Alkylating Analogs of Bradykinin[1a]

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Three analogs of bradykinin have been synthesized which bear a bromoacetyl function on the α-amino group or on an anilinic amino group at position 5 or 8. It was hoped that one or more of these analogs might act as an irreversible bradykinin antagonist or as a long-acting converting enzyme inhibitor. Although none of the analogs exhibited the desired pharmacological properties, the methods described for the synthesis and characterization of peptides bearing anilinic bromoacetyl groups are of potential utility in the development of antagonists of other tyrosine—or phenylalanine—containing peptides.

The activity of the kallikrein–kinin system is known to be altered in a wide variety of physiological and pathological processes. The system may play an important role in inflammation, tissue repair, and inflammatory disease;[2-4] some hypotensive states, such as those due to gram-negative septicemia, anaphyllaxis, and acute pancreatitis;[5-7] the response to myocardial ischemia;[8-10] ion and fluid balance and some forms of hypertension;[11,12] and in a variety of other human diseases.[13]

Despite the intriguing association of the kallikrein–kinin system with so many areas of active current inquiry, its actual role in most of them is far from clear. One reason for the slow progress in establishing or excluding a causative role for bradykinin with respect to any of the many phenomena with which it is known to be associated is that a basic pharmacological tool needed to probe the function of the kallikrein–kinin system is missing, namely a receptor-specific inhibitor of bradykinin. To gain some idea of the impact that the development of such an inhibitor could have on this area of investigation, one need only consider the rapid progress made in the study of the renin–angiotensin system since the appearance of potent, specific competitive inhibitors of angiotensin II (AII).[14,15]

A large number of analogs of bradykinin have been synthesized,[16,17] some of them in a conscious attempt to produce a competitive inhibitor of the parent peptide.[18,19] No generally useful inhibitor has yet been described, however. The chief problem in such a search is that even after the synthesis and biological characterization of hundreds of analogs of many biologically active peptides, few general rules relating the structure and activity of analogs to that of the parent molecule have emerged, and the development of inhibitory analogs remains an empirical, serendipitous process.

One direct approach to developing an inhibitor is to incorporate alkylating functions into BK analogs which might form a covalent link with the receptor or nearby functions. This would be expected to result in a long-lasting antagonism of BK activity. This approach was originally applied to BK by Freer and Stewart.[20] The series of peptides made by those workers bore chlorambucil residues attached to the N terminus of BK sequences n-9, where n was varied from 1 to 9. None of the peptides exhibited inhibitory properties, though some produced long-lasting potentiation of BK activity or partial inactivation of some of the pulmonary kininases.

A variation of this approach that was employed in this study is to retain the whole BK sequence in all analogs and to vary instead the site of attachment of the alkylating function. In addition, the bromoacetyl function was employed, rather than a nitrogen mustard function like that of chlorambucil, since the former group is more stable in the aqueous media employed in routine peptide purification procedures and is more easily characterized than is chlorambucil.

Walter et al. have shown that attachment of the bromoacetyl function to the α-amino group of oxytocin produces an analog that selectively attaches itself to neurohypophyseal hormone receptors in membrane preparations from rabbit kidney and toad bladder.[21] Model substrates of carboxypeptidase A bearing bromoacetyl groups have been shown to irreversibly inactivate that enzyme by selectively alkylating a glutamic acid side chain in the enzyme's active site.[22] A variety of α-halo esters, amides, and ketones have been shown to selectively modify active site residues in chymotrypsin (Met), trypsin (His), and ribonuclease (His). Other amino acid side chains that could be alkylated by such a function include Lys, Asp, and Cys.[23]

It was decided to prepare [p-NH_2-Phe^5]-BK and [p-NH_2-Phe^8]-BK and to attach bromoacetyl groups to the anilinic and α-amino groups of these molecules and to the α-amino group of native bradykinin. These analogs were chosen since it was known that acetylating the α-amino group or placing fairly bulky para substituents on the position 8 aromatic ring resulted in analogs with nearly full activity[16,17] and that [Tyr^5]-BK retained some activity.[16,17] In addition, it has been shown that angiotensin I converting enzyme isolated from lung cleaves the 5–6 and 7–8 bonds of bradykinin,[24] and the above analogs would place an alkylating function on each side of one of the scissile bonds. This could result in a long-acting converting enzyme inhibitor. Such a molecule might assume special importance in the light of recent clinical studies on the use of converting enzyme inhibitors in hypertensive patients.[25] Finally, in the absence of any real information about the possible location of a nucleophile in the bradykinin receptor, it seemed reasonable to attach alkylating functions at the N terminus, near the middle, and near the C terminus of the peptide in order to probe as much of the binding site as possible.

Experimental Section

Materials. The bradykinin used in this study was synthesized manually by the solid-phase protocol of Hancock et al.[26] All *tert*-butyloxycarbonylamino acids were obtained from Bachem except Boc-p-NO_2-Phe, which was prepared by the method of Ragnarsson et al.[27] and melted in the same range (107–108°) observed by Coy et al.[28] The *tert*-butyloxycarbonylnitro-L-arginyl-substituted resin was prepared by the method of Marshall and Merrifield.[29]

Analytical Methods. Thin-layer chromatography (TLC) was performed on 0.25-mm silica gel G plates (Analtech) in two systems: 1-butanol–pyridine–acetic acid–H_2O (15:10:3:12) and 2-butanol–3% NH_4OH (100:44). Mobilities in these systems will be designated R_{f1} and R_{f2}, respectively. The load of peptide was 50 μg per plate. Thin-layer plates were developed by ninhydrin and Clorox–starch sprays,[30] and the spots so visualized were positive to both reagents unless otherwise indicated. Paper electrophoreses were performed in a Gilson Model D high-voltage electrophorator for 2 kV hr at pH 3.5. Electrophoretic mobility relative to arginine will be designated E_A. Uv spectra were determined on a Cary 60 uv spectrophotometer. Amino acid analyses were performed on a Spinco 120C analyzer. Both p-NO_2-Phe and p-NH_2-Phe eluted from the 6-cm column at pH 5.25 between Phe and Lys and their

color constants were approximately equal to that for Phe. The peak for p-NH_2-Phe was insufficiently resolved from that for Phe under these conditions, however, and could not be accurately calculated. Sufficient resolution could be obtained by first eluting the neutral amino acids from the column at pH 4.25 and then eluting p-NH_2-Phe and Arg from the column at pH 5.25. The reason for the more satisfactory separation under these conditions is that the anilinic amino bears a charge at the lower pH, but not at the higher pH, since the pK_a of the function is 4.62.

Syntheses. *tert*-Butyloxycarbonylnitro-L-arginyl-L-prolyl-L-propylglycyl-L-phenylalanyl-*O*-benzyl-L-seryl-L-prolyl-*p*-nitro-L-phenylalanylnitro-L-arginyl-resin (I). A total of 2 g of 0.50 mequiv/g of *tert*-butyloxycarbonylnitro-L-arginyl resin was placed in the reaction vessel, deprotected, neutralized, and reacted with the appropriate amino acids in the standard cycle of operations.[29] Shrinking and swelling the resin was accomplished by three washings with 95% *tert*-butyl alcohol in dichloromethane (DCM) and with DCM, respectively. Deprotection was effected by treatment with 25% trifluoroacetic acid in DCM, and the free amino groups were neutralized with 10% triethylamine in DCM. All coupling steps were for 2 hr with threefold excesses of *tert*-butyloxycarbonylamino acid and dicyclohexylcarbodiimide in DCM, except for Boc-NO_2-Arg, which was coupled in dimethylformamide–DCM (1:1).

L-Arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-*p*-nitro-L-phenylalanyl-L-arginine ([*p*-NO_2-Phe^8]-BK). Compound I was dried and placed in a polypropylene reaction vessel of an HF apparatus.[31] Anisole (3 ml) and HF (25 ml) were added, and the suspension was stirred at 0° for 50 min. The HF was removed by a stream of nitrogen and then in vacuo. The peptide and resin were then washed with ethyl acetate and extracted with 1% acetic acid. The extract was lyophilized to give 920 mg (84%) of crude peptide which was subjected to 190 transfers in a 1-butanol–2% trifluoroacetic acid (1:1) countercurrent distribution (CCD) system. The peptide was located by the method of Lowry et al.[32] and was found to have distributed itself symmetrically in tubes 108–132 (k = 1.71). Thin-layer chromatography was performed on the contents of these tubes, and the contents of tubes 112–128 were combined, concentrated under reduced pressure, diluted with H_2O, and lyophilized to give 410 mg (37%) of peptide. This material was then subjected to 300 transfers in a 1-butanol–pyridine–acetic acid–H_2O (8:2:1:9) CCD system, and the location of uncontaminated peptide (k = 0.32) was determined as above. Tubes 56–88 yielded 350 mg (32%) of purified peptide. Amino acid analysis showed Ser 1.03, Pro 2.87, Gly 1.03, Phe 1.00, p-NO_2-Phe 0.90, Arg 2.06. The peptide content was 74%. One spot was seen on TLC and paper electrophoresis: R_{f1} 0.61, R_{f2} 0.10, E_A = 0.69. The optical activity of the peptide was $[\alpha]^{25}D$ −73.0° (*c* 0.43, methanol).

L-Arginyl-L-prolyl-L-prolylglycyl-*p*-nitro-L-phenylalanyl-L-seryl-L-propyl-L-phenylalanyl-L-arginine ([*p*-NO_2-Phe^5]-BK). The appropriate protected peptidyl-resin was prepared in the same manner as compound I and was subjected to the same cleavage-deprotection procedure, yielding 970 mg (88%) of crude peptide. This material was then subjected to the same purification procedure as [p-NO_2-Phe^8]-BK, yielding 400 mg of peptide (from tubes 124–148, k = 2.51) after the first step and 310 mg (tubes 52–96, k = 0.30) after the second step for an overall yield of 28%. Amino acid analysis showed Ser 0.93, Pro 3.00, Gly 1.03, Phe 0.95, p-NO_2-Phe 0.95, Arg 2.02. The peptide content was 67%. One spot was seen on TLC and electrophoresis: R_{f1} 0.62, R_{f2} 0.11, E_A = 0.69. The optical activity of the peptide was $[\alpha]^{25}D$ −77.0° (*c* 0.35, methanol).

Reduction of [*p*-NO_2-Phe^8]-BK to [*p*-NH_2-Phe^8]-BK. [p-NO_2-Phe^8]-BK (500 mg) and 500 mg of palladium on barium sulfate were combined in 30 ml of methanol–acetic acid–H_2O (10:1:1) and hydrogenated at 30 psi. The reduction was found to proceed to completion in less than 10 min by monitoring the uv absorbance due to p-NO_2-Phe at 278 nm. The solution was filtered, concentrated under reduced pressure, diluted with H_2O, and lyophilized to yield 430 mg of peptide. Amino acid analysis showed Ser 0.95, Pro 2.96, Gly 1.00, Phe 1.00, p-NH_2-Phe 1.04, Arg 2.00. The peptide content was 70%. The peptide had virtually no mobility in either of the CCD systems mentioned above but appeared to be homogeneous on TLC and electrophoresis: R_{f1} 0.51, R_{f2} 0.06, E_A = 0.90. The optical activity of the peptide was $[\alpha]^{25}D$ −79.3° (*c* 0.63, methanol).

Reduction of [*p*-NO_2-Phe^5]-BK to [*p*-NH_2-Phe^5]-BK. An exactly similar series of operations was applied to 500 mg of [p-NO_2-Phe^5]-BK to yield 450 mg of [p-NH_2-Phe^5]-BK. Amino acid analysis showed Ser 0.92, Pro 3.20, Gly 1.03, Phe 1.03, p-NH_2-Phe 1.00, Arg 2.00. The peptide content was 72%. One spot was seen on TLC and electrophoresis: R_{f1} 0.54, R_{f2} 0.06, E_A = 0.90. The optical activity of the peptide was $[\alpha]^{25}D$ −74.5° (*c* 0.35, methanol).

Bromoacetylation of Bradykinin to Form *N*-Bromoacetylbradykinin (1-BrAc-BK). Bradykinin (90 mg) was dissolved in 10 ml of 1 *N* $NaHCO_3$ and allowed to react at 0° for 60 min with 1.0 g of bromoacetyl bromide dissolved in 10 ml of 1,4-dioxane. (This reaction does not proceed well in dioxane purified by passage over an alumina column.) The pH was maintained at 8.5 on a Radiometer pH-stat with 1.5 *N* Na_2CO_3. The reaction mixture was then filtered, neutralized with glacial acetic acid, concentrated slightly under reduced pressure, applied to a 100 × 1.8 cm Sephadex G-25 column, and eluted with 1% acetic acid. The flow rate was adjusted to 15 ml/min and 3-ml fractions were collected. The OD at 255 nm was monitored in order to locate the peptide, which was found to be symmetrically distributed in fractions 60–80. These fractions were combined and lyophilized, yielding 70 mg of peptide (78%). This material was then subjected to 190 transfers in the 1-butanol–2% trifluoroacetic acid (1:1) CCD system, and 48 mg of peptide was harvested from tubes 168–184 (k = 12.60) for a final yield of 53%. Amino acid analysis showed Ser 0.91, Pro 3.03, Gly 1.00, Phe 1.88, Arg 1.87. The peptide content was 71%. The ninhydrin-negative peptide exhibited streaking on TLC plates, but only one spot was seen on electrophoresis: E_A = 0.35. (It is necessary to store bromoacetyl peptides frozen in a carefully evacuated desiccator to avoid slow loss of bromine from the material.)

Treatment of 1-BrAc-BK with Ammonia to Form Gly-BK. 1-BrAc-BK (10 mg) was dissolved in 5 ml of dimethyl sulfoxide, and anhydrous ammonia was bubbled through the solution for 1 hr at room temperature. The solution was then concentrated in vacuo, diluted with 1% acetic acid, applied to a 100 × 1.8 cm Sephadex G-15 column, and eluted with the same solvent. Peptide (6 mg) was harvested from fractions 43–53, and this material was homogeneous on electrophoresis: E_A = 0.89. Amino acid analysis showed Ser 1.00, Pro 2.80, Gly 2.10, Phe 1.88, Arg 1.87. The peptide content was 73%.

Bromoacetylation of [*p*-NH_2-Phe^8]-BK to Form *N*-Bromoacetyl-[*p*-bromoacetyl-NHPhe^8]-BK (1,8-BrAc-BK). [p-NH_2-Phe^8]-BK (90 mg) was bromoacetylated in the same manner as BK, and the reaction product was subjected to the same purification procedure. Peptide (68 mg) (tubes 69–80) was recovered after column chromatography and 39 mg (tubes 164–184, k = 10.90) after the CCD for an overall yield of 43%. The ninhydrin-negative material was homogeneous on electrophoresis: E_A = 0.35. Amino acid analysis showed Ser 0.95, Pro 2.95, Gly 0.95, Phe 1.00, p-NH_2-Phe 0.97, Arg 2.02. The peptide content was 76%.

Treatment of 1,8-BrAc-BK with Ammonia to Form Gly-[*p*-Gly-NHPhe^8]-BK (1,8-Gly-BK). 1,8-BrAc-BK (10 mg) was treated with ammonia, and the product was purified in the manner described for 1-BrAc-BK. Peptide (7 mg) (tubes 52–68) was recovered from the column and was homogeneous on electrophoresis: E_A = 0.89. Amino acid analysis showed Ser 0.98, Pro 2.96, Gly 3.00, Phe 1.02, p-NH_2-Phe 0.98, Arg 2.00. The peptide content was 70%.

Formation of 1,5-BrAc-BK from [*p*-NH_2-Phe^5]-BK and Its Conversion to 1,5-Gly-BK. 1,5-BrAc-BK was prepared from 90 mg of [p-NH_2-Phe^5]-BK and isolated as described above. Peptide (70 mg) was recovered from the column (tubes 70–79) and 40 mg (tubes 168–184, k = 12.50) from the CCD, for an overall yield of 45%. The ninhydrin-negative material migrated as one spot on electrophoresis: E_A = 0.34. Amino acid analysis showed Ser 0.90, Pro 3.04, Gly 1.02, Phe 1.03, p-NH_2-Phe 1.03, Arg 2.00. The peptide content was 72%. 1,5-BrAc-BK (10 mg) was treated with ammonia, and the reaction product was isolated as above. Peptide (6 mg) was recovered from the column (tubes 46–63) which showed one spot on electrophoresis: E_A = 0.90. Amino acid analysis showed Ser 0.96, Pro 3.02, Gly 3.04, Phe 1.00, p-NH_2-Phe 1.12, Arg 2.12. The peptide content was 74%.

Bioassays. The oxytocic activity of the analogs was measured in rat uterus as previously described.[33] All analogs were assayed for agonistic and antagonistic activity. Candidates for long-acting antagonists were added to the organ bath at a concentration of 10 μg/ml and were incubated with the tissue at 37° for 45 min while bubbling 95% O_2–5% CO_2 (pH 6.8) through the bath. Identical experiments were also performed using 100% O_2 (pH 8.6). The sensitivity of the strips to BK was then reexamined and compared to the sensitivity before the treatment and to that of a control strip that had been incubated with 100 ng/ml of BK.

Blood pressures were measured in urethane anesthetized rats (1.25 g/kg) by recording from the left femoral artery (P-1000A

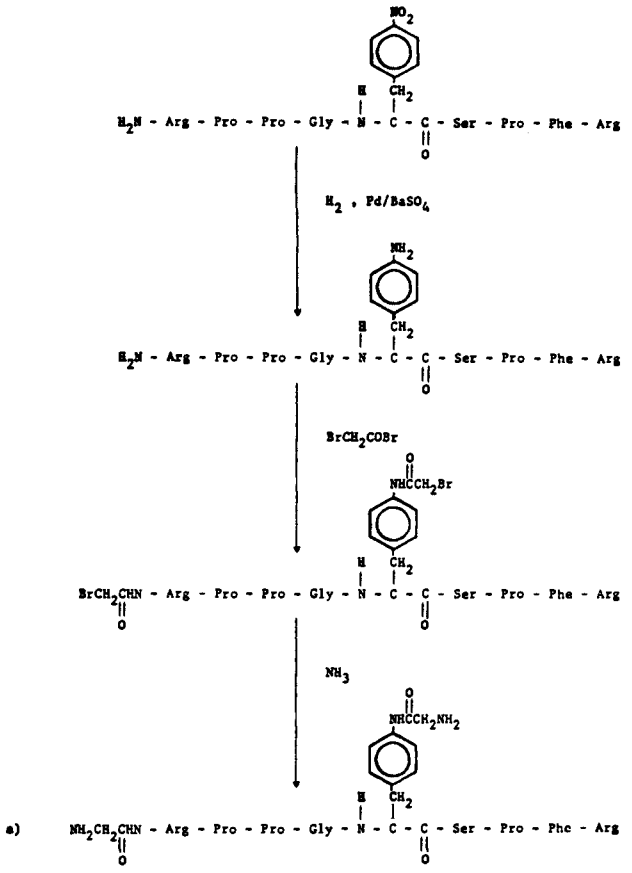

Figure 1. (a) Chemical route employed in the synthesis and characterization of 1,5-BrAc-BK. (b) Structures of 1,8-BrAc-BK and 1-BrAc-BK.

pressure transducer, physiograph). The agent was injected into the cannulated right jugular vein or left carotid artery by means of a Hamilton syringe automatic dispensing apparatus. Intraarterial (ia) and intravenous (iv) dose–response curves for BK were determined before and after the administration of each analog so that each animal served as its own control. Each analog was assayed for agonistic (ia and iv) and competitive antagonistic (ia) activity. Candidates for long-acting antagonists were infused into the left jugular vein at a rate of 40 μg/min for 1 hr. Ia and iv sensitivity to BK was determined before, during, and after the infusion. The amount of displacement on the dosage axis of the iv dose–response curve from the ia dose–response curve reflects pulmonary degradation. Any change in that displacement thus reflects altered activity of the pulmonary kininases.

Results and Discussion

A summary of the synthetic route to and structures of the desired BK analogs is presented in Figure 1. For the sake of convenience, a somewhat unorthodox nomenclature has been employed to designate the newly synthesized peptides. The chemical structures of [*p*-NO_2-Phe^5]-BK and of its reduction product [*p*-NH_2-Phe^5]-BK should be clear.

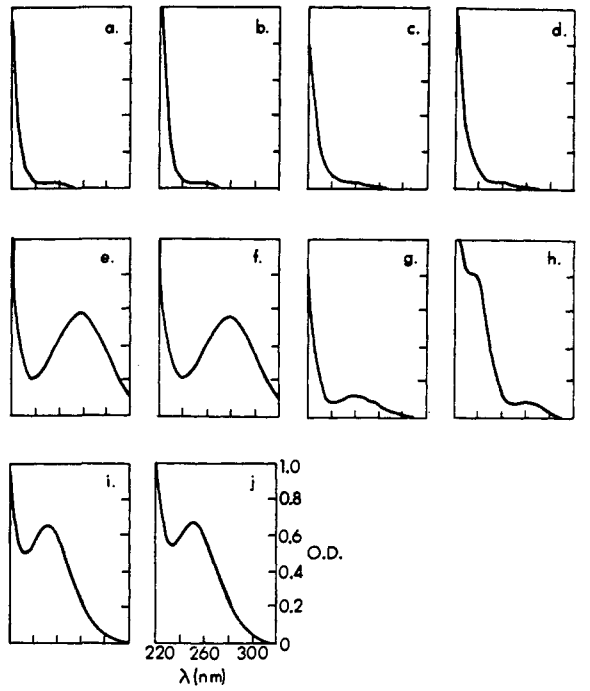

Figure 2. pH dependence of the uv spectra of BK (a and b), 1-BrAc-BK (c and d), [*p*-NO_2-Phe^5]-BK (e and f), [*p*-NH_2-Phe^5]-BK (g and h), and 1,5-BrAc-BK (i and j). The concentration of all the samples was 0.10 mg/ml. Samples a, c, e, g, and i were dissolved in glycine buffer, pH 2.5. Samples b, d, f, h, and j were dissolved in borate buffer, pH 9.5.

The product of the reaction of [*p*-NH_2-Phe^5]-BK and bromoacetyl bromide has a bromoacetyl function attached to the α-amino group of Arg^1 and to the anilinic amino group of *p*-NH_2-Phe^5. This compound has been designated 1,5-BrAc-BK. Treatment of that compound with ammonia results in a compound with a Gly residue attached to the same groups that previously bore bromoacetyl functions. This compound has been designated 1,5-Gly-BK (see Figure 1). Similar conventions were adopted for the position 8 analogs. *N*-Bromoacetyl-BK is designated 1-BrAc-BK.

Analogs of angiotensin II (AII) and of luteinizing hormone-releasing hormone (LH-RH) that contain *p*-NO_2-PHe and *p*-NH_2-Phe in the place of Phe or Tyr have been synthesized previously in a similar manner by other workers.[28,34] An analog of oxytocin bearing a bromoacetyl function on the α-amino group of the peptide has also been prepared by treatment of oxytocin with bromoacetyl bromide, as mentioned before, and this peptide was also characterized by the conversion of the bromoacetyl function to a glycine residue by treatment with ammonia.[21] The peptides prepared in this study are apparently the first that bear a bromoacetyl function on an anilinic amino group, however.

A convenient method of determining the success of attempted modifications of substituents of an aromatic ring is to examine the uv spectra of the reactants and products. The pH dependence of the spectra can be particularly informative if the substituent is an ionizable function. The uv studies of the newly synthesized peptides are presented in Figure 2. As expected, BK has only a weak absorption at 257 nm (due to the phenylalanine side chain) that is affected neither by pH (Figure 2, a and b) nor by bromoacetylation of the α-amino group (Figure 2, c and d). [*p*-NO_2-Phe^5]-BK bears a substituent which extends the system of π conjugation, and its spectrum is characterized by a much stronger absorption at 278 nm which is not pH dependent (Figure 2, e and f). Upon reduction of the nitro

Table I. Bradykinin Analogs[a]

Analog	% activity, rat uterus	% activity, rat depressor	% pulmonary degradation
BK	100	100	99 ± 1
1-BrAc-BK	62 ± 9	80 ± 9	99 ± 1
Gly-BK	80 ± 11	–	–
[p-NO_2-Phe^5]-BK	4.3 ± 1.1	3 ± 1.7	95 ± 3
[p-NH_2-Phe^5]-BK	0.7 ± 0.3	2 ± 0.8	91 ± 4
1,5-BrAc-BK	< 0.01	< 0.1	–
1,5-Gly-BK	< 0.01	< 0.1	–
[p-NO_2-Phe^8]-BK	100 ± 10	84 ± 11	99 ± 1
[p-NH_2-Phe^8]-BK	53 ± 9	18 ± 4	99 ± 1
1,8-BrAc-BK	0.9 ± 0.4	0.4 ± 0.2	91 ± 4
1,8-Gly-BK	0.5 ± 0.3	< 0.1	–

[a] Agonistic activity is expressed as the (ratio of the dose of BK required to produce a half-maximal response to that of the analog required to produce the same response) × 100. Pulmonary inactivation is expressed as $[1 - (\text{ia } ED_{30}'/\text{iv } ED_{30}')] \times 100$, where ia ED_{30}' and iv ED_{30}' are the intraarterial and intravenous doses of the analog required to produce a 30-mm fall in mean blood pressure. Figures shown are the mean of 16 separate determinations in four different animals. Standard deviations are indicated. Dashes indicate that the parameter in question was not determined.

group to the ionizable amino group, the strong absorption at 278 nm is replaced by a weaker one at 258 nm (at pH 2.5), and the spectrum is now pH dependent. At pH 9.4 a strong absorption appears at 233 nm and a weaker one at 276 nm (Figure 2, g and h). With the masking of the anilinic amino by the bromoacetyl group, the aromatic substituent is no longer ionizable, and the system of conjugation has been reextended due to the partial double-bond character of the amide bond. This is reflected in the lack of pH dependence of the spectra and the appearance of a strong absorption at 252 nm (Figure 2, i and j). The uv spectra for the series of peptides modified in position 8 are identical with those for the peptides modified in position 5 and are not presented.

Further substantiation of the success of the syntheses is provided by the fact that 1 mol of glycine is generated for every mole of bromine expected in the bromoacetylated peptides when they are treated with ammonia (see the Experimental Section). In addition, the peptides which are expected to have a net charge of +2 at pH 3–4 (BK, [p-NO_2-Phe^5]-BK, [p-NO_2-Phe^8]-BK) migrate less rapidly on paper electrophoresis than those with an expected net charge of +3 ([p-NH_2-Phe^5]-BK, [p-NH_2-Phe^8]-BK, 1,5-Gly-BK, 1,8-Gly-BK) and more rapidly than those with a net charge of +1 (1-BrAc-BK, 1,5-BrAc-BK, 1,8-BrAc-BK). In countercurrent distribution and thin-layer chromatography the opposite relation between charge and mobility is observed, as expected (see Experimental Section).

The agonistic activity of the new analogs is summarized in Table I. None of these peptides exhibited the properties of a competitive or of an irreversible antagonist either in vivo or in vitro, nor did any of them prolong the duration of the response to BK or produce long-lasting interference with its pulmonary degradation (data not shown). 1-BrAc-BK shows strong agonistic activity both in vivo and in vitro, which is consistent with the fact that a free α-amino group is not required for the activity of BK.[16,17] The fact that the presence of a bromoacetyl function on the α-amino group seemed to interfere so little with binding is the reason that the α-amino groups of [p-NH_2-Phe^5]-BK and [p-NH_2-Phe^8]-BK were not protected during the bromoacetylation of their anilinic amino groups.

The high activities of [p-NO_2-Phe^8]-BK and [p-NH_2-Phe^8]-BK are consistent with the observation that a fairly bulky para substituent on the aromatic ring in position 8 is well tolerated: [Tyr(-OCH_3)^8]-BK is more active than BK.[17] The much lower activity of the position 5 analogs is consistent with the reduced activity of [Tyr^5]-BK (0.2–1%[16]). In both positions, note that as the bulk of the para substituent increases, activity decreases both in vivo and in vitro. The drop in activity that occurs with the reduction of [p-NO_2-Phe^8]-BK to [p-NH_2-Phe^8]-BK may reflect an increased bulk of the hydrated para substituent, the relative increase in the electron sufficiency of the aromatic ring, or the ability of the anilinic amino to participate in a hydrogen bond that is slightly unfavorable to the preferred binding orientation.

The apparently decreased pulmonary degradation of [p-NO_2-Phe^5]-BK, [p-NH_2-Phe^5]-BK, and 1,8-BrAc-BK is probably not significant, since these analogs are only weakly active, and the intravenous (iv) levels required to see a depressor response probably approach saturation levels of the pulmonary kininases. All three bromoacetyl analogs produced an apparent partial competitive inhibition of the pulmonary degradation of BK, but this was only at high infusion rates, and the effect was not long-lasting (data not shown).

The lack of inhibitory activity of the bromoacetyl analogs suggests that appropriate nucleophiles necessary for covalent bond formation are not adjacent to the α-amino group or the side chains of residues 5 and 8 when the BK molecule is bound to its receptors or to the pulmonary kininases. It could be suggested on this basis that future attempts to synthesize irreversible antagonists of BK should concentrate on placing the alkylating function on side chains that might be expected to lie close to a nucleophilic center in the receptor, such as the guanidinium groups of Arg^1 and Arg^9. The aromatic rings of Phe^5 and Phe^8 may be sequestered in hydrophobic environments at the binding site. Analogs of angiotensin II which bear alkylating functions on aromatic rings have been shown to act as irreversible antagonists in some systems,[35,36] however, suggesting that such rings are not always sequestered in hydrophobic environments. In addition, since the α-amino group of BK bears a positive charge at physiologic pH, it might have been expected to be directed toward a nucleophile in the receptor, and, yet, 1-BrAc-BK also failed to antagonize the action of BK. An ionized α-amino group seems to be less important for BK activity than ionized guanidino groups in the 1 and 8 positions,[16,17] however, and so it is possible that these positions are more promising points to attach alkylating functions.

While no useful tool for the physiologist or pharmacologist interested in the actions of the kallikrein–kinin system has emerged from this study, the methods employed to synthesize and characterize peptides bearing a bromoacetyl function on an anilinic amino group may prove to be of use in preparing irreversible antagonists of other biologically active peptides containing Phe or Tyr. In particular, in cases where chlorambucil or melphalen containing analogs of peptides have been shown to act as irreversible antagonists (as in the case of angiotensin II), the synthetic methods described here may offer a more convenient route to molecules with similar biological activity since the radii of the nitrogen mustard and bromoacetyl groups are nearly identical.

References and Notes

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Notes

Analogs of Bradykinin with Restricted Conformational Freedom[1a]

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Three analogs of bradykinin have been synthesized which bear an α-methyl group in the place of an α proton at position 4, 5, or 8. Such analogs possess restricted conformational freedom and are of interest for three reasons. (1) They may provide information about the receptor-bound conformation of the peptide. (2) They may provide a route to antagonists of the native peptide. (3) They may be degraded slowly by proteolytic enzymes. None of the analogs described here antagonized the action of bradykinin, but one exhibited tissue specificity and decreased pulmonary inactivation in the rat.

Peptides which bear methyl groups in the place of protons at various positions on the backbone of the peptide chain may be of unusual interest for a number of reasons. Theoretical studies of the allowed dihedral angles of model peptides have shown that substitution of a methyl group for the proton on the α carbon or on the amide nitrogen of an amino acid residue results in a dramatic reduction of the conformational space available to the backbone of the peptide chain at the position where that residue occurs.[2-5] Peptide hormone analogs containing α-methyl or N-methylamino acids should therefore have a sterically rigid backbone conformation at those positions. The term "conformational analogs" has been suggested for such peptides,[6,7] since they would have a primary structure essentially identical with that of the native hormone but could assume only a more restricted set of conformations.

Should such an analog be biologically active, important constraints on the receptor-bound conformation of the parent molecule might be provided, since high biological activity would imply that the conformational restrictions imposed by the methyl substitution were compatible with the conformational requirements for binding to the receptor. Analogs whose conformations are restricted by the presence of a backbone methyl group also provide a route to a novel class of inhibitors. Schemes of analog generation that have been applied to bradykinin and other peptides nor-