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Short communication

FURTHER STUDIES ON THE STRUCTURE-ACTIVITY RELATIONSHIPS OF BRADYKININ-POTENTIATING PEPTIDES

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Several pentapeptides were synthesized and tested for bradykinin-potentiating activity. From these and previous data it appeared that an (L)-aromatic amino acid residue (preferably Trp) in position 3 is essential for high activity. Position 3 represents a stereospecific pillar function, whereas the other positions and the lipophilicity/hydrophilicity balance are important for additional activity. So far, BPP_{5a} seems to have the optimal structure for a bradykinin-potentiating pentapeptide.

Bradykinin-potentiating pentapeptides Structure-activity relationships

1. Introduction

In order to establish the nature of the active group(s) or structural characteristics of certain bradykinin-potentiating pentapeptides, a number of A-VI-5 (Val-Glu-Ser-Ser-Lys) analogues and fragments were synthetized and tested for bradykinin-potentiating activity (Ufkes et al., 1978; Heuver et al., 1980). It was concluded that (1) the polar groups of the side-chains are not essential; (2) the chain length (at least 5 amino acid residues) and the lipophilicity are of much more importance; (3) the free N-terminal NH_2 group is not essential; (4) aromatic amino acid residues in position 3 result in highly active peptides. The present paper reports on further studies made to confirm the importance of position 3 and the contribution of the other positions as well, to assess the requirement of a certain balance between lipophilicity and hydrophilicity and to search for the optimal sequence of a bradykinin-potentiating pentapeptide.

2. Materials and methods

The bradykinin-potentiating activity of the peptides was determined on the isolated guinea pig ileum and expressed as the relative activity index. This index is the ratio of the molar concentration of A-VI-5 and the molar concentration of the peptide under test in relation to an equivalent bradykinin potentiation as described in detail by Ufkes et al. (1978).

Most of 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 followed by gel chromatography and freeze-drying. Thin layer chromatography in three solvent systems was used as a test for purity. The amino acid compositions were confirmed by amino acid analysis performed by Dr. A.O. Muijsers and J.K.P. Post, Department of Biochemistry, University of Amsterdam. Peptides nr. 23, 24 and 25 were obtained from Bachem (Bubendorf,

TABLE 1

The amino acid sequences of A-VI-5, BPP_{5a}, the synthetized and purchased * pentapeptides and their bradykinin-potentiating activity expressed as the relative activity index.

Peptide	Structure	Relative activity index
A-VI-5	^{1}Val – ^{2}Glu– ^{3}Ser – ^{4}Ser – ^{5}Lys	1.0
BPP_{5a}	<Glu – Lys – Trp – Ala – Pro	920.0
23	* Ala – Ala – Ala – Ala – Ala	0.8
24	* Gly – Gly – Gly – Gly – Gly	0.0
25	* Ala – Ala – Tyr – Ala – Ala	2.9
26	Ala – Ala – Trp – Ala – Ala	5.6
27	Val – Ala – Trp – Ala – Ala	26.8
28	Val – Ala – Trp – Ala – Lys	27.9
29	Val – Lys – Trp – Ala – Ala	51.1
30	Val – Trp – Ala – Ala – Lys	1.1
31	Val – Ala – Ala – Trp – Lys	1.7
32	<Glu – Lys – Phe – Ala – Pro	192.2
33	<Glu – Lys – D-Trp – Ala – Pro	6.9
34	<Glu – Lys – Nal(2) – Ala – Pro **	93.3
35	Glu – Lys – Trp – Ala – Pro	20.1
36	Val – Lys – Trp – Ala – Pro	222.1
37	Val – Lys – fTrp – Ala – Pro ***	262.1
38	Arg – Lys – Trp – Ala – Pro	96.6
39	<Glu – Glu – Trp – Ala – Pro	63.8
40	<Glu – Lys – Phe – Arg – Pro	54.7
41	Val – Glu – Trp – Val – Lys	51.5
42	<Glu – Lys – Trp – Ala – Lys	11.7
43	<Glu – Lys – Phe – Ala – Phe	33.3
44	Pro – Gly – Phe – Ser – Pro	7.9
45 Bk-fragments	Phe – Ser – Pro – Phe – Arg	4.4
46 (proctolin)	Arg – Tyr – Leu – Pro – Thr	2.5

** Nal(2) = 2-naftyl-3-alanine.
*** fTrp = N^{in}-formyl-Trp.

Switzerland). Bradykinin was obtained from Schwarz-Mann (New York, U.S.A.).

3. Results

The pentapeptides were tested on the isolated guinea pig ileum for their bradykinin-potentiating activity. Among them were peptides with a more 'neutral' character with or without Trp incorporated in different positions, A-VI-5 and BPP_{5a} hybrid analogues, pentapeptide fragments of bradykinin and pentapeptides with rather divergent structures. The amino acid sequences of the peptides are listed in table 1, together with the bradykinin-potentiating activity, expressed as the relative activity index as compared to that of A-VI-5 (considered as 1.0).

4. Discussion

The influence of certain amino acids within the pentapeptide chain on the bradykinin-potentiating activity can be described as follows: A peptide with a 'neutral' character such as penta-alanine (23, table 1) showed only a slight decrease in activity as compared to A-VI-5. However, the peptide backbone per se does not contribute to the activity since pentaglycine (24) appeared to be inactive. Substitution with Tyr (25) or Trp (26) in position 3 increased the activity but much less so than

expected from the enhanced activity of A-VI-5 analogues with an aromatic residue in position 3 (Ufkes et al., 1978). This was probably not due to the very poor water-solubility of these peptides, since introduction of the more lipophilic residue Val in position 1 (27), as in A-VI-5, further enhanced the activity by a factor of 5. The introduction of Lys, thereby increasing hydrophilicity, had no effect on the activity when substitution was in position 5 (28), as in A-VI-5, but doubled the activity when substitution was in position 2 (29), as in BPP_{5a}. Incorporating Pro in position 5 (36) also resulted in a marked increase in activity. These findings already indicate that in BPP_{5a}, in addition to Trp in position 3, each amino acid residue may contribute to the total biological activity of the peptide molecule. To confirm the importance of the presence of an aromatic amino acid in position 3, Trp was also substituted in position 2 (30) and 4 (31). As compared to peptides 28 or 29, the activity of both peptides decreased enormously. In order to specify the characteristic requirement of Trp in position 3 of BPP_{5a}, Phe (32) and D-Trp (33) were introduced. Peptide 32 was 5 times less active whereas peptide 33 was 130 times less active as compared to BPP_{5a}. Apparently position 3 represents a stereospecific pillar function determining the activity to a large extent. Substitution of BPP_{5a} with 2-naftyl-3-alanine in position 3 (34) caused a considerable decrease in bradykinin-potentiating activity as compared to BPP_{5a}. Thus the 2-naftyl group appeared to be less effective than the indolyl group in BPP_{5a} or the phenyl group in peptide 32. The indolyl NH-function does not play a role here, as masking the indolyl N-atom in the Trp-containing peptides with a formyl-group (a standard procedure during the synthesis of Trp-containing peptides, in order to protect the indolyl group against chemical attack) had hardly any effect on the potentiating activity (37) as compared to peptide 36. The requirements regarding the size of the aromatic ring system or the degree of aromaticity (or electron-donating capacity) are probably quite precise.

In order to obtain more insight in the contribution of the other positions of the peptide chain, several BPP_{5a} analogues were synthetized. Introduction of Glu in position 1 resulted in a peptide (35) with a considerable loss in activity as compared to BPP_{5a}. This had already been found by Greene et al. (1970). Val in position 1 (36,37) or Arg (38) also caused a decrease in activity though to a smaller extent. The terminal pyroglutamyl residue in position 1 together with Trp in position 3 seemed to be an optimal combination for the activity. Introduction of Glu in position 2, as present in A-VI-5, also resulted in a peptide (39) with diminished activity as compared to BPP_{5a}. The same applied to substitutions in position 4 with Arg (40) as compared to peptide 32 and Val (41) as compared to Val-Glu-Trp-Ala-Lys (peptide 18, see Ufkes et al., 1978). Of the amino acids Ser, Ala, Gly, Val and Arg in position 4 (see also Ufkes et al., 1978) Ala seems to be the best choice. Lys (42) and Phe (43) were introduced to determine the importance of position 5. Both peptides were considerably less active than BPP_{5a} and peptide 32 respectively. Therefore, Pro in position 5 seems to be the best choice in BPP_{5a}. Besides the enormous significance of position 3 the other positions appeared to be important for additional contributions to the activity. In this respect < Glu-Lys-Trp-Ala-Pro (BPP_{5a}) so far seems to be the optimal structure for a bradykinin-potentiating pentapeptide.

Additionally, two pentapeptide fragments of bradykinin (44 and 45) and proctolin (46), a highly biologically active pentapeptide in insects, were synthetized and tested on the ileum for their bradykinin-potentiating activity. Both bradykinin fragments appeared to be active which suggests a possible (patho-)physiological function of these peptides in situations where there is a high bradykinin turnover. However, no additional information about the structure-activity relationships was obtained.

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