SYNTHESIS AND SOME PHARMACOLOGICAL PROPERTIES OF THREE NEW BRADYKININ ANTAGONISTS

Bernard LAMMEK, Yi-Xin WANG and Haralambos GAVRAS

Department of Medicine, Boston University Medical Center, 80 East Concord Street, Boston, MA 02118, U.S.A.

Received October 4, 1990
Accepted October 14, 1990

Three new analogues of bradykinin (BK) were designed, synthesized and bio assayed in conscious, unrestrained rats. The analogues were designed by modifying the [D-Arg^0,Hyp^3,Thi^{5,8},D-Phe^7]-BK which was previously synthesized by the Stewart group and was considered to be one of the most potent antagonists of BK known to date. It has been reported herein that the introduction of additional D-Arg residue or acylation with propionic acid of the N-terminus of [D-Arg^0, Hyp^3,Thi^{5,8},D-Phe^7]BK (model peptide) results in analogues with similar or weaker antagonistic potency as compared to the model, respectively. However, the acylation with 1-adamantaneacetic acid results in an analogue with more than ten times enhanced potency and efficacy. All peptides were synthesized by the solid phase method.

The mechanism of bradykinin (BK)* activity and its interactions with other vaso-active systems were investigated by various methods. In one approach, the effect of bradykinin was studied by blocking its action via antibodies[2]. Another approach, made possible recently, consists in the use of bradykinin analogues with BK-receptor binding properties. By this approach some bradykinin analogues were found to be effective BK inhibitors.

Two major classes of bradykinin receptors, B_1 and B_2 have been defined. In general, compounds which are B_2 antagonists are nonselective, and will act both on B_1 and B_2 receptors. The B_1 receptors are sensitive to the bradykinin metabolite des--Arg^9-BK, and B_1-mediated effects are blocked by the specific antagonist des-Leu^8, Arg^9-BK. Effects mediated by B_2 receptors cannot be blocked by this type of peptides. The key modification that confers B_2-antagonistic properties to an analogue consists in the replacement of the proline residue at position 7 of BK with a D-phenylalanine residue. Other substitutions, such as the replacement of proline by hydroxyproline in positions 2 or 3, substitutions of phenylalanine in positions 5 and 8 with β-2--thienyl-L-alanine (Thi), or addition of one or two amino acid residues to the N-terminus, can potentiate the antagonistic properties of the analogues[3].

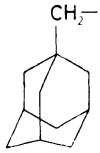
* Unless stated otherwise, all chiral amino acids belong to the L-series. The nomenclature and symbols of the amino acids, their derivatives and peptides obey the published IUPAC recomendations[1]. Hyp and Thi denote hydroxy-L-proline and β-(2-thienyl)-L-alanine, respectively.

Antagonists of bradykinin have been used to study various cardiovascular effects exerted by BK either directly or via interactions with other vasoactive substances, by assessing the effects of chronic B_2 receptor inhibition of BK on systematic or regional hemodynamics, cardiac function and other vasoactive systems. One of the commonly used and most potent antagonists of bradykinin has the sequence: D-Arg--Arg-Pro-Hyp-Gly-Thi-Ser-D-Phe-Thi-Arg ([D-Arg^0,Hyp^3,Thi^{5,8},D-Phe^7]BK). This analogue combines the chemical modifications that confer bradykinin antagonistic capacity at the B_2 receptor and a number of other desirable properties, such as high selectivity for vascular tissues and a relative resistance to enzymatic degradation which prolongs its plasma half-life up to several minutes. The plasma half-life of bradykinin itself is only a few seconds. However, this analogue has relatively low potency which neccesitates the use of high concentrations during the experiments. This may in turn result in the stimulation of the BK-receptors of other tissues e.g., in the adrenals to release catecholamines[4,5]. Thus, more effective and efficient bradykinin antagonists would be useful for studying the physiological effects of BK and for diagnostic and therapeutic applications in bradykinin-mediated vascular disorders.

We report herein the synthesis and pharmacological evaluation of three new analogues of BK *I*–*III*. We designed them by modifying the [D-Arg^0,Hyp^3,Thi^{5,8},D--Phe^7]BK (model peptide) which was previously synthesized by the Stewart group and was considered to be one of the most potent antagonists of BK known to date[6,7]. For comparison with our new analogues we also synthesized and bioassayed the model peptide.

D - Arg - D - Arg - Arg - Pro - Hyp - Gly - Thi - Ser - D - Phe - Thi - Arg *I*

CH_3—CH_2—CO - D - Arg - Arg - Pro - Hyp - Gly - Thi - Ser - D - Phe - Thi - Arg *II*



CO - D - Arg - Arg - Pro - Hyp - Gly - Thi - Ser - D - Phe - Thi - Arg *III*

The antagonistic potency of the analogues was assessed by their ability to inhibit the vasodepressor response of exogenous BK in awake rats. A preliminary report on analogue *III* has been published[8].

The antagonistic potencies of new bradykinin analogues with the properties of [D-Arg^0,Hyp^3,Thi^{5,8},D-Phe^7]BK (model peptide) used for comparison are presented

in Table I. All new analogues are bradykinin antagonists in vivo. In order to compare the antagonistic potency of analogues in low and high doses we used ED_{20} and ED_{90}, respectively. ED_{20} and ED_{90} represent the doses of bradykinin antagonist (nmol/kg/min) that inhibit 20% and 90% of the vasodepressor response to the agonist (250 ng of BK), respectively. The comparison of the antagonistic properties of our peptides and the model peptide indicates that only peptide *III* shows superior antagonism as compared to the model. The peptides *I* and *II* show similar or weaker antagonistic potency then the model, respectively. In low doses, the antagonistic activity of peptide *I* is similar, peptide *II* is about five times less potent, only analogue *III* is approximately twice as potent as the model peptide. However, ED_{90} is a parameter which is much more useful then ED_{20} in the actual use since most of the investigators choose the dose in this range, i.e. the dose of the antagonist which could inhibit 90% or more of the response to its agonist. The comparison of the ED_{90} values for the analogues shows that peptide *III* is over ten times stronger then the model one. The activity of peptides *I* and *II* is similar or slightly lower, respectively. We found that analogue *III* did not exhibit any agonistic vasodepressor effects at doses up to 50 nmol/kg/min, it also had no intrinsic effect on systemic blood pressure. The exceptionally high activity of adamantaneacetic acid derivatized bradykinin antagonist compared to that of propionic acid derivative may be due to the presence of bulky substituent at the N-terminus of the peptide, which it appears, modifies the interaction of the peptide and its B_2 receptor.

Our discovery suggests new possibilities for the design of experiments aimed at clarifying the role of BK in various processes. Moreover, similar modifications might be useful in the design of even more potent and selective bradykinin antagonists.

TABLE I

Pharmacological data of new bradykinin analogues

Analogue	N^a	Antagonistic potency (nmol/kg/min)[b]		
		ED	ED_{20}	ED_{90}
I	5	7·8 ± 2·8	9·9 ± 4·0	572 ± 90·7
II	5	28·2 ± 3·9	25·6 ± 3·8	774 ± 141·1
III	7	2·7 ± 0·4	2·4 ± 0·28	39·7 ± 5·3
Model peptide[c]	7	5·5 ± 1·3	4·6 ± 0·75	439 ± 63·3

[a] Number of rats tested with each compound; [b] for definitions of ED, ED_{20} and ED_{90} see Experimental; [c] peptide designed by Stewart[6].

EXPERIMENTAL

Apparatus and Methods

The optical rotations were measured with a Hilger–Watts polarimeter with an accuracy of 0·01°. For amino acid analysis, the peptides were hydrolyzed with constantly boiling hydrochloric acid (400 μl), containing phenol (20 μl), in evacuated, sealed ampoules placed for 18 h at 110°C. The analyses were performed on a Mikrotechna type AAA881 analyser. TLC was carried out on silica plates (Merck), and the spots were visualized by iodine. The following solvent systems were used: *A* 1-butanol–acetic acid–water (4 : 1 : 5, v/v, upper phase), *B* ethyl acetate–pyridine–acetic acid–water (5 : 5 : 1 : 3, v/v). N,N-Dimethylformamide was distilled under reduced pressure, triethylamine (NEt_3) was distilled from ninhydrin. Other solvents and reagents were of analytical grade.

Synthesis of the Peptides

Three new analogues of bradykinin *I*—*III* and the model peptide were synthesized using the solid phase method by stepwise coupling of Boc-amino acids to the growing peptide chain on a Merrifield resin. Boc-Arg(Tos)-resin (Sigma) was converted to protected peptidyl or acylpeptidyl resins in ten cycles of solid phase synthesis. The Boc group was removed with 35% solution of trifluoroacetic acid in methylene chloride in the presence of 0·2% indole. Coupling reactions were mediated by the DCC or DCC/HOBt method[9]. Boc-D-Arg, propionic acid and 1-adamantaneacetic acid (Sigma) were each used in the final coupling steps. The completion of all coupling reactions was monitored by the Kaiser test[10]. After completing the synthesis, 1 g of the protected peptidyl or acylpeptidyl resin was treated with 10 ml of liquid HF at 0°C for 50 min in the presence of 1 ml of anisole. After removal of HF in vacuo, the mixture was washed with anhydrous diethyl ether and extracted three times with 40 ml of 15% acetic acid. The combining extracts were lyophilized to give crude analogue. This material was desalted by gel filtration on Sephadex G-15 (50% acetic acid) and purified twice on Sephadex LH-20 (30% acetic acid). The purity and identity of the peptides were ascertained by the thin-layer chromatography

TABLE II

Physico-chemical characteristics of new bradykinin analogues

Peptide	R_F		$[\alpha]_D$ (*c* 0·5, 1M AcOH)	Amino acid analysis			
	A	*B*		Phe Pro	Hyp Arg	Gly Ser	Thi
I	0·39	0·52	−75·4°	1·02 1·04	0·97 3·92	1·00 0·96	1·97
II	0·32	0·73	−109·2°	1·03 1·04	0·96 3·04	1·00 0·95	2·01
III	0·30	0·77	−114·6°	1·01 1·02	0·95 3·05	1·00 0·97	1·98

in two different solent systems (*A* and *B*), and by the amino acid analysis. The physico-chemical properties of the analogues are summarized in Table II.

Pharmacological Methods

The antagonistic potency was assessed by the ability to inhibit the vasodepressor response to exogenous bradykinin in awake rats. Intact male Wistar rats (Charles River Breeding Laboratories, Wilmington, MA, U.S.A.) weighing 225–250 g were used and maintained on a regular Purina chow diet, as well as tap water in a room at constant temperature (23 ± 1°C) with 12 h dark/12 h light cycles. One day before the experiment, the right carotid and the iliac artery in the rats were catheterized with polyethylene tubing (PE 50) under light ether anesthesia. A "Y" type conection was attached to the carotid artery catheter with two ends: one for injecting bradykinin and the other for infusing the bradykinin analogues. All of the catheters were externalized subcutaneously at the back of the neck. On the day of the experiment, the rats were maintained conscious and unrestrained in plastic cages. Arterial pressure (AP), mean of arterial pressure (MAP) and heart rate (HR) were monitored through a Gould-statham P23 ID pressure transducer (Gould, Cleveland, OH, U.S.A.) connected to the iliac catheter and recorded on a Gould 2200S paper chart recorder. An half-hour period was allowed before the actual initiation of the experiment. A 1 mg/kg amount of angiotensin converting enzyme inhibitor, enalapril (Merck Sharp and Dohme Research Lab., Rahway, NJ, U.S.A.) was injected into the iliac catheter. Thirty to sixty minutes later, when a stable blood pressure was obtained, two doses of bradykinin acetate salt (Sigma) of 125 ng and 250 ng, dissolved in 5% dextrose to a concentration of 2·5 μg/ml, were injected randomly every 4 to 5 min into one branch of the carotid catheter. Each dose was repeated twice to three times until the vasodepressor responses to exogenous bradykinin were stable. Two average values of the vasodepressor response to these two doses of bradykinin were taken as the control response. The bradykinin analogue dissolved in 5% dextrose solution, was infused starting from a low dose (0·5 μg/min) with a volume no more than 0·2 ml/min, via another branch of the carotid catheter. During the infusion of each dose of the analogue, 250 ng of bradykinin was injected into the carotid artery, repeating twice to three times until the vasodepressor responses were stable. The average value was assumed as the response to 250 ng of BK at the given dose of infusion of the analogue. The dose of BK antagonist infusion was increased and the same procedure was repeated until the vasodepressor response to 250 ng of exogenous BK decreased to less than 10% of the control response. The infusion was then ceased and 250 ng of BK was injected until the vasodepressor response to exogenous BK returned to over 90% of the control level.

The antagonistic potency of the bradykinin analogues was quantitatively expressed by the effective dose (ED), ED_{20} and ED_{90}. The ED is defined as the dose (in nmol/kg/min) of antagonist that reduces the response to two units of agonist (250 ng of bradykinin) to equal the response to one unit of agonist (125 ng of bradykinin) in the absence of antagonist. ED_{20} and ED_{90} represent the doses of bradykinin antagonist (nmol/kg/min) that inhibit 20% and 90% of the vasodepressor response to its agonist (250 ng of bradykinin). Results are reported as mean values ± standard error (SE). The comparison of two analogues was accomplished by Student's nonpaired *t*-test. Differences were considered to be significant for $p < 0{\cdot}05$.

This work was supported by the National Institutes of Health.

REFERENCES

1. *Biochemical Nomenclature and Related Documents.* International Union of Biochemistry, London 1978.
2. Carretero O. A., Miyazaki S., Scicli A. G.: Hypertension *3*, 18 (1981).
3. Stewart J. M., Vavrek R. J. in: *Kinins IV* (L. M. Greenbaum and J. S. Margolis, Eds), p. 537. Plenum, New York 1986.
4. Mulinari R., Benetos A., Gavras I., Gavras H.: Hypertension *11*, 754 (1988).
5. Mulinari R., Franco R., Gavras I., Gavras H.: Hypertension *13*, 960 (1989).
6. Schachter M., Uchida Y., Longridge D. J., Labedz T., Whalley E., Vavrek R. J., Stewart J. M.: Br. J. Pharmacol. *92*, 851 (1987).
7. Beierwalters W. H., Carretero O. A., Scicli A. G., Vavrek R. J., Stewart J. M.: Proc. Soc. Exp. Biol. Med. *186*, 79 (1987).
8. Lammek B., Wang Y. X., Gavras I., Gavras H.: Peptides, in press.
9. König E., Geiger R.: Chem. Ber. *103*, 788 (1970).
10. Kaiser E., Colesott R. L., Bossinger C. D., Cook P. I.: Anal. Biochem. *34*, 595 (1970).