Novel non-peptide lead structures for Bradykinin B2-receptor antagonists

L. Felipe Pineda a,*, Claus Liebmann a, Sabine Hensellek a, Inge Paegelow b, Torsten Steinmetzer a, Andrea Schweinitz a, Jörg Stürzebecher c & Siegmund Reissmann a

a Institute of Biochemistry and Biophysics, Faculty of Biology and Pharmacology, Friedrich-Schiller University of Jena, Philosophenweg 12, D-07743 Jena, Germany; b Institute of Experimental and Clinical Pharmacology and Toxicology, University of Rostock, Schillingallee 70, D-18057 Rostock, Germany; c Centre for Vascular Biology and Medicine, Clinic of the Friedrich-Schiller University of Jena, Nordhäuserstrasse 78, D-99089 Erfurt, Germany

Received 10 July 1999; Accepted 30 September 1999

Key words: 3D-pharmacophore model, guinea pig ileum, IMR-90, peptide hormones, radioligand binding assay

Summary

A series of new non-peptide Bradykinin (BK) B2-receptor antagonists is reported. These new leads belong to a larger set including both commercially or otherwise available potential candidates found by proprietary database searches using 3D-pharmacophore models as query, and several bis-benzamidino compounds selected from our tryptase-like protease inhibitor library on the basis of topological considerations, derived from the same models. Some of these compounds show functional competitive antagonistic activity, inhibiting in vitro the BK-induced contraction of isolated guinea-pig ileum (GPI) and rat uterus with a pA2 up to 5.3 and 7.0, respectively. They display also binding affinity (IC50 up to 0.56 μM) to the BK B2-receptor in radioligand binding assays on GPI membrane preparations and on human IMR-90 fetal lung fibroblast cells expressing this receptor subtype. Furthermore, the results with the commercially available compounds, in some cases developed as therapeutics, show that the used 3D-pharmacophore model allows to predict to some certainty possible side actions of potential drugs.

Abbreviations: BK, Bradykinin; BSA, bovine serum albumin; DMEM, Dulbecco’s modified Eagle’s medium; DTE, dithioerythritol; EDTA, ethylenediaminetetraacetic acid; FCS, fetal calf serum; GPI, guinea pig ileum; Hyp, trans-4-hydroxy-Pro; Nas, 2-naphthylsulfonyl; Oic, L-[(3aS, 7aS)-octahydroindole-2-carboxylic acid]; PBS, phosphate-buffered saline; PyBOP, benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate; RUT, rat uterus; TES, 2-[[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino]ethane-sulfonic acid; Thi, β-2-thienyl-Ala; Tic, L-(1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid).

Introduction

Bradykinin (BK: RPPGFSFPR) is an endogenous nonapeptide hormone released from kininogens by tissue and plasma kallikreins upon tissue injury or trauma. It induces a variety of biological activities and is involved in numerous pathophysiological processes like pain, hyperalgesia, inflammation and symptoms of the common cold. Its biological activity is mostly mediated by the B2-receptor, a G-protein-coupled receptor. Because of its strong proinflammatory properties, great effort has been put in developing B2 receptor antagonists as potential therapeutic agents for the treatment of the disorders mentioned above. As a result, several ‘generations’ of such antagonists (most of them are linear peptides, sequence related to BK) differing in potency, tissue selectivity and stability against enzymatic degradation, have been synthesized and characterized by several groups over the past 14 yr (for a review see Reference 1). On the other hand, different series of clinically
more promising non- and pseudo-peptide antagonists, mostly designed on the basis of mass screening results or using a novel combinatorial chemistry approach, respectively, were recently reported. Some years ago, Salvino and co-workers [2] reported a series of non-peptide molecules to be competitive BK B2 receptor antagonists, displaying binding affinities to this receptor in human IMR-90 fetal lung fibroblasts ranging from 0.06 to >100 μM. In the course of a structure optimization, well-defined structure-activity relationships were established for these compounds [3,4], mostly based on an α-amino acid scaffold. Although they (especially the lead compound WIN 64338, which was announced to be the first relatively high affinity non-peptide B2 receptor antagonist) were promising for the design of other compounds with improved pharmacological characteristics, they were significantly poorer than peptide antagonists regarding their potency, selectivity and bio-availability. No further progress seems to have taken place in this direction.

A novel class of potent, selective, and orally active non-peptide BK B2 receptor antagonists, incorporating an 8-[[3-(N-acylglycyl-N-methylamino)-2,6-dichlorobenzyl]-oxy]-3-halo-2-methylimidazo-pyridine skeleton as the basic framework, were recently disclosed by Fujisawa Pharmaceutical Co. [5-9]. Two representative lead compounds, FR173657 and FR167344, display binding activities in the nanomolar range and are the first orally active non-peptide BK B2 antagonists reported to date.

In an effort to elucidate the essential structural features for BK antagonism we used the expert system Catalyst™ [10] and performed Consensus Molecular Dynamics simulations to extract 3D-pharmacophore models from a training set consisting of selected Sterling-Winthrop non-peptide BK B2 receptor antagonists mentioned above (the more potent Fujisawa compounds were not disclosed at that time), taking into account the conformational properties of two linear (including [D-Phe7]BK and the potent B2 receptor antagonist Hoe 140) and two cyclic peptide antagonists synthesized in our laboratory, and of BK itself, as well. As a result, we obtained a relatively small number of pharmacophore models, which were used to search five proprietary 3D-databases (MDL’s Available Chemical Directory V. MDL-95.2, BioByte Master File, Derwent Drug Index, Maybridge, and National Cancer Institute data bases) for new potential candidates. These searches yielded about 200 non-peptide hits. Details on these calculations were published elsewhere [11,12].

Herein we present the results of binding and functional pharmacological studies on some of these and related compounds, which support the predictive capabilities of our 3D-pharmacophore models and led to the discovery of a novel class of non-peptide lead structures for BK B2 receptor antagonists.

Methods

Compound selection

On the basis of the 200 non-peptide search hits mentioned above, additional on-line searches on other relevant chemical databases (ChemCats, Beilstein, CAS, Medline, Chemfinder) were carried out in order to find commercially or otherwise available substances among those compounds. The latter are relatively small molecules with a molecular weight ranging from 280 to 1650. Interestingly, these included a large number of compounds with anti-malarial or antiproliferative/cytotoxic properties. Unfortunately, only three substances among them (Figure 1) could be directly purchased. These are the tertiary amines (DHQ)zPHAL (hydroquinine 1,4-phthalazinediyl diether), 1, N,Nf-bis(2-diethylaminoethoxycarbonyl)-4-methyl-1,3-phenylenediamine, 2, and POE (2) coco amine, 3, with no reported biological activity. An additional compound (Cyclochin, 4-(7-chloro-quinolin-4-ylamino)-2,6-bis-diethylamino methyl-phenol, 4), reported to display anti-malarial activity against different Plasmodium strains in vitro [13], was kindly donated to us by G.B. Barlin.

Because of the very small number of test candidates, additional considerations concerning the topology of the proposed 3D-pharmacophore models led us to search for molecules possessing in their structures two positive ionizable (basic) groups, one of them preferably surrounded by two hydrophobic moieties, attached at different sites of a more or less rigid scaffold. This scaffold acts primarily as a spacer between the charges but could play the function of the ring aromatic group contained in the pharmacophore models (Figure 2) [11,12]. Alternatively, such an aromatic grouping should be attached to the same scaffold. A series of bis-benzamidine compounds from our protease inhibitor pool seem to fulfill these topological requirements and some of them (Figure 3) were therefore selected for the pharmacological tests. Together with the four compounds mentioned earlier, they form