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 herein, with emphasis on drug development, receptor expression, signaling, and adaptation to persistent stimulation. Peptide and non-peptide 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. 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. Both receptor subtypes, mainly coupled to protein G G_q, phospholipase C and calcium signaling, 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. This disease is the therapeutic showcase of the kallikrein-kinin system, with an orally bioavailable B_2R antagonist under development, as well as other agents that inhibit the kinin forming protease, plasma kallikrein. Other clinical applications are still elusive despite the maturity of the medicinal chemistry efforts applied to kinin receptors.

Keywords: bradykinin; B_1 receptor; B_2 receptor; receptor ligands; receptor signaling; receptor adaptation.

1. 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 endeavor 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 40 years. There will be a certain autobiographic bias in the selection of the illustration material, as our group has been particularly active in exploring and designing novel ligands and study kinin receptor adaptation via cell imaging.

2. 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 enzymatically removed (des-Arg^9-BK, Lys-des-Arg^9-BK, respectively; Fig. 1) [4]. Often overlooked, the only agonist of the human form of the B_1R that possesses a subnanomolar affinity is Lys-des-Arg^9-BK, also called des-Arg^{10}-kallidin [5]. This peptide can be generated from Lys-BK (kallidin), itself derived from the cleavage of kininogens by tissue kallikrein (KLK1 gene product). Therefore, the B_1R may be irrelevant to conditions associated

with hyperactive plasma kallikrein (KLKB1), such as hereditary angioedema (HAE), as this protease generates BK. The early peptide antagonists of the B_1R 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, are selective agonists of the physiologically prominent B_2R subtype that has been consolidated with specific peptide antagonists created in J. M. Stewart's laboratory in the 1980s [6]. 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®, Hoe 140) [7] 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 HAE attacks in a self-injected form [8]. Icatibant is apparently a competitive (surmountable) antagonist at the human B_2R, but the peptide may be insurmountable and/or a partial agonist in other mammalian species [9, 10]. The des-Arg^9 form of icatibant, while not a spontaneous metabolite of this drug, 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 [11] (Fig. 1). As a polycationic polymer, icatibant has been recently identified as a direct histamine releaser from mast cells via a GPCR termed MRGPRX2 [12], explaining a local and common skin reaction at the site of subcutaneous injection.

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 [13] (Fig. 1), has distinctive effects on B_2R cycling (see below). Another selective B_2R agonist resistant to carboxypeptidases, labradimil (lobradimil, Cereport®, 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 [14]. Subsequent 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 [15].

Docking models for peptide and some non-peptide ligands have been proposed for both the B_1R and B_2Rs [1, 16]. 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, Lajos Gera's laboratory has developed the full set of fluorescent agonists and antagonists for both subtypes of receptors by prolonging their structure at the N-terminus with the green-emitting fluorescein or, for B_2R ligands, the infrared-emitting Cy7 [17-21] (Fig. 1; see also below). The same docking considerations have led to the hypothesis that the agonists BK or Lys-des-Arg^9-BK could be positioned at the C-terminus of fusion proteins, separated by a spacer sequence from a functional protein. The amphibian sequence maximakinin has the full BK sequence at the C-terminal of a 10-residue hydrophilic extension (Fig. 1); this is a sufficient spacer-agonist module for successful fusion with enhanced green fluorescent protein (EGFP): EGFP-maximakinin is a high affinity agonist ligand of the B_2R which supported cell imaging studies [22, 23]. Another uncharged but hydrophilic spacer, $(Asn\text{-}Gly)_{15}$, has been used to create the fluorescent ligand of the human B_1R,

EGFP-$(Asn\text{-}Gly)_{15}$-Lys-des-Arg^9-BK [24] or combined to maximakinin to produce APEX2-$(Asn\text{-}Gly)_{15}$-maximakinin [25] (table 1). APEX2 is an engineered peroxidase compatible with readily available detection systems for histochemistry and optical detection, allowing a direct detection of the rat B_2R with this enzymatic ligand. However, a limitation of ligands based on the maximakinin sequence was their unexpected low affinity for the human form of the B_2R; optimization of the spacer sequence is warranted. This line of investigation may define novel diagnostic and analytical tools alternative to antibodies.

Another type of B_2R ligands has been recently explored: cleavable sequences with low receptor affinity that release a high affinity agonist after reaction with a specific peptidase. The goal of this effort is to extract cardiovascular benefits from the controlled stimulation of endothelial B_2Rs, such as vasodilation, while avoiding extravascular sites of actions where an agonist may produce major side effects (e.g., afferent nerve terminals, gastrointestinal or respiratory epithelia). The best in vitro and in vivo validated prototypes were BK-Arg and D-Arg^0-BK-Arg-Arg, both substrates of arginine-carboxypeptidases (sequences in Fig. 1) [26, 27].

The pharmaceutical industry has developed a large number of non-peptide antagonists for both BK receptor subtypes [28, 29]; let us mention only one or two for each. The B_2R antagonist anatibant [30] (LF 16-0687; Fig. 1) was used in a clinical trial for the prevention of brain edema after head injury (an inconclusive trial) [31]. Anatibant is a relatively heavy molecule not suitable for oral administration; oral bioavailability and increased potency has been reached in a recent B_2R antagonist prototype, PHA-022121 [32]. Such a drug is likely to improve the quality of life

of patients with HAE. The clinical development of an orally bioavailable and brain penetrant B_1R antagonist, MK-0686 [33], as an analgesic was interrupted after trials suggested lack of efficacy in inflammatory pain indication [28]. Other efforts to develop B_1R antagonists for clinical use have also disappointed [29]. 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 [34]. In the course of structure-activity studies of non-peptide B_2R antagonists, Fujisawa scientists have discovered several non-peptide 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 [35]. The docking of non-peptide antagonists to both kinin receptor subtypes has been modelled [36-38].

3. Overview of the expression and signaling of bradykinin receptors

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 in vascular cells, notably under the influence of tissue injury, cytokines, and the signaling systems mentioned in Figure 2 [39, 40]. The injection of bacterial lipopolysaccharide in laboratory animals, including a primate species, is a traditional 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*) [41, 42]. 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) [40]. 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) [40], 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 [39, 40], 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, interferon-γ, interleukin-1, epidermal growth factor, etc.) clearly upregulates the B_1R protein expression and function in these models. Cellular studies have evidenced that the newly formed B_1R glycoproteins do not transit readily through the secretory pathway to the cell surface. Homo-oligomerization may be necessary for B_1R maturation [43]; cell-permeant non-peptide B_1R antagonists also facilitate the surface expression of glycosylated B_1Rs, a form of molecular chaperoning [44]. Further, the inflammatory transition from B_2R to B_1R signaling may be facilitated by the heterodimer formation leading to the selective proteolytic degradation of the B_2R partner [45].

Both B_1R and B_2Rs are mainly coupled to protein G 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 MAP kinases and c-Fos expression and phosphorylation [35, 46]. 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 [40, 47]; 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 [48].

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 [49]. 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 (e.g., in airway

mucosae), neurogenic inflammation driven by the antidromic release of proinflammatory neuropeptides and tissue leukocytes responsive to them can amplify edema [50].

The proinflammatory effects of BK-related peptides are largely asymmetric, favoring the vascular manifestations of the process over leukocyte recruitment; there are few validated 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 [51].

4. 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 [52], 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 [53]. 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) [35].

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) [17]. 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 of 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 [54]. 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 non-peptide partial agonist, compound 47a (structures in Fig. 1) [13, 35]. The amphibian BK homologue maximakinin (Fig. 1) also acts as an inactivation-resistant agonist [55]. 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 (Fig. 3, bottom).

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 [1]. Accordingly, the B_1R is not phosphorylated on agonist stimulation [56] 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 the 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 the plasma membrane plane (confocal microscopy, Fig. 3, top) [57]. 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; 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) [18].

Microscopic studies support that caveolin-1 is colocalized with B-10378 [57] and with agonist-stimulated B_1R-YFP [18, 57]. In addition, cell fractionation studies that aimed to recover 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 [57] or to cultured

human vascular smooth muscle cells expressing endogenous B_1Rs (unpublished data from our laboratory). Caveolae, particularly prominent in vascular endothelial cells, are signaling platforms of interest where the protein G $G_{\alpha q}$, 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 it clear that it is economically managed, as well as long-lived [58]. Like other gene products induced during inflammatory conditions (e.g., 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 [58]. It has been argued that the signaling of the agonist-stimulated B_1Rs is dependent on this irreversible endocytosis, an inherently transient phenomenon [59].

Some potentially important molecular issues have not been discussed in this overview, such as additional modes of receptor heterodimerization or the 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 (see above) [18].

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, HAE presently being the showcase of the kallikrein-kinin system in human therapeutics. Indeed, the B_2R antagonist icatibant and the plasma kallikrein inhibitors ecallantide and lanadelumab are now clinically used for this indication and several investigational products related to the kallikrein-kinin system are being developed [60]. Despite the disappointing analgesic effect of BK receptor antagonists in humans [28], other therapeutic avenues are worth pursuing in applications such as inflammation. Further areas requiring research include the potential salutary effects of endogenous kinins in the circulation, especially during stressful situations such as ischemia, renal injury and perhaps hypertension; therapeutic BK receptor agonists may prove useful in such situations. Conversely, accidental and catastrophic side effects of BK antagonists may arise in individuals for whom kinins are compensatory. In conclusion, despite the wide amount of work to gain a better understanding of the pharmacology of BK receptors, much remains to be uncovered.

Author contributions

J.B., J.-P.F., G.M., M.-T.B., X.C.-M. generated and analyzed experimental data reviewed in this text, including the construction of receptor variants and biotechnological ligands. L.G. designed and produced numerous peptide ligands. F.M., H.B. and L.G. are the senior authors of the manuscript. All authors read and approved the final manuscript.

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Declaration of Competing Interest

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Figure legends

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. EGFP: enhanced green fluorescent protein; APEX2: genetically modified soybean peroxidase. 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. The compounds represented in green or red are fluorescent (in synthetic peptides, green or infrared fluorescence conferred by N-terminally positioned fluorescein or Cy7, respectively).

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*. Briefly, *1* is a schematic representation of the human genomic BK receptor locus; *2* refers to the selective expression of the B1R following tissue injury and immunopathology; *3* summarizes selected pharmacologic experiments applied to cultured human endothelial cells; *4* is a schematic representation of kinin receptor signaling; *5* emphasizes the kinin-induced responses of microvessels, including endothelial production of vasodilator mediators and enhanced permeability. The graph to the right of marker *3* is reproduced from Koumbadinga et al. [40] 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;

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 [19, 53, 57]. Activated B_2Rs are endocytosed along its agonist (modeled with B_2R-GFP and CF-εACA-BK, respectively), while the β-arrestin is translocated from its uniform cytosolic distribution to the endosomes containing phosphorylated receptors. The B_1R-YFP construction is not importantly endocytosed upon 30 min agonist stimulation, but condenses in structures that remain associated with the membrane. The fluorescent B_1R ligands essentially remain associated with the cell membrane, while the cytosolic distribution of the fluorescent β-arrestin is not affected by B_1R stimulation. Imaging of B_1R-YFP reproduced from Sabourin et al. [57] with the permission of ASPET. Bottom: schematic representation of BK receptor kinin adaptation. See main text for a description of B_2R cycling. 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.

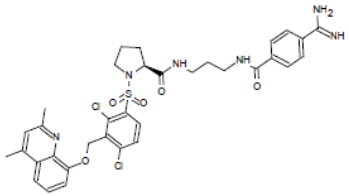
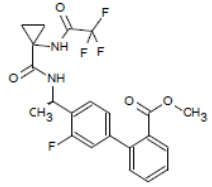
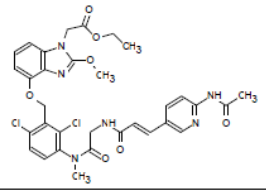
Ligands	Developed Structure	B_2R Ligands	Fluorescent Version	B_1R Ligands	Developed Structure
Natural peptide agonists	H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH H-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH H-Asp-Leu-Pro-Lys-Ile-Asn-Arg-Lys-Gly- Pro-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH	BK* → Lys-BK maximakinin [21]	**CF-εACA-BK** [17] **B-10378** [18] ←	Lys-des-Arg^9-BK* des-Arg^9-BK	H-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-OH H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-OH
Peptidase-resistant agonists	[Hyp^3, Thi^5, 4-Me-$Tyr^8\Psi(CH_2\text{-}NH)$-$Arg^9$]-BK D-Arg-[$Hyp^3$, Igl^5, Oic^7, Igl^8]-BK	labradimil [14] B-9972 [11] →	**FTC-B-9972** [19] **B-10666** [20]	Sar-[D-Phe^8]desArg^9-BK [61] Sar-Lys[D-Phe^8]desArg^9-BK [15]	H-Sar-Arg-Pro-Pro-Gly-Phe-Ser-Pro-D-Phe-OH H-Sar-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-D-Phe-OH
Peptidase-activated agonists	H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-Arg-OH H-D-Arg-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-Arg-Arg-OH	BK-Arg [26] D-Arg^0-BK-Arg-Arg [27]			
Peptide antagonists	D-Arg-[Hyp^3, Thi^5, D-Tic^7, Oic^8]-BK D-Arg-[Hyp^3, Igl^5, D-Igl^7 ,Oic^8]-BK	icatibant [7] B-9430 →	**B-10376** [18] ← **B-10380** [21] **FTC-B-9430** [19] **B-10665** [20]	B-9958 [11] Lys-[Leu^8]des-Arg^9-BK*	Lys-Lys-[Hyp^3, CpG^5, D-Tic^7, CpG^8]des-Arg^9-BK H-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu-OH
Fusion protein agonists	**EGFP-maximakinin** [22] APEX2-$(Asn\text{-}Gly)_{15}$-maximakinin [25]				**EGFP-$(Asn\text{-}Gly)_{15}$-Lys-des-Arg^9-BK** [24]
Nonpeptide antagonists		anatibant [30] ←		MK-0686 [33] →	
Nonpeptide partial agonist		compound 47a [35] ←			

Fig. 1

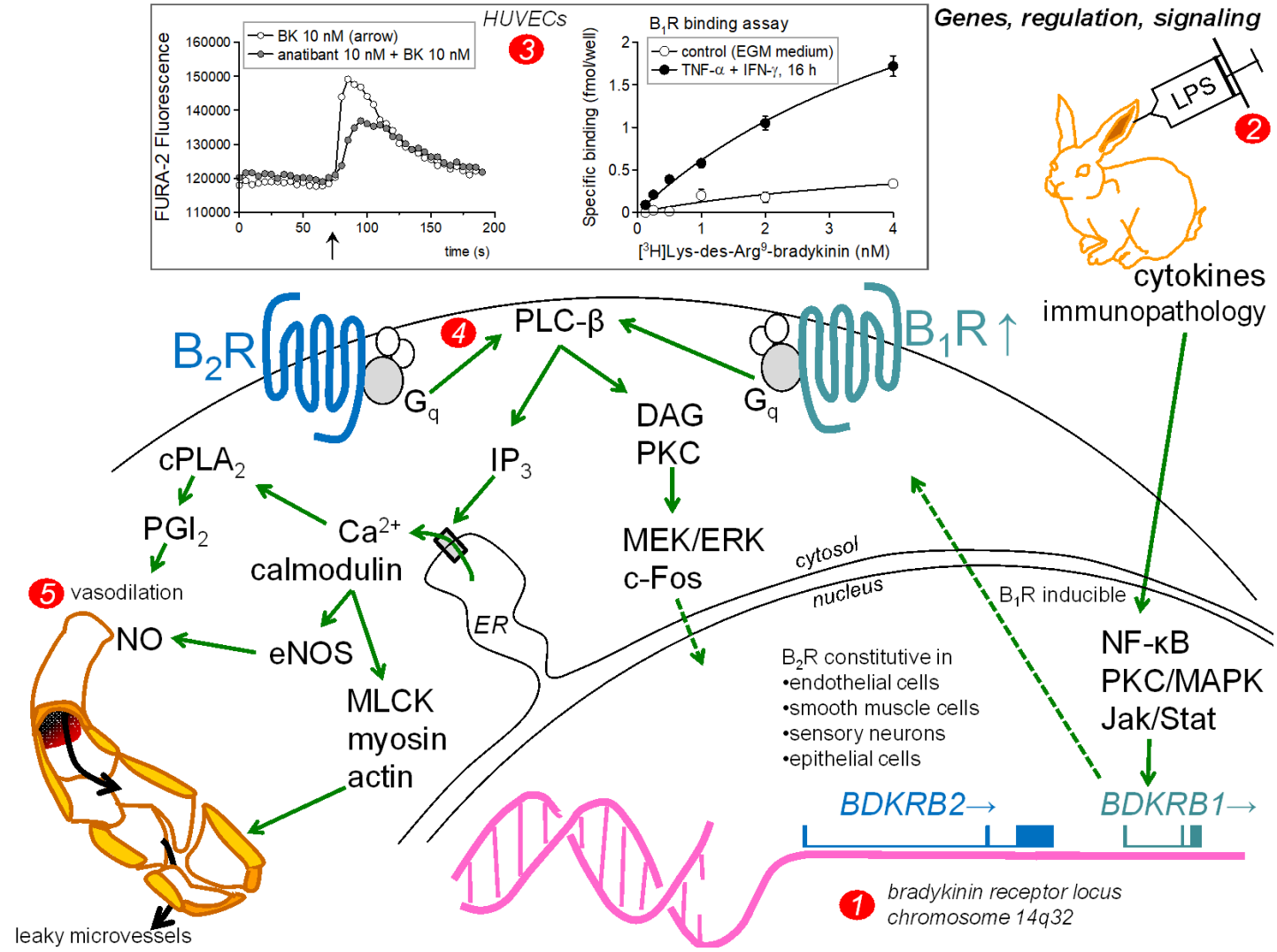

HUVECs
3
BK 10 nM (arrow)
anatibant 10 nM + BK 10 nM
FURA-2 Fluorescence
time (s)
B1R binding assay
control (EGM medium)
TNF-α + IFN-γ, 16 h
Specific binding (fmol/well)
[3H]Lys-des-Arg9-bradykinin (nM)
Genes, regulation, signaling
LPS
2
cytokines
immunopathology
B2R
Gq
4
PLC-β
B1R ↑
DAG
PKC
cPLA2
IP3
PGI2
Ca2+
calmodulin
MEK/ERK
c-Fos
cytosol
nucleus
B1R inducible
5 vasodilation
NO
eNOS
ER
MLCK
myosin
actin
NF-κB
PKC/MAPK
Jak/Stat
B2R constitutive in
•endothelial cells
•smooth muscle cells
•sensory neurons
•epithelial cells
BDKRB2→
BDKRB1→
1 bradykinin receptor locus
chromosome 14q32
leaky microvessels

Fig. 2

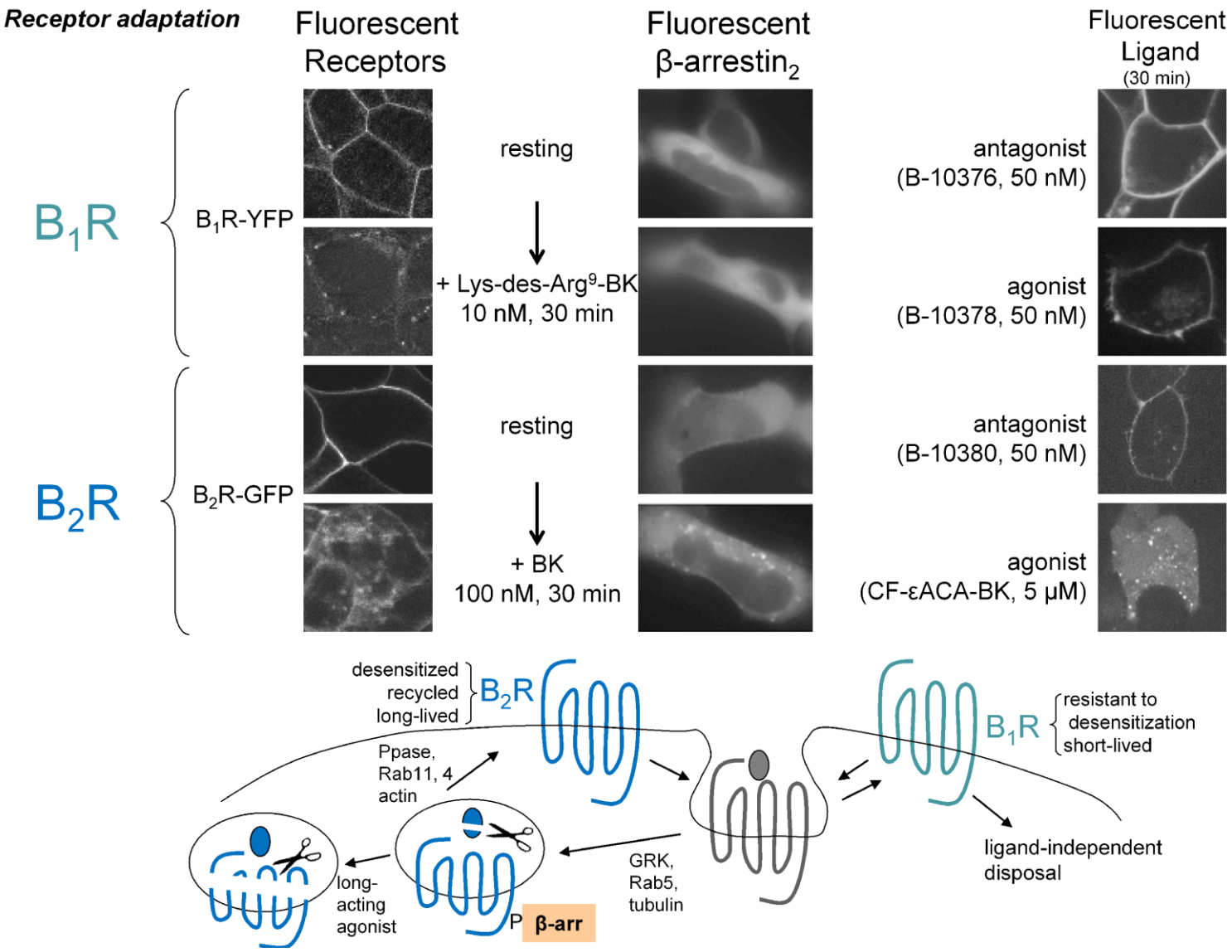

Receptor adaptation
Fluorescent Receptors
Fluorescent β-arrestin2
Fluorescent Ligand (30 min)
B1R
B1R-YFP
resting
+ Lys-des-Arg9-BK 10 nM, 30 min
antagonist (B-10376, 50 nM)
agonist (B-10378, 50 nM)
B2R
B2R-GFP
resting
+ BK 100 nM, 30 min
antagonist (B-10380, 50 nM)
agonist (CF-εACA-BK, 5 μM)
desensitized recycled long-lived
B2R
Ppase, Rab11, 4 actin
B1R
resistant to desensitization short-lived
ligand-independent disposal
GRK, Rab5, tubulin
long-acting agonist
P β-arr

Fig. 3