STRUCTURAL ANALOGUES OF BRADYKININ AND OTHER VASOACTIVE PEPTIDES IN THE VENOM OF THE WASP *Polistes gallicus*

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Six vasoactive peptides have been isolated in the homogeneous state from the venom of the wasp Polistes gallicus. Their full amino acid sequences have been determined. It has been shown that four of them are structural analogues of bradykinin with additional N- and C-terminal sequences and intramolecular substitutions of amino acids. Some structure-function relationships in bradykinin analogues are discussed.

In investigations by a series of authors, the existence in the composition of hymenoptera venoms of various structural elements of bradykinin (BK) possessing the properties of kinins has been shown [1-4]. In addition, vasoactive peptides not containing the amino acid sequence of BK in their structure have been isolated from natural sources [5, 6].

On the basis of the results obtained, certain conclusions have been drawn concerning structural-functional interrelationships in vasoactive peptides [6]. However, the problem is far from a definitive solution. It is obvious that a structural-functional investigation of natural vasoactive peptides is necessary for the synthesis of new peptide bioregulators.

In the present paper we give results on the structure and functional properties of vasoactive peptides from the venom of the wasp P. gallicus.

As a result of the successive chromatography of the venom on TSK gel HW-40, SE-cellulose, and Sephadex G-10, and rechromatography, six vasoactive peptides have been isolated in the homogeneous state [7].

From the results of an investigation of interaction with antibodies to BK and from the amino acid compositions of the peptides isolated (Table 1) it was concluded that four of them were BK analogues. Two others did not interact with the antibodies and are most probably representatives of other classes of vasoactive peptides.

The N-terminal amino acid sequences of the peptides isolated were determined by Edman's method with identification of the amino acids in the form of their DNS derivatives on polyamide plates [8]. The results so obtained (number of cycles of degradation of each peptide) are given in Table 2.

It must be mentioned that the number of cycles of degradation with the reliable identification of the N-terminal amino acids for each peptide will depend mainly on the structure of the peptide remaining after each successive step. To prevent the loss of peptide remaining after each cycle of degradation, in the intermediate washing with ether from the PTH-amino acid it is necessary to use the minimum amounts of ethyl acetate with monitoring of the completeness of extraction of the PTH derivatives.

The C-terminal amino acid sequences of the peptides were determined in a study of the kinetics of hydrolysis using carboxypeptidases A, B, and Y (CPA, CPB, and CPY) followed by analysis of the amino acids split off with the aid of an analyzer and microthin-layer chromatography.

As a rule, the analysis of a sequence began with the use of carboxypeptidase B (Sigma)

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TABLE 1. Amino Acid Compositions of Vasoactive Peptides from the Wasp P. gallicus

Amino acid	Peptide					
	I	II	III	IV	V	VI
Asp	—	—	—	—	—	—
Thr	—	—	—	—	1	1
Ser	—	1	1	1	1	2
Glu	—	—	—	—	—	—
Pro	—	4	3	3	2	2
Gly	2	2	1	1	1	1
Ala	1	1	—	—	—	—
Val	1	—	1	1	2	1
Met	—	—	—	—	—	—
Ile	2	1	—	1	—	1
Leu	2	1	1	—	1	—
Tyr	—	—	—	—	—	—
Phe	—	4	3	2	2	2
His	—	—	—	—	—	—
Lys	3	—	2	—	3	—
Arg	—	2	2	2	—	2
Cys	—	1	—	—	—	—

TABLE 2. N-Terminal Amino Acid Sequences of Vasoactive Peptides from the Venom of the Wasp P. gallicus

Peptide	Terminal sequence
I	Ile^{1}-Leu^{2}-Ala^{3}-Gly^{4}-Gly^{5}-Ile^{6}-Val^{7}-Lys^{8}-
II	Leu^{1}-Ala^{2}-Ile^{3}-Pro^{4}-Phe^{5}-Cys^{6}-Phe^{7}-Gly^{8}-Arg^{9}-Pro^{10}-Pro^{11}-Gly^{12}
III	Phe^{1}-Lys^{2}-Leu^{3}-Val^{4}-Lys^{5}-Arg^{6}-Pro^{7}-Pro^{8}-Gly^{9}-Phe^{10}
IV	Ile^{1}-Arg^{2}-Pro^{3}-Pro^{4}-Gly^{5}-Phe^{6}-Ser^{7}-Pro^{8}-
V	Phe^{1}-Lys^{2}-Val^{3}-Pro^{4}-Lys^{5}-Lys^{6}-Gly^{7}-Val^{8}-Phe^{9}-
VI	Ile^{1}-Arg^{2}-Pro^{3}-Val^{4}-Gly^{5}-Phe^{6}-Ser^{7}-Pro^{8}-Phe^{9}

that had been treated with diisopropyl phosphorofluoridate and was free from impurities having CPA, trypsin, and chymotrypsin activities. The absence of foreign activities in the preparation was confirmed in a study of the kinetics of the hydrolysis of bradykinin (Sigma); this liberated only arginine from the peptide. In addition, in preliminary controlled experiments, the optimum ratio of enzyme and substrate and also the intervals of time for taking aliquots for analysis were determined. In this way, it was shown that only in peptides (II) and (III) was the C-terminal amino acid arginine.

After exhaustive hydrolysis of the peptides with CPB, the samples were hydrolyzed with carboxypeptidase A (FMSF-treated preparation free from trypsin and chymotrypsin activities). This showed that the C-terminal amino acid in peptide (I) was Leu while at later periods of incubation Lys was detected in the hydrolysis products. Arg and Phe were found in the products of the hydrolysis of peptides (II) and (III) (CPB and CPA); i.e., the C-terminal sequence in each of them was —Phe—Arg. The first product of the hydrolysis of peptide (IV) by CPA was Val; at later periods of incubation Arg was liberated (action of CPB in the sample). Finally, on the combined hydrolysis by CPA and CPB of the peptides (V) and (VI), Leu (V) and Ser and Thr (VI) were found in aliquots.

The most complete information on the structures of the C-terminal sequences of the peptides isolated was obtained in a study of the kinetics of hydrolysis by carboxypeptidase Y, which confirmed the results of the experiments using CPA and CPB. The hydrolysis of the peptides with CPY was carried out in triethanol acetate buffer, pH 5.5, at a molar ratio of enzyme to substrate of 1:100,000. The amino acids liberated were analyzed with the aid of an amino acid analyzer. On the basis of an analysis of the kinetic curves, the C-terminal amino acid sequences of the peptides are: peptide (I) —Val-Lys-Lys-Lys-Leu; (II) —Gly-Phe-Ser-Pro-Phe-Arg; (III) —Gly-Phe-Ser-Pro-Phe-Arg; (IV) —Ser-Pro-Phe-Arg-Val; (V) —Val-Phe-Thr-Ser-Pro-Leu; (VI) —Pro-Phe-Arg-Thr-Ser.

Thus, on comparing the results on the amino compositions and the N-terminal and C-terminal sequences of the peptides their primary structures were determined (Table 3).

As follows from the results given in Table 3, peptides (I) and (V) are set apart from the other vasoactive peptides (which are structural analogues of BK). Vasoactive peptides

TABLE 3. Amino Acid Sequences of the Vasoactive Peptides from the Venom of the Wasp P. gallicus

Peptide	Amino acid sequence
I	Ile-Leu-Ala-Gly-Gly-Ile-Val-Lys-Lys-Lys-Leu
II	Leu-Ala-Ile-Pro-Phe-Cys-Phe-Gly-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
III	Phe-Lys-Leu-Val-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
IV	Ile-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-Val
V	Phe-Lys-Val-Pro-Lys-Lys-Gly-Val-Phe-Thr-Ser-Pro-Leu
VI	Ile-Arg-Pro-Val-Gly-Phe-Ser-Pro-Phe-Arg-Thr-Ser

of this type have also been detected in other venoms [5, 10] and some other natural sources [6] and have been studied. The most characteristic properties of these peptides are their chain lengths (11-14 amino acid residues), and the substantial proportion of hydrophobic amino acids in their structures, and also the considerable positive charge. Peptides (I) and (V) exhibited a myotropic action (0.061 and 0.0027 μg-equivalent of BK/μg) and hypotensive properties. Particular interest is presented by the hypotensive effect of peptide (I): the duration of its hypotensive effect was approximately 20% greater than that of BK; i.e., a prolonged effect was observed, and the beginning of the action set in more rapidly, while the equidepressor dose differed only slightly (28 and 10 μg/kg, respectively).

Among the structural analogues of BK that have been detected in venoms, peptides containing additional N- and C-terminal sequences are known [1, 2]. A structural analogue of BK having an intramolecular amino acid substitution – Ala-Arg(Thr^6-BK) – has been isolated from the venom of the wasp Polistes rothneyi. Analogues containing hydroxyproline in place of proline in the fourth position of the amino acid sequence of BK (vespakinin-M) are also known. Furthermore, vasoactive glycopeptides having carbohydrate components and a block of arginine residues in an additional N-terminal sequence to BK have been found in the venom of the wasp Vespula maculifrons [3]. The myotropic and hypotensive activities of these peptides are substantial.

BK analogues containing both additional N-terminal (peptides (II) and (III)) and C-terminal ((IV) and (VI)) sequences have been isolated from the venom of P. gallicus (see Table 3). In addition, an analogue having the intramolecular replacement of Pro^3 by Val in the structure of BK (peptide (VI)) has been detected.

The vasoactive peptides from the venom of the wasp P. gallicus exhibit myotropic activity and hypotensive properties. The myotropic effect of the isolated peptides is lower than that of BK in all cases [7], which corresponds to a general law revealed for kinins with additional N-terminal sequences. As follows from the literature, the functional role of the additional C-terminal sequences consists in an increased resistance to the action of kinin-activating enzymes. However, with respect to the half-period of their hypotensive effects, the peptides isolated form the sequence (I) > BK > (II) = (VI) > (IV) > (V) > (III) [7].

Thus, the multiplicity of vasoactive components and, in particular, the presence of peptides with additional sequences and intramolecular substitutions makes the venom of the wasp P. gallicus a promising object for systematic structural-functional investigation.

EXPERIMENTAL

The isolation of the peptides was carried out by a method described previously [7].

The homogeneity of the peptides obtained was shown by the method of gradient electrophoresis under denaturing conditions [9], isoelectric focusing, N-terminal amino acid analysis [8], and TLC. Thin-layer chromatography was conducted on silica gel plates (Merck) in the solvent system: butan-1-ol–acetic acid–water (63:10:27).

Amino acid analysis was performed in a T-339 analyzer (Czechoslovakia) with an Ostion AB column. The peptides were hydrolyzed with 5.7 M HCl–TFA (2:1) at 166°C for 60 min.

The N-terminal analysis of the peptides was carried out as described by I. B. Nazimov [11].

Determination of Amino Acid Sequences. For the determination of N-terminal sequences we used degradation by Edman's method with identification of the amino acids in the form of their DNS-derivatives on polyamide plates [8]. The polyamide plates were prepared from polyester films using polyamide powder for TLC (Woelm Pharma). The defatted and dried film

was immersed in a solution of polyamide in ethylene glycol (10% w/w) at 145°C and was dried in the air at room temperature and then at 100°C for 1 h.

The amino acid DNS-derivatives were detected in UV light with the deposition on the opposite side of the plate of a standard mixture of DNS-amino acids. All the reagents used for analyzing amino acid sequences were purified as described by I. B. Nazimov [11].

The C-terminal amino acid sequences were determined in a study of the kinetics of the hydrolysis of the peptides using carboxypeptidases A, B, and Y (Sigma).

The hydrolysis of the peptides by carboxypeptidase Y was carried out in 0.1 M triethanol acetate buffer, pH 5.5, at a molar ratio of enzyme to substrate of 1:100,000 with aliquots being taken after various intervals of time (0-5 h) and the amino acids that had been split out being analyzed in an amino acid analyzer and by microthin-layer chromatography.

Hydrolysis by carboxypeptidase B was carried out at pH 7.7-7.8 (weight ratio of enzyme to substrate 1:100). The reaction was stopped by acidifying the reaction mixture with acetic acid to pH 3.

Hydrolysis with Carboxypeptidase A. To eliminate chymotrypsin and trypsin activities, a preparation of CPA, type I (Sigma), was treated with phenylmethanesulfonyl fluoride (FMSF, 10^{-7} M). The enzyme was dissolved in the following way. A suspension containing 100 μg of CPA was diluted to 100 ml with water and was centrifuged at 6000 rpm for 5 min. The deposit was resuspended in 0.1 ml of a 1% solution of $NaHCO_3$, which was cooled to 0°C and, with stirring, 0.1 N NaOH was added dropwise until the enzyme had dissolved. Then the pH was brought to 8-9 with the aid of 0.1 N HCl. The solution obtained was used for the hydrolysis of the peptide with an enzyme to substrate ratio of 1:100 (by weight).

Determination of Myotropic Activity. Bioanalysis was based on the determination of the contractile effect of isolated rat uterus neck in de Jalon's solution under the action of the vasoactive peptide, with BK as standard. The influence of antibodies to BK on the myotropic effect of the peptides was determined at a 50-fold excess of immunoglobulins by weight.

The hypotensive action of the peptides isolated was investigated under the conditions of an acute experiment with intravenous injection into nembutal-narcotized cats.

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