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PEPTIDE INHIBITORS OF ANGIOTENSIN I-CONVERTING ENZYME FROM THE VENOM OF *Echis multisquamatus*

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Using bradykinin (BK) as substrate and ACE from ox kidneys we have studied the inhibitory properties of eight peptides isolated in the pure form from viper venom. The values of IC-50 for them proved to be between 1.6 and 265.0 μg/ml. It was shown simultaneously that the inhibitors isolated, consisting of hydrophobic positively charged deca- to pentapeptides, potentiate the contractile activity of BK on smooth-muscle preparations in vitro. *For a set of six of the peptides, practically complete coincidence of a number of potentiating activites and inhibitory properties has been demonstrated (correlation coefficient $r = 0.86 \pm 0.14$). The inclusion of the two other peptides in the analysis leads to a marked fall in the corrrelation and shows the existence in their case of additional mechanisms for the realization of bradykinin-potentiating action.*

Angiotension I-converting enzyme (ACE, EC 3.4.15.1; synonyms: dipeptidyl dipeptidase, kininase II) is a key enzyme of the metabolism of the hypertensive angiotensins and hypotensive kinins. The inhibition of ACE *in vivo* causes a hypotensive effect. Natural inhibitors of the enzyme (ACEIs) are therefore considered as molecular models for the creation of new specific antihypotensive drugs.

Low-molecular-mass hydrophobic (enriched with proline) peptides consisting of 5-12 amino acid residues that effectively suppress the activity of ACE have been found in the venoms of the snakes *Agkistrodon halys blomhoffii*, *A. h. pallas* and *Bothrops jararaca* [1, 2]. Together with the inhibition effect, these peptides potentiate the depressor activity of bradykinin. The potentiating action of the peptides has a specific nature in relation to BK and is not exhibited in the presence of other contractile agents. The mechanism of the effect, in view of which the peptides are called bradykinin-potentiating peptides (BPPs) is, in general, mediated by the inhibition of the angiotensin-converting enzyme [3]. However, among the BPPs there are exceptions not obeying this law in full measure [4]. On the whole, the question of the relationship between the potentiating properties and inhibitory activities among the BPPs has not been definitively elucidated and requires a detailed study.

In the present paper we consider the inhibitory properties and the mechanisms of the bradykinin-potentiating action of eight peptides that have been isolated in the pure form from viper venom. The peptides were obtained in the individual state with the aid of multistage chromatography, including gel filtration, and adsorption and ion-exchange methods [5]. The isolated inhibitors consisted of hydrophobic positively charged deca- to pentapeptides differing from one another in their physicochemical and functional properties.

Table 1 gives information on the inhibitory action of the peptides in relation to ACE, which are compared with their bradykinin-potentiating properties on isolated preparations of rat uterus and guinea-pig ileum.

A comparison of the results obtained reveals a specificity of the peptides in relation to the muscle preparations used as test systems. Thus, some peptides, such as E-IIIa-2-3 and E-IIIb-2 were more active on the uterus preparation, while others (E-IIIa-2-4 and E-IIIa-2-2) acted more effectively on the ileum. An analogous specificity for muscle preparations has been demonstrated for BPPs for the venom of the mamushi *Ag. halys blomhoff* [1]. This phenomenon is characteristic for BPPs and obviously affects the link between the structure of the peptides and the nature of factors interacting with them. The peptides most active with respect to their inhibiting effect, E-IIIa-2-3 and E-IIIb-2, exhibit a high efficiency on smooth-muscle preparations and can be compared with peptide P from the venom of *Brothrops jaracussu*, which is characterized by an inhi-

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TABLE 1. Bradykinin-potentiating Activities and Inhibitory Properties of Viper Venom Peptides

Peptide	PU (μg of substance in 1 ml) on action on preparations of		IC-50, μg/ml
	uterus	ileum	
E–IIIa–1–1–1	16.7 ± 0.3	13.3 ± 0.3	33.0
E–IIIa–1–2–1	None	16.0 ± 0.3	44.0
E–IIIa–1–3–1	None	65.3 ± 0.3	210.0
E–IIIa–2–1–1	8.7 ± 0.2	8.7 ± 0.1	21.0
E–IIIa–2–2	>400	27.0 + 0.3	265.0
E–IIIa–2–3	1.07 ± 0.02	2.13 ± 0.02	1.6
E–IIIa–2–4	4.7 ± 0.1	3.9 ± 0.1	10.8
E–IIIb–2	6.5 ± 0.1	10.7 ± 0.2	1.7

biting activity (DE-50) of 1.4 μg [6]. At the same time, we have detected peptides inferior to those mentioned above in inhibitory activity but fairly effective on one of the smooth-muscle preparations.

When the peptides under consideration are arranged in order of their decreasing potentiating and inhibitory activities (see Table 1), the following conclusions may be drawn. If we exclude from consideration the peptides E-IIIb-2 and E-IIIa-2-2, all three activity series practically coincide. In other words, there is a direct relationship between the inhibitory activity of a peptide in relation to the angiotensin I-converting enzyme and the bradykinin-potentiating effect on both smooth-muscle preparations. This phenomenon corresponds to the generally accepted point of view that the basic mechanism of the potentiation of the depressor action of bradykinin is the suppression of ACE [1, 2, 7].

However, an example of a contrary situation is peptide E-IIIb-2, which is second with respect to inhibitory activity but appears in the fourth place in the series of potentiating effect (ileum). An analogous pattern is observed for peptide E-IIIa-2-2: while possessing substantial potentiating activity in the test on the ileum (PU = 27.0 ± 0.3), it is a fairly weak inhibitor of the enzyme. In this respect, these two peptides are similar to already known BPPs [4] for which an additional receptor mechanism of the realization of the bradykinin-potentiating action has been demonstrated.

Such a qualitative consideration is confirmed by the results of a correlation analysis of the magnitudes PU and IC-50 performed by generally accepted methods [8]. Thus, for example, if peptides E-IIIb-2 and E-IIIa-2-2 are excluded from consideration, practically absolute correlation of the magnitudes characterizing the two effects appears ($r = 0.86 \pm 0.14$). The relatively high mean error in the calculation of the correlation coefficient is theoretically substantiated and is connected with the small set of results ($n = 6$), i.e., for this series of peptides the predominant mechanism of potentiation is inhibition of the enzyme. The inclusion of the two other peptides in the analysis leads to the sharp fall in the correlation efficient ($r = 0.68 \pm 0.2$) and shows the existence of additional, most probably receptor, mechanisms in addition to the inhibition of ACE.

Correlation analysis of the magnitudes PU and IC-50 in the series of inhibitors from viper venom shows a nonuniformity of the mechanisms of the bradykinin-potentiating effect. The series of peptides obtained is of great interest for structural–functional investigation.

EXPERIMENTAL

We used venom of the viper *Echis multisquamatus* Ch., obtained from the Central Asian Zonal Zoological Combine that had been dried over calcium chloride; bradykinin from Reanal (Hungary); Sephadexes from Farmacia (Sweden); and TSK gels from Toyo-Soda (Japan).

The isolation of the peptides was effected in the way described previously [5].

The isolation of ACE from ox kidneys was done as described in [9].

The contractile activities of the venom and its fractions were determined by their capacity for contracting an isolated muscle preparation of rat uterus and of guinea-pig ileum, with bradykinin as standard [10].

The bradykinin-potentiating activities of the venom and its fractions were investigated from their capacity for enhancing the contractile effect of kinins [1], this being characterized in potentiating units (PU), i.e., the concentrations (μg/ml) at which a doubling of the contractile effect takes place.

The inhibitory activities of the peptides in relation to ACE were analyzed in the following way: the enzyme (4.25 μg) was incubated with 4.0 μg of the substrate in the presence of various concentrations of a BPP (pH 8.0, 37°C, 60 min). Control samples contained no ACE. The reaction was stopped with the aid of TCAA, and samples were neutralized with alkali and diluted with oxalic acid (0.0004 M) and the residual BK–substrate contractile activity in them was determined. The values of IC-50, i.e., the concentration of inhibitory peptide (μg/ml) at which the activity of ACE was 50% inhibited were calculated from inhibition curves. Each point of the curve represented the mean of three measurements.

The correlation analysis of the results was made in accordance with [8].

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