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Structural modification of the highly potent peptide bradykinin B1 receptor antagonist B9958

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Abstract

Bradykinin (BK)-related peptides stimulate two major classes of receptors, B1 and B2. The B1 receptor (B1R) plays an important role in various pathophysiological states including chronic inflammation, pain, hypotension, trauma and proliferation of cancer. Therefore, there is interest in the development of highly potent peptide BK B1R antagonists. We previously developed a highly potent and selective BK B1R receptor antagonist, B9958 (Lys-Lys-[Hyp^3, CpG^5, D-Tic^7, CpG^8]des-Arg^9-BK) (Hyp, *trans*-4-hydroxyproline; CpG, α-cyclopentylglycine; Tic, tetrahydroisoquinoline-3-carboxylic acid). We now report on new BK B1R antagonist analogs of B9958 with N-terminal basic residues in the D-configuration, or Lys-, Orn- derivatives (NiK, ε-nicotinoyllysine; PzO, 3-pyrazinoylornithine) and/or having hindered unusual amino acids at position 5 (Igl, α-(2-indanyl)glycine). These changes were designed to prevent enzyme degradation while keeping an acceptable affinity. However, these new analogs do not show higher B1R antagonist activity than B9958, but its N-terminal acylated derivative with a bulky and hydrophobic 2,3,4,5,6-pentafluorocinnamic acid (F5c), B10324, retains a B1R antagonist activity close to that of B9958 and, in addition, has high inhibition *in vivo* against lung cancer (SCLC, 86 %) and moderate inhibition against prostate cancer (PC3, 43%) xenografts. This class of compounds offers hope for the development of new BK antagonist peptide drugs for lung or prostate cancer.

Abbreviations: APN, Aminopeptidase N; PBS, phosphate-buffered saline; Boc, *tert*-butyloxycarbonyl; BOP, (benzotriazol-1-yloxy)tris (dimethylamino)phosphoniumhexafluorophosphate; BK, bradykinin; B1R, B1 receptor; B2R, B2 receptor; CpG, α-cyclopentylglycine; DMSO, dimethyl sulfoxide; F5c, 2,3,4,5,6-pentafluorocinnamic acid; HOBT, 1-hydroxybenztriazole; Hyp, *trans*-4-hydroxyproline; HPLC, high performance liquid chromatography; Igl, α-(2-indanyl)glycine; LDMS, laser desorption mass spectroscopy; NEP, Neutral Endopeptidase; NiK, ε-nicotinoyllysine; Oic, octahydroindole-2-carboxylic acid; PzO, 3-pyrazinoylornithine; SCLC, small cell lung cancer; Thi, β-(2-thienyl)alanine; TLC, thin layer chromatography; Tic, tetrahydroisoquinoline-3-carboxylic acid.

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1. Introduction

The B1 bradykinin (BK) receptors (B1R) do not respond to full-chain BK; their natural agonists lack the C-terminal Arg residue. They produce chronic inflammation, pain, hypotension, proliferation of cancer and influence cell migration. The first B1R antagonist, [Leu^8]-des-Arg^9-BK was described by Regoli and coworkers in 1977 [1]. B2 receptors (B2R) require the full peptide chain and mediate most of the biological actions of BK: bronchoconstriction, vasodilation, plasma extravasation, stimulation of nociceptive neurons, release of various mediators and cytokines, growth stimulation and proliferation of cancer. The first B2R antagonist was developed in 1985 by Vavrek and Stewart [2]. On the basis of these strong proinflammatory properties, BK is believed to play an important role in a variety of inflammatory diseases represented by asthma, rhinitis, brain edema, hyperalgesia, septic shock and cancer. Therefore, the development of specific B1 and/or B2 BK receptor antagonists and agonists has been of great importance for developing novel classes of therapeutic drugs for these kinds of diseases. The first full-chain bradykinin antagonist which showed high affinity at both B1 and B2 receptors, B9430 (DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg, Hyp, *trans*-4-hydroxyproline; Oic, octahydroindole-2-carboxylic acid), was developed by Gera at the Stewart laboratory a decade ago with the introduction of α-(2-indanyl)glycine (Igl) [3]. As expected, the des-Arg^9-analog of B9430, B9858 (Lys-Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic) and B9958 (Lys-Lys-Arg-Pro-Hyp-Gly-CpG-Ser-DTic-CpG, Tic, tetrahydroisoquinoline-3-carboxylic acid) had a remarkably high B1R antagonist activity [4,5]. Interestingly, B9858 was an insurmountable B1R antagonist in the rabbit aorta contractility assay, whereas B9958 was competitive (surmountable) [5]. Another issue concerns the well-known ability of aminopeptidase N (APN) to limit the potency and lifetime of B1R antagonists, which typically have N-terminal lysine or arginine residues. To overcome the above limitation, in an earlier study we designed and synthesized several new BK B1R antagonists having N-terminal basic residues in the D-configuration (D-Arg, D-Lys, D-Orn) [6]. Among the new peptides B10356, D-Arg-Lys-Arg-Pro-Hyp-Gly-CpG-Ser-DTic-CpG was found to be a dual inhibitor of the B1R and of the enzymatic activity of aminopeptidase N. We have now designed and synthesized some new analogs with N-terminal basic residues, *e.g.*, Lys-, Orn-derivatives (NiK = ε-nicotinoyllysine, D-NiK, PzO = 3-pyrazinoylornithine) and having hindered unusual amino acids at position 5 (D-CpG, Igl). These changes were designed to prevent enzyme degradation at both positions.

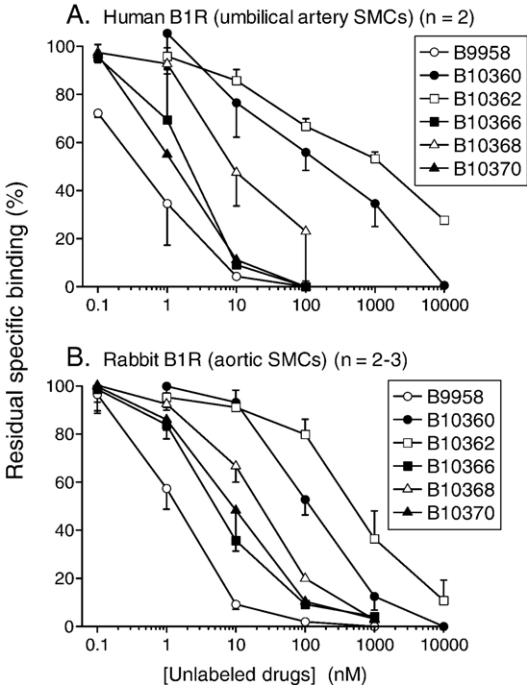

Figure 1 Competition of radioligand binding to B1 receptors by unlabeled peptide antagonists. The residual specific binding of 0.5–1 nM [^{3}H]Lys-des-Arg9-BK to human (A) or rabbit (B) vascular smooth muscle cells expressing the natural B1 receptor is presented: unlabeled drugs were coincubated at the indicated concentrations with the radioligand. Values are the mean ± S.E.M. of 2–3 experiments composed of duplicate determinations.

2. Materials and methods

2.1. Synthesis of compounds

The peptides were synthesized by solid-phase technology on Merrifield resin using conventional Boc-procedures (Boc, *tert*-butyloxycarbonyl) [7]. Boc-protected-amino acids were coupled

Table 1 Aligned structures of B1 receptor antagonists and some of their properties

Compound	Structure										Receptor affinity (Ki, nM) [a]		pA_2 rabbit aorta	
											Human	Rabbit	Control	+Amastatin
B9958	K	K	R	P	Hyp	G	CpG	S	D-Tic	CpG	0.089	0.29	8.27	9.11
B10324	F5c-K	K	R	P	Hyp	G	CpG	S	D-Tic	CpG		0.169	8.22	8.08
B10350	K	K	R	P	Hyp	G	Igl	S	D-Tic	CpG		0.74	7.4	8.3
B10360	K	K	R	P	Hyp	G	D-CpG	S	D-Tic	CpG	43.4	24.2	7.19	7.61
B10362	D-K	K	R	P	Hyp	G	D-CpG	S	D-Tic	CpG	308.6	101.1	7.24	7.12
B10366	Nik	K	R	P	Hyp	G	CpG	S	D-Tic	CpG	0.48	1.04		
B10368	D-Nik	K	R	P	Hyp	G	CpG	S	D-Tic	CpG	2.02	4.70		
B10370	PzO	K	R	P	Hyp	G	CpG	S	D-Tic	CpG	0.3	1.86		

[a] Based on competition of 1 nM (human) or 0.5 nM (rabbit) [^{3}H]Lys-des-Arg^9-BK binding (K_D estimates for the radioligand in the two species: 0.3 and 0.13 nM, respectively).