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SYNTHESIS AND BIOLOGICAL ACTIVITY OF NEW BRADYKININ PSEUDOPEPTIDE B_1 RECEPTOR AGONISTS CONTAINING ALKYLIC SPACERS

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Abstract: The tetrapeptide in the central portion of bradykinin and desArg[10]-kallidin was replaced by alkyl spacers of various lengths (-NH-$(CH_2)_n$-CO-, n=5-11).When tested as agonists at the kinins receptors, these pseudopeptides showed significant activity only at the B_1 receptor. In particular, the introduction of a dodecanoic spacer in the central portion of desArg[10]-kallidin reduces only tenfold the agonist activity at the B_1 receptor.

The two kinins, bradykinin (BK; H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH) and kallidin (KD; Lys-bradykinin) are generated as a result of the activity of the proteolytic enzymes kallikreins on kininogens. Once released, kinins elicit many pathophysiological responses, including pain and hyperalgesia and contribute to the inflammatory response[1]. Two types of kinin receptors, termed B_1 and B_2, have been proposed by Regoli and Barabè[2] on the basis of the different rank order of potency of various kinin analogues acting as agonists or antagonists. More recently, B_1 and B_2 receptors have been isolated and cloned[3]. BK and KD are relatively weak agonists at the B_1 receptor, while carboxypeptidase metabolites of kinins, such as desArg[9]-BK and desArg[10]-KD, are the most potent agonists[2].

Recently, a model of BK bound to the agonist site of its B_2 receptor was reported[4]. Combining structural homology modeling, molecular dynamics, systematic conformational searching methods and mutagenesis experiments, the authors hypothesized that agonist peptides may minimally require "an intact C-terminal β-turn structure with appropriate side chains in place and N-terminal amino and guanidine groups"[4]. The hypothesis was tested by designing an analogue of the B_2 receptor antagonist HOE 140 (H-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Oic-Arg-OH) with residues 3-6 replaced by a simple organic spacer: the 12-aminododecanoic acid. The resulting pseudopeptide (H-DArg-Arg-NH-$(CH_2)_{11}$-CO-Ser-DTic-Oic-Arg-OH) was reported to have a K_i of 360 nM against [^{3}H]-BK in the classical guinea pig ileum B_2 binding assay and to be a functional antagonist against BK-induced contractions in the same tissue (pA_2 = 5.5)[4]. Subsequently, the same authors studied the relationship between the length of the alkylic chain and the affinity to the B_2 receptor: a series of five analogues was described and their binding affinity to the human B_2 receptor measured[5]. The pseudopeptide containing the dodecanoic spacer was reported to have a K_i of 44 nM against [^{3}H]-BK in membrane preparations from a stable cell line that expresses the human B_2 receptor[5]. However, its functional behavior at the human B_2 receptor was different from that observed at the guinea pig B_2 receptor, as it was reported to be an agonist, as measured by its ability to stimulate IP turnover in a cell line stably transfected with the human B_2 receptor[6]. Correctly, the authors observed that this result is not completely surprising, since the pseudopeptide was designed on the basis of an agonist site of the receptor[6]. To the best of our knowledge, no attempt to perform the same modification on the structure of a full agonist, i.e. BK, was reported. We address this point in the present paper.

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Results

In the first series of analogues (peptides no. 1-4 in Tab. 1) the tetrapeptide Pro-Pro-Gly-Phe of BK was replaced by alkyl spacers of various lengths, namely 6-aminohexanoic acid (Ahx), 8-aminooctanoic acid (Aoc), 11-aminoundecanoic acid (Aun) and 12-aminododecanoic acid (Ado)[7]. All these analogues were found to be devoid of any agonist or antagonist activity up to 10 μM concentration, in the guinea pig ileum longitudinal smooth muscle, a preparation endowed only with the B_2 kinin receptor[8]. Surprisingly, when the same analogues were tested in a B_1 kinin receptor functional assay, the rat ileum longitudinal smooth muscle[8], two of them (no. 3 and 4) showed a weak agonist activity (Fig. 1, panel A). This unexpected result prompted us to design a second series of pseudopeptides, where the same modifications were performed in the structure of a selective B_1 receptor full agonist, namely desArg^{10}-KD. Testing of the resulting four compounds (no. 5-8 in Tab. 1) for their agonist activity at the B_1 receptor confirmed the results obtained with the first series. In fact, while peptide no. 5, bearing the shorter hexanoic spacer, was inactive, the other three compounds showed a clear-cut agonist activity (Fig. 1, panel B). In particular peptide no. 8, containing the dodecanoic spacer, has a EC_{50} values of 980±191 nM (n = 4), i.e. about tenfold higher as compared to the unmodified peptide desArg^{10}-KD, (EC_{50} = 85±25 nM, n = 8). Moreover, compound no. 8 was the only agonist whose concentration-response curve could be completed: at 30 μM concentration it produced a maximal effect amounting to 78±3 % of the maximal contraction to desArg^9-BK (10 μM) on the same preparation, thus suggesting a partial agonist behavior. All the tested compounds were devoid of antagonist activity.

Discussion

This work is based on the model of the interaction of BK with the B_2 receptor, proposed by Kyle et al.[4]. According to these authors, the key features for the interaction of the agonist with the B_2 receptor are located within its N- and C-termini and thus the central portion of the peptide can be replaced by a simple spacer. In order to test this hypothesis using an agonist as a template, we modified the BK molecule by replacement of the central tetrapeptide Pro^2-Pro^3-Gly^4-Phe^5 with a series of four alkylic spacers of various lengths, as shown in Tab. 1 (peptides no. 1-4). The lack of activity of these modified peptides at the B_2 receptor clearly indicates that

Table 1. Sequences of the pseudopeptides synthesized.

No	Sequence
1	H-Arg-**Ahx**-Ser-Pro-Phe-Arg-OH
2	H-Arg-**Aoc**-Ser-Pro-Phe-Arg-OH
3	H-Arg-**Aun**-Ser-Pro-Phe-Arg-OH
4	H-Arg-**Ado**-Ser-Pro-Phe-Arg-OH
5	H-Lys-Arg-**Ahx**-Ser-Pro-Phe-OH
6	H-Lys-Arg-**Aoc**-Ser-Pro-Phe-OH
7	H-Lys-Arg-**Aun**-Ser-Pro-Phe-OH
8	H-Lys-Arg-**Ado**-Ser-Pro-Phe-OH

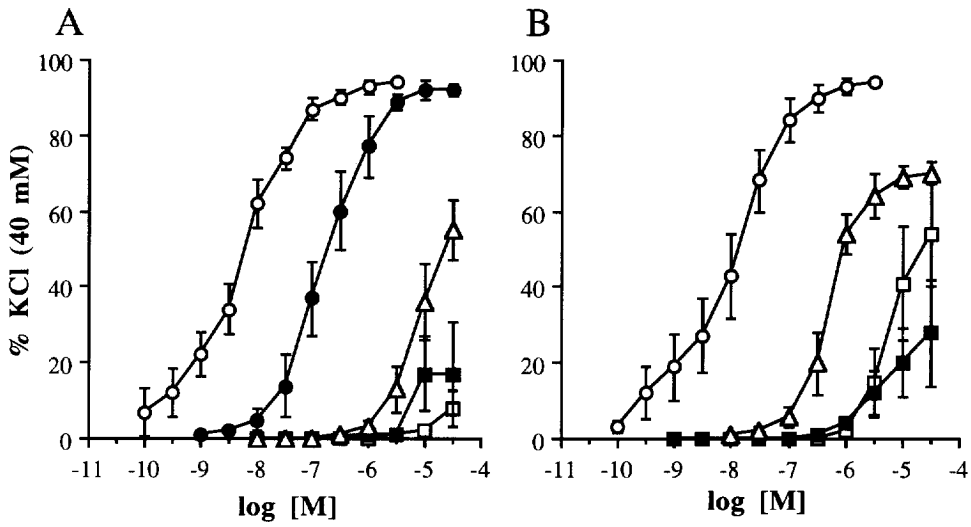

Figure 1. Agonist activity at the B_1 kinin receptor in rat isolated ileum longitudinal smooth muscle: natural kinins compared to pseudopeptides containing different alkylic spacers. Panel A. Concentration-related contractile responses to: desArg^9-BK (open circles), BK (closed circles) and peptides no. 2 (open squares), no. 3 (closed squares), no. 4 (open triangles). Panel B. Concentration-related contractile response to: desArg^{10}-KD (open circles) and peptides no. 6 (open squares), no. 7 (closed squares), no. 8 (open triangles). Data are expressed as % of response to KCl (40 mM), with each point representing the mean ± s. e. mean of at least 4 experiments.

the replaced tetrapeptide plays an important functional role in the interaction of BK with the B_2 receptor. Indeed, the functional role of these residues was previously shown by Regoli and Barabè[2]: the "alanine scan" of BK (i.e. the systematic replacement of each residue by Ala) showed that the Ala substitution of Phe^5, Gly^4 and, to a lesser extent, Pro^2 brings about a dramatic decrease of affinity at the B_2 receptor in various preparations. However, at least three of the four modified residues, i.e. Pro^2, Pro^3 and Gly^4, should also play an important conformational role, due to their intrinsic structural properties. Our results show that the putative bioactive conformation favoured by this tetrapeptide is theoretically accessible also to the very flexible alkylic spacers, at least when considering the undecanoic one (peptide no. 3), which is formally made up of the same number of atoms along the peptide backbone as the unmodified peptide. Taken together, these observations lead to the hypothesis that the aromatic side chain of Phe^5 should play a very important functional role for the interaction of BK with the agonist site of the B_2 receptor. However, the fact that differences might exist between kinin receptors across species should be also taken into account, as the original data of Kyle et al. have been obtained at the guinea pig[4] and human[5, 6] B_2 receptors, while the data reported in this paper refer to the rat B_2 receptor.

The results obtained at the B_1 receptor are much more interesting, particularly those obtained with the modified analogues of desArg^{10}-KD (peptides no. 5-8), as some of them maintain a good activity. The alanine scan of desArg^{10}-KD has not been reported in the literature, but in the case of desArg^9-BK, Regoli and Barabè[2] observed a much less dramatic effect on B_1 affinity after replacement of residues 2-5 by Ala, as compared to the effects exerted by the same modifications on the affinity of BK for the B_2 receptor (see above). In particular, the Ala^5 analogue maintains, in this case, as much as 5% of the affinity of unmodified desArg^9-BK.

An interesting correlation was observed between activity and length of the spacer. In fact, when the two series of modified peptides were tested at the B_1 receptor, the two analogues bearing the shorter spacer (amino-hexanoic acid, peptides no. 1 and 5) were inactive. In the case of the three other spacers, the correlation between affinity and length of the alkylic chain appears to be approximately linear. A similar correlation was observed also by Kyle et al.[5] in the case of the modified analogues of HOE 140. As observed by these authors, the correlation is more correctly expressed as a function of the hydrophobicity of the spacer, which increases with increasing length. Also in the case of HOE 140 analogues, higher affinity was observed in that case where the spacer was formally longer, in terms of number of atoms along the peptide backbone, as compared to the unmodified peptide. These results could be explained with two hypotheses: a) the optimal distance between the two pharmacophoric termini of the ligand requires the longer spacer in an extended conformation; b) the overall hydrophobicity of the central portion of the ligand plays a crucial role, even if the spacer is in a coiled conformation. However, the high flexibility of the alkylic spacers enables their easy interconversion among various conformations and thus the activity of our pseudopeptidic agonists could result from a combination of both the above mentioned effects.

In conclusion, we have shown that the central tetrapeptide Pro-Pro-Gly-Phe of the BK molecule has an important functional role in the agonist interaction with the B_2 receptor, while it is mostly structural in the case of the B_1 receptor. Starting from this observation, we have designed an analogue of desArg^{10}-KD which, in spite of a greatly simplified structure, maintains an affinity only tenfold lower as compared to the unmodified peptide. The design of this compound represents an important step toward the rational design of peptidomimetic ligands for the B_1 receptor.

References and Notes

1. Bhoola, K.D.; Figueroa, C.D.; Worthy, K. *Pharmacol. Rev.* **1992**, *44*, 1.
2. Regoli, D.; Barabè, J. *Pharmacol. Rev.* **1980**, *32*, 1.
3. Hess, J.F.; Borkowski, J.A.; Young, G.S.; Strader, C.D.; Ransom, R.W. *Biochem. Biophys. Res. Commun.* **1992**, *184*, 260. Menke, J.G.; Borkowski, J.A.; Bierilo, K.K.; MacNeil, T.; Derrik, A.W.; Schneck, K.A.; Ransom, R.W.; Strader, C.; Linemeyer, D.; Hess, J. *J. Biol. Chem.* **1994**, *269*, 21583.
4. Kyle, D.J.; Chakravarty, S.; Sinsko, J.A.; Stormann, T.M. *J. Med. Chem.* **1994**, *37*, 1347.
5. Chakravarty, S.; Connolly, M.A.; Kyle, D.J. *Pept. Res.* **1995**, *8*, 16.
6. Kyle, D.J. *Curr. Pharm. Design* **1995**, *1*, 233.
7. Peptides were synthesized as previously described in Rovero, P.; Viganò, S.; Pegoraro, S.; Quartara, L. *Lett. Pept. Sci.*, **1995**, *2*, 319. Briefly, syntheses were performed on a Milligen 9050 automatic synthesizer, using standard Fmoc chemistry on PEG-PS resin and continuous-flow strategy. Crude products were purified to homogeneity by preparative RP-HPLC and characterized by mass spectrometry.
8. Rat and guinea-pig ileum longitudinal smooth muscles were prepared as previously described in Meini, S.; Lecci, A; Maggi, C.A. *Br. J. Pharmacol.*, **1996**, 117, 1619. Briefly, isolated preparations were placed in oxygenated (95% O_2, 5% CO_2) Krebs solution containing indomethacin, guanethidin (3 μM), clorpheniramine and atropine (1 μM). The contractile responses to various agonists were carried out in the presence of peptidases inhibitors (thiorphan, bestatin and captopril, 1 μM). Responses to B_1 agonists in the rat ileum were performed at 5 h from set-up.

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