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BRADYKININ POTENTIATING PEPTIDE PCA-LYS-TRP-ALA-PRO

AN INHIBITOR OF THE PULMONARY INACTIVATION OF BRADYKININ AND CONVERSION OF ANGIOTENSIN I TO II*

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Abstract—The synthesis of a bradykinin potentiating peptide isolated from *Bothrops jararaca* venom [S. H. FERREIRA, D. C. BARTELT and L. J. GREENE, *Biochemistry* **9**, 2583 (1970)] is described. The synthetic pentapeptide BPP_{5a}, PCA-Lys-Trp-Ala-Pro, had the same spectrum of pharmacological properties as previously reported for Bradykinin Potentiating Factor [S. H. FERREIRA, *Br. J. Pharmac. Chemother.* **24**, 163 (1965)] and had the same specific activity as the natural pentapeptide in the guinea-pig ileum assay. The peptide potentiated the depressor effect of bradykinin on blood pressure and also potentiated the effect of bradykinin on capillary permeability.

Intravenous infusion of the peptide into the rat substantially reduced the pulmonary inactivation of bradykinin and inhibited the conversion of angiotensin I to angiotensin II.

BRADYKININ Potentiating Factor, BPF‖, is a peptide fraction from *Bothrops jararaca* venom which inhibits the enzymes that normally inactivate bradykinin and the enzyme responsible for the conversion of angiotensin I to angiotensin II. We have described the isolation, amino acid composition, and relative activities in the guinea-pig ileum assay of nine biologically active peptides from the venom. The amino acid sequence of

* A preliminary report of this work was presented at the International Symposium on Cardio, vascular and Neuro-Actions of Bradykinin and Related Kinins, Fiesole, Florence, Italy, July 21–25-1969. L. J. GREENE, J. M. STEWART and S. H. FERREIRA, *Pharm. Res. Comm.* **1**, 159 (1969). The preceding paper in this series is Ref. 1.

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‖ Abbreviations used: BPF, Bradykinin Potentiating Factor; BPP, Bradykinin Potentiating Peptide; Boc, *t*-butyloxycarbonyl; PCA, pyrrolidone carboxylic acid (pyroglutamic acid) as both free acid and in peptide bond.

the smallest active peptide, BPP_{5a}* was shown to be PCA-Lys-Trp-Ala-Pro.[1] In this paper we report the synthesis of this peptide and related peptides by the solid phase method and the activity of BPP_{5a} in five biological assay systems.

EXPERIMENTAL PROCEDURE

Materials. Chloromethylated polystyrene-2%-divinyl benzene resin, dicyclohexylcarbodiimide, Boc-L-proline, Boc-L-alanine, Boc-L-tryptophan, α-Boc-ε-carbobenzoxy-L-lysine and α-Boc-γ-benzyl-L-glutamate were purchased from Schwarz BioResearch, Orangeburg, New York. L-Pyrrolidone carboxylic acid was purchased from Mann Laboratories, Orangeburg, New York. Bradykinin and angiotensin I (Asp[1]-Ile[5]-angiotensin I) and angiotensin II (Asp[1]-Ile[5]-angiotensin II) were synthesized by one of us (J.M.S.).

Peptide synthesis. The peptides were synthesized by the solid phase method[3,4] using both manual and automatic procedures.[5] Boc proline and α-Boc-ε-carbobenzoxy lysine were esterified to 2% crosslinked chloromethyl resin by the standard procedure (Ref. 3, p. 32). One g of Boc proline resin (0·4 mmole proline/g resin) was used for each synthesis of peptides 2, 4 and 6, and 1·4 g of α-Boc-ε-carbobenzoxy lysine resin (0·28 mmole/g) was used for peptide 7 (cf. Table 1). The Boc protecting group was removed at each stage of the synthesis by a 30-min treatment with 4 M anhydrous hydrogen chloride in peroxide-free dioxane (Ref. 3, p. 38). This solution contained 2-mercaptoethanol (1 mg/ml) to prevent acid degradation of tryptophan. The α-amine hydrochloride form of the peptide-resin was neutralized by treatment with 10% triethylamine in chloroform for 10 min. Boc alanine, α-Boc-ε-carbobenzoxy lysine and Boc glutamic acid-γ-benzyl ester were coupled to the resin by treating the peptide-resin with 2·5 equivalents of the Boc amino acids and dicyclohexylcarbodiimide in dichloromethane for 2 hr. Because of the low solubility of Boc tryptophan and pyrrolidone carboxylic acid in dichloromethane, these amino acids were coupled using the same proportions in a mixed solvent consisting of 60 per cent purified (Ref. 3, p. 35) dimethylformamide and 40 per cent dichloromethane. Due to the low atmospheric pressure at the altitude of Denver (1620 m), some difficulty was experienced in pumping dichloromethane solutions with the automatic instrument. For this reason the coupling solvent used in later automatic runs was chloroform or 1,2-dichloroethane instead of dichloromethane.

After assembly of the peptide on the resin was complete, the peptide-resin was treated with 4 M HCl-dioxane for 30 min, washed thoroughly with dioxane, ethanol and dichloromethane, and dried *in vacuo*. Peptides were simultaneously deblocked and cleaved from the resin by treatment with anhydrous hydrogen fluoride in the

* We suggest the following system of nomenclature for the peptides of known structure having the same spectrum of pharmacological activity as Bradykinin Potentiating Factor, BPF (low molecular fraction from *Bothrops jararaca* venom, Ref. 1). All peptides, whether naturally occurring, synthetic peptides of the same structure as naturally occurring peptides or synthetic analogues of naturally occurring peptides, would be identified by the letters BPP. A subscript number indicates the number of amino acid residues per molecule. A lower case letter would be used to complete the nomenclature because different peptides with the same number of residues per molecule have been described. Changes in the position or substitution of residues in synthetic peptides would follow the current peptide nomenclature. The pentapeptide described in this paper, PCA-Lys-Trp-Ala-Pro is peptide V-3-A in Ref. 1. It will be referred to as BPP_{5a}. The peptide PCA-Gly-Leu-Pro-Pro-Arg-Pro-Lys-Ile-Pro-Pro, potentiator B described by Kato and Suzuki[2] would be BPP_{11a} in this system of nomenclature.

TABLE 1. PROPERTIES OF BRADYKININ POTENTIATING PEPTIDES

Peptide	Relative specific activity (Molar)	Electrophoretic mobility	Dowex 50-X2 elution pH	Distribution coefficient (CCD)	Amino acid composition moles per mole peptide					Yield (%)
					Glx	Lys	Trp	Ala	Pro	
1. PCA-Lys-Trp-Ala-Pro (Natural)	100·0	1·64	3·75		1·01	1·00	0·81	0·97	1·02	
2. PCA-Lys-Trp-Ala-Pro (Synthetic)	100·0	1·64	3·75	0·076	1·00	0·99	0·91	0·97	1·03	35
3. PCA-Lys-Trp-Ala-Pro (Synthetic yellow peptide)	0·50		4·05		1·01	1·01	0·38	0·98	1·00	2
4. Glu-Lys-Trp-Ala-Pro	6·25	3·10	4·05	0·33	1·00	0·97	0·70	1·00	1·02	15
5. PCA-Lys-Trp- - - - - Pro	< 0·44	1·66	3·75	0·182	0·98	0·98	0·75		1·04	23
6. - - - - - - - - Trp-Ala-Pro	0·25	2·21	3·90				0·89	1·00	0·99	67
7. PCA-Lys - - - - - - - - - - - -	0	1·85	3·40		1·01	0·99				43

Relative specific activity: Potentiation of bradykinin in the guinea-pig ileum assay. The values are expressed as specific activity on a molar basis relative to Peptide 1 which has been taken as 100. The concentration of each peptide was determined by amino acid analyses. *Electrophoretic mobility:* The mobility of the peptide relative to glycine on high voltage paper electrophoresis in pyridine acetic acid buffer, pH 3·5 at 44 V/cm. *Dowex 50-X2 Elution pH:* The elution pH of the peptide from a Dowex 50-X2 column developed with a linear pH gradient of pyridine acetic acid buffer. *Distribution coefficient:* Partition coefficient in the system used for purification. Peptides 2 and 5 were separated by 240 transfers in a system employing 1-butanol–0·4 M ammonium acetate (pH 7·0). Peptide 4 was purified in 100 transfers using the solvent system 1-butanol–0·5% trifluoracetic acid. *Amino acid composition:* The average for all amino acids except tryptophan was used as the basis for the calculation of molar ratios of the constituent amino acids. No corrections have been applied for tryptophan destruction. *Yield:* Per cent yield of pure peptide obtained based on the amount of amino acid resin used. Peptide 5 was prepared as a byproduct in a synthesis of Peptide 2 (cf. Methods). Peptide 2 was synthetized in 18 per cent yield in the same experiment.

presence of anisole at 0° for 30 min (Ref. 3, p. 44). After thorough evaporation of the hydrogen fluoride, the mixture of resin and peptide was extracted with ethyl acetate to remove the last traces of anisole and its reaction products. Peptides were extracted from the resin with 1 M acetic acid.

Purification and characterization of synthetic peptides. The peptides were purified by countercurrent distribution (CCD) in a 200 tube H. O. Post Instrument (10 ml/phase) using the solvent systems listed in Table 1 or by gradient elution chromatography on Dowex 50-X2.[6] PCA-Lys-Trp-Ala-Pro and PCA-Lys-Trp-Pro were separated from each other either by CCD or by gel filtration on a 0·9 × 400 cm column of Sephadex G-25 equilibrated and developed with pyridine acetic acid buffer, pH 3·1. The procedures used for chromatography on Dowex 50-X2, gel filtration on Sephadex G-25, detection of peptides in chromatographic effluent and high voltage electrophoresis at pH 3·5 and pH 6·5 are given in Ref. 1.

Peptides (0·1–0·2 μmoles) were hydrolyzed in an evacuated sealed tube with 1 ml of 6 N constant boiling HCl containing 1 mg/ml each of phenol and 2-mercaptoethanol* for 22 hr at 110°. Amino acid analysis of hydrolysates was performed by the method of Spackman, Moore and Stein[7] on an automatic instrument with provisions for multiple sample application.[8] No corrections have been made for destruction of tryptophan during acid hydrolysis.

Isolated guinea-pig ileum. The bradykinin potentiating activity of the peptides was estimated in the guinea-pig ileum assay described in Ref. 1, except that the temperature of the tissue bath was 30°. Potentiating activity was estimated in terms of the increase in the response of the assay tissue to a standard dose of bradykinin. One unit of potentiation is the concentration, per ml of bath fluid, required to increase the effect of a single dose (b) of bradykinin to match that elicited by twice the dose (2b) of bradykinin.

Blood pressure. Mean arterial blood pressure in the dog (three animals), cat (three animals) and rabbit (four animals) was recorded with a Beckman–Offner Dynograph using a Statham pressure transducer attached to a cannula inserted in the carotid artery. Single injections of bradykinin, BPP_{5a}, and BPF were made into the jugular vein.[9]

Pulmonary inactivation of bradykinin in the rat.[10] Male rats, 200 g, were anesthetized with phenobarbital (65 mg/kg) or dial-urethane (1·3 ml/kg of a solution containing 10 g diallyl barbituric acid, 40 g urethane and 40 g monomethyl urea per 100 ml). Blood pressure was measured from a femoral artery with a Statham transducer and a Grass polygraph. Intravenous injections were made into a polyethylene cannula inserted through the left jugular vein so that its tip lay in the right atrium. Intra-arterial injections were made into a polyethylene cannula inserted through the right carotid artery so that its tip lay in the ascending aorta. Pulmonary disappearance of bradykinin was measured by determining the difference in the amounts of bradykinin required by the two routes, intravenous and intra-arterial, to produce a standard depressor response of 25 mm Hg from the usual basal pressure of 100–125 mm Hg. Peptides to be assayed for BPF activity were dissolved in isotonic saline and the solution was infused through a femoral vein at 0·1 ml/min.

Pulmonary conversion of antiotensin I in the rat. The systemic response method[10] was also used as above to measure the inhibition of the pulmonary angiotensin I

* J. M. Stewart, unpublished procedure.

converting enzyme by synthetic BPP_{5a} in normal rats. The amounts of synthetic angiotensin I and angiotensin II required to produce a standard pressor response (25 mm Hg) by intravenous and intra-arterial routes of administration was determined in rats before and during infusion of different amounts of synthetic BPP_{5a}.

Capillary permeability in the rat. The assay was performed on rats weighing 150–200 g, anesthetized with ethyl ether. Evans blue (200 mg) was injected into the dorsal penis vein 5 min after the intradermal injection (0·1 ml) of bradykinin (0·1–3 μg), BPP_{5a} (1–5 μg), or a mixture of both, in saline. The animals were sacrificed 10 min after dye injection by exsanguination. The size of the blue spot on the inner surface of the skin at the site of intradermal injection was used as a measure of the bradykinin-induced increase of capillary permeability.

RESULTS

Synthetic peptides. The data presented in Table 1 summarize the chemical and physical properties of natural BPP_{5a} and six synthetic peptides. With the exception of peptide 3 (see below) the peptides were homogeneous by the criterion of high voltage electrophoresis at pH 3·5 and pH 6·5 and on the basis of the integral molar ratios of the constitutive amino acids. Complete correspondence in chemical and physical properties as well as susceptibility to enzymatic hydrolysis was found for natural (Peptide 1) and synthetic (Peptide 2) BPP_{5a}.

Peptide 3 (synthetic yellow peptide) was a minor byproduct of the synthesis of PCA-Lys-Trp-Ala-Pro (Peptide 2) and was isolated in 2 per cent yield by chromatography on Dowex 50-X2. Amino acid analysis of the peptide hydrolysate showed equimolar amounts of glutamic acid, lysine, alanine and proline, but the amount of tryptophan present after acid hydrolysis was lower than expected. Several fluorescent components were separated by high voltage electrophoresis at pH 3·5 under conditions where the other tryptophan containing peptides behaved as single components. Peptide 3 apparently is PCA-Lys-Trp-Ala-Pro containing one or more oxidized forms of tryptophan. When Peptide 2 was rechromatographed on Dowex 50-X2 it was recovered as a single component in high yield. This result indicates that Peptide 3 was not an artifact produced from Peptide 2 during chromatography.

The tetrapeptide PCA-Lys-Trp-Pro (Peptide 5) was prepared during one automatic synthesis of PCA-Lys-Trp-Ala-Pro (Peptide 2) when poor pumping of Boc alanine occurred due to vaporization of the dichloromethane solvent in the pump. The crude product contained both peptides. They were satisfactorily separated by CCD (cf. Table 1) and by gel filtration. The peptides were not separated by high voltage electrophoresis at pH 3·5 or by chromatography on Dowex 50-X2.

Peptides 6 and 7 which correspond to the tryptic cleavage products of BPP_{5a} also had the same physical properties (cf. Table 1) and susceptibility to enzymatic digestion as the tryptic peptides prepared from natural BPP_{5a}.[1]

Potentiation of bradykinin in guinea-pig ileum assay. The relative specific activity (molar basis) of each peptide for the potentiation of bradykinin in the guinea-pig assay is presented in Table 1. In these experiments one unit of potentiation was achieved with the peptides at the concentration $0{\cdot}5\text{–}1{\cdot}0 \times 10^{-7}$ M. Thus the specific activity was of the order of 10 bradykinin potentiating units per nmole per ml bath fluid. The specific activities of natural and synthetic BPP_{5a} were identical when assayed on the same tissue. The potentiating activity of BPP_{5a} on ileum is independent of the

time of contact of BPF with the tissue; thus the same result was obtained by pre-incubation of the tissue with BPP_{5a} (up to 3 min) or by addition of BPP_{5a} to the tissue when it was at the height of a bradykinin-induced contraction. Identical results were obtained by isotonic or isometric recording of contractions. BPP_{5a} alone produced no effect on the guinea-pig ileum at concentrations up to 10 μg/ml.

Peptide 3, BPP_{5a} containing one or more oxidized forms of tryptophan, had less than 1 per cent of the activity of the unmodified peptide. Comparison of the activity of Peptide 4 (Glu-Lys-Trp-Ala-Pro) with BPP_{5a} indicates that replacement of the cyclized form of glutamic acid with glutamic acid (which introduces an α-amino and a γ-carboxyl group not present in BPP_{5a}) reduces the potency of the peptide by a factor of 16. The tryptic fragments of BPP_{5a} PCA-Lys (Peptide 7) and Trp-Ala-Pro (Peptide 6) as well as Des-Ala-BPP_{5a} (Peptide 5) had little or no activity.

Potentiation of the hypotensive effect of bradykinin. A single intravenous injection of BPP_{5a}, 2 mg/kg, produced no or only transitory hypotension. The same dose potentiated the hypotensive effect of intravenously injected bradykinin in the dog, cat and rabbit, Fig. 1. The intensity and duration of response to bradykinin was greater with BPF than BPP_{5a} in the three species tested as illustrated for the rabbit in Fig. 1. After injection of BPP_{5a} in these species the response to bradykinin returned to control levels within 15 min. In contrast to this result with BPP_{5a}, bradykinin potentiation could be detected for more than 30 min after treatment with BPF.

Inhibition of pulmonary inactivation of circulating bradykinin in the rat. The pulmon-

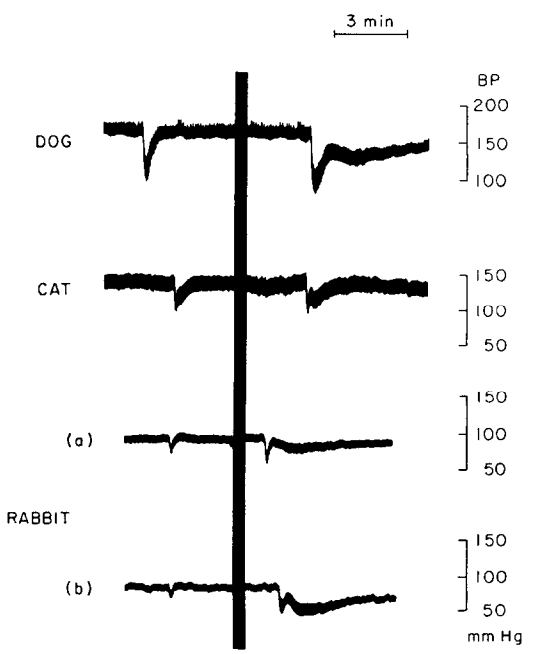

FIG. 1. Potentiation of the hypotensive effect of bradykinin by BPP_{5a} and BPF. The intravenous doses of bradykinin were 0·25 μg/kg for the dog (4 kg), 1·0 μg/kg for the cat (3 kg) and 0·2 μg/kg for the rabbit (3 kg). The bar separates the effects of bradykinin before (left side) and 5 min after a single intravenous injection 2 mg/kg of BPP_{5a} or BPF (tracing b, rabbit). Tracing b was obtained 20 min after injection of BPP_{5a} (tracing a).

ary inactivation of bradykinin was studied before and during intravenous infusion of BPP_{5a}. No significant direct effect on the blood pressure was observed at rates up to 2·5 mg/min of BPP_{5a}. Although infusion of BPP_{5a} did not appreciably modify the effect of intra-aortically injected bradykinin it potentiated bradykinin administered intravenously as described above for other species.

In normal rats 98 per cent of the circulating bradykinin disappears in one passage through the pulmonary bed; i.e. in order to produce a standard depressor response the intravenous dose of bradykinin required is 50 times the intra-aortic dose.[20] The effects of intravenous infusions of various amounts of BPP_{5a} on the pulmonary inactivation of intravenously injected bradykinin are shown as open circles in Fig. 2. Maximum

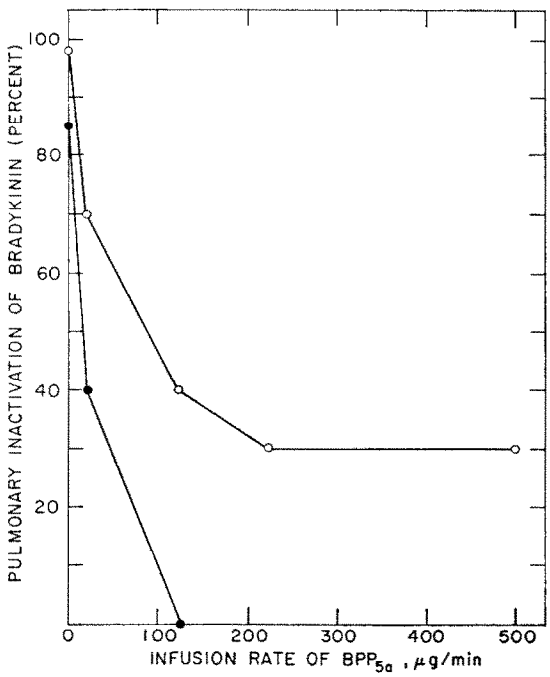

FIG. 2. Protection of bradykinin in rat pulmonary circulation by intravenous infusion of BPP_{5a}. ○—○, BPP_{5a} alone (five animals); ●—●, BPP_{5a} after 2-mercaptoethanol treatment (three animals).

protection of bradykinin was obtained by infusion of BPP_{5a} at the rate of 250 μg/min. Under these conditions the pulmonary inactivation of bradykinin was 30 per cent, i.e. equal bradykinin responses were obtained by intravenous doses 1·5 times the intra-aortic dose. Pretreatment of rats with 2-mercaptoethanol (one injection, 15 mg, intramuscularly 5–30 min before challenge) gave partial protection of bradykinin; 85 per cent rather than 98 per cent pulmonary inactivation was observed. When BPP_{5a} and 2-mercaptoethanol were used together the effect of BPP_{5a} was much more pronounced (closed circles, Fig. 2). Under these conditions, infusion of 125 μg/min of BPP_{5a} completely protected bradykinin from pulmonary inactivation.

The effect of BPP_{5a} on the pulmonary inactivation of bradykinin vanished within 10 min after the end of the infusion.

Inhibition of the conversion of angiotensin I and angiotensin II. The intra-aortic dose of angiotensin I required to produce a standard pressor response was usually 1·5 times that required intravenously thereby indicating the participation of the lung in the conversion of angiotensin I to angiotensin II. During infusion of BPP_{5a} the amount of angiotensin I, but not angiotensin II, required to produce a standard pressor response by either route of administration was greatly increased, Fig. 3. Infusion of BPP_{5a}

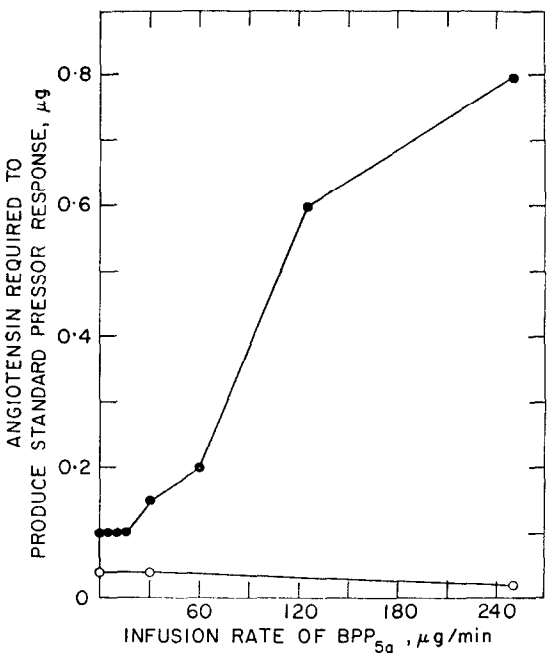

FIG. 3. Action of BPP_{5a} on the pressor response to angiotensin I and II in the rat. Intravenous injections of angiotensin I (●—●) and angiotensin II (○—○) to a 200 g male rat.

at 250 μg/min completely blocked the response to the standard intravenous dose of angiotensin I. However, this effect could be overcome by increasing the amount of angiotensin I. In the experiment illustrated in Fig. 3 an increase in the amount of angiotensin I of eight times was required. In other rats up to 50 times the dose of angiotensin I was required to overcome the effect of BPP_{5a} infusion. The effect of BPP_{5a} on the pulmonary conversion of angiotensin I vanished within 10 min after the end of the infusion.

Capillary permeability. In rats injected intravenously with Evans blue dye the bluing effect of 1 μg of bradykinin was greatly enhanced when it was mixed with 1·0 μg of BPP_{5a}, Fig. 4. When injected alone, intradermal doses of synthetic BPP_{5a} greater than 5 μg were required to produce any significant skin bluing.

DISCUSSION

Synthesis. The synthesis of this group of tryptophan peptides demonstrates the use of this acid sensitive amino acid in the solid phase method. Addition of mercaptoethanol to the HCl–dioxane reagent used for the removal of the Boc protecting groups protected the tryptophan from oxidation under acidic conditions and also prevented

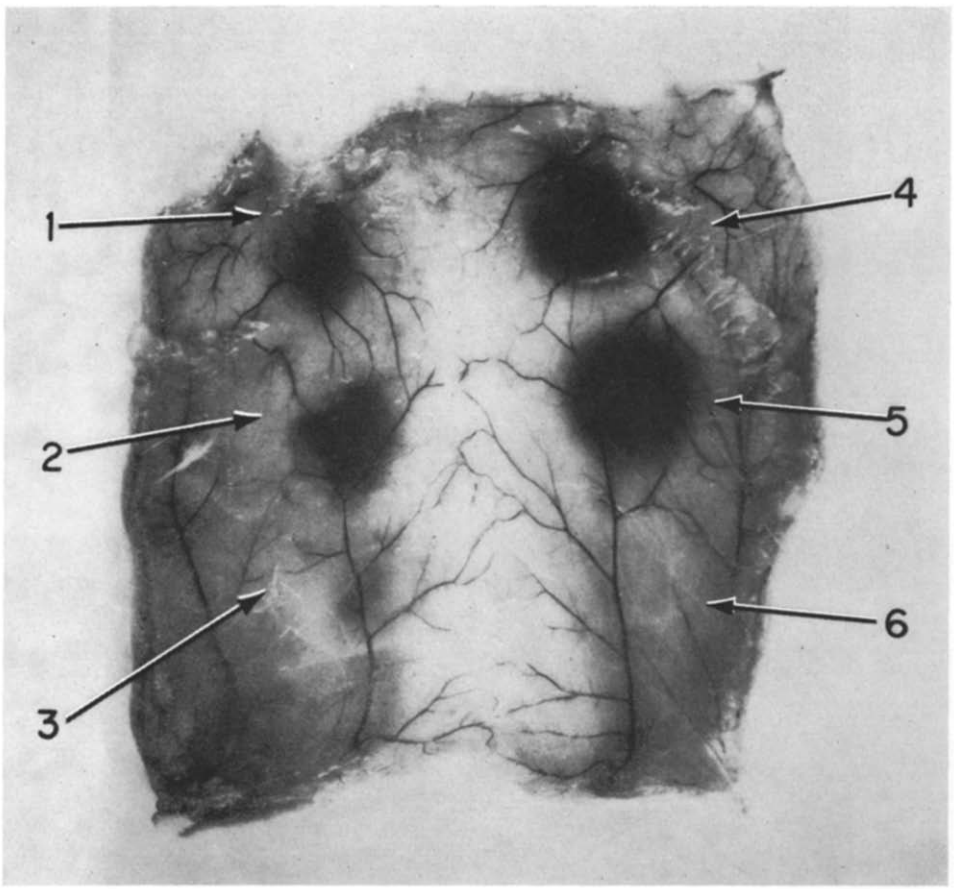

FIG. 4. Action of BPP_{5a} on the effect of bradykinin on capillary permeability. The photograph shows areas of bluing in the skin of a rat which had received intradermal injections (0·1 ml) of (1) 1 μg bradykinin; (2) 2 μg bradykinin; (3) saline; (4) 1 μg bradykinin + 1 μg BPP_{5a}; (5) 2 μg bradykinin + 1 μg BPP_{5a}; (6) 1 μg BPP_{5a}.

the gradual accumulation of peroxides in this reagent which usually occurs on standing. Although all filtrations of the peptide resins during synthesis were done by suction in the usual way, i.e. by drawing air through the resin to insure complete removal of solvents, no oxidation of tryptophan was observed (as was indicated by absence of color formation in the resins). In the case where some oxidized product was obtained the oxidation probably occurred during the work-up before chromatography.

The only other published account of the use of tryptophan in solid phase synthesis is that of Marshall,[11] who used HCl in acetic acid containing mercaptoethanol for removal of Boc groups. In some cases[12,13] the dioxane–HCl reagent has given results superior to those obtainable with acetic acid–HCl, and it is important to point out that these advantages can be applied to tryptophan containing peptides.

Activity of BPP_{5a} analogs. PCA-Lys-Trp-Ala-Pro and PCA-Gly-Leu-Pro-Pro-Arg-Pro-Lys-Ile-Pro-Pro[2] are the two bradykinin potentiating peptides from snake venom whose structures have been determined. It is likely that pyrrolidone carboxylic acid is a structure common to all of the other bradykinin potentiating peptides isolated from *Bothrops jararaca*[1] and *Agkistrodon halys blomhoffi*[14] venom since they are ninhydrin negative and not susceptible to Edman degradation. The results obtained with the BPP_{5a} analogs reported here (Table 1) indicate that opening the pyrrolidone carboxylic acid ring (Peptide 4), oxidation of tryptophan (Peptide 3) or tryptic cleavage of BPP_{5a} (Peptides 6 and 7) lead to a loss of activity. Opening the pyrrolidone ring in thyrotropin releasing factor, PCA-His-Pro-NH_2 also produces relatively inactive peptide Gln-His-Pro-NH_2 which had up to 5 per cent of the activity of the parent compound.[15] The presence of pyrrolidone carboxylic acid and tryptophan in a small peptide is not sufficient for biological activity; PCA-Lys-Trp-Pro (Peptide 5) as well as PCA-Gln-Trp and PCA-Asn-Trp[16] do not potentiate the activity of bradykinin on isolated guinea-pig ileum.

Pulmonary inactivation of bradykinin. Many tissues including blood are capable of inactivating bradykinin.[17] However, it has been shown by the blood-bathed organ technique[18] and confirmed by the systemic response method[10] that the lungs play the major role in the inactivation of circulating bradykinin (cf. Ref. 19 for a recent review). Ryan, Roblero and Stewart[20] demonstrated that the pulmonary disappearance of bradykinin in rats is the result of the action of peptidases which hydrolyze the peptide chain in several positions. The suggestion that the potentiation of the depressor effect of bradykinin *in vivo* by thiol compounds[21,22] and by BPF[23,24] is due to the inhibition of bradykinin destroying enzymes, has recently been confirmed for the pulmonary circulation. Both 2-mercaptoethanol[25] and BPF* protect bradykinin, at least partially, from pulmonary inactivation.

There are at least two primary cleavage sites, Arg^1-Pro^2 and Ser^6-Pro^7, as well as several further cleavages, in the bradykinin molecule when it passes through the pulmonary circulation. A single cleavage at any of these sites is sufficient to cause loss of biological activity. In the presence of 2-mercaptoethanol only the Ser^6-Pro^7 bond is hydrolyzed; the enzymes responsible for all other cleavages are inhibited.[26] The bradykinanase activity present in fractions derived from homogenates of dog lung is also partially inhibited by 2-mercaptoethanol and BPF.[27]

High rates of infusion of synthetic BPP_{5a} alone were not able to protect bradykinin completely in the pulmonary circulation of the rat. However, infusion of lower doses

* J. Roblero and J. M. Stewart, unpublished result.

of BPP_{5a} in 2-mercaptoethanol-treated rats resulted in complete protection (Fig. 2). Since the only site of bradykinin cleavage after 2-mercaptoethanol treatment is Ser^6-Pro^7, it seems likely that BPP_{5a} is inhibiting the enzyme which cleaves bradykinin at the Ser^6-Pro^7 bond (Fig. 5).

Bradykinin	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg (↓ at Ser-Pro)
BPP_{5a}	PCA-Lys-Trp-Ala-Pro ↓
Angiotensin I	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu

FIG. 5. Amino acid sequences of bradykinin, BPP_{5a}, and angiotensin I. The arrows indicate the peptide bond in bradykinin cleaved by the 2-mercaptoethanol resistant, BPP_{5a} sensitive pulmonary kinase in the rat and the peptide bond in angiotensin I cleaved by the pulmonary angiotensin converting enzyme.

BPP_{5a} may be a competitive inhibitor of the kininase. Comparison of the amino acid sequence of BPP_{5a} and bradykinin (Fig. 5, compare residues 3-7 of bradykinin with BPP_{5a}) indicates that a structural similarity exists. The requirement for the constant infusion of large amounts of BPP_{5a} to achieve complete inhibition of the enzyme in 2-mercaptoethanol-treated rats (125 μg/min) may indicate that BPP_{5a} is serving as an alternate substrate for the enzyme rather than as a reversible competitive inhibitor. An alternate explanation is that BPP_{5a} is an effective inhibitor of the pulmonary enzyme, but that it is rapidly removed from the circulation by the peripheral vascular bed.

Conversion of angiotensin I to angiotensin II. The comparatively inactive decapeptide angiotensin I, is converted to the active octapeptide, angiotensin II, on the passage through the pulmonary circulation.[28,29] Bakhle[27] showed that bradykininase and angiotensin-converting enzyme activity present in subcellular fractions derived from dog lung are both inhibited by BPF, while only the bradykininase was partially inhibited by 2-mercaptoethanol. Ng and Vane have recently confirmed this pattern of inhibition *in vivo*.[30]

Our experiments show that the angiotensin-converting enzyme is inhibited by BPP_{5a} in the rat *in vivo*. This inhibition is reversed by excess substrate, thereby appearing to be competitive. The great inhibition of the response to angiotensin I in the rat during infusion of BPP_{5a} may mean that multiple recirculation through the pulmonary vascular bed or conversion of angiotensin I in other tissues are normally important for complete activation of angiotensin I in the rat. The angiotensin-converting enzyme, which hydrolyzes angiotensin I at the Phe^8-His^9 peptide bond, and the 2-mercaptoethanol resistant bradykininase, which hydrolyzes bradykinin at the Ser^6-Pro^7 peptide bond, have the same pattern of response to BPP_{5a}, BPF and 2-mercaptoethanol. It is not known whether one or more enzymes are responsible for these selective proteolytic cleavages of bradykinin and angiotensin I in the pulmonary circulation.[31]

Synthetic BPP_{5a} has been shown to reflect the complete spectrum of activity observed for the mixture of peptides naturally occurring in *Bothrops jararaca* venom.[9] However, in the blood pressure system when the activities of BPF and BPP_{5a} were compared differences in potency (on a weight basis) and duration of action were observed. These differences probably reflect the fact that BPF is a mixture of at least nine different active peptides and that BPP_{5a} represents less than 5 per cent of the peptides present in BPF.

The experiments described in this paper have been concerned primarily with the effect of BPP_{5a} on the metabolism of bradykinin and angiotensin I. However, these experiments do not exclude other possible mechanisms of action, i.e. interaction of potentiating peptides with receptors, to explain the physiological actions of bradykinin-potentiating peptides.

It is noteworthy that the venom of *Bothrops jararaca* contains not only materials which produce and maintain profound hemodynamic effects (bradykinin liberating enzymes[32,33] and bradykinin-potentiating peptides), but also peptides which block the enzymatic conversion of angiotensin I to antiotensin II, one of the physiological mechanisms available for overcoming the depressor effect of bradykinin.

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