Peptides 105 (2018) 37–50

Contents lists available at ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

Review

Bifunctional ligands of the bradykinin B_2 and B_1 receptors: An exercise in peptide hormone plasticity

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ARTICLE INFO

Keywords:
Bradykinin
B_2 receptors
B_1 receptors
Synthetic peptides
Fluorescent ligands
Biotechnological agents

ABSTRACT

Kinins are the small and fragile hydrophilic peptides related to bradykinin (BK) and derived from circulating kininogens via the action of kallikreins. Kinins bind to the preformed and widely distributed B_2 receptor (B_2R) and to the inducible B_1 receptor (B_1R). B_2Rs and B_1Rs are related G protein coupled receptors that possess natural agonist ligands of nanomolar affinity (BK and Lys BK for B_2Rs, Lys-des-Arg^9-BK for B_1R). Decades of structure-activity exploration have resulted in the production of peptide analogs that are antagonists, one of which is clinically used (the B_2R antagonist icatibant), and also non-peptide ligands for both receptor subtypes. The modification of kinin receptor ligands has made them resistant to extracellular or endosomal peptidases and/or produced bifunctional ligands, defined as agonist or antagonist peptide ligands conjugated with a chemical fluorophore (emitting in the whole spectrum, from the infrared to the ultraviolet), a drug-like moiety, an epitope, an isotope chelator/carrier, a cleavable sequence (thus forming a pro-drug) and even a fused protein. Dual molecular targets for specific modified peptides may be a source of side effects or of medically exploitable benefits. Biotechnological protein ligands for either receptor subtype have been produced: they are enhanced green fluorescent protein or the engineered peroxidase APEX2 fused to an agonist kinin sequence at their C-terminal terminus. Antibodies endowed with pharmacological actions (agonist, antagonist) at B_2R have been reported, though not monoclonal antibodies. These findings define classes of alternative ligands of the kinin receptor of potential therapeutic and diagnostic value.

1. Formation, signaling, metabolism and medical importance of kinins

The bradykinin (BK) sequence, H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH, is imbedded in domain 4 of 2 circulating proteins, the low and high molecular weight kininogens, produced by the liver from a single gene subjected to mRNA alternative splicing [1–3]. Kinins, the BK-related peptides, are generated from these kininogens, mainly by two major types of serine proteases, plasma and tissue kallikreins, that generate the native kinins BK and Lys-BK (also known as kallidin). Several constituents of the kallikrein-kinin system are found in the blood plasma, but also at the surface of cells or in their secretions (e.g., tissue kallikrein = KLK-1) [1,3].

Two related receptors belong to the kallikrein-kinin system, the B_1 and B_2 receptors (B_1R, B_2R). Those are “type A” G protein coupled receptors (GPCRs). While the B_2R is expressed at a rather constant level in many tissues and cell types and submitted to a classical endocytosis/reexpression cycle following stimulation, the B_1R is highly regulated by tissue injury, notably via cytokines or the innate immune system [1,2]. Both receptor types are mainly coupled to the G_q protein (also G_i), with ensuing stimulation of a phospholipase C and generation of second messengers such as calcium and diacylglycerol [2] (schematic representation, Fig. 1B). The B_2R is widely distributed, the vascular endothelial cells being a privileged physiological site of BK action, but the receptor is also expressed in sensory neurons, smooth muscle cells, epithelial (intestinal, respiratory) and some types of leukocytes. B_1R

Abbreviations: ACE, angiotensin converting enzyme; ε-ACA, ε-aminocaprolyl; APEX2, engineered soya bean peroxidase; Arg-CP, arginine carboxypeptidase; B_1R, B_1 receptor; B_2R, B_2 receptor; B_2R-GFP, rabbit B_2 receptor fused to green fluorescent protein; BK, bradykinin; CF, 5(6)-carboxyfluorescein; Cy7, cyanine dye 7; EGFP, enhanced green fluorescent protein; FTC, fluorescein-5-thiocarbamoyl; GPCR, G protein coupled receptor; GRK, G protein coupled receptor kinase; HSA, human serum albumin; KLK-1, tissue kallikrein; MK, maximakinin; NG, asparaginyl-glycyl; PTH, parathyroid hormone; TM, transmembrane (domain)

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https://doi.org/10.1016/j.peptides.2018.05.007
Received 22 March 2018; Received in revised form 14 May 2018; Accepted 15 May 2018
Available online 23 May 2018
0196-9781/

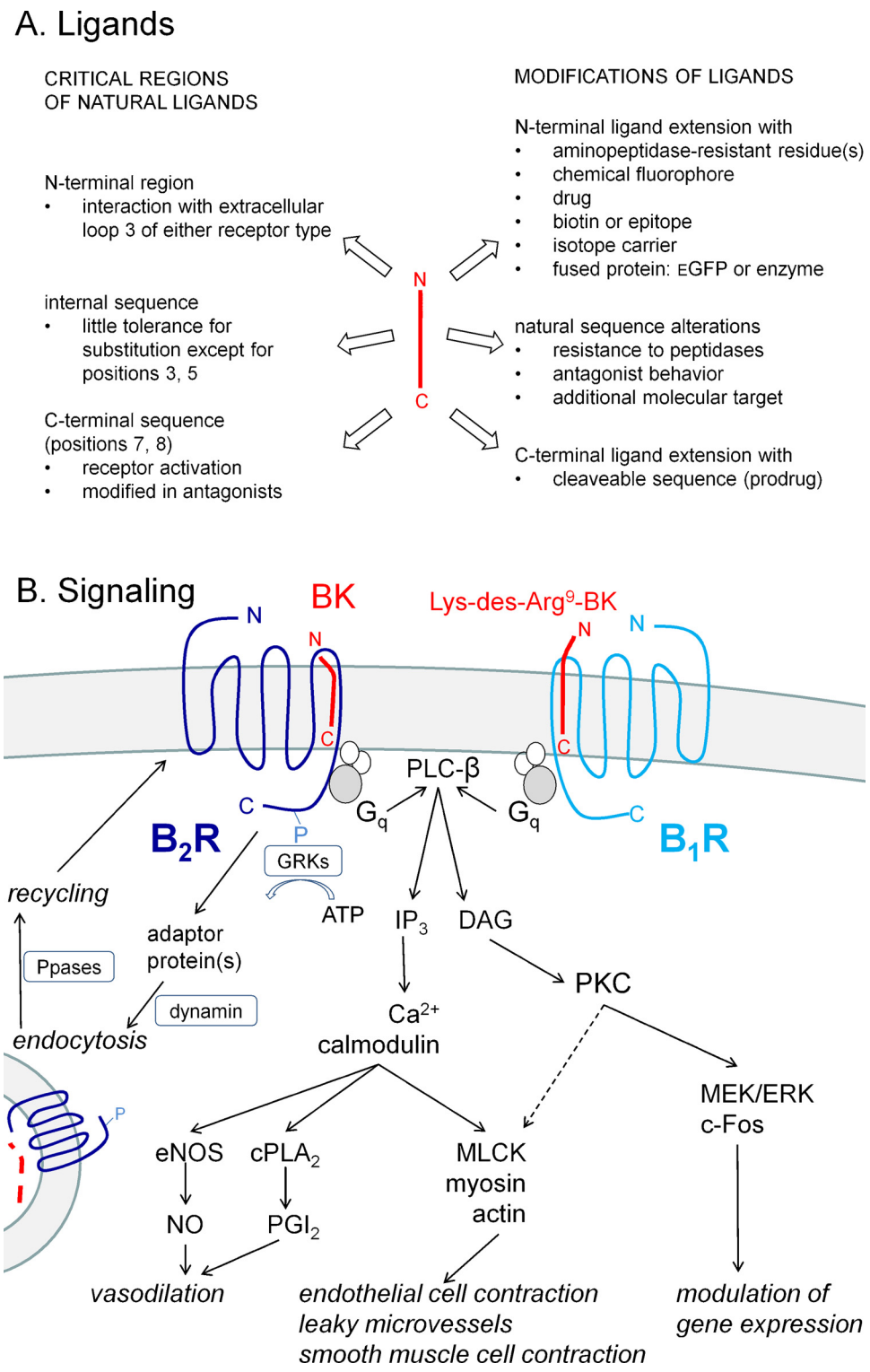

Fig. 1. A. Overview of the molecular pharmacology of natural and synthetic ligands of both B_2R and B_1R, related GPCRs. B. Overview of the signaling and cycling of those receptors and of selected ensuing physiological responses (*italics*). See main text for details. Abbreviations: $cPLA_2$, cytosolic phospholipase A_2; DAG: diacylglycerol; eNOS: endothelial nitric oxide synthase; ERK: one of the mitogen-activated protein kinases; G_q: protein G_q; GRKs: G protein coupled receptor kinases; IP_3: inositol triphosphate; MEK: one of the mitogen-activated protein kinases; MLCK, myosin light chain kinase; PGI_2, prostacyclin; PKC, protein kinase C; PLC-β, phospholipase C-β; Ppases, phosphatases.

tend to be present in the same cell types when expressed.

Kinins are often considered inflammatory mediators, with such effects as edema, pain, diarrheic states [4] and flu-like airway irritation [5], but are as well compensatory vasodilator autacoids that release nitric oxide (NO) and prostanoids from vascular endothelial cells, with salutary effects in the circulation of the heart, kidney, brain and the promotion of angiogenesis [1,6]. Cultured human umbilical vein endothelial cells (HUVECs) are a conventional model where both NO production and actin reorganization, predictive of microvascular leakage, can be recorded in response to BK (Supplementary Figs. 1 and 2).

Kinins are fragile peptides, being degraded by several metallopeptidases present both in blood plasma and at the cell surface. Angiotensin converting enzyme (ACE), present at the surface of endothelial cells and also circulating under a cleaved form, is by far the major kinin-destroying enzyme in the extracellular compartment both in human blood plasma and *in vivo* in rats [7,8]. Second in importance for BK inactivation in both milieus is aminopeptidase P that cleaves Arg^1 and inactivates the peptides. Arginine carboxypeptidases (Arg-CPs), such as plasma carboxypeptidase N and membrane