being collected in the light of a biotest. Bioanalysis was based on the determination of the myotropic activity of the isolated rat uterus neck [8] with bradykinin as standard. Three active fractions selected according to the biotest were subjected to rechromatography on columns of Sephadexes G-25 and G-10. In some cases, the peptides were purified by chromatography on columns of TSK gel HW 40 or by preparative thin-layer chromatography in the solvent system butan-1-ol—water—pyridine—acetic acid (5:12:10:3) and were eluted with 50% pyridine.

The Homogeneity of the peptides obtained was shown by gradient electrophoresis under denaturing conditions [9], by isoelectrofocusing, by TLC, and by N-terminal amino acid analysis.

Amino Acid Compositions were analyzed on a Biotronic amino acid analyzer. N-terminal acid sequences were determined by Edman degradation with identification of the amino acids in the form of their dansyl derivatives on polyamide plates [10].

The Trypsin Hydrolysis of the Peptides was carried out in 0.2 M Tris-HCl buffer, pH 8.3, at an enzyme concentration of 10 μg in an incubation mixture with a column of 200 μl at 37°C for 2 h. Hydrolysis with chymotrypsin and with kininase II was carried out in the same conditions.

Antibodies to Bradykinin were obtained by immunizing animals with BK—bovine serum albumin and BK—ovalbumin conjugates obtained with the aid of the synthesized bifunctional reagent 2,4-toluylene diisocyanate. BK was labeled with the isotope ^{125}I by a method that we have developed.

Experiments on the Competitive Interaction of the ^{125}I-labeled BK analog and the peptides with antibodies to BK were performed with the use of polyethyleneglycol to separate the complex and the unbound peptide [11].

The Liberation of Histamine from rat mast cells under the action of the hornet venom peptides was determined by independent methods on guinea-pig ileum [8] and by a fluorimetric method [12].

The Influence of the Vasoactive Peptides on the Blood Pressure was tested under the conditions of an acute experiment with intravenous injection into nembutal-narcotized cats.

SUMMARY

1. Two peptides possessing kinin activity have been isolated in the homogeneous state from the venom of the hornet Vespa orientalis. One of them is a close structural analog of bradykinin.

2. The vasoactive hornet venom peptides exhibit a myotropic effect and hypotensive and histamine-liberating properties while differing from bradykinin in their resistance to proteolytic enzymes.

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ENZYMATIC SYNTHESIS OF MULTIPLE TRITIUM-LABELED NUCLEOSIDES AND NUCLEOTIDES FROM NITROGEN BASES AND RIBOSE

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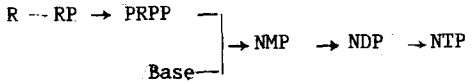
A wide range of highly active nucleotides containing tritium labels in the heterocycle nucleus and ribose residue has been synthesized with the aid of an enzyme preparation from E. coli, starting from tritium labeled nitrogen bases and ribose. In individual cases, the complex enzyme preparation was modified by the addition of nucleotide kinases or phosphatases.

Recently, considerably interest has been aroused by the biosynthesis of nucleotides from nitrogen bases with elimination of the stage of forming nucleosides. Analogous reactions in vitro provide the possibility of synthesizing nucleoside 5'-triphosphates (NTPs) from bases and 5-phosphoribosyl 1-pyrophosphate (PRPP) without the isolation of the intermediate products; the phosphoribosyl pyrophosphate can be replaced by its precursors – ribose 5-phosphate (RP) or ribose (R):



The complex (multifunctional) enzyme preparation used must contain enzymes catalyzing the conversion of ribose into ribose 5-phosphate (ribokinase, EC 2.7.1.15), ribose 5-phosphate into phosphoribosyl pyrophosphate (ribose phosphate pyrophosphokinase, EC 2.7.6.1), phosphoribosyl pyrophosphate and nitrogen bases into nucleoside 5'-monophosphates (adenine phosphoribosyltransferase, EC 2.4.2.7; uracyl phosphoribosyltransferase, EC 2.4.2.9, etc.); and nucleoside 5'-monophosphates into the corresponding di- and then triphosphates (specific nucleoside 5'-monophosphate kinases, EC 2.7.4, and nonspecific nucleoside 5'-diphosphate kinase, EC 2.7.4.6). This set of enzymes is present in various cultures – E. coli [2-8], Saccharomyces cerevisiae [9, 10], Brevibacterium ammoniagenes [11, 12], Corynebacterium species [13], etc. Depending on the ratio of the activities of the individual enzymes in the preparation used and the nature of the nitrogen base, the predominating reaction product consists of nucleoside 5'-monophosphates or nucleoside 5'-triphosphates. In order to increase the yield of nucleoside 5'-triphosphates, the activity of the ATP-regenerating system is usually enhanced by the supplementary addition to the incubation mixture of creatine phosphate and creatine phosphokinase (EC 2.7.3.2) or phosphoenol pyruvate and pyruvate kinase (EC 2.7.1.40) [12].

As applied to the synthesis of labeled nucleotides, the majority of investigations have been limited to the use of phosphoribosyl pyrophosphate [5, 6, 9-10, 12] and to the presence

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