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Bradykinin Receptors: Agonists, Antagonists, Expression, Signaling, and Adaptation to Sustained Stimulation

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ABSTRACT

Bradykinin-related peptides, the kinins, are blood-derived peptides that stimulate 2 G protein–coupled receptors, the B_1 and B_2 receptors (B_1R, B_2R). The pharmacologic and molecular identities of these 2 receptor subtypes will be succinctly reviewed, with emphasis on drug development, receptor expression, signaling, and adaptation to persistent stimulation. Peptide and nonpeptide antagonists and fluorescent ligands have been produced for each receptor. The B_2R is widely and constitutively expressed in mammalian tissues, whereas the B_1R is mostly inducible under the effect of cytokines during infection and immunopathology. Both receptor subtypes mediate the vascular aspects of inflammation (vasodilation, edema formation). On this basis, icatibant, a peptide antagonist of the B_2R, is approved in the management of hereditary angioedema attacks. Other clinical applications are still elusive despite the maturity of the medicinal chemistry efforts applied to kinin receptors. While both receptor subtypes are mainly coupled to the G_q protein and related second messengers, the B_2R is temporarily desensitized by a cycle of phosphorylation/endocytosis followed by recycling, whereas the nonphosphorylable B_1R is relatively resistant to desensitization and translocated to caveolae on activation.

INTRODUCTION

The pharmacology of bradykinin (BK)-related peptides, the kinins, has come a long way in recent decades, with the pharmacologic and then the molecular definition of 2 G protein–coupled receptors (GPCRs) that mediate their cellular actions, the B_1 and B_2 receptors (B_1R, B_2R).[1] Additional modern research tools include mouse strains in which one or both of the genes coding for kinin receptors have been deleted ("gene knockout" models).[2] The formation of kinins and their degradation by interesting peptidases, such as angiotensin-converting enzyme, will not be covered in the present text. The arduous nature of the analytic biochemistry of BK-related peptides is outlined elsewhere.[3] We rather propose an excursion into the pharmacology of kinins, the first author having been a spectator and an actor in the field for the past 35 years. There will be a strong autobiographic bias in the selection of the illustration material. We hope this partiality will be forgiven in light of the generous invitation he has received to summarize the field during an oral presentation at the 8th C1-Inhibitor Deficiency Workshop.

BRADYKININ RECEPTOR LIGANDS: AGONISTS AND ANTAGONISTS

The receptors for bradykinin were initially defined in the late 1970s and 1980s using pharmacologic criteria. The B_1R was historically the first defined using both a typical potency order of agonists and a class of specific antagonists. This somewhat atypical receptor subtype is optimally responsive to fragments of the native kinins (BK and Lys-BK) in which the Arg^9 residue has been removed (des-Arg^9-BK, Lys-des-Arg^9-BK, respectively; Fig. 1).[4] The early peptide antagonists were simply des-Arg^9 sequences in which Phe^8 was replaced with a residue possessing an aliphatic side chain, such as Leu. In retrospect, it is now clear that the native kinins produced by the kallikreins, either BK or Lys-BK (kallidin), are selective agonists of the physiologically prominent B_2R subtype that has been consolidated with specific peptide antagonists in the 1980s by Professor John M. Stewart and colleagues. The B_2R antagonists typically possess a constrained peptide backbone due to the inclusion of bulky nonnatural amino acids that also confer resistance

to inactivation by peptidases (Fig. 1). Icatibant (Firazyr® Shire Human Genetic Therapies, Inc, Lexington, Massachusetts, Hoe 140)[5] is an excellent prototype of the peptide B_2R antagonists exploited in hundreds of basic science studies; it is presently approved for the management of hereditary angioedema attacks.[6] Icatibant is apparently a competitive (surmountable) antagonist at the human form of the B_2R, but the peptide may be insurmountable and/or a partial agonist in other mammalian species.[7,8] The des-Arg[9] form of icatibant is predominantly a B_1R antagonist,[1] showing that the constrained peptide design is also viable at this receptor subtype; other highly specific peptide antagonists of this type include B-9958 (Fig. 1).

Peptidase-resistant agonists selective for either receptor type are interesting laboratory tools, and one of the selective B_2R agonists of this category, B-9972, has distinctive effects on B_2R cycling (see below). Another selective B_2R agonist resistant to carboxypeptidases, labradimil (Cereport®, formerly manufactured by Alkermes, Inc. Cambridge, MA, RMP-7; Fig. 1), has reached clinical development as an adjuvant to chemotherapy for brain tumors. In this case, the proinflammatory effect of BK was deliberately exploited to temporarily open the blood-brain barrier. However, a trial of labradimil as an adjuvant of carboplatin was inconclusive in children with brain tumors.[9] Current preclinical research indicates that a B_1R agonist resistant to peptidases, Sar-Lys[D-Phe[8]]desArg[9]-BK, has a superior potential to open the blood-brain barrier at the level of gliomas, the B_1R being selectively expressed at lesion sites.[10]

The pharmaceutical industry has developed a large number of nonpeptide antagonists for both BK receptor subtypes[11]; let us mention only one for each. The B_2R antagonist anatibant (LF 16-0687; Fig. 1) was used in a clinical trial for the prevention of brain edema after head injury (an inconclusive trial).[12] The clinical development of the brain penetrant B_1R antagonist MK-0686[13] as an analgesic was interrupted after at least 2 trials for inflammatory pain, apparently because of lack of efficacy.[11] The anti-inflammatory effect of kinin receptor antagonists, well established in numerous preclinical studies, has not been evaluated in humans, but is of considerable potential interest. For instance, in inflammatory bowel disease, diarrheal symptoms may be driven by both BK receptor subtypes that mediate chloride and water secretion at the level of the intestinal epithelium.[14]

In the course of structure-activity studies of nonpeptide B_2R antagonists, Fujisawa scientists have discovered several nonpeptide partial agonists of this receptor. Compound 47a, illustrated in Figure 1 , is one of them; it has limited structural commonalities with anatibant and is an intriguing laboratory tool.[15]

Docking models for peptide and some nonpeptide ligands have been proposed for both the B_1 and B_2Rs.[1] They notably explain why the B_1R excludes peptides possessing the Arg[9] residue and also some species discrepancies, for example, the fact that the presence of Lys at the position "zero" is critical for good affinity at the human form of the B_1R. The models predict that the C-terminus of the ligands plunges into the central receptor cavity, whereas the N-terminus remains closer to the extracellular fluid. Based on these premises, Professor Lajos Gera has developed the full set of fluorescent agonists and antagonists for both the B_1R and B_2R by prolonging their structure at the N-terminus (Fig. 1).[16]

BRADYKININ RECPTORS: EXPRESSION AND SIGNALING

In mammalian genomes, the 2 genes encoding the BK B_2R and B_1R, termed *BDKRB2* and *BDKRB1*, respectively, are located next to each other, in tandem and in this order (Fig. 2, marker *1*). In the human chromosome 14q32 region schematically represented (about 70 kb, from position 94.66 to 94.73 Mb), the 3 major exons of each gene are shown (not precisely to scale).[1] B_2R expression is constitutive in a large number of tissues. The B_1R is an exceptional GPCR that is inducible, notably under the influence of tissue injury, cytokines, and the signaling systems mentioned in Figure 2.[17,18] The injection of bacterial lipopolysaccharide in laboratory animals, including a primate species, is a historic model to sensitize the whole cardiovascular system to B_1R agonists, with such responses as hypotension, vasodilation, and increased vascular permeability (Fig. 2, marker *2*).[19,20] This can be modeled at the cellular level: for instance, the B_1R is synergistically upregulated in human umbilical vein endothelial cells (HUVECs) by cotreatment with tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) (mRNA measurements or radioligand binding assay, Fig. 2, marker *3*, graph on right).[18] Thus, B_1R induction is clearly regulated at the transcriptional level, with very small concentrations in healthy tissues, and extends the organism's response to a broader spectrum of kinin metabolites as a function of time and physiological state. Although the cellular concentration of the mRNA coding for B_2R often varies in parallel with that of B_1R in immunopathology or in response to cytokines (as in HUVECs),[18] the former receptor subtype does not seems much regulated at the level of transcription, possibly because the B_2R protein is constitutive, recycled, and long-lived, as we will see below. Baseline populations of B_1Rs are also observed in cultured cells that are assumed to be resting, as in radioligand binding studies applied to human vascular cells,[17,18] for example, in HUVECs maintained in commercial endothelial cell growth medium (Fig. 2, marker *3*). However, it seems to be an artifact linked to cell stimulation with serum, growth factors, and culture conditions. Stimulation with various cytokines (TNF-α, IFN-γ, interleukin-1, epidermal growth factor etc.) clearly upregulates the B_1R protein and function in these models.

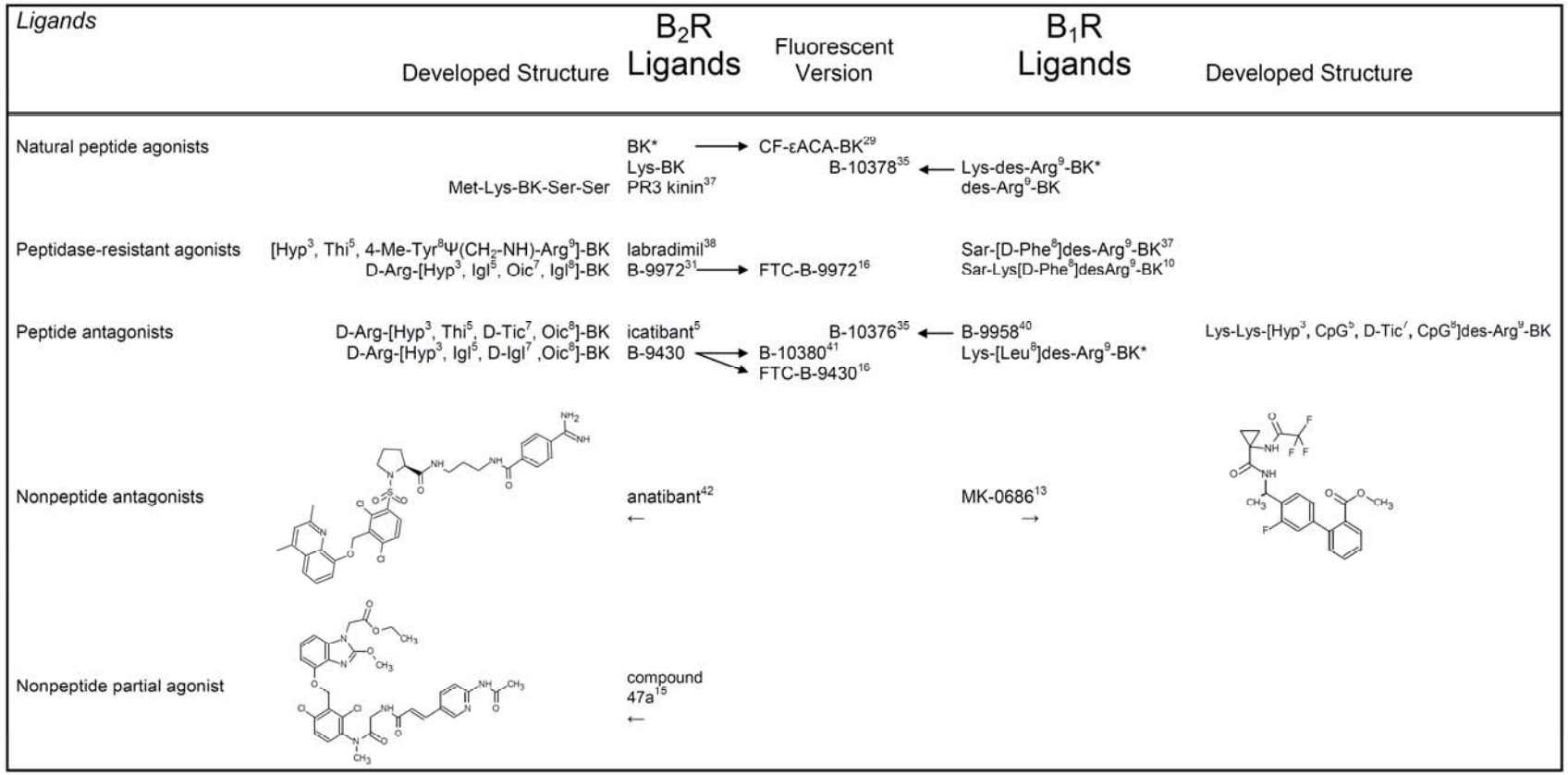

Figure 1. Selected ligands of the bradykinin receptors. For peptides, the reference structure is the nonapeptide bradykinin (BK; H-Arg^1-Pro^2-Pro^3-Gly^4-Phe^5-Ser^6-Pro^7-Phe^8-Arg^9-OH). Des-Arg^9 indicates a peptide with the Arg^9 residue removed. Nonnatural amino acids: CF, 5(6)-carboxyfluorescein; CpG, α-cyclopentylglycine; ε-ACA, ε-aminocaproic acid; FTC, fluorescein-5-thiocarbamoyl; Hyp, trans-4-hydroxyproline; Igl, α-(2-indanyl)glycine; Oic: (3as,7as)-octahydroindole-2-carboxylic acid; Sar, sarcosine; Thi, β-2-thienylalanine; Tic, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid. *Commercially available as a tritiated radioligand.

Both B_1 and B_2Rs are mainly coupled to protein G_q, itself activating a phosphoinositide phospholipase C-β that hydrolyzes phosphatidylinositol 4,5-bisphosphate present in the plasma membrane into inositol 1,4,5-trisphosphate (IP_3) + diacylglycerol (Fig. 2, marker *4*). The latter lipid stays in the membrane and recruits various isoforms of protein kinase C that may sequentially determine the activation of the MEK/ERK mitogen-activated protein kinases and c-Fos expression and phosphorylation.[15,21] The phosphosugar IP_3 is the activator of the IP_3 receptor of the endoplasmic reticulum (ER) that is a high conductance ligand-gated calcium channel. Thus, stimulation of either BK receptor subtype rapidly releases ER Ca^{2+} stores to raise cytosolic Ca^{2+} concentrations. This was assessed by the variation of FURA-2 fluorescence read at 510 nm under excitation at 340 nm in HUVECs cultured as described[18,22]; cells were stimulated with BK with or without a submaximal concentration of the B_2R antagonist anatibant (unpublished results from our laboratory, Fig. 2, marker *3*, left graph). Other mechanisms may contribute to late Ca^{2+} entry from the extracellular fluid in activated vascular cells.[23]

Ca^{2+} activates relevant enzymatic systems in endothelial cells, either directly (cytosolic phospholipase A_2) or via its binding to calmodulin (endothelial nitric oxide synthase; myosin light chain kinase, MLCK). Messenger molecules produced by the endothelium, of which NO and prostaglandin I_2 are prime examples, relax the arteriolar smooth muscle cells (Fig. 2, marker *5*), supporting the vasodilation that is the basis of specific cardinals signs of inflammation (*rubor*, *calor*). The MLCK-mediated activation of the contraction of actin-myosin cytoskeleton leads to the retraction of endothelial cells, making them leaky, particularly at the level of postcapillary venules, and to the exocytosis of Weibel-Palade bodies containing P-selectin.[24] This leads to the extravasation of fluid, proteins and, eventually, inflammatory cells that supports *tumor*, tissue edema. As kinins stimulate the abundant afferent nerves ending present in tissues (eg, in airway mucosae), neurogenic inflammation driven by the antidromic release of proinflammatory neuropeptides and tissue leukocytes responsive to them can amplify edema.[25]

The proinflammatory effects of BK-related peptides are largely asymmetric, favoring the vascular manifestations of the process vs leukocyte recruitment; there are few credible effects of kinins on blood leukocytes. The B_1R gene knockout mouse exhibits a minor deficit of phagocyte migration at inflammatory sites; this effect may be primarily vascular as des-Arg^9-BK increases chemokine production in the endothelium, the likely explanation for the B_1R-mediated facilitation of neutrophil extravasation.[26]

BRADYKININ RECEPTOR ADAPTATION

The adaptation function of the agonist-stimulated B_2R is a typical feature of GPCRs in general in the sense that it follows a sequence of events well documented for a number of other receptors[1]: a Ser/Thr rich domain of the intracellular C-terminal tail of the B_2R sequence is phosphorylated by several GPCR kinases; the 2 nonvisual arrestins (β-arrestin$_1$ and $_2$) can associate with the phosphorylated receptor and compete with the G protein, thus desensitizing the receptor. Adaptor and structural proteins, which probably include AP-2 and clathrin in the case of the B_2R,[27] direct the receptor to a pit that can leave the plasma membrane and acquire the properties of the early endosome (Fig. 3, bottom, schematic representation). These events can be modeled using fluorescent receptors, arrestins, and ligands in HEK 293 (a) cells (Fig. 3, top). The rabbit B_2R fused to the green fluorescent protein (GFP) is translocated from the cell surface to multiples and polymorphic endosomes on BK stimulation; various approaches (microscopy, binding assays, immunoblots) show the complete recycling of the receptor in 1 to 3 hours.[28] The β-arrestins have a smooth cytosolic distribution in resting cells but, in cells expressing a nonfluorescent B_2R that are further stimulated with BK, the fluorescence associated with the fusion protein β-arrestin$_2$–GFP condenses in endocytic structures (Fig. 3).[15]

Other cells that express nonfluorescent B_2Rs can be labeled with the fluorescent antagonist B-10380 or agonist CF-εACA-BK (peptide structures given in Fig. 1, confocal imaging of HEK 293a cells in Fig. 3), but the subcellular distribution of the fluorescence is very different. While the antagonist labels the resting B_2R essentially at the cell surface, the fluorescent agonist labels endosomal structures, and evidence of endosomal breakdown of the agonist is provided by the fact that some of the carboxyfluorescein label of the peptide is transferred as a function of time in the cytosol (Fig. 3).[29] Thus, the B_2R transports the agonist ligands into cells and this leads to their degradation. Microscopic studies in short-term experiments (30-min incubation periods with the agonist) fully support the colocalization of the agonist-B_2R-arrestin triad in early endosomes; Rab5 is also colocalized at this stage. Then, protein phosphatases dephosphorylate the B_2Rs, which are ready to be recycled to the cell surface (Fig. 3, bottom).[1]

Recent evidence based on cytoskeletal-disrupting drugs and dominant negative (GDP-locked) Rab GTPases points out that the progression of the ligand-B_2R-arrestin cargo from the cell surface to the perinuclear space is dependent on Rab5-mediated displacement of early endosomes along tubulin fibers and that the recycling process is entirely different, dependent on Rab4 and Rab11 and the actin cytoskeleton.[30] The intraendosomal inactivation of BK is also critical for the time course of B_2R recycling as shown by an inactivation-resistant peptide agonist, B-9972, or by a nonpeptide partial agonist,

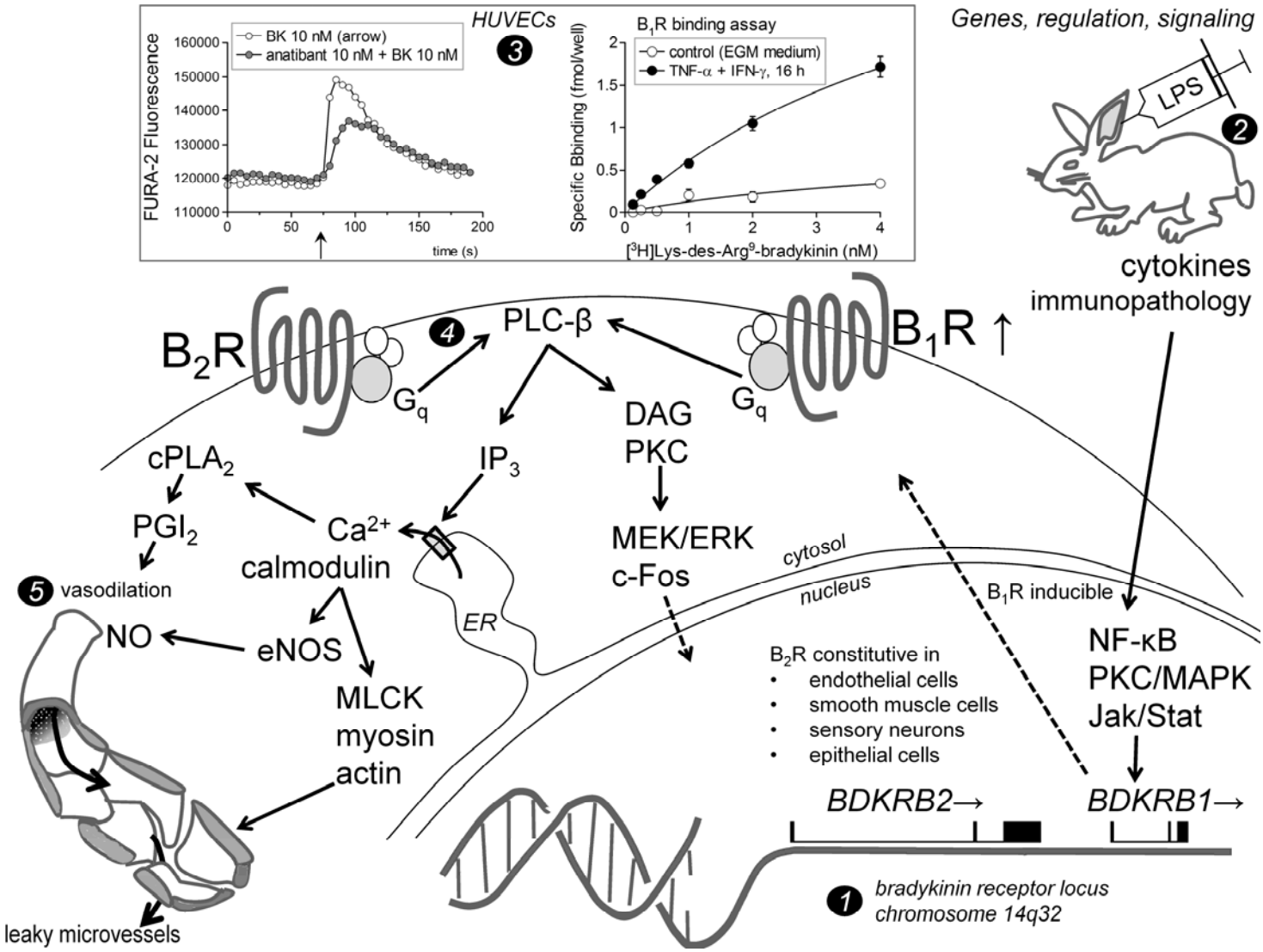

Figure 2. Bradykinin (BK) receptor genes, regulation, and signaling. The schematic representations and experimental data are described in the main text using markers *1-5*. The graph to the right of marker *3* is reproduced from Koumbadinga et al[18] with the permission of Elsevier. B_1R, B_1 receptor; B_2R, B_2 receptor; $cPLA_2$,cytosolic phospholipase A_2, DAG, diacylglycerol; EGM, endothelial growth medium; eNOS, endothelial nitric oxide synthase; ERK, extracellular signal-regulated kinase; G, glycoprotein; HUVECs, human umbilical vein endothelial cells; IFN-γ, interferon-γ; IP_3, inositol 1,4,5-trisphosphate; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MEK, MAP kinase kinase; MLCK, myosin light chain kinase; NF-κB, nuclear factor κB; PG, prostaglandin; PKC, protein kinase C; PLC-β, phospholipase C-β; TNF-α, tumor necrosis factor-α;

compound 47a (structures in Fig. 1).[15,31] An amphibian BK homologue termed maximakinin also acts as an inactivation-resistant agonist.[32] In all these cases, no or little B_2R recycling is observed in the 12-hour incubation period following cell stimulation, and a sizeable proportion of the receptor is destroyed (immunoblot evidence), presumably after progression into the late endosome-lysosome continuum.

The B_1R has an intracellular C-terminal tail that is not well conserved in sequence between mammalian species and that is very short in some mammalian species.[1] Accordingly, the B_1R is not phosphorylated on agonist stimulation[33] and does not promote condensation of the cytosolic β-arrestins at endosomal or plasma membrane levels (Fig. 3 top, unpublished results). Thus B_1Rs lack a basic desensitization mechanism of the B_2R and may signal for prolonged periods in some systems.[1] However, an unexpected discovery based on a fusion protein of the rabbit B_1R with the yellow fluorescent protein (YFP) was the lateral agonist-induced translocation of the B_1R into structures that remain close to the plasma membrane plane (confocal microscopy, Fig. 3, top).[34] These structures are disrupted by cholesterol extraction from the cells and are likely to be caveolae, the lipid rafts that can be resolved in optical microscopy. This view is supported by the labeling of nonfluorescent rabbit or human B_1Rs by fluorescent ligands (Fig. 3, top; the human B_1Rs are illustrated using confocal microscopy). While the antagonist B-10376 essentially labels resting B_1Rs in the plasma membrane in a continuous manner, the agonist B-10378 has no significant endosomal distribution, but a rather spotty plasma membrane location (Fig. 3, top; peptide structures in Fig. 1).[35]

Microscopic studies support that caveolin-1 is colocalized with B-10378[33] and with agonist-stimulated B_1R-YFP.[34] In addition, cell fractionation studies that aimed to recover

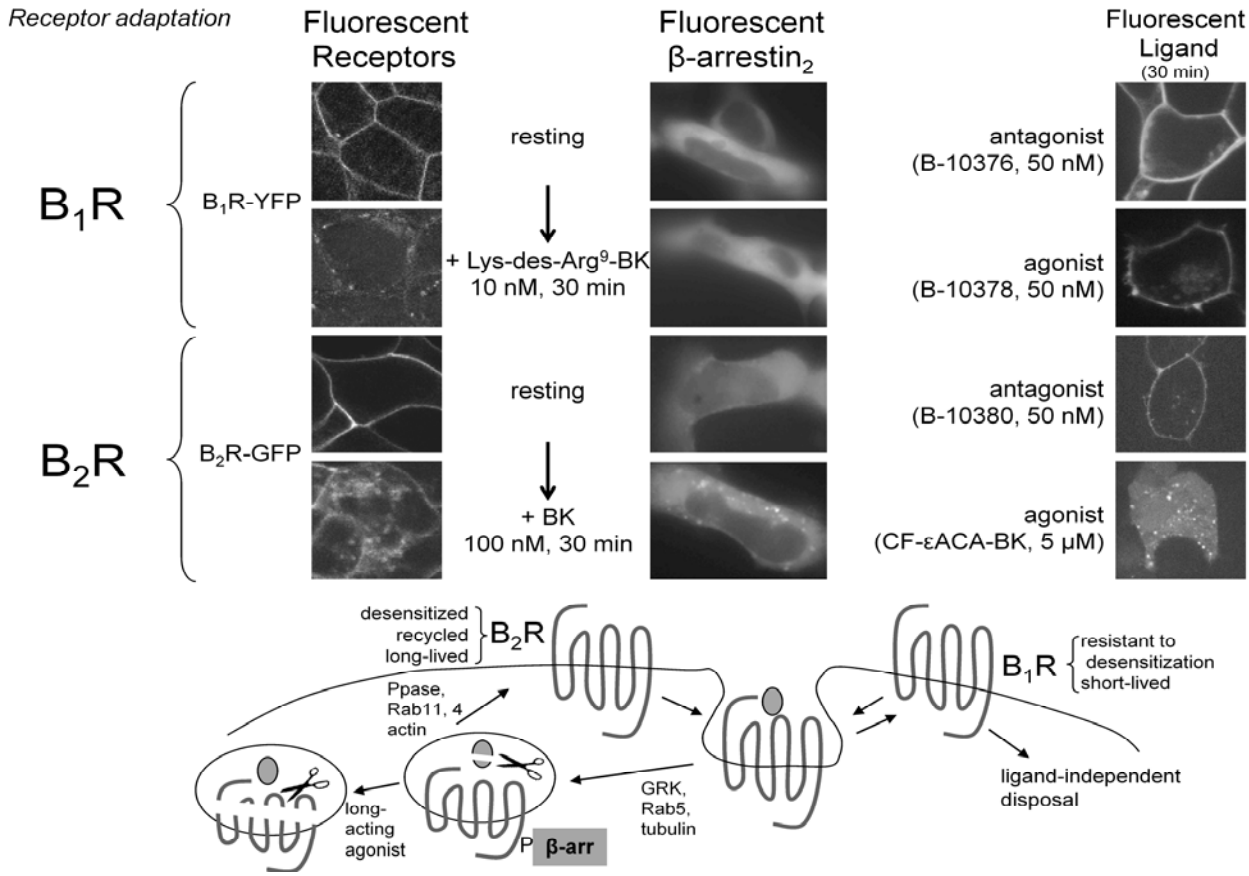

Figure 3. Bradykinin (BK) receptor adaptation. Top: Subcellular location of receptors, β-arrestin$_2$, and ligands in HEK 293 (left-most column) or HEK 293a cells (2 right-most columns) expressing recombinant bradykinin receptors. The fluorescent receptors or the distribution of fluorescent ligands were examined using confocal microscopy, as described elsewhere.[16,28,34] Imaging of B_1R-YFP reproduced from Sabourin et al[34] with the permission of ASPET. Bottom: schematic representation of BK receptor kinin adaptation. B_1R, B_1 receptor; B_2R, B_2 receptor; β-arr, β-arrestin; CF, carboxyfluorescein; ε-ACA, ε-aminocaproic acid; GFP, green fluorescent protein; GRK, G protein–coupled receptor kinase; Ppase, protein phosphatase; YFP, yellow fluorescent protein.

buoyant caveolae-related lipid rafts from cells prelabeled with either an agonist or an antagonist radioligand showed that the agonist version is highly enriched in such rafts, as opposed to the antagonist. This applied both to HEK 293 cells overexpressing B_1R-YFP[33] or to cultured human vascular smooth muscle cells expressing endogenous B_1Rs (see supplemental material). Caveolae, particularly prominent in vascular endothelial cells, are signaling platforms of interest where the protein $G_{\alpha q}$, a signaling partner of the B_1R, is enriched.

Another noticeable difference of interest between the 2 BK receptor subtypes is their apparent half-life as mature proteins at the cell surface. The discussion above about the B_2R cycling makes clear that it is economically managed and it is also long-lived.[36] Like other gene products induced during inflammatory conditions (eg, inducible nitric oxide synthase), the B_1R is short-lived ($t_{1/2}$ 2-4 h), being eliminated from the cell surface in a ligand-independent manner (schematic representation, Fig. 3, bottom). This difference of half-life was established for both recombinant and naturally expressed receptors.

Some potentially important molecular issues have not been discussed in this text, such as the possible homo- and heterodimerization of receptors or development of biased agonists, because of the uncertain nature of the limited reported findings applied to the B_1Rs and B_2Rs. Suffice it to say that B-9972 behaves as an apparent biased agonist of the B_2R simply because it changes the kinetics of receptor cycling.[36]

CONCLUSIONS

Kinins have emerged as inflammatory mediators particularly implicated in the development of the cardinal signs of inflammation that are dependent on vascular responses (vasodilation, increased microvascular permeability). Despite the maturity of the medicinal chemistry efforts aimed at antagonizing the BK receptors, clinical applications remain limited, hereditary angioedema presently being the showcase of the kallikrein-kinin system in human therapeutics. Indeed, the B_2R antagonist icatibant and the plasma kallikrein inhibitor ecallantide (Kalbitor™, Dyax Corp, Burlington, Massachusetts; DX-88) are now clinically used for this indication. Despite the disappointing analgesic effect of BK receptor antagonists in humans, other therapeutic avenues are worth pursuing (eg, anti-inflammatory effects). Potential salutary effects of endogenous kinins in the circulation are also of great interest, especially during stressful situations (ischemia, renal injury, etc). Whether accidental and catastrophic side effects of BK antagonists may arise in individuals for whom these mechanisms are compensatory is of interest for the clinical development of such drugs.

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The authors have no financial interests in the materials or the subject dealt with in this article.

REFERENCES

1. Leeb-Lundberg LMF, Marceau F, Müller-Esterl W, Pettibone DJ, Zuraw BL. International Union of Pharmacology. XLV. Classification of the kinin receptor family: from molecular mechanisms to pathophysiological consequences. *Pharmacol Rev.* 2005;57:27-77.

2. Sales VMT, Turaça LT, Pesquero JB. Animal models in the kinin field. In: *Kinins.* Bader M, ed. Berlin, Germany: De Gruyter; 2011:51-68.

3. Blais C, Marceau F, Rouleau JL, Adam A. The kallikrein -kininogen-kinin system: lessons from the quantification of endogenous kinins. *Peptides.* 2000;21:1903-1940.

4. Regoli D, Barabé J. Pharmacology of bradykinin and related kinins. *Pharmacol Rev.* 1980;32:1-46.

5. Hock FJ, Wirth K, Albus U, et al. Hoe 140 a new potent and long acting bradykinin-antagonist: in vitro studies. *Br J Pharmacol.* 1991;102:769-773.

6. Maurer M, Aberer W, Bouillet L, et al. Hereditary angioedema attacks resolve faster and are shorter after early icatibant treatment. *PLoS One.* 2013;8:e53773.

7. Marceau F, Levesque L, Drapeau G, et al. Effects of peptide and nonpeptide antagonists of bradykinin B_2 receptors on the venoconstrictor action of bradykinin. *J Pharmacol Exp Ther.* 1994;269:1136-1143.

8. Houle S, Larrivée JF, Bachvarova M, et al. Antagonist-induced intracellular sequestration of the rabbit bradykinin B_2 receptor. *Hypertension.* 2000;35:1319-1325.

9. Warren K, Jakacki R, Widemann B, et al. Phase II trial of intravenous lobradimil and carboplatin in childhood brain tumors: a report from the Children's Oncology Group. *Cancer Chemother Pharmacol.* 2006;58:343-347.

10. Côté J, Bovenzi V, Savard M, et al. Induction of selective blood-tumor barrier permeability and macromolecular transport by a biostable kinin B1 receptor agonist in a glioma rat model. *PLoS One*. 2012;7:e37485.

11. Fincham CI, Bressan A, Paris M, et al. Bradykinin receptor antagonists—a review of the patent literature 2005-2008. *Expert Opin Ther Pat.* 2009;19:919-941.

12. Shakur H, Andrews P, Asser T, et al. The BRAIN TRIAL: a randomised, placebo controlled trial of a Bradykinin B_2 receptor antagonist (Anatibant) in patients with traumatic brain injury. *Trials.* 2009;10:109.

13. Kuduk SD, Di Marco CN, Chang RK, et al. Development of orally bioavailable and CNS penetrant biphenylaminocyclopropane carboxamide bradykinin B_1 receptor antagonists. *J Med Chem.* 2007;50:272-282.

14. Stadnicki A. Intestinal tissue kallikrein-kinin system in inflammatory bowel disease. *Inflamm Bowel Dis.* 2011;17:645-654.

15. Bawolak MT, Fortin S, Bouthillier J, et al. Effects of inactivation-resistant agonists on the signalling, desensitization and down-regulation of bradykinin B_2 receptors. *Br J Pharmacol.* 2009;158:1375-1386.

16. Gera L, Roy C, Bawolak MT, Charest-Morin X, Marceau F. N-terminal extended conjugates of the agonists and antagonists of both bradykinin receptor subtypes: structure-activity relationship, cell imaging using ligands conjugated with fluorophores and prospect for functionally active cargoes. *Peptides.* 2012;34:433-446.

17. Moreau ME, Bawolak MT, Morissette G, Adam A, Marceau F. Role of nuclear factor-κB and protein kinase C signaling in the expression of the kinin B_1 receptor in human vascular smooth muscle cells. *Mol Pharmacol.* 2007;71:949-956.

18. Koumbadinga GA, Désormeaux A, Adam A, Marceau F. Effect of interferon-γ on inflammatory cytokine-induced bradykinin B_1 receptor expression in human vascular cells. *Eur J Pharmacol.* 2010;647:117-125.

19. Regoli D, Marceau F, Lavigne J. Induction of the B_1-receptor for kinins in the rabbit by a bacterial lipopolysaccharide. *Eur J Pharmacol.* 1981;71:105□115.

20. deBlois D, Horlick RA. Endotoxin sensitization to kinin B_1 receptor agonist in a non-human primate model: haemodynamic and pro-inflammatory effects. *Br J Pharmacol.* 2001;132:327-335.

21. Vidal MA, Astroza A, Matus CE, et al. Kinin B_2 receptor-coupled signal transduction in human cultured keratinocytes. *J Invest Dermatol.* 2005;124:178-186.

22. Morissette G, Houle S, Gera L, Stewart JM, Marceau F. Antagonist, partial agonist and anti-proliferative actions of B-9870 (CU201) as a function of the expression and

density of the bradykinin B_1 and B_2 receptors. *Br J Pharmacol* 2007;150:369-379.

23. Mathis SA, Criscimagna NL, Leeb-Lundberg LMF. B_1 and B_2 kinin receptors mediate distinct patterns of intracellular Ca^{2+} signaling in single cultured vascular smooth muscle cells. *Mol Pharmacol.* 1996;50:128-139.

24. Bogatcheva NV, Verin AD. The role of the cytoskeleton in the regulation of vascular endothelial barrier function. *Microvasc Res.* 2008;76:202-207.

25. Lacroix JS, Landos BN. Neurogenic inflammation of the upper airway mucosa. *Rhinology.* 2008;46:163-165.

26. Duchene J, Lecomte F, Ahmed S, et al. A novel inflammatory pathway involved in leukocyte recruitment: role for the kinin B_1 receptor and the chemokine CXCL5. *J Immunol.* 2007;179:4849-4856.

27. Hamdan FF, Rochdi MD, Breton B, et al. Unraveling G protein-coupled receptor endocytosis pathways using real-time monitoring of agonist-promoted interaction between β -arrestins and AP-2. *J Biol Chem.* 2007;282:29089-29100.

28. Bachvarov DR, Houle S, Bachvarova M, Bouthillier J, Adam A, Marceau F. Bradykinin B_2 receptor endocytosis, recycling, and down-regulation assessed using green fluorescent protein conjugates. *J Pharmacol Exp Ther.* 2001;297:19-26.

29. Gera L, Bawolak MT, Roy C, Lodge R, Marceau F. Design of fluorescent bradykinin analogs: application to imaging of B_2 receptor-mediated agonist endocytosis and trafficking and of angiotensin-converting enzyme. *J Pharmacol Exp Ther.* 2011;337:33-41.

30. Charest-Morin X, Fortin S, Lodge R, et al. Inhibitory effects of cytoskeleton disrupting drugs and GDP-locked Rab mutants on bradykinin B_2 receptor cycling. *Pharmacol Res*. 2013;71:44-52.

31. Bawolak MT, Gera L, Stewart JM, Marceau F. B-9972 (D-Arg-[Hyp^3, Igl^5, Oic^7, Igl^8]-bradykinin) is an inactivation-resistant agonist of the bradykinin B_2 receptor derived from the peptide antagonist B-9430 (D-Arg-[Hyp^3, Igl^5, D-Igl^7, Oic^8]-bradykinin): pharmacologic profile and effective induction of receptor degadation. *J Pharmacol Exp Ther.* 2007;323:534-546.

32. Bawolak MT, Roy C, Gera L, Marceau F. Prolonged signalling and trafficking of the bradykinin B_2 receptor stimulated with the amphibian peptide maximakinin: insight into the endosomal inactivation of kinins. *Pharmacol Res.* 2012;65:247-253.

33. Blaukat A, Herzer K, Schroeder C, Bachmann M, Nash N, Müller-Esterl W. Overexpression and functional characterization of kinin receptors reveal subtype-specific phosphorylation. *Biochemistry.* 1999;38:1300-1309.

34. Sabourin T, Bastien L, Bachvarov DR, Marceau F. Agonist-induced translocation of the kinin B_1 receptor to caveolae-related rafts. *Mol Pharmacol.* 2002;61:546-553.

35. Bawolak MT, Gera L, Morissette G, et al. Fluorescent ligands of the bradykinin B_1 receptors: pharmacologic characterization and application to the study of agonist-induced receptor translocation and cell surface receptor expression. *J Pharmacol Exp Ther.* 2009;329:159-168.

36. Fortin JP, Bouthillier J, Marceau F. High agonist-independent clearance of rabbit kinin B_1 receptors in cultured cells. *Am J Physiol Heart Circ Physiol.* 2003;284:H1647-H1654.

37. Gera L, Roy C, Bawolak MT, Bouthillier J, Adam A, Marceau F. Met-Lys-bradykinin-Ser-Ser, a peptide produced by the neutrophil from kininogen, is metabolically activated by angiotensin converting enzyme in vascular tissue. *Pharmacol Res.* 2011;64:528-534.

38. Packer RJ, Krailo M, Mehta M, et al. A phase I study of concurrent RMP-7 and carboplatin with radiation therapy for children with newly diagnosed brainstem gliomas. *Cancer.* 2005;104:1968-1974.

39. Morissette G, Sabourin T, Adam A, Marceau F. Inhibition of human and rabbit arterial smooth muscle cell migration mediated by the kinin B_1 receptor: role of receptor density and released mediators. *Can J Physiol Pharmacol.* 2006;4:1107-1118.

40. Gera L, Stewart JM, Fortin JP, et al. Structural modification of the highly potent peptide bradykinin B1 receptor antagonist B9958. *Int Immunopharmacol*. 2008;8:289-292.

41. Bawolak MT, Gera L, Bouthillier J, Stewart JM, Adam A, Marceau F. A fluorescent version of the bradykinin B_2 receptor antagonist B-9430: pharmacological characterization and use in live cell imaging. *Peptides.* 2008;29:1626-1630.

42. Pruneau D, Paquet JL, Luccarini JM, et al. Pharmacological profile of LF 16-0687, a new potent non-peptide bradykinin B_2 receptor antagonist. *Immunopharmacology.* 1999;43:187-194.

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