Synthesis and Pharmacological Properties of [5-Isoleucine]-, [8-Isoleucine]-, and [5,8-Diisoleucine]bradykinin

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Three bradykinin analogues have been synthesized in which the phenylalanine residue(s) at positions 5 and/or 8 have been substituted by isoleucine. All these analogues have weak bradykinin-like activity in isolated rat uterine smooth muscle or in rat blood pressure assay. No antagonistic activity was observed with any of these analogues. The importance of phenylalanine at positions 5 and 8 is discussed.

Development of specific receptor antagonists for the endogenous hormones has contributed profoundly to the understanding of their physiological functions and their involvement in disease. Numerous examples are found in the renin/angiotensin system,[1,2] the autonomic nervous system,[3,4] and for histamine[5,6] to name a few. Although the structure–activity relationships for bradykinin have been extensively studied for the past 20 years, no useful receptor antagonist has been identified.[7,8] Obviously, the development of a bradykinin antagonist is necessary in order to establish or exclude the participation of bradykinin in a wide variety of physiological and pathological conditions.[9]

Among the numerous analogues of bradykinin which have been synthesized, only a few have been reported to show some indications of antagonizing the action of bradykinin in in vitro systems.[8,10,11] These are the analogues of bradykinin (BK) in which phenylalanine at position 5 and 8 has been modified [e.g., [Tyr(OMe)^{5,8}]-, [Thr^{6},Leu^{5,8}]-, and [AcArg^{1},Gly^{6},Tyr(OMe)^{8}]BK]. However, all these analogues are partial agonists and, in addition, their inhibitory properties are highly variable, being observed only in specific assay systems. Partially encouraged by these previous observations, as well as the observation that in the renin/angiotensin system substitution of phenylalanine at position 8 yielded an angiotensin II antagonist,[1,2] we systematically replaced the phenylalanine residues in bradykinin with isoleucine. The results are reported herein.

Results and Discussion

The three peptides were synthesized by solid-phase synthesis and tested for bradykinin-like activity on isolated, estrous rat uterus and in vivo on rat blood pressure. As shown in Table I, biological activities of these analogues of bradykinin are drastically reduced by the substitution of isoleucine at either or both the 5 and 8 positions. In accordance with previous observations,[8,12] substitution of the phenylalanine at position 5 results in greater reductions in biological activity than substitution of the phenylalanine at position 8. Thus, the order of potency is seen again to be the following: [Ile^{8}]BK > [Ile^{5}]BK > [Ile^{5,8}]BK. To our disappointment, however, none of these analogues showed any antagonism to the action of bradykinin on either assay system, even at concentrations up to 10^{-5} M. Apparently, substitution of phenylalanine with isoleucine, in addition to markedly decreasing intrinsic activity, also drastically reduces the affinity of the analogues for the receptor.

In the past, it was suggested that the aromatic rings of the phenylalanine residues were important for binding to the receptor, possibly owing to the interaction between the π electron clouds of the aromatic rings with a similar area on the receptor site.[8] This is probably not the case, however, since saturation of the phenylalanine rings to give [5,8-cyclohexylalanoyl]bradykinin ([Cha^{5,8}]BK) did not abolish the biological activity on guinea pig ileum. This analogue, in fact, was reported to retain the full biological activity of bradykinin in this tissue.[13-17] We have also prepared this analogue and confirmed its high potency on guinea pig ileum as reported initially by Schafer et al.[13] (Table I). In addition, when tested on the rat uterus, it was found to be one-third as potent, while in vivo vasodepressor activity was one-twentieth that of bradykinin. It retains, therefore, significant bradykinin-like activity in contrast to other modifications at these positions.

Interestingly, the [Cha^{5,8}]BK was equipotent with BK when administered via the intravenous route. This apparent discrepancy is due to total lack of pulmonary inactivation of this analogue vs. >95% inactivation of BK itself. In addition, the [Cha^{5,8}]BK produced a somewhat delayed but marked pressor response. This was probably due to catecholamine release, since it was abolished following infusion of phentolamine mesylate (Ciba-Geigy) at 100 $\mu g/kg^{-1}min^{-1}$ in saline.

All the bradykinin analogues so far studied[7] indicate that either one or both of the phenylalanine residues can be substituted with amino acids containing bulky or rigid side chains (e.g., tyrosine, tyrosine methyl ether, *p*-fluorophenylalanine, *p*-nitrophenylalanine, *p*-aminophenylalanine, thienylalanine, and cyclohexylalanine) without

Table I. Biological Activity of Isoleucyl and Cyclohexylalanyl Analogues of Bradykinin[a]

variat in posit	compd	amino acid sequence	rat uterus	vaso depressor	guinea pig ileum	anti-BK act. (uterus)	
5	[Ile^{5}]BK	H Arg Pro Pro Gly Ile Ser Pro Phe Arg OH	1/3600	1/5600		none	~2.5 × 10^{-6} M
8	[Ile^{8}]BK	H Arg Pro Pro Gly Phe Ser Pro Ile Arg OH	1/490	1/1800		none	~1 × 10^{-5} M
5 + 8	[Ile^{5,8}]BK	H Arg Pro Pro Gly Ile Ser Pro Ile Arg OH	<1/5000	<1/5600		none	~1 × 10^{-5} M
5 + 8	[Cha^{5,8}]BK	H Arg Pro Pro Gly Cha Ser Pro Cha Arg OH	1/3	1/20[b]	1		

[a] Myotropic activity is expressed as the ratio of the dose of bradykinin/dose of analogues to give 50% maximum response. The vasodepressor activity is expressed as the ratio of the intraarterial dose of bradykinin/dose of analogues that produced a 20 mmHg fall in mean blood pressure. Cha = β-cyclohexyl-L-alanine. [b] [Cha^{5,8}]BK was equipotent with BK when administered intravenously (see text for explanation).

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Table II. Analytical Data for Isoleucyl Bradykinin Analogues

compd	TLC R_f syst A	TLC R_f syst[a] B	high-volt. electrophor[b] R_{Arg}, pH 5	high-volt. electrophor[b] R_{Arg}, pH 1.9	amino acid anal.:[c] molar ratios Arg	Pro	Gly	Phe	Ser	Ile
[Ile^5]BK	0.71	0.60	0.65	0.63	2.12	3.00	1.02	1.04	0.77	1.10
[Ile^8]BK	0.67	0.57	0.65	0.63	2.07	2.97	1.06	1.06	0.90	1.00
[Ile^{5,8}]BK	0.64	0.60	0.68	0.68	2.18	3.10	1.09		0.87	2.00

[a] System A = butanol-acetic acid-water (4:1:1), system B = butanol-acetic acid-water-pyridine (15:3:12:10). [b] R_f expressed as ratio of R_f peptide/R_f arginine. [c] Amino acid analysis on Beckman 119C analyzer. Samples hydrolyzed in 6 N HCl (constant boiling) for 22 h at 110 °C.

drastic loss of activity. In fact, bradykinin analogues containing β-2-thienylalanine have significantly greater (2–10×) biological activity than bradykinin itself.[16] However, replacement of the phenylalanine by unrestricted aliphatic residues (e.g., glycine, alanine, leucine, and isoleucine) results in essentially inactive peptides. Unfortunately, as with our analogues reported here, none of the above compounds show any consistent antagonism to the action of bradykinin, although the Tyr(Me) derivatives and leucyl derivatives were reported to have variable and inconsistent inhibitory properties.

In conclusion, substitution of phenylalanine residues in bradykinin with isoleucine results in drastic loss of both inherent activity and receptor affinity. No antagonism to bradykinin was observed. In view of the high potency of the cyclohexylalanine and thienylalanine derivatives of bradykinin, future attempts in the development of bradykinin antagonists may be more fruitful in substituting the phenylalanine residues with rigid cyclic amino acids with the aim of retaining binding affinity for the receptor while eliminating intrinsic activity.

Experimental Section

Materials. Boc amino acids were purchased from Peninsula Laboratories, San Carlos, CA. All other reagents and solvents were of the highest quality available.

Peptide Synthesis. Peptides were prepared by solid-phase synthesis as previously described[17,18] using the *tert*-butyloxycarbonyl (Boc) protecting group. The final product was removed from the resin by treatment with anhydrous HF for 45 min at 0 °C in the presence of 50 equiv of anisole. Crude peptides were purified by countercurrent distribution in 1% TFA/*n*-BuOH, 100 transfers. Purity was determined by high-voltage electrophoresis, thin-layer chromatography, and quantitative amino acid analysis (Beckman 119C). Analytical data are recorded in Table II.

Hydrogenation of bradykinin (20 mg) was carried out under 40 psi at room temperature for 65 h in 80% AcOH using a platinum oxide catalyst (120 mg). The reaction mixture was filtered and dried, and the absence of phenylalanine in the product was confirmed by amino acid analysis and UV absorption.

Bioassays. All analogues of bradykinin were tested for their agonistic and antagonistic properties in at least two bioassay systems, namely, rat uterus, rat blood pressure, or guinea pig ileum. The procedure and conditions of these assays have been previously described.[19,20]

In brief, rat uteri obtained from estrous virgin females were suspended in a modified deJalon's solution containing only 20 mg/L of $CaCl_2$. Isotonic contractions were measured under 1 g of tension. The bathing medium was kept at 30 °C and gassed continually with 98% O_2–2% CO_2. Guinea pig ileum assays were carried out with 2–3-cm strips of terminal ileum suspended in Tyrode's solution, kept at 36 °C, and bubbled with 95% O_2–5% CO_2. Again, isotonic contractions were monitored under 1 g of tension. Full dose–response curves were obtained for bradykinin and its analogues. To test for antagonism, the analogues were added to the bath 2 min before the addition of various doses of bradykinin. The potency ratio of bradykinin vs. the analogues was calculated at the concentration which gave 50% maximum contraction.

The rat blood pressure assays were carried out in Dial-urethan-anesthetized male rats.[20] Mean arterial blood pressure was recorded from the femoral artery, and all drugs were administered, in saline, via intracarotid injection. In studies for possible antagonism, analogues were administered by continuous infusion (0.018 mL/min in 0.9% NaCl) into the jugular vein.

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