European Journal of Pharmacology, 50 (1978) 119—122

STRUCTURE—ACTIVITY RELATIONSHIPS OF BRADYKININ POTENTIATING PEPTIDES

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Received 28 December 1977, revised MS received 17 March 1978, accepted 24 March 1978

J.G.R. UFKES, B.J. VISSER, G. HEUVER and C. VAN DER MEER, *Structure—activity relationships of bradykinin potentiating peptides*, European J. Pharmacol. 50 (1978) 119—122.

A number of A-VI-5 (Val-Glu-Ser-Ser-Lys) analogues and fragments were synthetized and tested on bradykinin potentiating activity so as to establish the nature of the active group(s) or structural characteristics of some bradykinin potentiating pentapeptides. It could be concluded that (1) the polar groups of the side-chains, such as the two hydroxyl groups of the serine residues, the ω-carboxyl group of the glutamic acid residue and the ω-amino group of the C-terminal lysine, are not essential for the bradykinin potentiating activity; (2) the chain length (at least 5 amino acids) and the lipophilicity of the N-terminal amino acid as well as the whole peptide are of much more importance; (3) the free N-terminal NH_2-group is not essential; (4) aromatic amino acids in position 3 of the peptide chain result in highly active bradykinin potentiating peptides.

Bradykinin potentiating peptides Bradykinin A-VI-5 BPP_{5a}
Structure—activity relationship

1. Introduction

Bradykinin potentiating activity is found in a large number of compounds from various chemical classes, among these compounds are a number of peptides from different sources. In a previous investigation (Ufkes et al., 1976) two specific bradykinin potentiating peptides, A-VI-5 (Val-Glu-Ser-Ser-Lys) and BPP_{5a} (Pyr-Lys-Trp-Ala-Pro), were compared with respect to their potentiation of several effects of bradykinin on various isolated smooth muscle preparations. Apart from a considerable difference in effective concentration, no essential qualitative difference was observed between these two peptides. In a further investigation (Ufkes et al., 1977) the mechanism of action underlying the peptide-induced potentiation of the bradykinin effect on isolated smooth muscle was studied. The results showed that both A-VI-5 and BPP_{5a} induced sensitization of bradykinin receptors by increasing the affinity of the receptors for bradykinin. There was thus no evidence for differences in the mechanism of action of the two peptides. This raises the question why molecules with quite different structures exert the same highly specific effect, i.e. potentiation of the bradykinin response.

In order to gain more insight into the nature of the active group(s) or structural characteristics involved, a number of peptides, mostly A-VI-5 analogues and fragments, were synthetized and tested for bradykinin potentiating activity.

2. Materials and methods

2.1. General

Female guinea pigs (Cpb: albino) of 450—700 g body weight were stunned and bled. A piece about 6 cm long was taken from the terminal ileum and suspended in a 5 ml

organ bath containing Krebs Ringer bicarbonate solution with 0.2% glucose at 35°C and gassed with 5% CO_2 in O_2. A 1.2 g load was used. Contractions were recorded with a displacement transducer (7DCDT-1000, Hewlett Packard, California, U.S.A.) and a Kipp recorder (10-fold augmentation, Micrograph BD9, Delft, Holland).

2.2. Potentiating activity

Bradykinin (BRS 640, kindly supplied by Sandoz, Basel, Switzerland) was added to the bath fluid in concentrations of 1—40 ng/ml. In order to test the bradykinin potentiating activity of the peptides, dose—response curves for bradykinin were made during the experiment. The potentiating factor Pf was defined as: Pf = bradykinin equivalent of bradykinin plus potentiating peptide/actual dose of bradykinin added. Pf values were expressed as the geometric mean.

The peptides were added to the bath fluid 30 sec prior to the addition of bradykinin and in concentrations sufficient to cause detectable bradykinin potentiation. The relative activity for each peptide could be established from the final molar concentration and from the Pf value. The activity of A-VI-5 was taken as 1.0. The relative activity index was given by the ratio of the molar concentration of A-VI-5 and the equivalent molar concentration of the peptide. This ratio could be calculated from the (linear) relation between the log concentration and the log Pf found with A-VI-5.

2.3. Peptides

The peptides were synthetized according to the solid-phase technique developed by Merrifield (Stewart and Young, 1969). The synthetized peptides were purified by ion-exchange chromatography (Dowex AG 50W-X4 or Dowex 1-X2), followed by gel filtration (Sephadex G-10) and freeze-drying. Thin-layer chromatography was used as a test for purity. Amino acid compositions were confirmed by amino acid analysis performed by Dr. A.O. Muijsers, Department of Biochemistry, University of Amsterdam, Plantage Muidergracht 13, Amsterdam. Amino acid sequences were checked by mass spectrometry, performed by Dr. H.A.H. Craenen and Ir. E.R.J. Wils, Chemical Laboratory TNO, Lange Kleiweg 137, Rijswijk.

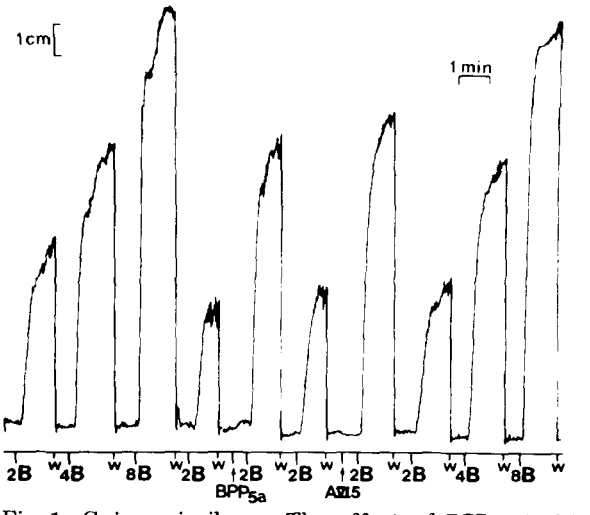

Fig. 1. Guinea-pig ileum. The effect of BPP_{5a} (0.04 μg/ml) and A-VI-5 (55 μg/ml) on the bradykinin (B, in ng/ml bath fluid-induced contractions. w = washing 3 times.

3. Results

A number of A-VI-5 analogues and fragments were tested on the isolated guinea-pig ileum for their bradykinin potentiating activity. A sample recording (fig. 1) shows the effect of A-VI-5 and BPP_{5a} on the bradykinin-induced contraction. The amino acid sequences of A-VI-5, of the A-VI-5 analogues and fragments and of BPP_{5a} are listed in table 1 together with the concentration used, the bradykinin potentiating activity and the relative activity index.

4. Discussion

The effect of replacing certain amino acids in the A-VI-5 peptide chain on the bradykinin

TABLE 1

The amino acid sequences of A-VI-5, the A-VI-5 analogues and fragments and BPP_{5a}, the concentrations used, the potentiating activity (Pf) and the relative activity index.

Peptide	Structure	Concentration (mol/l)	Pf	Relative activity index
A-VI-5	Val-Glu-Ser-Ser-Lys 1 2 3 4 5	1.00×10^{-4}	2.4	1.0
1	Val-Glu-Ser-Ala-Lys	1.03×10^{-4}	3.2	1.9
2	Val-Glu-Ala-Ser-Lys	1.03×10^{-4}	3.0	1.6
3	Val-Glu-Ala-Ala-Lys	1.06×10^{-4}	4.1	3.2
4	Val-Nva-Ala-Ala-Lys	1.13×10^{-4}	3.5	2.2
5	Val-Ala-Ala-Ala-Lys	1.20×10^{-4}	2.3	0.8
6	Val-Glu-Ala-Ala-Nle	1.10×10^{-4}	3.0	1.6
7	Gly-Glu-Ala-Ala-Lys	2.30×10^{-4}	1.9	0.3
8	Leu-Glu-Ala-Ala-Lys	1.04×10^{-4}	3.6	2.5
9	Phe-Glu-Ala-Ala-Lys	0.97×10^{-4}	3.2	2.0
10	Val-Glu-Gly-Gly-Lys	2.26×10^{-4}	1.2	0.1
11	* Iva-Glu-Ala-Ala-Lys	0.55×10^{-4}	2.9	2.8
12	Glu-Ala-Ala-Lys	1.32×10^{-4}	1.3	0.2
13	Nva-Ala-Ala-Lys	1.42×10^{-4}	1.4	0.2
14	Ala-Ala-Lys	3.82×10^{-4}	1.5	0.1
15	His-Ala-Lys	2.82×10^{-4}	1.8	0.2
16	Pyr-Glu-Ala-Ala-Lys	1.04×10^{-4}	2.7	1.4
17	Val-Lys-Ala-Ala-Lys	1.07×10^{-4}	2.8	1.3
18	Val-Glu-Trp-Ala-Lys	1.58×10^{-7}	2.2	534.0
19	Val-Glu-Ala-Ala-Pro	1.13×10^{-4}	2.9	1.5
20	Val-Glu-Phe-Ala-Lys	1.69×10^{-6}	1.9	37.2
21	Val-Glu-His-Ala-Lys	3.43×10^{-6}	2.5	34.0
22	Val-Glu-Leu-Ala-Lys	0.18×10^{-4}	2.0	3.9
BPP_{5a}	Pyr-Lys-Trp-Ala-Pro	1.63×10^{-7}	2.8	920.0

* Iva = isovaleric acid.

potentiating activity can be summarized as follows. Replacement of the serine residue in position 3 or 4 by alanine as in peptides 1 and 2, slightly increased the bradykinin potentiating activity. The activity was considerably enhanced if both serine residues were replaced by alanine as in peptide 3. Apparently the presence of both hydroxyl groups is not only redundant but even diminishes the bradykinin potentiating activity. When the glutamic acid residue in position 2 was replaced by norvaline, resulting in peptide 4, the activity was only slightly reduced. The ω-carboxyl group in position 2 therefore appears not to be essential. However, shortening the side chain in this position (peptide 5) decreased the activity. Replacement of the lysine residue in position 5 by norleucine yielded peptide 6 without an ω-amino group, resulting in a small loss in bradykinin potentiating activity. It can be concluded from these data that all polar groups of the side chains are not essential for the bradykinin potentiating activity of A-VI-5. Therefore, the assumption of Weyers et al. (1972) that the bradykinin potentiating activity of peptides derived from plasma proteins might be determined by the juxtaposition of an amino acid with an ω-carboxyl group and an amino acid with a hydroxyl group is not valid.

Three peptides (7, 8 and 9) were subsequently synthetized, each with a different N-terminal amino acid, the rest of the peptide chain remaining identical to peptide 3.

There was a considerable loss in activity when glycine was introduced (peptide 7) and only a slight reduction when leucine (peptide 8) or phenylalanine (peptide 9) were introduced. Replacement of both alanine residues in position 3 and 4 by glycine as in peptide 10, greatly reduced the activity. Obviously the N-terminal amino acid as well as the peptide as a whole must be somewhat lipophilic for the bradykinin potentiating activity to exist. Replacement of the valine residue by isovaleric acid (peptide 11) caused no loss in activity and therefore a free N-terminal amino group is not essential.

Shortening of the peptide chain from pentapeptides to tetrapeptides (peptides 12 and 13) or tripeptides (peptides 14 and 15) caused a drastic fall in bradykinin potentiating activity. A chain-length of at least five amino acids seems to be essential.

In order to establish why BPP_{5a} was so much more active than A-VI-5, a number of peptides were synthetized on the basis of the structure of peptide 3 but with each containing one amino acid residue present in BPP_{5a}. Substitution with pyroglutamyl, lysyl, or prolyl in position 1, 2 or 5 (peptide 16, 17 or 19 respectively) did not greatly affect the bradykinin potentiating activity. However, substitution with tryptophan in position 3 (peptide 18) resulted in an enormous increase in activity. Apparently the bradykinin potentiating activity is largely determined by the amino acid residue in position 3. To specify the characteristic requirement for the amino acid in position 3, other amino acids were substituted. It appeared that aromatic amino acids (peptides 20 and 21) in this position were much more efficient than an aliphatic amino acid (peptide 22) with a lipophilic side-chain. In this respect tryptophan is by far the best choice for position 3. The high bradykinin potentiating activity of BPP_{5a} is therefore largely due to the presence of tryptophan in position 3.

Acknowledgements

The authors wish to express their gratitude to Mrs J. Visser-Berdenis van Berlekom and R. de Boer for technical assistance during the purification of the peptides, Dr. A.O. Muijsers for the amino acid analysis and Dr. H.A.H. Craenen and Ir. E.R.J. Wils for the mass spectrometry.

References

Stewart, J.M. and J.D. Young, 1969, Solid Phase Peptide Synthesis (Freeman and Company, San Francisco).

Ufkes, J.G.R., P.N. Aarsen and C. van der Meer, 1976, The bradykinin potentiating activity of two pentapeptides on various isolated smooth muscle preparations, European J. Pharmacol. 40, 137.

Ufkes, J.G.R., P.N. Aarsen and C. van der Meer, 1977, The mechanism of action of two bradykinin potentiating peptides on isolated smooth muscle, European J. Pharmacol. 44, 89.

Weyers, R., P. Hagel, B.C. Das and C. van der Meer, 1972, Tryptic peptides from rabbit and bovine albumin enhancing the effect of bradykinin, Biochim. Biophys. Acta 279, 331.