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Differentiation of Bradykinin Receptors and of Kininases with Conformational Analogues of Bradykinin

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SUMMARY

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Two bradykinin (BK) analogues with restricted conformational freedom, α-methylphenylalanine[5] bradykinin ([Phe(αMe)[5]]-BK) and α-methylphenylalanine[8] bradykinin ([Phe(αMe)[8]]-BK), were compared with BK with respect to potency on a variety of biological preparations. [Phe(αMe)[5]]-BK and [Phe(αMe)[8]]-BK were equipotent (1.5–3.0% of the activity of BK) in their hypotensive action in rats *in vivo* and (3.0% of the activity of BK) in their ability to contract isolated rat stomach strips *in vitro*. [Phe(αMe)[5]]-BK was 10 and 50 times more potent than [Phe(αMe)[8]]-BK in releasing prostaglandins from isolated, perfused rabbit kidney and heart, respectively, and was 5 times more potent in contracting isolated, superfused cat jejunum. [Phe(αMe)[8]]-BK was 10 times more potent than [Phe(αMe)[5]]-BK in contracting isolated rat uterus and, moreover, possessed 31% of the potency of BK. The degradation of the BK analogues in a variety of systems was also studied. BK and [Phe(αMe)[5]]-BK were degraded by guinea pig plasma and by purified rabbit lung converting enzyme, and degradation was inhibited by bradykinin-potentiating peptide (BPP_{9a}). Although not a substrate for the purified rabbit lung converting enzyme, [Phe(αMe)[8]]-BK was degraded by guinea pig plasma kininase and was protected by BPP_{9a}. The isolated, perfused rabbit kidney efficiently degraded all three peptides, whereas both α-methylated analogues were more resistant to degradation by heart. Pulmonary destruction in rats was 95% for BK and 77% for [Phe(αMe)[5]]-BK; infusion of BPP_{9a} inhibited this destruction. Pulmonary destruction of [Phe(αMe)[8]]-BK was not observed, and infusion of BPP_{9a} had no effect. Since degradation of [Phe(αMe)[8]]-BK by other kininases (e.g., one in guinea pig plasma) was inhibited by BPP_{9a}, it appears that BPP_{9a} is a more general inhibitor of kininases than previously thought. Its failure to potentiate the hypotensive action of [Phe(αMe)[8]]-BK in rats indicates the dominance of converting enzyme as the major physiologically active kininase in this species.

INTRODUCTION

Peptide hormone analogues in which a methyl group has been substituted for the hydrogen on an α carbon have restricted rotational mobility about the adjacent bonds (1, 2) and decreased susceptibility to proteolytic degradation (3). Such analogues therefore are useful tools for evaluating the heterogeneity of hormone receptors in terms of preferred conformations of the hormone. Analogues with α-methyl substitutions can also be used to analyze degradative enzymes on the basis of the site of bond cleavage or preferred conformation of

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the substrate.

Bradykinin is a potent vasoactive agent with multiple actions, including vasodilation (4), increase of vascular permeability (4), release of prostaglandins (5), and contraction of some smooth muscles such as cat jejunum (6) and rat uterus (7). Whether BK[1] receptors all have the same structural requirements for optimal binding of BK, and what such requirements are, is unknown.

The major sites of BK degradation are the pulmonary circulation (8) and plasma (9); however, bradykininase activity can be demonstrated in almost every tissue so far investigated. The bradykininase dipeptidyl carboxypeptidase (EC 3.4.15.1) has been purified from a variety of sources—rabbit lung (10), hog lung (11), hog kidney (12), and human plasma (where it was called kininase II) (9)—and is currently believed to be identical with angiotensin-converting enzyme (13). Fluorescent antibody-labeling techniques demonstrate the presence of this enzyme in blood vessels (13, 14). Kininase activities forming a variety of peptide products have been described in both perfused lung (15) and a partially purified lung microsomal enzyme (16). Other kininases have also been described; for example, kininase I is a plasma carboxypeptidase (17). Analysis of the relative contributions of different kininases to the inactivation of BK *in vivo* awaits identification of more specific antagonists or substrate analogues of these enzymes than are presently available.

In this study two α-methylated BK analogues, α-methylphenylalanine[5] bradykinin and α-methylphenylalanine[8] bradykinin, were compared with BK for biological potency in a variety of smooth muscle and endocrine systems and for their susceptibility to hydrolysis by a variety of bradykininase preparations. The discovery that [Phe(αMe)[8]]-BK was completely resistant to purified pulmonary converting enzyme allowed us to study the contribution of other kininases to the physiological regulation of BK concentration.

Bradykinin-potentiating peptide (Pyr-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro), a nonapeptide originally isolated from the venom of *Bothrops jararaca* (18), is well known as an inhibitor of dipeptidyl carboxypeptidase. The ability of BPP_{9a} to potentiate the actions of kininases-resistant BK analogues on guinea pig ileum has led to the suggestion that BPP_{9a} may also act by increasing the affinity of the BK receptor for BK (19, 20). We have evaluated the ability of BPP_{9a} to potentiate the actions and inhibit degradation of [Phe(αMe)[8]]-BK in light of the demonstrated resistance of that analogue to hydrolysis by dipeptidyl carboxypeptidase.

MATERIALS AND METHODS

Materials. Bradykinin, α-methylphenylalanine[5] bradykinin, and α-methylphenylalanine[8] bradykinin were synthesized by Dr. John Turk (21). BPP_{9a} was synthesized in the laboratory of Dr. Garland Marshall. Rabbit lung converting enzyme was generously provided by Dr. Richard L. Soffer (10). Prostaglandin E_2 was a gift from the Upjohn Company, and indomethacin, from Merck Sharp & Dohme.

Rat blood pressure determinations. Male Sprague-Dawley rats weighing 250–300 g were anesthetized with 200 mg/kg of phenobarbital (Merck) intraperitoneally, and blood pressures were recorded from the left femoral artery (Statham transducers, Beckman R-511 Dynograph). Injections of BK, [Phe(αMe)[5]]-BK, and [Phe(αMe)[8]]-BK were made (in multiples of 5-μl aliquots by means of a Hamilton syringe automatic dispensing apparatus) into the cannulated (PE 50 tubing) right jugular vein or left carotid artery. We assessed the effects of BPP_{9a} by comparing dose-response curves for intravenous and intra-arterial bolus injections of the BK agonists determined before or during BPP_{9a} infusion through the cannulated left femoral vein at 25 μg/kg/min.

Bradykininase studies in vitro. In studies of the kininase activity of guinea pig plasma, equal volumes of peptide solution and guinea pig plasma (diluted 1:100 in 0.9%

[1] The abbreviations used are: BK, bradykinin; [Phe(αMe)[5]]-BK, α-methylphenylalanine[5] bradykinin; [Phe(αMe)[8]]-BK, α-methylphenylalanine[8] bradykinin; BPP_{9a}, bradykinin-potentiating peptide; PGE_2, prostaglandin E_2.

NaCl solution) were combined and incubated at room temperature. The final peptide concentrations, chosen so that similar volumes of each peptide mixture could be tested in the bioassay system, were: BK, 100 ng/ml; [Phe(αMe)^5]-BK, 10 μg/ml; and [Phe(αMe)^8]-BK, 10 μg/ml. Immediately after plasma and peptide solutions had been combined (zero time) and at appropriate time intervals, aliquots of the incubation mixture were assayed. Degradation of the peptides was assessed by following the loss of biological activity on cat jejunum (see below for details of bioassay). In experiments measuring enzyme inhibition by BPP_{9a}, the converting enzyme inhibitor was added to a final concentration of 100 μg/ml in the plasma just before addition of the BK or analogue solution.

Purified lung converting enzyme (10) was used as another source of bradykininase activity. BK, [Phe(αMe)^5]-BK, and [Phe(αMe)^8]-BK were made up in 0.1 M Tris, pH 7.5, to final concentrations of 5 μg/ml (5 μM). Purified lung kininase was added to the peptide solution at a final concentration of 2.7 or 270 nM. The mixture was incubated at room temperature, and degradation of the peptides was followed by bioassay on cat jejunum. In some experiments BPP_{9a} was added to a final concentration of 100 μg/ml in the BK or analogue solution immediately before the reaction was started by adding kininase.

Perfused organ studies. Male New Zealand rabbits weighing 1.5–2 kg were anesthetized with sodium pentobarbital (30 mg/kg intravenously) and given heparin (250 units/kg intravenously). After the abdominal cavity had been opened, a polyethylene catheter (PE 160, Clay-Adams) was tied into the renal artery. The kidney was removed, placed in a warming jacket, and perfused with oxygenated (95% O_2–5% CO_2) Krebs-Henseleit solution (37°) at 15 ml/min. Hearts were removed and perfused retrograde through an aortic cannula with oxygenated (95% O_2–5% CO_2) Krebs-Henseleit solution (37°) at 15 ml/min. The venous effluent from the isolated, perfused organ was bioassayed by superfusion. Identification of the substance detected in the heart or kidney effluents as a prostaglandin was based on the specificity of the bioassay system and was substantiated by the disappearance of activity after treatment of the perfused organ with indomethacin, a specific inhibitor of prostaglandin synthesis. The prostaglandin in the cardiac effluent has recently been characterized as prostacyclin, or PGI_2 (22), although the prostaglandin in the kidney effluent appears to be genuine PGE_2 (23).

Bioassays. A series of smooth muscle tissues sensitive to BK (rat stomach and cat jejunum) or to prostaglandins (rat stomach and chick rectum) were superfused at 37° with oxygenated (95% O_2–5% CO_2) Krebs-Henseleit solution at 10–15 ml/min. Tissues were made more specific in sensitivity by infusing solutions of antagonists to histamine, serotonin, acetylcholine, and catecholamines over the assay tissues as previously described (24); indomethacin at 10 μg/min was also infused to inhibit endogenous synthesis of prostaglandins. Changes in smooth muscle tension were measured with F-50 linear core myographs (Narco). The bradykinin or prostaglandin content of the kininase incubation mixtures or of the perfused organ effluent was estimated by comparison of their smooth muscle activity with that of PGE_2 and BK standards, which were tested as bolus injections directly over the assay tissues or through the heart or kidney. In studies of peptide degradation by the heart or kidney, indomethacin was infused through the organ to inhibit peptide-stimulated synthesis of prostaglandins that would interfere with the assay of the BK agonists on the rat stomach strip.

RESULTS

Pulmonary destruction in vivo. Bradykinin and the two analogues studied produced transient falls in mean arterial blood pressure when administered as bolus intra-arterial or intravenous doses to rats; parallel dose-response curves were obtained for the agonists (Figs. 1–3). The responses to both BK and [Phe(αMe)^5]-BK depended upon the route of administration. Intra-arterial doses of BK were 20 times more potent than intravenous doses (Fig. 1), and intra-arterial doses of [Phe(αMe)^5]-BK

were 2–3 times more potent than the same doses intravenously (Fig. 2). There was no difference between the intravenous and intra-arterial dose-response curves for [Phe(αMe)^8]-BK (Fig. 3). The potencies of the analogues relative to BK were determined from the ratios of the BK dose giving a 20-mm-Hg fall in blood pressure to the dose of each analogue producing a response of equal magnitude. Comparing intravenous doses, we calculated that [Phe(αMe)^8]-BK has 24% and [Phe(αMe)^5]-BK has 11% of the potency of BK. However, comparison of intra-arterial doses of the agonists indicated that the methylated analogues are equipotent but are only 2% as potent as BK.

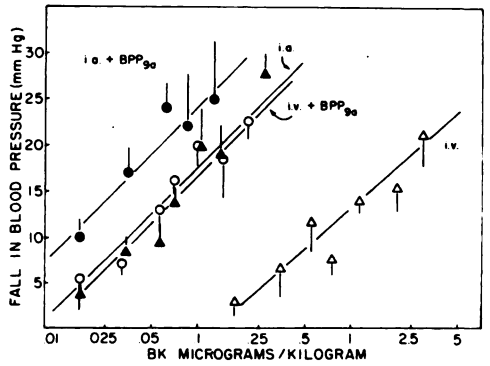

FIG. 1. *Dose-response curves for rat blood pressure depression induced by intra-arterial (i.a., ●, ○) and intravenous (i.v., ▲, △) bolus injections of BK*

Solid symbols are dose-response curves obtained during intravenous infusion of BPP_{9a} at 25 μg/kg/min; open symbols are dose-response curves obtained before BPP_{9a} infusion. The values are means ± standard errors for a minimum of five determinations made in nine animals.

The ratio of intra-arterial (i.a.) to intravenous (i.v.) doses required to give the same response is a measure of the survival of an agonist across the pulmonary circulation, and using the doses producing a 20-mm-Hg fall in blood pressure, we calculate

$$\% \text{ degradation} = \left(1 - \frac{\text{i.a. dose}}{\text{i.v. dose}}\right) \times 100$$

Degradation of BK in the lungs is 95%, and of [Phe(αMe)^5]-BK, 77%. The difference between intravenous and intra-arterial doses of [Phe(αMe)^8]-BK is not statistically significant.

Infusion of BPP_{9a} at 25 μg/kg/min enhanced the effects of both intravenous and intra-arterial doses of BK and [Phe(αMe)^5]-BK (Figs. 1 and 2) but had no effect on either intravenous or intra-arterial doses of [Phe(αMe)^8]-BK (Fig. 3). From these data it appears that the potentiation of the activity of a BK agonist by BPP_{9a} is related to the capacity of that agonist to act as a substrate for the lung bradykininase.

Guinea pig plasma kininase. Several explanations are possible for the potentiation by BPP_{9a} of intra-arterial doses of BK and

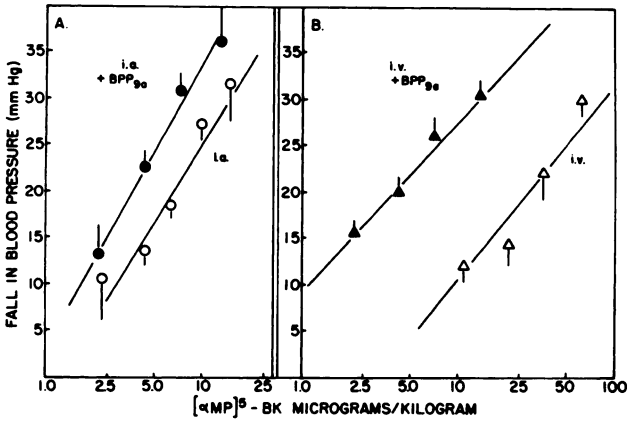

FIG. 2. *Dose-response curves for rat blood pressure depression induced by intra-arterial (A) and intravenous (B) bolus injections of [αMP^5]-BK ([Phe(αMe)^5]-BK)*

Solid symbols are dose-response curves obtained during intravenous infusion of BPP_{9a} at 25 μg/kg/min; open symbols are dose-response curves obtained before BPP_{9a} infusion. The values are means ± standard errors for a minimum of six determinations made in eight animals.

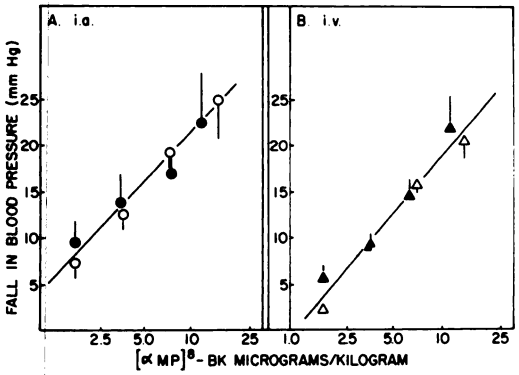

FIG. 3. *Dose-response curves for rat blood pressure depression induced by intra-arterial (A) and intravenous (B) bolus injections of [αMP⁸]-BK ([Phe(αMe)⁸]-BK)*

Solid symbols are dose-response curves obtained during intravenous infusion of BPP_{9a} at 25 μg/kg/min; open symbols are dose-response curves obtained before BPP_{9a} infusion. The values are means ± standard errors for a minimum of six determinations made in nine animals.

[Phe(αMe)⁵]-BK (Figs. 1 and 2). Because the circulation time of the rat is less than the duration of the hypotensive effect, intra-arterially administered peptide that circulates through the systemic vasculature, including the lungs, may return to the site of action and contribute to the observed response. Thus inhibition by BPP_{9a} of pulmonary degradation would result in increased survival of peptide and increased biological response. Alternatively, BPP_{9a} might act at the receptor site or inhibit a plasma kininase. Accordingly, the susceptibilities of BK, [Phe(αMe)⁵]-BK, and [Phe(αMe)⁸]-BK to degradation by plasma were studied. Guinea pig plasma was used because of its very high kininase activity (25).

In the presence of guinea pig plasma, loss of biological activity was observed for each of the three BK agonists (Fig. 4). The slow degradation of [Phe(αMe)⁸]-BK by guinea pig plasma suggests that the amount of peptide degraded by plasma enzymes was probably not significantly different within the time of the biological response, whether administered intravenously or intra-arterially to rats. This would explain why there was no potentiation of intra-arterial doses of [Phe(αMe)⁸]-BK in the presence of BPP_{9a} (Fig. 3) despite inhibition of the plasma kininase. No loss of activity was seen for NaCl or boiled plasma controls over the same time period (data not shown). Higher concentrations of [Phe-(αMe)⁵]-BK and [Phe(αMe)⁸]-BK were required to produce detectable contraction of the cat jejunum; thus the degradation of these peptides was studied at 100-fold greater concentrations than that of BK for parallel studies. Later experiments showed that increasing concentrations of peptide are reflected in increasing half-lives ($t_{1/2}$) because of the very much greater amounts of peptide hydrolysis required to achieve loss of half the total peptide originally present. Because of this effect, and the possibility that different substrate concentrations may have contributed to different rates of enzymatic action, the $t_{1/2}$ of BK cannot be compared directly with the $t_{1/2}$ values calculated for the two α-methylated analogues. However, it can readily be seen that all three peptides are substrates of plasma

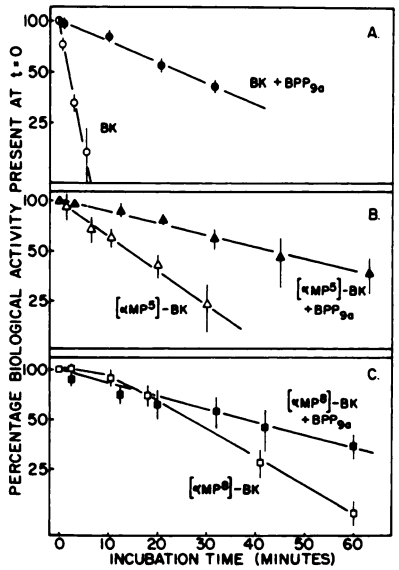

FIG. 4. *Loss of biological activity, assayed on cat jejunum, of 100 nM BK (A), 10 μM [αMP⁵]-BK ([Phe(αMe)⁵]-BK) (B), and 10 μM [αMP⁸]-BK ([Phe(αMe)⁸]-BK) (C) as a function of time of incubation with guinea pig plasma at room temperature in the absence (open symbols) and presence (solid symbols) of BPP_{9a}, 100 μg/ml*

Values are means ± standard errors (n = 4 for BPP_{9a}; n = 8 for BK peptides alone).

kininases. Furthermore, hydrolysis of all three peptides is inhibited by BPP_{9a}.

Purified rabbit lung converting enzyme. The use of purified rabbit lung converting enzyme (10) made it possible to assess unequivocally the role of converting enzyme in the metabolism of BK, [Phe(αMe)^5]-BK, and [Phe(αMe)^8]-BK. Incubation of either 5 μM BK or 5 μM [Phe(αMe)^5]-BK with enzyme led to a time-dependent loss of biological activity (Fig. 5A and B); the rate of degradation was decreased by inclusion of BPP_{9a} (100 μg/ml) in the incubation medium. No degradation of 5 μM [Phe(αMe)^8]-BK was observed during incubation times of up to 1 hr (Fig. 5C), even in the presence of 100 times more enzyme than was used in studies of BK and [Phe(αMe)^5]-BK. From this we conclude that [Phe(αMe)^8]-BK is not a substrate for the purified lung converting enzyme. Simultaneous incubation of BK and excess [Phe(αMe)^8]-BK with purified enzyme did not affect the rate of hydrolysis of BK (data not shown). This finding suggests that [Phe(αMe)^8]-BK has a lower binding affinity than BK for the active site of the enzyme. Prior incubation of purified enzyme and [Phe(αMe)^8]-BK for 1 hr at room temperature also did not lead to any inhibition of BK degradation when the incubated enzyme and BK were subsequently combined (Fig. 5C).

Smooth muscle activity. To compare the potency of the BK peptides agonists on superfused cat jejunum or rat stomach, the doses required to produce a 3-cm contraction (ED_{50} over the dose range studied) of the smooth muscles were calculated from parallel dose-response curves (Table 1). [Phe(αMe)^5]-BK and [Phe(αMe)^8]-BK were equipotent in contracting rat stomach; their potency was 3% of that of BK. On cat jejunum, [Phe(αMe)^5]-BK was 5-fold more potent than [Phe(αMe)^8]-BK, but again both analogues had very low activity ([Phe(αMe)^5]-BK = 3.5% and [Phe(αMe)^8]-BK = 0.7% of the activity of BK).

In only one system studied was the potency of [Phe(αMe)^8]-BK greater than that of [Phe(αMe)^5]-BK: [Phe(αMe)^8]-BK was 10-fold more potent in contracting isolated rat uterus (Table 1) (21). Remarkably, on this preparation, [Phe(αMe)^8]-BK also showed high potency relative to that of BK (31%).

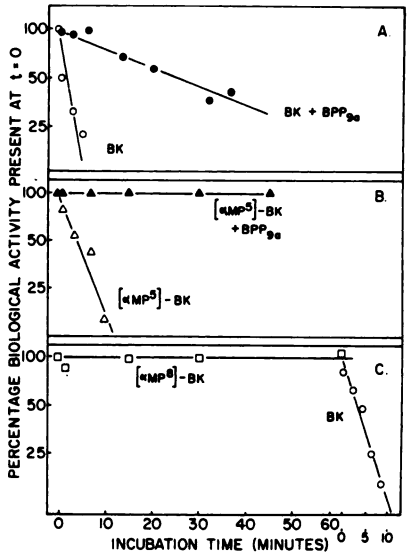

FIG. 5. *Loss of biological activity, assayed on cat jejunum, of 5 μM BK (A), 5 μM [αMP^5]-BK ([Phe(αMe)^5]-BK) (B), and 5 μM [αMP^8]-BK ([Phe(αMe)^8]-BK) (C) as a function of time of incubation with purified rabbit lung converting enzyme at room temperature in the absence (open symbols) and presence (solid symbols) of BPP_{9a}, 100 μg/ml*

The concentration of rabbit lung converting enzyme was 2.7 nM (A and B) or 270 nM (C). In panel C is shown the effect of adding an aliquot of the enzyme–[Phe(αMe)^8]-BK mixture at the end of 1 hr to 5 μM BK. Results from a typical experiment are shown.

Prostaglandin release by isolated, perfused organs. All three agonists, BK, [Phe(αMe)^5]-BK, and [Phe(αMe)^8]-BK, produced dose-dependent release of a prostaglandin-like substance when injected through the isolated, perfused rabbit heart or kidney (Fig. 6). To compare the potencies of the BK agonists, the doses required to elicit release of 50 ng of prostaglandin-like substance were calculated (Table 1). BK was equipotent in heart and kidney; however, [Phe(αMe)^5]-BK was more potent in heart than in kidney (Table 1), having 10% and 5% of the potency of BK, respectively. [Phe(αMe)^8]-BK, on the other hand, was even less potent in heart (0.2% of the potency of BK) than in kidney (0.5% of the potency of BK). [Phe(αMe)^5]-BK is thus 50

TABLE 1

Activity of BK analogues on smooth muscle and in releasing prostaglandin-like substance (PLS)

Agonist	Intra-arterial dose producing 15-mm-Hg fall in rat blood pressure	Dose producing 3-cm contraction of		Activity on rat uterus ($n = 4$)[a]	Dose causing release of 50 ng of PLS from rabbit	
		Rat stomach ($n = 3$)	Cat jejunum ($n = 3$)		Kidney	Heart
	μg	μg	μg	%	μg	μg
BK	0.07 ± 0.02	0.130 ± 0.045	0.012 ± 0.003	100	0.18 ± 0.07	0.18 ± 0.04
[Phe(αMe)^5]-BK	4.5 ± 0.9	4.8 ± 0.9	0.38 ± 0.06	3.5 ± 1.6	4.0 ± 1.5	1.9 ± 1
[Phe(αMe)^8]-BK	4.5 ± 1.4	4.0 ± 1.4	1.55 ± 0.2	31 ± 6	43 ± 17	95 ± 20

[a] From Turk *et al.* (21).

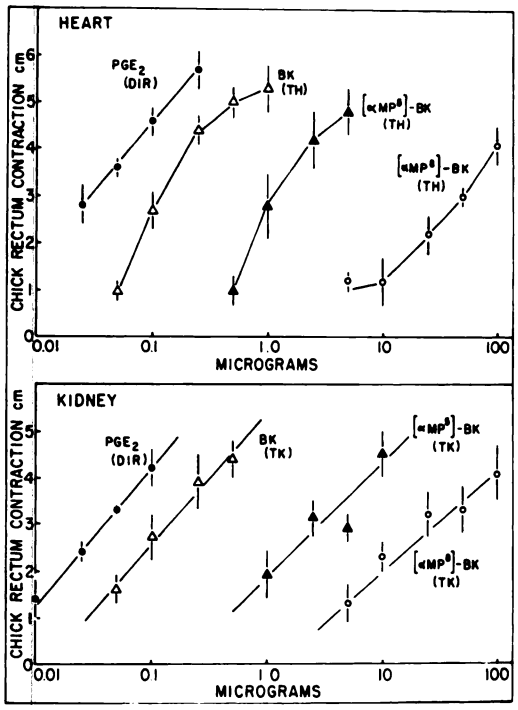

FIG. 6. *Dose-response curves for contraction of chick rectum by PGE_2 standards (DIR) or by coronary (upper) or renal (lower) venous effluent after BK, [αMP^5]-BK ([Phe(αMe)^5]-BK), or [αMP^8]-BK ([Phe(αMe)^8]-BK) injected through the organ (TH or TK)*

Values are means ± standard errors for a minimum of five determinations.

times more potent than [Phe(αMe)^8]-BK in heart, but only 10 times more potent in kidney.

The cardiac or renal degradation of BK and the analogues was determined by comparing the contraction of superfused assay strips (cat jejunum, rat stomach) produced either by direct application of the peptides or following intra-arterial injections of the peptides into the perfused organs. All three BK peptides were rapidly (one transit through the organ) and efficiently (81–91%) (Table 2) destroyed in transit through the kidney. On the other hand, the perfused heart readily degraded BK, but the 5-position analogue and especially the 8-position analogue were more resistant to destruction. Thus [Phe(αMe)^8]-BK may possess even less than 0.2% of the potency of BK in the heart.

DISCUSSION

In this study we investigated BK analogues with conformational restrictions and with peptide bonds potentially resistant to hydrolysis for activity as ligands at receptor sites and as substrates for bradykininases in a variety of systems both *in vitro* and *in vivo*. [Phe(αMe)^8]-BK is resistant to rabbit lung dipeptidyl carboxypeptidase and also appears resistant to the action of rat lung converting enzyme *in vivo*. Thus degradation of [Phe(αMe)^8]-BK indicates the presence of kininases other than converting enzyme. We therefore used this peptide to study the physiological importance, distribution, and inhibition by BPP_{9a} of kininases other than converting enzyme.

Some investigators (19, 20) have postulated a role for BPP_{9a} as a cooperative ligand that increases receptor affinity for BK in addition to its well-established role as a competitive inhibitor of the dipeptidyl carboxypeptidase known as converting enzyme (18). The suggestion that BPP_{9a} acts as receptor sites as well as converting enzyme arises from experiments using BK

analogues [(D-Pro^7)-BK and (des-Arg^9)-BK] that are stable to kininases in guinea pig ileum but whose effects are enhanced by BPP_{9a} in these systems (19). On the other hand, Ondetti and Engel (26) also found BPP_{9a} potentiation of a BK analogue that was not a substrate for converting enzyme and postulated the existence of at least two enzymatic systems, both inhibited by BPP_{9a}, but only one with the specificity of converting enzyme. Results presented here show that although [Phe(αMe)^8]-BK is not a substrate for converting enzyme (Fig. 5C), its degradation in guinea pig plasma is inhibited by the presence of BPP_{9a} (Fig. 4C). We conclude, therefore, that BPP_{9a} acts on another kininase in addition to converting enzyme. This is consistent with the finding of two BK analogues containing β-homoamino acids resistant to hydrolysis by converting enzyme and the paradoxical potentiation of the vasodepressor action of one of these analogues by BPP_{9a} (26). Although it appears likely that the inhibition of kininases other than converting enzyme by BPP_{9a} may explain potentiation of BK activity in tissues lacking converting enzyme, the data we present do not rule out the possibility that BPP_{9a} acts on the BK receptor as well. If such an action on the receptor exists, however, it is hard to understand why potentiation of [Phe(αMe)^8]-BK activity was not seen in several cases.

TABLE 2

Degradation of BK analogues by rabbit heart and kidney

Values are means ± standard errors of independent measurements of the percentage degradation in five or six hearts or kidneys. Doses (direct or through the perfused organ) required to produce a given contraction were calculated from parallel dose-response curves, and the following expression was used to calculate degradation:

$$1 - \left(\frac{\text{direct dose}}{\text{TH or TK dose}}\right)$$

where TH and TK denote injection through the heart and kidney, respectively.

Peptide	Percentage degraded	
	Heart	Kidney
	%	%
BK	80 ± 3	91 ± 2
[Phe(αMe)^5]-BK	60 ± 6	85 ± 2
[Phe(αMe)^8]-BK	51 ± 5	81 ± 2

Although we have demonstrated the presence in both isolated tissues and plasma of bradykininases that differ from purified converting enzyme, it is desirable to evaluate the relative contributions of converting enzyme and of other kininases to the inactivation of BK *in vivo* or in isolated tissues. The small differences between the amounts of BK, [Phe(αMe)^5]-BK, and [Phe(αMe)^8]-BK degraded in the kidney indicate that, in addition to converting enzyme, there is a high concentration of kininases with different specificities in that tissue. In the heart, on the other hand, the large difference in the metabolism of BK and [Phe(αMe)^8]-BK indicates that dipeptidyl carboxypeptidase and carboxypeptidase represent a much higher proportion of the total kininase activity in heart than in kidney. *In vivo* the major site of BK degradation is the lungs (27, 28). The lack of difference between intravenous and intra-arterial doses of [Phe(αMe)^8]-BK required to produce a comparable fall in blood pressure indicates that most or all of the kininase activity in rat lung is due to dipeptidyl carboxypeptidase or enzymatic cleavage adjacent to position 8, e.g., carboxypeptidase activity. Other peptidase activities to which BK is susceptible have been described in lung homogenates (16); however, the substrate may not reach these enzymes under conditions *in vivo*.

Actions at the receptor site. The analogues appear to be fully agonistic, since they generate dose-response curves parallel to that for BK. Further evaluation of [Phe(αMe)^8]-BK for possible antagonistic actions on the heart or kidney receptor was precluded by its agonistic activity. Although the differences in the order of potency of [Phe(αMe)^5]-BK and [Phe(αMe)^8]-BK in different preparations indicates that BK receptors are heterogeneous, it is not possible to discriminate between distinct classes of receptors on the basis of these studies. The heterogeneity of the BK receptors observed here indicates that it may be possible to design BK agonists or antagonists with target specificity. The rat uterine receptor appears most different,

unique both in the very high agonistic activity demonstrated by [Phe(αMe)^8]-BK (31% of that of BK) and in the finding that [Phe(αMe)^8]-BK is more potent than [Phe(αMe)^5]-BK.

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