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PULMONARY METABOLISM OF BRADYKININ ANALOGUES AND THE CONTRIBUTION OF ANGIOTENSIN CONVERTING ENZYME TO BRADYKININ INACTIVATION IN ISOLATED LUNGS

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1 The activity and pulmonary metabolism of two peptides, 7-homo Pro-bradykinin and 8-homo Phe-bradykinin were studied in isolated systems.

2 Both analogues were about 50–70 times less active than bradykinin on the guinea-pig ileum and 70–160 times less active on isolated strips of cat terminal ileum.

3 The action of both analogues on guinea-pig ileum was potentiated (2.5–3.0 fold) by a bradykinin potentiating peptide (BPP_{9a}) but less so than the action of bradykinin (4–5 fold).

4 Like bradykinin, the 8-homo Phe analogue was extensively inactivated (>90%) in a single passage through the pulmonary circulation of guinea-pig or rat isolated lungs and this inactivation was prevented by pre-treatment of the lungs with BPP_{9a}.

5 The 7-homo Pro analogue was inactivated to a lesser degree in guinea-pig lungs (58%) and in rat lungs (89%) and its inactivation was not affected by BPP_{9a}.

6 It is concluded that the 8-homo Phe analogue is a substrate for the dipeptidylcarboxypeptidase (angiotensin I converting enzyme) of lung, whereas the 7-homo Pro analogue is not a substrate.

7 There is about four times as much dipeptidylcarboxypeptidase activity in guinea-pig isolated lungs as there is in rat isolated lungs.

Introduction

Bradykinin is extensively inactivated during passage through the pulmonary circulation by the action of peptidases. The original findings *in vivo* (Ferreira & Vane, 1967) were extended using isolated lungs of various species. For example, in rat isolated lungs, bradykinin perfused through the pulmonary circulation suffers hydrolysis at several bonds (Figure 1; Ryan, Roblero & Stewart, 1970). Since the hydrolysis of any peptide bond of bradykinin leads to its virtual inactivation (Suzuki, Abiko, Endo, Kameyama, Sasaki & Nabeshima, 1969), any one of the cleavages shown in Figure 1 could represent the action of the fastest acting peptidase and all the other cleavages due to other peptidases would be relatively unimportant in terms of the pulmonary inactivation. One of the pulmonary bradykininases, angiotensin converting enzyme (dipeptidylcarboxypeptidase, E. C. 3. 4. 15. 1) has been well characterized (Soffer, 1976) but its importance relative to the other pulmonary bradykininases has not been assessed. Since two recently synthesized analogues, 7-homo Pro-bradykinin and 8-homo Phe-bradykinin, were said to be resistant to hydrolysis by converting enzyme (Ondetti & Engel, 1975), it was decided to investigate their metabolism relative to that of bradykinin in isolated lungs.

Methods

Isolated lungs of rats and guinea-pigs of either sex were perfused via the pulmonary artery with oxygenated Krebs solution at 37°C as described earlier (Bakhle, Reynard & Vane, 1969). The composition of the Krebs solution was (mM): $NaHCO_3$ 25, NaCl 120, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2 and glucose 5.6. The lung perfusate was directed over isolated smooth muscle preparations for bioassay and the contractions of the assay tissues recorded via isotonic transducers coupled to a multi-channel Watanabe recorder. The assay tissues were pieces of guinea-pig ileum, and longitudinal strips of cat

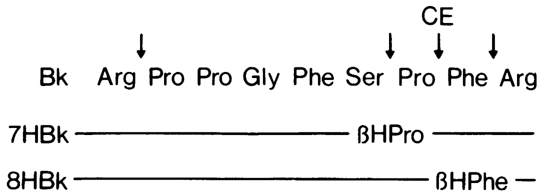

Figure 1 Structure of bradykinin and the two β-homo-analogues. The arrows show the points of cleavage in bradykinin perfused through rat isolated lungs (Ryan *et al.*, 1970). CE = angiotensin converting enzyme.

terminal ileum (Alabaster & Bakhle, 1972); they responded to injections of bradykinin of 2–50 ng. The assay tissues were also constantly superfused (0.1 ml/min) with a mixture of antagonists (methysergide, 0.56 μM; mepyramine, 0.35 μM; hyoscine, 0.33 μM; phenoxybenzamine, 0.33 μM; propranolol, 7.5 μM; the final concentrations are given).

The drugs used were: 2-mercaptoethanol (Sigma); methysergide bimaleate (Sandoz); mepyramine hydrochloride (May & Baker); hyoscine hydrobromide (BDH); phenoxybenzamine hydrochloride (Smith, Kline & French); (±)-propranolol hydrochloride (ICI); bradykinin (Sandoz); 7-β-homo Pro-bradykinin (SQ 22516), 8-β-homo Phe-bradykinin (SQ 22515) and Pyr-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro, BPP_{9a} (SQ 20881) (Dr Ondetti, Squibb Institute for Medical Research, Princeton, New Jersey, U.S.A.).

Results

Activity of bradykinin analogues on isolated tissues

The first experiments were designed to assess the potency of the analogues relative to the parent peptide on the two isolated smooth muscle preparations that were to be used later to study the pulmonary metabolism of the peptides. 7-Homo Pro-bradykinin, 8-homo Phe-bradykinin, and bradykinin were assayed on the same preparations, guinea-pig ileum and cat terminal ileum (Figure 2). The potency of the analogues was expressed as bradykinin equivalents (Table 1). On the cat terminal ileum the β-homo analogues had different potencies whereas on the guinea-pig ileum they were effectively equipotent.

The responses of the guinea-pig ileum to bradykinin are potentiated by the nonapeptide, BPP_{9a} (Ferreira, Greene, Alabaster, Bakhle & Vane, 1970). The effects of a constant infusion of BPP_{9a} on the responses of the guinea-pig ileum to bradykinin and the two analogues are shown in Table 2. The β-homo analogues were potentiated to a lesser degree than bradykinin. The responses of the cat terminal ileum to the β-homo analogues, like those to bradykinin (Alabaster & Bakhle, 1972), were not potentiated by BPP_{9a}.

Inactivation in isolated lungs

Like bradykinin, both β-homo analogues were inactivated on a single passage through guinea-pig and

Table 1 Agonist potency on isolated smooth muscles of 7-homo Pro- and 8-homo Phe-bradykinin relative to bradykinin

Analogue	Dose (μg)	Bradykinin equivalent (ng) on guinea-pig ileum (mean ± s.e.)	cat terminal ileum
7-homo Pro	0.5	7.7 ± 0.8 (10)†	6.5 ± 0.5 (10)
	1.0	19.7 ± 1.8 (10)	13.7 ± 1.1 (14)
	2.0	33.3 ± 2.4 (8)	29.0 ± 1.3 (9)
8-homo Phe	0.5	8.0 ± 0.9 (12)	—
	1.0	14.1 ± 0.9 (10)	6.1 ± 0.3 (9)
	2.0	25.3 ± 3.8 (10)	11.1 ± 0.8 (15)
	3.0	—	19.6 ± 2.1 (10)

† Numbers in parentheses refer to number of experiments.

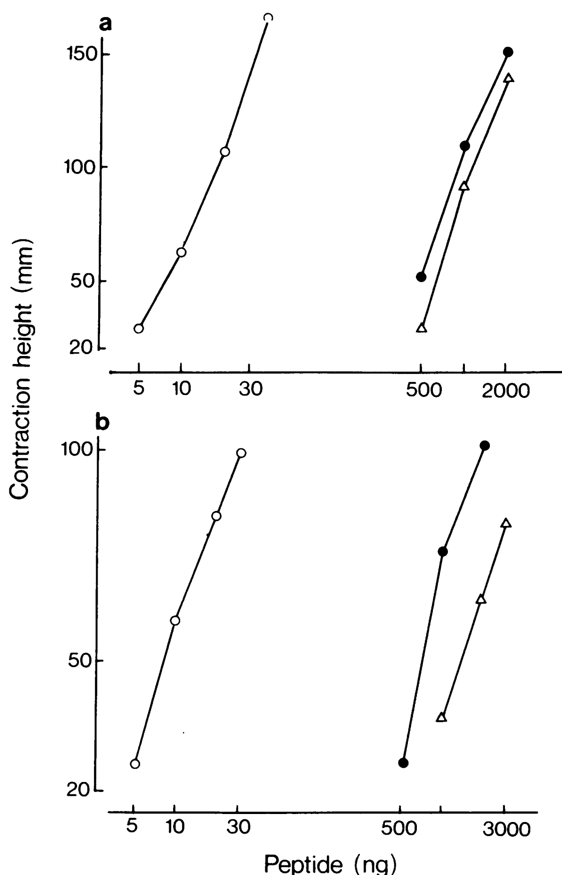

Figure 2 Dose-response relationships for bradykinin (○), 7-homo Pro-bradykinin (●) and 8-homo Phe-bradykinin (△) on (a) the guinea-pig isolated ileum and (b) cat terminal ileum. The tissues were superfused with Krebs solution (8 ml/min) and the peptides administered by injection (0.1–0.2 ml) into the superfusing fluid.

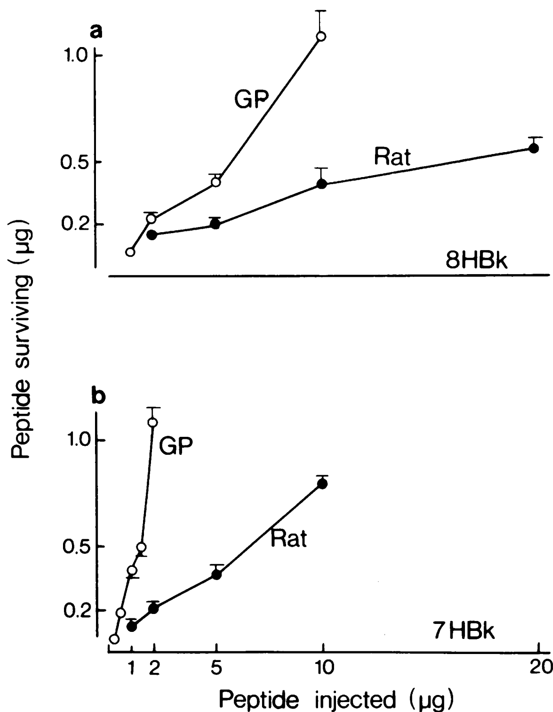

Figure 3 Survival of (a) 8-homo Phe-bradykinin (8HBk) and (b) 7-homo Pro-bradykinin (7HBk) after a single passage through the pulmonary circulation of isolated lungs of guinea-pig and rat. The amount of peptide surviving a single passage through the pulmonary circulation was measured by bioassay and each point represents the mean of at least four assays. Vertical lines show s.e. means.

rat isolated lungs, though there were important quantitative differences. The relationship between the amount of β-homo analogue injected into the pulmonary artery and the amount of activity surviving passage through the pulmonary circulation of guinea-pig and rat lungs is shown in Figure 3. In both species, 7-homo Pro-bradykinin showed a higher survival than 8-homo Phe-bradykinin. Furthermore, although the 8-homo Phe analogue, like bradykinin itself, was over 90% inactivated in both guinea-pig and rat lungs, over 40% of the 7-homo Pro analogue survived in guinea-pig lungs, more than 4 times its survival in rat lungs.

The bradykinin potentiating peptide, BPP_{9a}, is also a specific inhibitor of the dipeptidylcarboxypeptidase that hydrolyses angiotensin I and bradykinin. Figure 4 shows that in guinea-pig lung, an infusion of BPP_{9a} (100 ng/ml) increased survival of bradykinin and 8-homo Phe-bradykinin five-fold ($P < 0.05$), whereas the already high survival of 7-homo Pro-bradykinin was not affected. At the higher concentration of BPP_{9a} (500 ng/ml) there was a small further increase in survival of bradykinin and its 8-homo Phe analogue and a non-significant change in the survival of 7-homo Pro-bradykinin. In rat lung, survival of all three peptides is much lower than in guinea-pig lung but BPP_{9a} again increased survival of bradykinin and 8-homo Phe-bradykinin ($P < 0.05$), whereas survival of the 7-homo Pro analogue was not significantly changed even at the higher concentration of BPP_{9a} (500 ng/ml).

Discussion

The change in the molecule brought about by the substitution of a β-homo-amino acid for the normal α-amino acid is comparatively small, extending the

peptide backbone by one carbon atom in the chain of eight peptide links, but it reduces the potency of both analogues as agonists on intestinal smooth muscle. The analogues were probably acting via bradykinin receptors since the dose-response relationships were roughly parallel to those for bradykinin and the potentiating peptide BPP_{9a} also potentiated the analogues. The potentiation of bradykinin by the peptides from *Bothrops jararaca*, of which BPP_{9a} is one, was originally attributed to inhibition of bradykinin degradation by tissues (Ferreira, 1965), but subsequently a direct interaction of the potentiator with the receptor has been postulated (Camargo & Ferreira, 1971). If this is so, then the changes in the receptor brought about by BPP_{9a} are not as effective for the analogues as they are for bradykinin. Furthermore, although the analogues are equally active on the guinea-pig ileum, the 8-homo Phe analogue was more potentiated by BPP_{9a} than the 7-homo Pro analogue.

The analogues were both designed to be resistant to the major bradykinin hydrolysing enzyme in lung, the dipeptidylcarboxypeptidase or angiotensin converting enzyme. This enzyme (see Figure 1) is known to cleave bradykinin initially at the Pro^7-Phe^8 bond (Dorer, Kahn, Lentz, Levine & Skeggs, 1974) and therefore substitutions at Pro^7 or Phe^8 were expected to interfere with the action of the enzyme. Furthermore, BPP_{9a} which protects bradykinin from inactivation by dipeptidylcarboxypeptidase should have no effect on the inactivation, if any, of the analogues. Even under conditions of maximal inhibition of converting enzyme, some inactivation of the analogues would be expected, as the other bradykinin hydrolysing peptidases (see Figure 1) would not be inhibited.

The expected resistance to dipeptidylcarboxypeptidase was found only with 7-homo Pro-bradykinin. This analogue was more resistant than bradykinin to inactivation in the isolated lung and the inactivation that did occur was unaffected by concentrations of BPP_{9a} that gave near maximal protection to bradykinin. 8-Homo Phe-bradykinin was, like bradykinin, rapidly inactivated by the lung and protected by BPP_{9a}. These results lead to the conclusion that 7-homo Pro-bradykinin was not a substrate or at best a very poor substrate, for converting enzyme, whereas 8-homo Phe-bradykinin was a substrate.

These conclusions are at variance with those of Ondetti & Engel (1975). They found that a purified converting enzyme preparation from rabbit lung liberated the C-terminal dipeptide Phe-Arg from bradykinin, but not from the two analogues and concluded that neither analogue was a substrate for converting enzyme. However, the results of their experiments *in vivo* were not compatible with this conclusion, as 8-homo Phe-bradykinin was a less potent vasodepressor than bradykinin and its effects, like those of bradykinin, were enhanced by a previous

Table 2 Potentiation by BPP_{9a} of the contractor effects of bradykinin and two analogues on guinea-pig isolated ileum

	Dose of agonist peptide	*Dose equivalent after treatment*†	*Potentiation (fold)*
Bradykinin (ng)	1	5 (2)	5.0
	2	8.1 ± 1.2 (4)	4.0
	5	23.6 ± 3.0 (8)	4.7
	10	40.2 ± 2.7 (8)	4.0
7-homo Pro (µg)	0.2	0.5 ± 0.1 (5)	2.5
	0.5	1.1 ± 0.1 (8)	2.3
	1.0	2.4 ± 0.1 (8)	2.4
	2.0	4.7 ± 0.2 (8)	2.4
8-homo Phe (µg)	0.2	0.6 ± 0.1 (7)	3.1
	0.5	1.6 ± 0.1 (8)	3.1
	1.0	2.8 ± 0.2 (4)	2.8

The BPP_{9a} in a final concentration of 100 ng/ml was superfused continuously over the isolated tissues for at least 15 min before, and during the administration of the agonist peptides.

† Dose equivalent is the amount of agonist that would be required to give an equivalent contraction on untreated tissue; thus 1 ng of bradykinin causes a contraction of treated ileum equivalent to that given by 5 ng of bradykinin on the ileum before treatment (mean ± s.e. mean).

Number of experiments is given in parentheses.

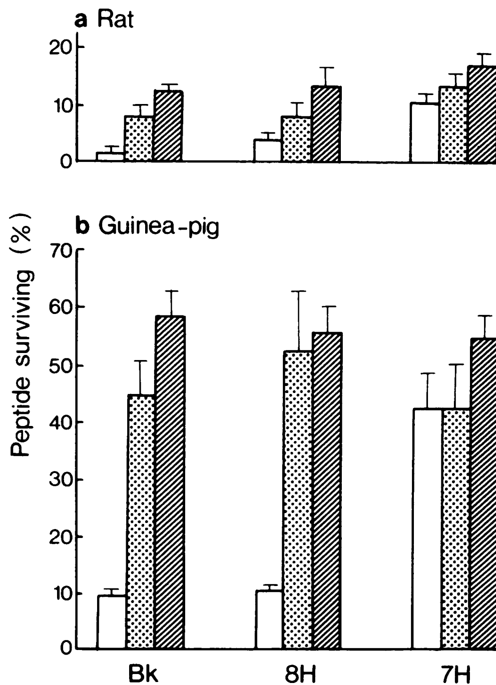

Figure 4 Effect of BPP_{9a} 100 ng/ml (dotted columns) and 500 ng/ml (hatched columns) on survival of bradykinin (Bk), 7-homo Pro-bradykinin (7H) and 8-homo Phe-bradykinin (8H) in (a) rat and (b) guinea-pig isolated lungs. The height of the columns represents the mean (±s.e. mean) survival in 6 or more assays, expressed as a percentage of the amount injected into the pulmonary circulation. The open columns represent survival under control conditions. BPP_{9a} increased survival of bradykinin and the 8-homo analogue ($P<0.05$) but not that of the 7-homo Pro-bradykinin ($P>0.1$).

injection of BPP_{9a}. 7-Homo Pro-bradykinin, however, was 30 times as potent as the 8-homo Phe analogue and its effects were not enhanced by BPP_{9a}.

These differences between the properties of the analogues *in vivo* agree well with the findings that 8-homo Phe-bradykinin, but not 7-homo Pro-bradykinin, was a substrate for converting enzyme. My findings also suggest that, *in vivo*, relatively more 7-homo Pro-bradykinin survives the pulmonary, blood and peripheral converting enzymes and is recirculated. This would explain the longer hypotension (2.1 min) produced by 7-homo Pro-bradykinin, compared with bradykinin or 8-homo Phe-bradykinin (0.5–0.4 min) (Ondetti & Engel, 1975).

The present study also provides information on the level of angiotensin converting enzyme activity in isolated lungs. The inactivation of 7-homo Pro-bradykinin can be taken as an indication of the proportion of converting enzyme relative to the other bradykinin hydrolysing peptidases; thus in guinea-pig lung about half, and in rat, nearly all of the total bradykinin hydrolysing activity was due to peptidases other than converting enzyme. These results also suggest that these other peptidases inactivate bradykinin and the two analogues at very similar rates, possibly because these enzymes are acting at bonds not involving the Pro^7 or Phe^8 residues (see Figure 1). This indirect estimate of a low level of converting enzyme activity in rat isolated lungs agrees with the direct estimation of the conversion of angiotensin I to angiotensin II in isolated lungs (Bakhle, Reynard & Vane, 1969) and with results obtained *in vivo* (Kreye & Gross, 1971) suggesting that in the rat, conversion of angiotensin I in the lung is less than in the peripheral circulation. This large difference (four-fold) in converting enzyme activity in the lungs of two common laboratory animals means that results obtained in one species cannot be compared directly with those obtained in the other species.

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References

ALABASTER, V.A. & BAKHLE, Y.S. (1972). The inactivation of bradykinin in the pulmonary circulation of isolated lung. *Br. J. Pharmac.*, **45**, 299–309.

BAKHLE, Y.S., REYNARD, A.M. & VANE, J.R. (1969). Metabolism of the angiotensins in isolated perfused tissues. *Nature, Lond.*, **222**, 956–959.

CAMARGO, A. & FERREIRA, S.H. (1971). Action of bradykinin potentiating factor (BPF) and dimercaprol (BAL) on the responses to bradykinin of isolated preparations of rat intestines. *Br. J. Pharmac.*, **42**, 305–307.

DORER, F.E. KAHN, J.R., LENTZ, K.E., LEVINE, M. & SKEGGS, L.T. (1974). Hydrolysis of bradykinin by angiotensin converting enzyme. *Circulation Res.*, **34**, 824–827.

FERREIRA, S.H. (1965). A bradykinin-potentiating factor (BPF) present in the venom of *Bothrops jararaca*. *Br. J. Pharmac. Chemother.*, **24**, 163–169.

FERREIRA, S.H., GREENE, L.J., ALABASTER, V.A., BAKHLE, Y.S. & VANE, J.R. (1970). Activity of various fractions of Bradykinin Potentiating Factor against angiotensin I converting enzyme. *Nature, Lond.*, **225**, 379–380.

FERREIRA, S.H. & VANE, J.R. (1967). The disappearance of bradykinin and eledoisin in the circulation and vascular beds of the cat. *Br. J. Pharmac. Chemother.*, **30**, 417–424.

KREYE, V.A.W. & GROSS, F. (1971). Conversion of angiotensin I to angiotensin II in peripheral vascular beds of the rat. *Am. J. Physiol.*, **220**, 1294–1296.

ONDETTI, M.A. & ENGEL, S.L. (1975). Bradykinin analogs containing β-homo-amino acids. *J. med. Chem.*, **18**, 761–763.

RYAN, J.W., ROBLERO, J. & STEWART, J.M. (1970). Inactivation of bradykinin in rat lung. *Adv. exp. Med. Biol.*, **8**, 263–271.

SOFFER, R.L. (1976). Angiotensin converting enzyme. *Ann. Rev. Biochem.*, **45**, 73–94.

SUZUKI, Z., ABIKO, T., ENDO, N., KAMEYAMA, T., SASAKI, K. & NABESHIMA, J. (1969). Biologically active synthetic fragments of bradykinin. *Jap. J. Pharmac.*, **19**, 325–327.

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