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VASOACTIVE PEPTIDES FROM VENOM OF THE WASP Polistes gallicus. ISOLATION AND PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES

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Six vasoactive peptides have been isolated in the homogeneous form from the venom of the wasp Polistes gallicus. Their physicochemical characteristics have been investigated. The wasp kinins exhibit myotropic and hypotensive effects. One of the kinins shows a prolonged hypotensive action.

The biological properties of vasoactive peptides and, in particular, kinins are shown in very low concentrations [1] and are mediated by their specific interaction with receptor structures concentrated in the smooth tissue of the blood vessels and capillaries [2, 3]. The structural-functional laws of the differential activity of the kinins have scarcely been studied, but numerous attempts have been made at the chemical modification of the molecules of vasoactive peptides with the aim of obtaining analogs with a prolonged action [4]. 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 information on the isolation and the physicochemical and functional characteristics of vasoactive peptides from the venom of the wasp Polistes gallicus.

The vasoactive peptides forming components of the venom were identified by a biotest [5] based on measuring the myotropic activity of the venom on the neck of the rat uterus or on guinea-pig ileum. To distinguish between the contractions caused by peptides and by biogenic amines, we used treatment with proteolytic enzymes or spectific antagonists of histamine and of serotonin (atropine sulfate, cyproheptadine). It is interesting to note that when antibodies to bradykinin (BK) were present in the incubation medium a partial elimination of the myotropic activity of the venom was observed. In other words, the venom contained peptides (BK analogues), exhibiting a substantial myotropic activity.

As a result of the fractionation of the wasp venom on a TSK Hw-40 column (separation of the peptides according to molecular mass) and under conditions of low ionic strength (on the

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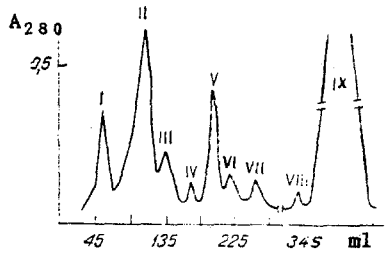

Fig. 1. Chromatographic separation of wasp venom on a TSK Hw-40 column. On the axis of abscissas) the fractions, ml. On the axis of ordinates) the absorption at 280 nm. Fractions II-V contained kinin activity.

TABLE 1. Amino Acid Compositions of the Peptides from Wasp Venom

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

TABLE 2. Hypotensive Activities of the Peptides

Peptide	Beginning of the change, sec	Normalized pressure, sec	Equidepressor dose, μg/kg	Half-life, sec
BK	12	90	10	51
I	5	120	28	62
II	11	58	60	35
III*	—	—	—	—
IV	3	51	134	27
V	7	28	120	18
VI	17	52	27	35

*No change in pressure was detected up to a dose of 200 μg/kg.

basis of hydrophobic-hydrophilic properties), four fractions possessing the biological activity of kinins were obtained (Fig. 1). Then the active fractions were, separately, subjected to ion-exchange chromatography on a SE-cellulose column. The biologically active subfractions were lyophilized and were analyzed by the TLC method on silica gel plates. They were finally purified and desalted on a column of Sephadex G-10. The separation on SE-cellulose of fractions II, III, IV, and V gave the biologically active subfractions II-1, II-2, and II-3, and also III-1, IV-1, and V-1 from which, by chromatography on Sephadex G-10, fractions II-1-1 (peptide I), II-2-2 (peptide VI), II-3-1 (peptide IV), III-1-2 (peptide III), IV-1-1 (peptide II), and V-1-1 (peptide V) were isolated. Thus, as a result of fractionation, six peptides (designated below as (I-VI)) exhibiting a pronounced active effect were isolated.

The homogeneity of the peptides isolated was shown by TLC in various solvent systems, by gradient electrophoresis under strongly denaturing conditions, and by isoelectric focusing, and also by the analysis of N-terminal amino acids. According to the results of the electrophoresis and isoelectric focusing, the wasp peptides migrated in the form of a single band stained by Coomassie and had molecular masses of 1500 ± 500 daltons and different isoelectric points in the range 10.5 ± 0.5. The N-terminal amino acids of peptides (I-VI) were Ile, Leu, Phe, Ile, Phe, and Ile, respectively.

The amino acid composition of the peptides are given in Table 1.

The deductions that can be made on the basis of the results given in Table 1 are as

follows. In the first place, all the peptides isolated had a high content of basic amino acids (Arg or Lys), which is characteristic of all known vasoactive peptides. Furthermore, all the peptides contained the amino acids typical for vasocactive peptides: Val, Pro, and Gly. With respect to their amino acid compositions, the peptides can be arbitrarily divided into two groups: containing and not containing Arg (peptides (II), (III), (IV), and (VI), and peptides (I) and (V), respectively).

In the second place, while the peptides of the first group contained amino acids typical for BK – Phe, Pro, Ser, and Gly – and were, most probably, analogues of BK, the peptides not containing Arg also lacked such amino acids as Phe and Pro. These peptides were apparently representatives of other classes of vasoactive peptides [4]. Finally, on comparing results on their amino acid compositions and N-terminal amino acids it is possible to observe that some peptides having relatively similar compositions (for example, peptides (IV) and (VI)) and identical N-terminal amino acids were nevertheless obtained from different subfractions (see above) and were, most probably, close analogues which is of additional interest for structural-functional investigation.

The myotropic activities of the peptides purified to a homogeneous state were determined by biological testing on isolated smooth-muscle preparations. The contractile effects of kinins (I-IV) were 0.0610, 0.0045, 0.0008, 0.0015, 0.0027, and 0.54 μg·eq BK/μg. Since the molecular masses of the peptides isolated exceeded the molecular dimensions of BK and their N-terminal amino acids were not Arg, then, most probably, they were lengthened from the N-end. The following correlation is known for kinins with different lengths: additional amino acids at the N-end of the BK decrease the myotropic activity of the peptide and increase its resistance to the action of proteolytic enzymes [5]. Thus, apparently, the lower myotropic activities of peptides (I-VI) as compared with BK correspond to this correlation. However, in experiments to study the stability of the peptides isolated under the action of a mixture of proteolytic enzymes (trypsin, chymotrypsin, and carboxypeptidase B – kininase (I)) it was shown that with respect to the half-period of this effect the peptides formed the following sequence: I > BK > II = VI > IV > V > III.

The results obtained agreed well with those of experiments to determine the hypotensive activity of the peptides isolated (Table 2).

On intravenous administration to cats of the vasoactive peptides from the venom, an equidepressor effect was observed at higher doses than that from BK in all cases. However, the hypotensive actions of the peptides set in faster (peptides (I), (II), (IV), and (V)), which is obviously connected with the higher permeability of the blood vessels and capillaries for them. Practically all the peptides isolated exhibited shorter action half-periods. Particular interest is presented by the results obtained for peptide (I). The duration of the hypotensive action for it was approximately 20% greater than for BK – i.e., a prolonged effect was observed – the beginning of its action appeared faster and the equidepressor dose differed only slightly.

In a comparison of the myotropic and hypotensive activities of the peptides isolated and of BK the following correlation was observed: peptides exhibiting a greater myotropic effect also possessed a greater hypotensive action.

On the other hand, with respect to the duration of their hypotensive action (see Table 2), the vasoactive peptides formed the following sequence: peptide (I) > BK > (II) and (VI) > (IV) > (V), which agrees completely with the sequence of resistance to the action of proteolytic enzymes.

EXPERIMENTAL

The venom of the wasp P. gallicus obtained by electrostimulation and lyophilized was investigated. The yield of dry venom from one wasp was 20-25 μg.

Fractionation of Venom. The first separation was carried out on a column (1.6 × 60 cm) of TSK gel Hw-40 in 0.01 M NH_4 formate buffer, pH 3.6, fractions being collected according to a biotest. The bioanalysis was based on the determination of the myotropic activities of the vasoactive peptides on isolated smooth-muscle preparations using BK as standard [5]. The active fractions were chromatographed on a column of SE-cellulose with elution by a gradient NH_4 formate buffer (0.01-1.00; pH 3.6-6.6). In some cases, final purification of the peptides was effected by chromatography on a column of Sephadex G-10 (0.3 × 115 cm) or by preparative thin-layer chromatography in the solvent system butan-1-ol—water—pyridine—acetic acid (15:12:10:3), with elution by 50% pyridine.

The homogeneity of the peptides obtained was shown by gradient electrophoresis under denaturing conditions [6], by isoelectric focusing on standard LKB plates, by analysis of N-terminal amino acids [7], and by TLC. Thin-layer chromatography was conducted on silica gel plates (Merk) in the solvent systems butan-1-ol—water—pyridine—acetic acid (15:12:10:3) and butan-1-ol—acetic acid—water (63:10:27).

Analysis of amino acid compositions was performed on a T-339 analyzer (Czechoslovakia) with an Ostion AB column. The peptides were hydrolyzed with 5.7 N HCl—TFAA (2:1) at 166°C for 60 min [8].

The hydrolysis of the peptides by enzymes was carried out in 0.2 M Tris—HCl buffer, pH 8.3, at a trypsin concentration of 10 μg in an incubation medium with a volume of 200 μl at 37°C for 2 h. Hydrolysis by chymotrypsin and carboxycathepsin (kininase II) were carried out under the same conditions. Hydrolysis by carboxypeptidases A and B was performed under optimum conditions for the action of enzymes [9], and hydrolysis by carboxypeptidase Y — in triethanol acetate buffer, pH 5.5.

Antibodies to BK were obtained by immunizing rabbits with BK-albumin and BK-ovalbumin conjugates obtained with the use of the synthesized bifunctional reagent 2,4-toluylene diisocyanate [10].

The testing of the influence of the vasoactive peptides on the blood pressure was carried out under the conditions of an acute experiment with intravenous administration to nembutal-narcotized cats.

SUMMARY

1. Six peptides possessing kinin activity have been isolated from the venom of the wasp Polistes gallicus. Their physicochemical characteristics have been studied.

2. The wasp vasoactive peptides exhibit a myotropic effect and hypotensive properties. One of them possesses a prolonged hypotensive action.

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