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Communicated February 4, 1991

A novel bradykinin antagonist with improved properties

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Abstract—Acylation of the *N*-terminus of [D-Arg^0, Hyp^3, Thi^{5,8}, D-Phe^7] bradykinin with 1-adamantanecarboxylic acid results in an analogue with enhanced potency of at least 33-fold. The new antagonist has potential as a pharmacological tool in the investigation of the role of endogenous bradykinin in cardiovascular regulation.

The physiological cardiovascular effects of endogenous bradykinin and its possible participation in the maintenance of normotension or the development of various hypertensive processes and cardiac diseases, as well as its contribution to the antihypertensive effects of angiotensin-converting enzyme (ACE) inhibition, have become increasingly the centre of research interest in recent years. As with many other vasoactive hormones (e.g. angiotensin II and vasopressin) the efforts towards elucidation of effects of bradykinin have acquired new impetus since the synthesis of bradykinin antagonists became possible. We now have the ability to study the various cardiovascular effects that bradykinin exerts either directly or via interaction with other vasoactive substances, by assessing the results of chronic B_2-receptor inhibition of bradykinin in terms of systemic or regional haemodynamic changes, changes in cardiac function and effects on other vasoactive systems. The fact that some bradykinin antagonists are being considered as therapeutic agents in man adds another dimension to the relevance of these studies.

Existing antagonists synthesized mostly by Stewart's group (Schachter et al 1987; Stewart & Vavrek 1991) have relatively low potency, which necessitates the use of high concentrations to inhibit the vascular effects of bradykinin. One of the most potent of these agents is [D-Arg^0, Hyp^3, Thi^{5,8}, D-Phe^7] bradykinin, designated here as peptide I. Recently we reported that the acylation of the *N*-terminus of peptide I with 1-adamantaneacetic acid results in an analogue (designated peptide II) with more than ten times enhanced potency (Lammek et al 1990). As a continuation of our effort to develop more effective bradykinin antagonists, we designed a new bradykinin analogue: 1-adamantanecarboxylic acid-[D-Arg^0, Hyp^3, Thi^{5,8}, D-Phe^7] bradykinin, designated peptide III. The structures of bradykinin analogues I–III are shown in Fig. 1.

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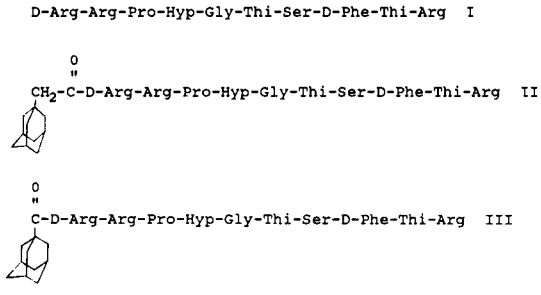

FIG. 1. Structures of bradykinin analogues.

Materials and methods

All peptides were synthesized using the solid phase method on a Merrifield resin (Stewart & Young 1984). After the protected peptides were assembled, they were cleaved from the support with simultaneous side-chain deprotection by acidolysis in anhydrous hydrogen fluoride at 0°C in the presence of 15% anisole. Crude materials were desalted by gel filtration on Sephadex G-15 and purified twice on Sephadex LJ-20. All analogues were shown to be homogeneous by thin-layer chromatography in three different solvent systems and gave the expected amino acid analysis ratios. The antagonistic potency of the analogues was assessed by their ability to inhibit the vasodepressor response to exogenous bradykinin in conscious rats (Lammek et al 1990, 1991), as follows.

Intact male Wistar rats (Charles River Breeding Laboratories, Wilmington, MA, USA) 225–250 g, were maintained on a regular Purina chow diet, as well as tap water in a room at constant temperature (23 ± 1°C) with a 12 h dark: 12 h light cycle. One day before the experiment, the right carotid and the iliac artery were catheterized with polyethylene tubing (PE50) under light ether anaesthesia. A 'Y' type connection was attached to the carotid artery for injection of bradykinin and for infusion of the bradykinin analogues. All catheters were exteriorized subcutaneously at the back of the neck.

On the day of the experiment, the rats were conscious and unrestrained in plastic cages. Mean arterial pressure (MAP) and heart rate (HR) were monitored through a Gould-Statham P23 ID pressure transducer (Gould, Cleveland, OH, USA) connected to the iliac catheter and recorded on a Gould 2200S paper

Table 1. Pharmacological data on bradykinin analogues.

Number and structure of analogue	n	Antagonistic potency ED20 (μg min^{-1})	ED90 (μg min^{-1})
I. D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Phe-Thi-Arg	7	1·49 ± 0·29	142·5 ± 26·44
II. R-CH_2-CO-D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Phe-Thi-Arg	7	0·84 ± 0·09	13·94 ± 1·69
III. R-Co-D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Phe-Thi-Arg	9	0·094 ± 0·02	4·23 ± 0·46

R = adamantyl. ED20 and ED90 represent the doses of bradykinin antagonist (μg min^{-1}) that inhibit by 20% and 90% the vasodepressor response to its agonist (250 ng of bradykinin) n, number of rats tested. The model analogue I was previously designed by Stewart's group (Schachter et al 1987). We assayed it to obtain reference data for comparison with our analogues.

chart recorder. A 30 min stabilization period was allowed before initiation of the experiment. Angiotensin-converting enzyme inhibitor, enalapril (Merck Sharp and Dohme Research Lab., Rahway, NJ, USA, 1 mg kg^{-1}) was injected into the iliac catheter. Thirty to sixty min later, when a stable blood pressure was obtained, bradykinin acetate salt (Sigma) (125 or 250 ng) dissolved in 5% dextrose to a concentration of 2·5 μg mL^{-1}, was injected every 4 to 5 min into one branch of the carotid catheter. Each dose was repeated two 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^{-1}) in a volume of 0·2 mL min^{-1}, via the other branch of the carotid catheter. During the infusion of each dose of the analogue, 250 ng of bradykinin was injected into the carotid artery, and the injection was repeated two or three times until the vasodepressor responses were stable. The average value was taken as the response to 250 ng of bradykinin at the given dose of infusion of the analogue. The dose of bradykinin antagonist infused was then increased and the same procedure was repeated until the vasodepressor response to 250 ng of exogenous bradykinin decreased to less than 10% of the control response. The infusion was then ceased and 250 ng of bradykinin was injected until the vasodepressor response to exogenous bradykinin returned to over 90% of the control level.

The antagonistic potency of the bradykinin analogues was quantitatively expressed as the effective dose (ED), ED20 and ED90. The ED is defined as the dose (μg min^{-1}) of antagonist that reduces the response to two units of agonist (250 ng of bradykinin) to equal the response obtained previously by one unit of agonist (125 ng of bradykinin) in the absence of antagonist. ED20 and ED90 represent the doses of bradykinin antagonist (μg min^{-1}) that inhibit by 20 and 90%, respectively, the vasodepressor response to its agonist (250 ng of bradykinin).

Results are reported as mean values ± s.e. Comparison of the two analogues was accomplished by Student's non-paired t-test. Differences were considered to be significant for $P < 0·05$.

Results and discussion

The antagonistic potencies of the new bradykinin analogue III in comparison with those of the model peptide (I) and our previously synthesized bradykinin antagonist (II; Lammek et al 1990) are presented in Table 1. The numbers clearly indicate that the new analogue shows superior antagonistic capacity as compared with I and II. In low doses it is about eight and fifteen times more potent than compounds II and I, respectively. At higher doses analogue III exhibits more than three and about 33 times higher potency than the peptides II and I, respectively. It should be pointed out that until recently peptide I was considered to be one of the most potent bradykinin antagonists tested both in-vivo and in-vitro (Beierwaltes et al 1987; Schachter et al 1987).

Our results suggest that even minor changes in the structure of bradykinin analogues may be of importance in the design of more potent B_2-receptor antagonists of bradykinin. This is shown by the substantial enhancement of B_2-antagonistic potency of analogue III which differs from the peptide II only in the lack of a CH_2 group in the N-acyl residue. It appears that the presence of a bulky substituent at the N-terminus of B_2-antagonists significantly influences the interaction of the peptide with the B_2-receptor and therefore increases its potency.

In summary, besides providing new information on structural factors required for B_2-antagonism, these studies yielded the most potent B_2-antagonist, in-vivo, known to date. This compound has potential as a valuable pharmacological tool for the investigation of the role of endogenous bradykinin in cardiovascular regulation (Gavras & Gavras 1991).

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