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Differential regulation of inducible and endothelial nitric oxide synthase by kinin B1 and B2 receptors

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Abstract

Kinins are vasoactive peptides that play important roles in cardiovascular homeostasis, pain and inflammation. After release from their precursor kininogens, kinins or their C-terminal des-Arg metabolites activate two distinct G protein-coupled receptors (GPCR), called B2 (B2R) or B1 (B1R). The B2R is expressed constitutively with a wide tissue distribution. In contrast, the B1R is not expressed under normal conditions but is upregulated by tissue insult or inflammatory mediators. The B2R is considered to mediate many of the acute effects of kinins while the B1R is more responsible for chronic responses in inflammation. Both receptors can couple to G α i and G α q families of G proteins to release mediators such as nitric oxide (NO), arachidonic acid, prostaglandins, leukotrienes and endothelium derived hyperpolarizing factor and can induce the release of other inflammatory agents. The focus of this review is on the different transduction events that take place upon B2R and B1R activation in human endothelial cells that leads to generation of NO via activation of different NOS isoforms. Importantly, B2R-mediated eNOS activation leads to a transient (~ 5 min) output of NO in control endothelial cells whereas in cytokine-treated endothelial cells, B1R activation leads to very high and prolonged (~90 min) NO production that is mediated by a novel signal transduction pathway leading to post-translational activation of iNOS.

Keywords

nitric oxide; nitric oxide synthase; bradykinin; kinin B1 receptor; kinin B2 receptor; eNOS; iNOS; endothelial cells

1. Introduction

The kallikrein/kinin system has been investigated since the early 1900s. In the 1920s and 1930s, Frey, Kraut, and Werle characterized a hypotensive factor in urine and they named this substance *kallikrein* from the Greek word *kallikrēas*, meaning pancreas, because it was enriched in that organ (Bhoola et al., 1992). By 1937, Werle and co-workers had established that kallikreins produce an active substance from an inactive precursor in plasma and this factor was called kallidin (KD) (Bhoola et al., 1992). Rocha e Silva, Beraldo and associates

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independently found that trypsin and snake venoms produced a substance derived from plasma globins that lowered blood pressure and caused a slow contraction of the gut (Bhoola et al., 1992). Because of this slow response, it was given the name *bradykinin* (BK). Later, KD was found to be a decapeptide identical with the nonapeptide BK, except for an additional N-terminal Lys residue. Pharmacological characterization of the receptors mediating kinin responses resulted in the definition of two receptor subtypes named B1 (B1R) and B2 (B2R) (Regoli and Barabe, 1980). The development of antagonists to investigate the functions of these receptors laid the groundwork for further characterization and cloning of the B2R and B1R in the late 1980s and early 1990s (Leeb-Lundberg et al., 2005).

A variety of insults, including pathogens, tissue damage and allergic reactions activate the proteolytic cascade that leads to cleavage of high- or low-molecular weight kininogen by the serine proteases plasma or tissue kallikrein to release BK or KD, respectively (Bhoola et al., 1992; Leeb-Lundberg et al., 2005) (Fig. 1). The released kinin peptides are algescic and have proinflammatory actions, but also have beneficial effects in the cardiovascular and renal systems.

2. B2R and B1R signal transduction

BK and KD are both specific agonists of the B2R (Fig. 1). These peptides can be further processed by membrane carboxypeptidase M or plasma carboxypeptidase N to remove the C-terminal Arg residue and produce des-Arg⁹-BK and des-Arg¹⁰-KD (Skidgel and Erdös, 1998; Skidgel and Erdös, 1998; Skidgel et al., 2006), which are specific agonists of the B1R (Leeb-Lundberg et al., 2005) (Fig. 1). Both the B1R and the B2R have been cloned from many different species and are members of the rhodopsin-like subfamily of G protein-coupled receptors (GPCRs). The crystal structure of bovine rhodopsin has been used as a template to model the kinin receptors (Blaukat, 2003). However, most of the structural information on these receptors has been based on pharmacological approaches utilizing chemical cross-linking and mutagenesis (Regoli et al., 1993; Nardone and Hogan, 1994; Herzig and Leeb-Lundberg, 1995; AbdAlla et al., 1996; Herzig et al., 1996).

Typically, B2Rs are constitutively expressed whereas B1R expression is induced (Leeb-Lundberg et al., 2005). However, some tissues and cells express B1Rs constitutively such as the spinal cord and some brain regions (Pesquero et al., 2000; Wotherspoon and Winter, 2000; Ma and Heavens, 2001; Shughrue et al., 2003) or bovine aortic or pulmonary artery endothelial cells (Wiemer and Wirth, 1992; Smith et al., 1995). In addition, B2R expression can be modulated by inflammatory cytokines. For instance, interleukin-1 and TNF- α enhanced B2R expression in osteoblasts, fibroblasts and pulmonary A549 cells (Newton et al., 2002; Brechter et al., 2008), TGF- β increased B2R mRNA and protein in cultured human airway smooth muscle (Kim et al., 2005) and IFN- γ enhanced B2R mRNA in T24 epithelial-like cells (Lung et al., 1998).

The B1R and B2R can both couple to G α_q and G α_i families of G proteins to cause increased intracellular Ca²⁺ and phosphoinositide (PI) turnover, activation of phospholipase C, arachidonic acid release and NO production (Issandou and Darbon, 1991; Tropea et al., 1993; Marceau et al., 1998; Prado et al., 2002; Leeb-Lundberg et al., 2005) (Fig. 1). Both kinin receptors have also been reported to activate the MAP kinase cascade upon agonist treatment (Dikic et al., 1996; Blaukat et al., 1999; Christopher et al., 2001). However, the B2R is rapidly desensitized and internalized but the B1R is not and produces prolonged signaling (Lamb et al., 2002; Prado et al., 2002; Leeb-Lundberg et al., 2005). For example, B2R activation generates a transient increase in PI that plateaus in five minutes whereas stimulation of the B1R leads to an accumulation of PI that is linear for up to sixty minutes (Austin et al., 1997). The B1R also has high basal constitutive activity as demonstrated by PI turnover in the absence

of agonist that is equivalent to that generated by B2Rs after agonist treatment (Leeb-Lundberg et al., 2001). Nevertheless, the B2R does have low but detectable constitutive activity. In addition, despite higher basal B1R activity, B1R agonist still greatly enhances PI turnover (Leeb-Lundberg et al., 2001).

2.1. B2R signal transduction

The B2R is constitutively expressed in many cell types, including endothelial cells, and is commonly referred to as a prototypical $G_{\alpha q/11}$ receptor (Bhoola et al., 1992; Liao and Homcy, 1993; Marceau et al., 2002). However, the B2R has also been shown to interact with $G_{\alpha i/o}$ in bovine aortic endothelial cells (BAEC), rat-1 cells and HEK293 cells (Voyno-Yasenetskaya et al., 1989; Liao and Homcy, 1993; Yang et al., 1999). B2R coupling to $G_{\alpha q/11}$ or $G_{\alpha i/o}$, can result in downstream activation of PLC, leading to the formation of diacylglycerol and inositol 1, 4, 5-trisphosphate (IP_3), which increases cytoplasmic Ca^{2+} concentrations (Fig. 1). BK induces a biphasic rise in intracellular Ca^{2+} , which results from an initial transient peak generated from intracellular stores, followed by a sustained plateau phase caused by extracellular Ca^{2+} entry through calcium channels in the plasma membrane (Morgan-Boyd et al., 1987; Luckhoff et al., 1988; Schilling et al., 1989; Leung et al., 2006). Calcium signaling is involved in the regulation of many vascular processes including the generation of NO. B2R agonists also activate PLA_2 , which releases arachidonic acid, leading to formation of vasodilator prostaglandins such as PGI_2 (Leeb-Lundberg et al., 2005). NO and PGI_2 are thought to be major endothelium-derived vasodilators, but endothelial derived hyperpolarizing factors also play a role (Luksha et al., 2009). For example, arachidonic acid released by B2R agonist can be converted to epoxyeicosatrienoic acids by cytochrome P450 epoxygenases in vascular endothelium, which act as hyperpolarizing factors to open calcium-activated potassium channels and relax vascular smooth muscle (Campbell and Falck, 2007).

B2R agonists also stimulate ERK activation that is dependent on coupling through both $G_{\alpha i/o}$ and $G_{\alpha q/11}$ in HEK293 cells whereas in vascular smooth muscle cells, B2R activation generates multiple second messenger pathways that converge to activate MAPK (Velarde et al., 1999; Blaukat et al., 2000). Activation of the B2R can also transactivate the EGFR that contributes to the pool of activate ERK (Adomeit et al., 1999).

Although it has been reported that the B2R couples to $G_{\alpha s}$ in human epidermoid carcinoma cells (Liebmann et al., 1996) and to $G_{\alpha 12/13}$ in fibroblasts (Gohla et al., 1999), these pathways have not been found in endothelial cells nor linked to B2R-dependent NO production.

The B2R undergoes rapid desensitization and internalization after agonist stimulation that is dependent on phosphorylation of the C-terminal tail by G protein-coupled receptor kinases (Leeb-Lundberg et al., 2005). Additionally, the B2R can coalesce in caveolae after agonist treatment, but can also internalize into endosomes and recycle to the membrane (Leeb-Lundberg et al., 2005).

2.2. B1R signal transduction

Atypical of most GPCRs, the B1R is constitutively internalized without agonist treatment, likely due to its basal constitutive activity, and once activated with ligand, is resistant to desensitization and internalization (Marceau et al., 2002; Leeb-Lundberg et al., 2005). Activation of the rabbit B1R, C-terminally tagged with yellow fluorescent protein, caused its lateral movement to form membrane-associated aggregates co-localized with caveolae (Marceau et al., 2002) whereas the human B1R was partially localized to caveolae basally (Lamb et al., 2001; Zhang et al., 2008) and its distribution did not change after agonist stimulation (Lamb et al., 2001).

B1R signaling is dependent on carboxypeptidase cleavage of the C-terminal Arg in BK or KD to generate its des-Arg⁹-BK and des-Arg¹⁰-KD agonists (Fig. 1). Carboxypeptidase M is ideally suited to carry out this function as it is membrane bound via a glycosylphosphatidylinositol anchor, has a neutral pH optimum and its catalytic domain is extracellular (Skidgel and Erdös, 1998). Based on its crystal structure, electrostatic surface potential and modeling, CPM may interact with the membrane so that its active center is well situated to interact with other membrane-bound proteins or peptide substrates (Reverter et al., 2004). In addition, carboxypeptidase M was recently shown to heterodimerize with the B1R on the cell membrane as demonstrated by coimmunoprecipitation and fluorescence resonance energy transfer (FRET) analysis (Zhang et al., 2008). Carboxypeptidase M and B1Rs are co-localized in caveolae/lipid raft membrane microdomains and disruption of their interaction with β -cyclodextrin or monoclonal antibody to CPM reduces B1R signaling in response to B2R agonists BK or KD (Zhang et al., 2008). Thus, the interaction of carboxypeptidase M and B1R on the membrane allows the efficient delivery of agonist to the receptor and facilitates B1R signaling (Fig. 1).

In CHO cells stably expressing human B1Rs, immunoprecipitation of photoaffinity-labeled G-proteins from membranes showed that B1Rs were coupled to both G α _q/11 and G α _{i1,2}, but not to G α _s or G α _o/i₃ (Austin et al., 1997). Furthermore, B1R agonist stimulated increased intracellular Ca²⁺, PLC activity and PI turnover which was not inhibited by PTX, indicating G α _q/11 coupling (Austin et al., 1997). Activation of these downstream signaling pathways has also been demonstrated in a variety of human and animal cells expressing native B1Rs (Marceau et al., 1998; Leeb-Lundberg et al., 2005). Interestingly, in bovine pulmonary artery endothelial cells, direct B1R stimulation by ACE inhibitor caused an increase in intracellular Ca²⁺ that was mediated by protein kinase C (PKC) activation and was primarily due to influx of extracellular Ca²⁺ whereas B1R peptide agonist stimulated intracellular Ca²⁺ mobilization that was independent of PKC (Ignjatovic et al., 2004). In addition, ACE inhibitor activation of B1R-dependent Ca²⁺ influx in bovine pulmonary artery endothelial cells was sensitive to cholera toxin, indicating coupling through G α _s whereas peptide agonist activation was not affected (Ignjatovic et al., 2004).

The B1R is also known to activate the ERK MAP kinase pathway (Leeb-Lundberg et al., 2005). In rat aortic vascular smooth muscle cells, B1R activation of ERK is G α _s-dependent and mediated through activation of PKC and Src kinase (Christopher et al., 2001). In our studies of cytokine-treated HLMVEC or transfected HEK cells, B1R-mediated ERK activation was dependent on coupling through G α _i and G β γ and resultant activation of Src, Ras, Raf and MEK (Brovkovich et al., 2009).

3. Nitric Oxide Synthases

NO is a ubiquitous signaling molecule that has important functions in most organ systems and tissues (Moncada et al., 1991; Kubes, 1995; Ignarro et al., 1999; Papapetropoulos et al., 1999). In neurons, for example, NO can function as a neurotransmitter whereas in the cardiovascular system, NO has many effects, including regulating vascular tone. In the immune system, high concentrations of NO are generated to help fight pathogens. Mammals express three isoforms of nitric oxide synthase (NOS) (Papapetropoulos et al., 1999; Fulton et al., 2001; Skidgel, 2002; Dudzinski et al., 2006): NOS 1 or neuronal NOS (nNOS), NOS 2 or inducible NOS (iNOS) and NOS 3 or endothelial NOS (eNOS). These three enzymes are distinct gene products that share 50–60% amino acid sequence similarity and are only functional as homodimers (Stuehr, 1997). All isoforms contain an N-terminal oxygenase and C-terminal reductase domain; the oxygenase domain contains the binding sites for substrate L-Arg, oxygen, tetrahydrobiopterin (BH₄) and heme, while the C-terminal domain binds the cofactors FMN, FAD and NADPH (Masters et al., 1996; Stuehr, 1997). A calmodulin-binding

site bridges the two domains and distinguishes the two constitutive isoforms nNOS and eNOS, from iNOS. In their basal states, both nNOS and eNOS have a low affinity for Ca^{2+} /calmodulin so their activation is dependent on increased intracellular Ca^{2+} levels. In contrast, iNOS binds calmodulin with a very high affinity; it is thus constitutively active at basal intracellular Ca^{2+} concentrations and is not further activated by elevation of Ca^{2+} (Forstermann et al., 1995; Stuehr, 1997). Endothelial cells are a key source of NO, and under normal conditions the primary NOS responsible for generation of NO in the vasculature is eNOS (Moncada et al., 1991; Kubes, 1995; Ignarro et al., 1999; Papapetropoulos et al., 1999; Venema, 2002). The activity of this constitutive isoform is tightly regulated by a variety of mechanisms, including changes in intracellular Ca^{2+} , phosphorylation at Ser, Thr and Tyr residues, S-nitrosylation, and interaction with other regulatory proteins (Papapetropoulos et al., 1999; Fulton et al., 2001; Skidgel, 2002; Andreeva et al., 2006; Dudzinski et al., 2006). Under inflammatory conditions, endothelial cells can also express iNOS. In contrast to eNOS, iNOS is considered to be regulated mainly at the level of expression, which is induced in response to inflammatory mediators such as lipopolysaccharide (LPS) or cytokines such as interleukin 1β , interleukin-6 and interferon- γ (Forstermann et al., 1995; Aktan, 2004; Ghalayini, 2004; Kleinert et al., 2004). Once expressed, iNOS is thought to continuously generate NO in the presence of sufficient cofactors and substrate and it is largely downregulated by protein degradation (Knowles and Moncada, 1994; Kleinert et al., 2004). These properties have led to the concept that iNOS generates high output NO in a relatively unregulated fashion (compared with eNOS and nNOS) with primarily cytotoxic functions, for example in the host defense response (Papapetropoulos et al., 1999). However, our recent findings contradict this notion and reveal a dynamic regulation of iNOS activity via B1R activation (see section 4.4).

4. Comparison of B2R versus B1R activation of NO production

4.1 Kinin regulation of nNOS

nNOS is localized primarily in neuronal, skeletal and cardiac tissue (Walker et al., 1995; Zhou and Zhu, 2009), and its activity is regulated by Ca^{2+} -calmodulin binding (Matsuda and Iyanagi, 1999; Guan and Iyanagi, 2003; Roman and Masters, 2006). Although B1Rs and B2Rs are expressed in the nervous system and induce Ca^{2+} mobilization, there is only limited evidence that nNOS is activated downstream. Recently, bradykinin generated in response to vincristine-induced hyperalgesia was found to activate nNOS (Bujalska and Makulska-Nowak, 2009), leading to speculation that B2R antagonist and NOS inhibitor might be used to treat neuropathic pain.

In the vascular system, nNOS expression was detected in capillary endothelial cells in the alveolar septa by immunofluorescence staining (Luhrs et al., 2002) and human umbilical vein endothelial cells were reported to express functional nNOS that is down-regulated in response to cytokines (Bachetti et al., 2004). Whether B1R or B2R can regulate vascular nNOS activity has not been well studied. Both eNOS and nNOS-selective inhibitors blocked the vasorelaxant effect of BK in the rat carotid artery, but the eNOS inhibitor had a more pronounced effect (Tirapelli et al., 2007). There have been no reports regarding activation of nNOS downstream of B1Rs. In our studies, nNOS could not be detected in either control or cytokine-treated HLMVEC and nNOS-specific inhibitor, N-propyl-L-arginine, did not inhibit B2R-mediated NO generation.

4.2 Mechanisms of eNOS regulation

Initially, eNOS was thought to be activated primarily by Ca^{2+} /calmodulin binding resulting from agonist-induced increases in intracellular Ca^{2+} . Indeed, in control HLMVEC, increasing only intracellular Ca^{2+} by the calcium ionophore A23187 stimulates a sharp but transient increase in NO production (in the continuous presence of ionophore) as measured in real time

with a porphyrinic electrode, reaching a maximum of ~200 nM NO and returning to baseline in about 2 min (Fig. 2). However, substantial evidence has accumulated showing eNOS is regulated in a highly complex manner (Moncada et al., 1991; Kubes, 1995; Ignarro et al., 1999; Papapetropoulos et al., 1999; Venema, 2002) (Fig. 2). For example, an increase in intracellular Ca^{2+} stimulated by BK also promotes activation of calcineurin which dephosphorylates eNOS at the inhibitory phosphorylation site Thr⁴⁹⁵, leading to further activation (Harris et al., 2001). In addition to Gαq/11-mediated signaling, dissociation of the Gβγ subunits leads to activation of the phosphoinositide 3-kinase/Akt pathway. Activated Akt phosphorylates eNOS at Ser¹¹⁷⁷, a major activation site (Dimmeler et al., 1999; Fulton et al., 1999; Michell et al., 1999), which is enhanced by its interaction with heat shock protein 90 (Fulton et al., 2001; Fontana et al., 2002; Venema, 2002). Additionally, adenosine monophosphate (AMP)-activated protein kinase (AMPK), protein kinase G (PKG), PKA and CaM-dependent kinase II (CaMKII) can activate eNOS by phosphorylating Ser¹¹⁷⁷ (Chen et al., 1999; Butt et al., 2000; Fleming et al., 2001). ERK has also been suggested as a direct regulator of eNOS activity but currently no ERK phosphorylation sites have been identified (Chen et al., 1999; Bernier et al., 2000; Schmidt et al., 2002; Chen et al., 2004; Cale and Bird, 2006). eNOS activity can also be regulated by phosphorylation at Ser⁶¹⁷, Ser⁶³⁵ and Tyr⁸³ or dephosphorylation at Ser¹¹⁶ (Michell et al., 2002; Venema, 2002; Dudzinski et al., 2006; Fulton et al., 2008).

In addition to phosphorylation and Ca^{2+} /calmodulin binding, eNOS activity can be regulated by localization, dimer formation and protein-protein interactions. For example, in endothelial cells, eNOS is localized to caveolae via irreversible, co-translational myristoylation of its N-terminal glycine residue and by reversible, post-translational palmitoylation of Cys¹⁶ and Cys²⁶ (Robinson and Michel, 1995; Shaul et al., 1996; Prabhakar et al., 2000; Shaul, 2002). Under basal conditions, eNOS activity is suppressed by its direct interactions with caveolin-1, a 22 kDa caveolar scaffolding protein that sterically blocks the calmodulin binding region (Garcia-Cardena et al., 1997; Ju et al., 1997; Michel et al., 1997). Basal eNOS activity is also constitutively suppressed by S-nitrosylation at Cys⁹⁶ and Cys¹⁰¹ and upon agonist stimulation with VEGF or insulin, eNOS becomes denitrosylated at two zinc-tetrathiolate cysteine residues that mediate dimer formation (Erwin et al., 2005). The mechanism by which S-nitrosylation affects eNOS activity is not entirely clear, but it is thought to alter eNOS subcellular localization and dimer stability mediated by zinc binding (Erwin et al., 2005).

After agonist stimulation, eNOS activity is eventually shut off by several mechanisms. As Ca^{2+} levels begin to reach baseline, eNOS becomes inactivated by Ca^{2+} /calmodulin dissociation, allowing for re-binding of caveolin-1 (Garcia-Cardena et al., 1997; Ju et al., 1997; Michel et al., 1997). In addition, the stimulatory phosphorylation sites become dephosphorylated, followed by re-phosphorylation at the inhibitory site, Thr⁴⁹⁵ (Venema, 2002; Dudzinski and Michel, 2007). Moreover, eNOS becomes re-nitrosylated (Erwin et al., 2005). Prolonged activation can result in eNOS becoming de-palmitoylated, which allows for its translocation from the plasma membrane to internal membranes (Robinson and Michel, 1995; Shaul et al., 1996; Prabhakar et al., 2000; Dudzinski and Michel, 2007).

4.3 B2R regulation of eNOS activity

Regulation of eNOS activity by the B2R has been the subject of many investigations. Besides causing a Gαq/11-mediated increase in intracellular Ca^{2+} , the B2R can activate eNOS by several other mechanisms (Fig. 1). This is clearly shown by measuring real time NO production in HLMVEC stimulated with BK alone or BK + A23187, which results in a larger and more prolonged (~5 min) output of NO than stimulation of increased intracellular Ca^{2+} alone with A23187 (Fig. 2).

Basal eNOS activity is suppressed by its direct interaction with intracellular domain 4 of the B2R which disrupts flavin to heme electron transfer (Ju et al., 1998; Golser et al., 2000). Upon B2R agonist stimulation, eNOS is thought to rapidly dissociate from both caveolin-1 and the B2R. However, it is not clear from published data whether caveolin-1 and eNOS completely dissociate from the B2R in response to BK. With other eNOS-activating agonists, eNOS translocates from the membrane to the cytosol, but caveolin-1 remains associated with the membrane (Michel et al., 1993). However, after B2R stimulation, eNOS does not translocate to the cytosol (Liu et al., 1995; Venema, 2002). Thus, it is possible that activation of the B2R elicits a conformational change in the eNOS/caveolin complex, without complete dissociation, allowing Ca^{2+} /calmodulin binding to eNOS. Stimulation of eNOS phosphorylation at Ser¹¹⁷⁷ and dephosphorylation at Thr⁴⁹⁵ is a predominant mechanism by which eNOS is activated by many agonists. These eNOS modifications also result from B2R stimulation (Venema, 2002) (Fig. 1). However, several studies utilizing bovine aortic endothelial cells suggest that phosphorylation at additional regulatory sites are involved in B2R-mediated eNOS activation. For instance, BK stimulates Akt-dependent phosphorylation of Ser⁶¹⁷ and PKA - dependent phosphorylation of Ser⁶³⁵ that are both associated with eNOS activation (Michell et al., 2002). Phosphorylation of Tyr⁸³, originally reported to be mediated by Src kinase in response to oxidant stress, was recently shown to be induced by BK in bovine pulmonary artery endothelial cells and intact blood vessel segments (Fulton et al., 2008). Although some eNOS-activating agents, such as VEGF, stimulate dephosphorylation at Ser¹¹⁶, BK appears to have no effect at this site (Michell et al., 2002).

B2R signaling is generally considered transient in nature because activated receptors undergo rapid desensitization and internalization (Prado et al., 2002; Leeb-Lundberg et al., 2005). This, coupled with the above regulatory mechanisms for eNOS activation and inactivation, results in B2R-mediated NO production that is relatively short, but still about twice as long as that generated by Ca^{2+} ionophore alone (Fig. 2).

4.4 B1R regulation of iNOS activity

Although B2R signaling leading to NO production via eNOS has been well studied, signaling pathways leading to NO production from B1R activation are poorly understood. It has been reported that eNOS transfected into adventitial fibroblasts of cerebral arteries can be activated by B1R stimulation (Tsutsui et al., 2000) and that the B1R stimulates eNOS-derived NO production in bovine pulmonary artery endothelial cells (Ignjatovic et al., 2004). However, we found that B1R-dependent NO generation in cytokine-treated human lung microvascular endothelial cells (HLMVEC) is mediated only through iNOS and not eNOS (Fig. 1). For example, B1R-mediated NO output was inhibited by 1400W, the highly specific inhibitor of iNOS, but not by L-NNA, which inhibits eNOS but not iNOS at the concentration used (Ignjatovic et al., 2004; Zhang et al., 2007). The B1R-mediated stimulation of NO production was also calcium independent as BAPTA-AM, an intracellular calcium chelator that blocks eNOS activation, had no effect (Brovkovich et al., 2009). In addition, transfection of B1Rs into control HLMVEC expressing eNOS (Zhang et al., 2007) or co-transfection of B1Rs and eNOS into HEK293 cells (Lowry, J., Brovkovich, V. and Skidgel, R.A., unpublished), did not result in significant NO production in response to B1R agonists. Furthermore, control HLMVEC (which constitutively express eNOS) transfected with only the B1R, did not generate significant NO when stimulated with B1R agonist (Zhang et al., 2007). Finally, eNOS protein expression and activity in response to calcium ionophore did not change after cytokine treatment of HLMVEC (Brovkovich et al., 2009). Taken together, the above data show that B1R-dependent NO production in cytokine-treated HLMVEC is due to activation of iNOS.

Interestingly, the profile of NO output generated by B2R-mediated activation of eNOS and B1R-mediated activation of iNOS is strikingly different in HLMVEC (Fig. 2). Thus, whereas

B2R activation results in a peak output of NO of ~165 nM that lasts for about 5 min, B1R-mediated iNOS activation results in a much more profound and prolonged response, reaching a maximum of ~800 nM NO and lasting almost 90 min. In both cases, agonists were present for the duration of the measurements. The specific B1R antagonist, des-Arg¹⁰-Leu⁸-KD, inhibited this response as did the iNOS specific inhibitor 1400W (Fig. 2), but 4 μ M L-NNA, the eNOS inhibitor, had no effect as we previously reported (Ignjatovic et al., 2004).

The most surprising finding was that iNOS activity could be acutely activated in a receptor-dependent process. As discussed above, iNOS was previously known to be regulated only by expression or by the supply of substrate or cofactors. iNOS activity is not regulated by a change in intracellular Ca²⁺ and the known phosphorylation sites on eNOS are not conserved in iNOS nor do kinases activating eNOS phosphorylate or activate iNOS (Fulton et al., 1999; Butt et al., 2000; Zhang et al., 2007). Thus, it was unclear how iNOS was being activated. Further investigation revealed that B1R and iNOS-mediated NO output is dependent on ERK activation, which results in ERK phosphorylation of iNOS at Ser⁷⁴⁵ (Zhang et al., 2007). iNOS mutants were generated to mimic the phosphorylated state (S745D) or prevent ERK-mediated phosphorylation (S745A). The S745D mutant basally produced large amounts of NO that could not be further activated by B1R stimulation. Furthermore, while the S745A mutant had the same basal activity as wild type iNOS, it could not be activated by B1R agonist (Zhang et al., 2007).

We have recently discovered unique aspects of the B1R signaling cascade that are required for stimulation of high output iNOS-derived NO. Although ERK activation is required for the response, it is not by itself sufficient to drive iNOS-mediated NO production. For example, epidermal growth factor robustly activated ERK, but did not stimulate iNOS-derived NO production nor did it alter B1R-mediated iNOS activation (F. Kuhr et al., submitted). Furthermore, activation of iNOS by the B1R is pertussis-toxin sensitive and can be mimicked by transfection of a constitutively active G α i mutant, showing that the signaling pathway is coupled through G α i and not G α q/11 (Brovkovich et al., 2009). Extensive analysis of the signaling pathway leading from B1R activation by peptide agonists or ACE inhibitor to iNOS-derived NO production in both cytokine-treated HLMVEC and transfected HEK293 cells showed that it requires G α i and G β γ -dependent activation of Src, Ras, Raf, MEK and ERK (Fig. 2). Finally, we have also recently demonstrated that this novel signaling pathway also depends on β -arrestin 2 (F. Kuhr et al., submitted). Recently, β -arrestins have been identified as mediators of prolonged ERK activation in response to activation of GPCRs (Lefkowitz and Shenoy, 2005), which is consistent with the prolonged nature of ERK-mediated iNOS activation we found in response to B1R signaling (Fig. 2) (Zhang et al., 2007).

4.5 Roles of B1R and B2R stimulation of NO production

Taken together, the above evidence indicates that B2Rs and B1Rs stimulate different signaling pathways leading to either eNOS or iNOS-mediated NO production (Fig. 1), very likely with different functions. The B2R-eNOS pathway delivers NO that is lower and of shorter duration, and in the vascular system likely regulates blood pressure or maintains normal endothelial barrier function. For example, eNOS knockout mice have elevated blood pressure (Shesely et al., 1996) and also exhibit increased vascular permeability (Predescu et al., 2005). In contrast, the B1R-iNOS pathway generates a much higher and prolonged output of NO. The role of B1R-mediated iNOS activation is currently under investigation and, although high levels of NO are often thought to promote inflammatory responses, the precise role of NO in sepsis and inflammation is still controversial. For example, both animal and clinical studies have shown that NO production may have both salutary and harmful effects (Gross et al., 1996; Artigas et al., 1998; Sittipunt et al., 2001; Wink et al., 2001; Zhu et al., 2001; Tasaka et al., 2002; McClintock et al., 2007; Brovkovich et al., 2008).

Studies utilizing receptor-specific antagonists and B1R or B2R knockout mice have revealed important functions that may relate to the roles of these receptors in regulating NOS activity and resulting changes in vascular tone or permeability. For example, B2R antagonist HOE140 reduced BK-induced cGMP formation and prostacyclin release from cultured endothelial cells (Hock et al., 1991). Inhaled HOE140 inhibited BK-induced increase in lung resistance and airway microvascular leakage in guinea-pigs (Sakamoto et al., 1994). In clinical trials, HOE140 shortened the duration of acute hereditary angioedema attacks (Rosenkranz et al., 2005). The potent pseudopeptide B2R antagonist, NPC-18884 inhibited paw edema and had antihyperalgesic effects in rats and mice (de Campos et al., 1999; Heitsch, 2000).

Experiments with B2R^{-/-} mice have confirmed the ability of BK to induce endothelium-dependent vasodilation (Berthiaume et al., 1997), plasma extravasation (Samadfam et al., 2000) or promote platelet aggregation (Labonte et al., 2001) which are diminished or abolished in B2R^{-/-} mice. B2R^{-/-} mice also exhibit increased hypertension in response to a high salt diet (Alfie et al., 1997). Both BK and NO are known to participate in the cardiac protective effects of ischemic preconditioning (Heitsch, 2000) and the prevention of ischemia/reperfusion injury was lost in B2R^{-/-} mice (Yang et al., 1997). B2R knockout also leads to hypertension, left ventricular remodeling and functional cardiac impairment (Emanuelli et al., 1999). The cardioprotective effects of ACE inhibitors and AT1 receptor antagonists are also dependent on B2R activation as they were reduced in B2R^{-/-} mice (Yang et al., 2001).

It was recently reported that B2R knockout leads to the upregulation of B1R expression and, likewise, B1R knockout results in upregulation of B2R expression and both receptor subtypes contribute to the cardiovascular protective effects mediated by ACE-inhibition (Duka et al., 2008). Another study found that endothelial cells lacking B2Rs resulted in decreased ACE activity (Sabatini et al., 2008), providing additional support for earlier findings that ACE and the B2R form a functional complex (Chen et al., 2006).

B1R-mediated iNOS activation likely plays an important role in inflammation as the inducible B1R is upregulated under the same inflammatory conditions that cause iNOS expression, for example in sepsis (Leeb-Lundberg et al., 2005; Campos et al., 2006; Pesquero and Bader, 2006). B1R^{-/-} mice are healthy, fertile and normotensive and are protected from lipopolysaccharide (LPS)-induced septic shock (Pesquero et al., 2000; Pesquero and Bader, 2006). Constitutive expression of B1R in transgenic mice (Ni et al., 2003) or endothelial-specific expression of B1Rs in transgenic rats (Merino et al., 2008) increased lethality in response to LPS challenge. B1R antagonists also reduced the hypotensive response to endotoxin challenge (McLean et al., 1999). However, the B1R signaling pathways that are activated in sepsis and their role in mediating the septic response have not been studied. Both B1R knockout and B1R antagonists were protective in preventing damage from renal ischemia/reperfusion injury (Wang et al., 2008).

Because B1Rs and B2Rs have overlapping signaling pathways, they may both contribute to some of the pathophysiological responses to kinins. For example, either B1 or B2R antagonists can attenuate the hypotensive response to LPS challenge in rats (Feletou et al., 1996; McLean et al., 1999) as did a combined B1R-B2R antagonist CP0127 (Whalley et al., 1992). In a recent study, there was marked hypotension in response to endotoxin in wild-type and B2R^{-/-} mice, moderate hypotension in B1R^{-/-} mice but blood pressure was unchanged in B1R/B2R^{-/-} mice (Cayla et al., 2007). However, the protection from hypotension in these mice did not increase their survival. In a model of capsaicin-induced cutaneous neurogenic inflammation, both B1R and B2R non-peptide antagonists (SSR 240612 and FR 173657) reduced the inflammatory response as did B1R/B2R^{-/-} double knockout (Pietrovski et al.). Surprisingly, a single knockout of either B1R or B2R had no effect, but inhibition was restored when B1R^{-/-} mice were treated with B2R antagonist FR 173657 or when B2R^{-/-} mice were treated with B1R

antagonist SSR 240612. These data suggest a combined role of the kinin receptors in neurogenic inflammatory responses.

Although B1R activation can have noxious consequences it also has beneficial effects, for example by improving cardiac function after myocardial infarction (Xu et al., 2009). In addition, the human B1R is directly activated by ACE inhibitors, even in the absence of ACE expression, to generate NO (Ignjatovic et al., 2002; Ignjatovic et al., 2004; Skidgel et al., 2006) by the same G α i/ERK pathway as with natural peptide agonists (Brovkovich et al., 2009). The increased enzymatic NO synthesis via B1R activation may also contribute to the cardiac benefits of ACE inhibitors including after myocardial infarction (McMurray et al., 2004; Donnini et al., 2006; Xu et al., 2009). We found that ACE inhibitor/B1R-mediated NO production inhibited PKC ϵ (Stanisavljevic et al., 2006), which could also have beneficial effects on the failing heart (Goldspink et al., 2004). Recently, it was found that B1R signaling prevents homing of encephalitogenic T-lymphocytes into the CNS (Schulze-Toppoff et al., 2009). B1R agonist markedly decreased symptoms of experimental autoimmune encephalomyelitis and a B1R antagonist caused earlier onset and greater severity of disease (Schulze-Toppoff et al., 2009).

Overall, stimulation of B2R and B1R signaling pathways can have both protective and detrimental effects in a variety of disease states. Thus, B2R or B1R agonists and/or antagonists may prove to be useful therapeutic agents depending on the disease or pathological condition (Heitsch, 2000; Leeb-Lundberg et al., 2005; Abraham et al., 2006; Campos et al., 2006; Feng et al., 2008).

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References

- AbdAlla S, Jarnagin K, Muller-Esterl W, Quitterer U. The N-terminal amino group of [Tyr8]bradykinin is bound adjacent to analogous amino acids of the human and rat B2 receptor. *J Biol Chem* 1996;271:27382–27387. [PubMed: 8910316]
- Abraham WM, Scuri M, Farmer SG. Peptide and non-peptide bradykinin receptor antagonists: role in allergic airway disease. *Eur J Pharmacol* 2006;533:215–221. [PubMed: 16455073]
- Adomeit A, Graness A, Gross S, Seedorf K, Wetzker R, Liebmann C. Bradykinin B(2) receptor-mediated mitogen-activated protein kinase activation in COS-7 cells requires dual signaling via both protein kinase C pathway and epidermal growth factor receptor transactivation. *Mol Cell Biol* 1999;19:5289–5297. [PubMed: 10409720]
- Aktan F. iNOS-mediated nitric oxide production and its regulation. *Life Sci* 2004;75:639–653. [PubMed: 15172174]
- Alfie ME, Sigmon DH, Pomposiello SI, Carretero OA. Effect of high salt intake in mutant mice lacking bradykinin-B2 receptors. *Hypertension* 1997;29:483–487. [PubMed: 9039146]
- Andreeva AV, Vaiskunaite R, Kutuzov MA, Profirovic J, Skidgel RA, Voyno-Yasenetskaya T. Novel Mechanisms of G Protein-Dependent Regulation of Endothelial Nitric-Oxide Synthase. *Mol Pharmacol* 2006;69:975–982. [PubMed: 16326932]
- Artigas A, Bernard GR, Carlet J, Dreyfuss D, Gattinoni L, Hudson L, Lamy M, Marini JJ, Matthay MA, Pinsky MR, Spragg R, Suter PM. The American-European Consensus Conference on ARDS, part 2: Ventilatory, pharmacologic, supportive therapy, study design strategies, and issues related to recovery and remodeling. Acute respiratory distress syndrome. *Am J Respir Crit Care Med* 1998;157:1332–1347. [PubMed: 9563759]

- Austin CE, Faussner A, Robinson HE, Chakravarty S, Kyle DJ, Bathon JM, Proud D. Stable expression of the human kinin B1 receptor in Chinese hamster ovary cells. Characterization of ligand binding and effector pathways. *J Biol Chem* 1997;272:11420–11425. [PubMed: 9111052]
- Bachetti T, Comini L, Curello S, Bastianon D, Palmieri M, Bresciani G, Callea F, Ferrari R. Co-expression and modulation of neuronal and endothelial nitric oxide synthase in human endothelial cells. *J Mol Cell Cardiol* 2004;37:939–945. [PubMed: 15522271]
- Bernier SG, Halder S, Michel T. Bradykinin-regulated interactions of the mitogen-activated protein kinase pathway with the endothelial nitric-oxide synthase. *J Biol Chem* 2000;275:30707–30715. [PubMed: 10899167]
- Berthiaume N, Hess F, Chen A, Regoli D, D'Orleans-Juste P. Pharmacology of kinins in the arterial and venous mesenteric bed of normal and B2 knockout transgenic mice. *Eur J Pharmacol* 1997;333:55–61. [PubMed: 9311661]
- Bhoola KD, Figueroa CD, Worthy K. Bioregulation of kinins: kallikreins, kininogens, and kininases. *Pharmacol Rev* 1992;44:1–80. [PubMed: 1313585]
- Blaukat A. Structure and signalling pathways of kinin receptors. *Andrologia* 2003;35:17–23. [PubMed: 12558525]
- Blaukat A, Barac A, Cross MJ, Offermanns S, Dikic I. G protein-coupled receptor-mediated mitogen-activated protein kinase activation through cooperation of G α (q) and G α (i) signals. *Mol Cell Biol* 2000;20:6837–6848. [PubMed: 10958680]
- Blaukat A, Pizard A, Rajerison RM, Alhenc-Gelas F, Muller-Esterl W, Dikic I. Activation of mitogen-activated protein kinase by the bradykinin B2 receptor is independent of receptor phosphorylation and phosphorylation-triggered internalization. *FEBS Lett* 1999;451:337–341. [PubMed: 10371216]
- Brechter AB, Persson E, Lundgren I, Lerner UH. Kinin B1 and B2 receptor expression in osteoblasts and fibroblasts is enhanced by interleukin-1 and tumour necrosis factor- α . Effects dependent on activation of NF- κ B and MAP kinases. *Bone* 2008;43:72–83. [PubMed: 18467203]
- Brovkovich V, Gao X-P, Ong E, Brovkovich S, Brennan M-L, Su X, Hazen SL, Malik AB, Skidgel RA. Augmented inducible nitric oxide synthase expression and increased NO production reduce sepsis-induced lung injury and mortality in myeloperoxidase-null mice. *Am J Physiol Lung Cell Mol Physiol* 2008;295:L96–103. [PubMed: 18424617]
- Brovkovich V, Zhang Y, Brovkovich S, Minshall RD, Skidgel RA. A novel pathway for receptor-mediated post-translational activation of inducible nitric oxide synthase. *Journal of Cellular and Molecular Medicine*. 2009 In press.
- Bujalska M, Makulska-Nowak H. Bradykinin receptors antagonists and nitric oxide synthase inhibitors in vincristine and streptozotocin induced hyperalgesia in chemotherapy and diabetic neuropathy rat model. *Neuro Endocrinol Lett* 2009;30:144–152. [PubMed: 19300402]
- Butt E, Bernhardt M, Smolenski A, Kotsonis P, Frohlich LG, Sickmann A, Meyer HE, Lohmann SM, Schmidt HH. Endothelial nitric-oxide synthase (type III) is activated and becomes calcium independent upon phosphorylation by cyclic nucleotide-dependent protein kinases. *J Biol Chem* 2000;275:5179–5187. [PubMed: 10671564]
- Cale JM, Bird IM. Inhibition of MEK/ERK1/2 signalling alters endothelial nitric oxide synthase activity in an agonist-dependent manner. *Biochem J* 2006;398:279–288. [PubMed: 16716148]
- Campbell WB, Falck JR. Arachidonic Acid Metabolites as Endothelium-Derived Hyperpolarizing Factors. *Hypertension* 2007;49:590–596. [PubMed: 17200437]
- Campos MM, Leal PC, Yunes RA, Calixto JB. Non-peptide antagonists for kinin B1 receptors: new insights into their therapeutic potential for the management of inflammation and pain. *Trends Pharmacol Sci* 2006;27:646–651. [PubMed: 17056130]
- Cayla C, Todiras M, Iliescu R, Saul VV, Gross V, Pilz B, Chai G, Merino VF, Pesquero JB, Baltatu OC, Bader M. Mice deficient for both kinin receptors are normotensive and protected from endotoxin-induced hypotension. *Faseb J* 2007;21:1689–1698. [PubMed: 17289925]
- Chen BC, Lin LL, Lin WW. Protein kinase C epsilon-dependent pathway of extracellular signal-regulated protein kinase activation by P2Y1 and P2Y2 purinoceptors that activate cytosolic phospholipase A2 in endothelial cells. *Eur J Pharmacol* 1999;373:101–110. [PubMed: 10408256]

- Chen DB, Bird IM, Zheng J, Magness RR. Membrane estrogen receptor-dependent extracellular signal-regulated kinase pathway mediates acute activation of endothelial nitric oxide synthase by estrogen in uterine artery endothelial cells. *Endocrinology* 2004;145:113–125. [PubMed: 14512434]
- Chen Z, Deddish PA, Minshall RD, Becker RP, Erdos EG, Tan F. Human ACE and bradykinin B2 receptors form a complex at the plasma membrane. *FASEB J* 2006;20:2261–2270. [PubMed: 17077303]
- Chen ZP, Mitchelhill KI, Michell BJ, Stapleton D, Rodriguez-Crespo I, Witters LA, Power DA, Ortiz de Montellano PR, Kemp BE. AMP-activated protein kinase phosphorylation of endothelial NO synthase. *FEBS Lett* 1999;443:285–289. [PubMed: 10025949]
- Christopher J, Velarde V, Jaffa AA. Induction of B(1)-kinin receptors in vascular smooth muscle cells: cellular mechanisms of map kinase activation. *Hypertension* 2001;38:602–605. [PubMed: 11566939]
- de Campos RO, Alves RV, Ferreira J, Kyle DJ, Chakravarty S, Mavunkel BJ, Calixto JB. Oral antinociception and oedema inhibition produced by NPC 18884, a non-peptidic bradykinin B2 receptor antagonist. *Naunyn Schmiedebergs Arch Pharmacol* 1999;360:278–286. [PubMed: 10543429]
- Dikic I, Tokiwa G, Lev S, Courtneidge SA, Schlessinger J. A role for Pyk2 and Src in linking G-protein-coupled receptors with MAP kinase activation. *Nature* 1996;383:547–550. [PubMed: 8849729]
- Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. *Nature* 1999;399:601–605. [PubMed: 10376603]
- Donnini S, Solito R, Giachetti A, Granger HJ, Ziche M, Morbidelli L. Fibroblast growth factor-2 mediates Angiotensin-converting enzyme inhibitor-induced angiogenesis in coronary endothelium. *J Pharmacol Exp Ther* 2006;319:515–522. [PubMed: 16868034]
- Dudzinski DM, Igarashi J, Greif D, Michel TM. The Regulation and Pharmacology of Endothelial Nitric Oxide Synthases. *Annu Rev Pharmacol Toxicol* 2006;46:235–276. [PubMed: 16402905]
- Dudzinski DM, Michel T. Life history of eNOS: partners and pathways. *Cardiovasc Res* 2007;75:247–260. [PubMed: 17466957]
- Duka A, Kintsurashvili E, Duka I, Ona D, Hopkins TA, Bader M, Gavras I, Gavras H. Angiotensin-converting enzyme inhibition after experimental myocardial infarct: role of the kinin B1 and B2 receptors. *Hypertension* 2008;51:1352–1357. [PubMed: 18347228]
- Emanuelli C, Maestri R, Corradi D, Marchione R, Minasi A, Tozzi MG, Salis MB, Straino S, Capogrossi MC, Olivetti G, Madeddu P. Dilated and failing cardiomyopathy in bradykinin B(2) receptor knockout mice. *Circulation* 1999;100:2359–2365. [PubMed: 10587341]
- Erwin PA, Lin AJ, Golan DE, Michel T. Receptor-regulated dynamic S-nitrosylation of endothelial nitric oxide synthase in vascular endothelial cells. *J Biol Chem* 2005;280:19888–19894. [PubMed: 15774480]
- Feletou M, Jamonneau I, Germain M, Thurieau C, Fauchere JL, Villa P, Ghezzi P, Canet E. Bradykinin B2 receptor involvement in rabbit and murine models of septic shock. *Journal of cardiovascular pharmacology* 1996;27:500–507. [PubMed: 8847866]
- Feng DM, DiPardo RM, Wai JM, Chang RK, Di Marco CN, Murphy KL, Ransom RW, Reiss DR, Tang C, Prueksaritanont T, Pettibone DJ, Bock MG, Kuduk SD. A new class of bradykinin B1 receptor antagonists with high oral bioavailability and minimal PXR activity. *Bioorganic & medicinal chemistry letters* 2008;18:682–687. [PubMed: 18240388]
- Fleming I, Fisslthaler B, Dimmeler S, Kemp BE, Busse R. Phosphorylation of Thr(495) regulates Ca(2+)-calmodulin-dependent endothelial nitric oxide synthase activity. *Circ Res* 2001;88:E68–75. [PubMed: 11397791]
- Fontana J, Fulton D, Chen Y, Fairchild TA, McCabe TJ, Fujita N, Tsuruo T, Sessa WC. Domain mapping studies reveal that the M domain of hsp90 serves as a molecular scaffold to regulate Akt-dependent phosphorylation of endothelial nitric oxide synthase and NO release. *Circ Res* 2002;90:866–873. [PubMed: 11988487]
- Forstermann U, Gath I, Schwarz P, Closs EI, Kleinert H. Isoforms of nitric oxide synthase. Properties, cellular distribution and expressional control. *Biochem Pharmacol* 1995;50:1321–1332. [PubMed: 7503779]

- Fulton D, Gratton JP, McCabe TJ, Fontana J, Fujio Y, Walsh K, Franke TF, Papapetropoulos A, Sessa WC. Regulation of endothelium-derived nitric oxide production by the protein kinase Akt. *Nature* 1999;399:597–601. [PubMed: 10376602]
- Fulton D, Gratton JP, Sessa WC. Post-translational control of endothelial nitric oxide synthase: why isn't calcium/calmodulin enough? *J Pharmacol Exp Ther* 2001;299:818–824. [PubMed: 11714864]
- Fulton D, Ruan L, Sood SG, Li C, Zhang Q, Venema RC. Agonist-stimulated endothelial nitric oxide synthase activation and vascular relaxation. Role of eNOS phosphorylation at Tyr83. *Circ Res* 2008;102:497–504. [PubMed: 18096817]
- Garcia-Cardena G, Martasek P, Masters BS, Skidd PM, Couet J, Li S, Lisanti MP, Sessa WC. Dissecting the interaction between nitric oxide synthase (NOS) and caveolin. Functional significance of the nos caveolin binding domain in vivo. *J Biol Chem* 1997;272:25437–25440. [PubMed: 9325253]
- Ghalayini IF. Nitric oxide-cyclic GMP pathway with some emphasis on cavernosal contractility. *Int J Impot Res* 2004;16:459–469. [PubMed: 15229623]
- Gohla A, Offermanns S, Wilkie TM, Schultz G. Differential involvement of Galpha12 and Galpha13 in receptor-mediated stress fiber formation. *J Biol Chem* 1999;274:17901–17907. [PubMed: 10364236]
- Goldspink PH, Montgomery DE, Walker LA, Urboniene D, McKinney RD, Geenen DL, Solaro RJ, Buttrick PM. Protein kinase Cepsilon overexpression alters myofilament properties and composition during the progression of heart failure. *Circ Res* 2004;95:424–432. [PubMed: 15242976]
- Golser R, Gorren AC, Leber A, Andrew P, Habisch HJ, Werner ER, Schmidt K, Venema RC, Mayer B. Interaction of endothelial and neuronal nitric-oxide synthases with the bradykinin B2 receptor. Binding of an inhibitory peptide to the oxygenase domain blocks uncoupled NADPH oxidation. *J Biol Chem* 2000;275:5291–5296. [PubMed: 10681501]
- Gross SS, Kilbourn RG, Griffith OW. NO in septic shock: good, bad or ugly? Learning from iNOS knockouts. *Trends Microbiol* 1996;4:47–49. [PubMed: 8820564]
- Guan ZW, Iyanagi T. Electron transfer is activated by calmodulin in the flavin domain of human neuronal nitric oxide synthase. *Arch Biochem Biophys* 2003;412:65–76. [PubMed: 12646269]
- Harris MB, Ju H, Venema VJ, Liang H, Zou R, Michell BJ, Chen ZP, Kemp BE, Venema RC. Reciprocal phosphorylation and regulation of endothelial nitric-oxide synthase in response to bradykinin stimulation. *J Biol Chem* 2001;276:16587–16591. [PubMed: 11340086]
- Heitsch H. Bradykinin B2 receptor as a potential therapeutic target. *Drug News Perspect* 2000;13:213–225. [PubMed: 12937626]
- Herzig MC, Leeb-Lundberg LM. The agonist binding site on the bovine bradykinin B2 receptor is adjacent to a sulfhydryl and is differentiated from the antagonist binding site by chemical cross-linking. *J Biol Chem* 1995;270:20591–20598. [PubMed: 7657637]
- Herzig MC, Nash NR, Connolly M, Kyle DJ, Leeb-Lundberg LM. The N terminus of bradykinin when bound to the human bradykinin B2 receptor is adjacent to extracellular Cys20 and Cys277 in the receptor. *J Biol Chem* 1996;271:29746–29751. [PubMed: 8939910]
- Hock FJ, Wirth K, Albus U, Linz W, Gerhards HJ, Wiemer G, Henke S, Breipohl G, König W, Knolle J, et al. Hoe 140 a new potent and long acting bradykinin-antagonist: in vitro studies. *Br J Pharmacol* 1991;102:769–773. [PubMed: 1364851]
- Ignarro LJ, Cirino G, Casini A, Napoli C. Nitric oxide as a signaling molecule in the vascular system: an overview. *J Cardiovasc Pharmacol* 1999;34:879–886. [PubMed: 10598133]
- Ignjatovic T, Stanisavljevic S, Brovkovich V, Skidgel RA, Erdös EG. Kinin B1 receptors stimulate nitric oxide production in endothelial cells: Signaling pathways activated by angiotensin I-converting enzyme inhibitors and peptide ligands. *Mol Pharmacol* 2004;66:1310–1316. [PubMed: 15304551]
- Ignjatovic T, Tan F, Brovkovich V, Skidgel RA, Erdös EG. Novel mode of action of angiotensin I converting enzyme inhibitors: direct activation of bradykinin B1 receptor. *J Biol Chem* 2002;277:16847–16852. [PubMed: 11880373]
- Issandou M, Darbon JM. Des-Arg9 bradykinin modulates DNA synthesis, phospholipase C, and protein kinase C in cultured mesangial cells. Distinction from effects of bradykinin. *J Biol Chem* 1991;266:21037–21043. [PubMed: 1939151]
- Ju H, Venema VJ, Marrero MB, Venema RC. Inhibitory interactions of the bradykinin B2 receptor with endothelial nitric-oxide synthase. *J Biol Chem* 1998;273:24025–24029. [PubMed: 9727019]

- Ju H, Zou R, Venema VJ, Venema RC. Direct interaction of endothelial nitric-oxide synthase and caveolin-1 inhibits synthase activity. *J Biol Chem* 1997;272:18522–18525. [PubMed: 9228013]
- Kim JH, Jain D, Tliba O, Yang B, Jester WF Jr, Panettieri RA Jr, Amrani Y, Pure E. TGF-beta potentiates airway smooth muscle responsiveness to bradykinin. *Am J Physiol Lung Cell Mol Physiol* 2005;289:L511–520. [PubMed: 15923209]
- Kleinert H, Pautz A, Linker K, Schwarz PM. Regulation of the expression of inducible nitric oxide synthase. *Eur J Pharmacol* 2004;500:255–266. [PubMed: 15464038]
- Knowles RG, Moncada S. Nitric oxide synthases in mammals. *Biochem J* 1994;298(Pt 2):249–258. [PubMed: 7510950]
- Kubes P. Nitric oxide affects microvascular permeability in the intact and inflamed vasculature. *Microcirculation* 1995;2:235–244. [PubMed: 8748947]
- Labonte J, Brochu I, Honore JC, D'Orleans-Juste P. Role of ETB and B2 receptors in the ex vivo platelet inhibitory properties of endothelin and bradykinin in the mouse. *Br J Pharmacol* 2001;132:934–940. [PubMed: 11181435]
- Lamb ME, De Weerd WF, Leeb-Lundberg LM. Agonist-promoted trafficking of human bradykinin receptors: arrestin- and dynamin-independent sequestration of the B2 receptor and bradykinin in HEK293 cells. *Biochem J* 2001;355:741–750. [PubMed: 11311137]
- Lamb ME, Zhang C, Shea T, Kyle DJ, Leeb-Lundberg LM. Human B1 and B2 Bradykinin Receptors and Their Agonists Target Caveolae-Related Lipid Rafts to Different Degrees in HEK293 Cells. *Biochemistry* 2002;41:14340–14347. [PubMed: 12450400]
- Leeb-Lundberg LM, Kang DS, Lamb ME, Fathy DB. The human B1 bradykinin receptor exhibits high ligand-independent, constitutive activity. Roles of residues in the fourth intracellular and third transmembrane domains. *J Biol Chem* 2001;276:8785–8792. [PubMed: 11134011]
- Leeb-Lundberg LM, Marceau F, Muller-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. [PubMed: 15734727]
- Lefkowitz RJ, Shenoy SK. Transduction of Receptor Signals by {beta}-Arrestins. *Science* 2005;308:512–517. [PubMed: 15845844]
- Leung PC, Cheng KT, Liu C, Cheung WT, Kwan HY, Lau KL, Huang Y, Yao X. Mechanism of non-capacitative Ca²⁺ influx in response to bradykinin in vascular endothelial cells. *J Vasc Res* 2006;43:367–376. [PubMed: 16791008]
- Liao JK, Homcy CJ. The G proteins of the G alpha i and G alpha q family couple the bradykinin receptor to the release of endothelium-derived relaxing factor. *J Clin Invest* 1993;92:2168–2172. [PubMed: 8227332]
- Liebmann C, Graness A, Ludwig B, Adomeit A, Boehmer A, Boehmer FD, Nurnberg B, Wetzker R. Dual bradykinin B2 receptor signalling in A431 human epidermoid carcinoma cells: activation of protein kinase C is counteracted by a GS-mediated stimulation of the cyclic AMP pathway. *Biochem J* 1996;313(Pt 1):109–118. [PubMed: 8546671]
- Liu J, Garcia-Cardena G, Sessa WC. Biosynthesis and palmitoylation of endothelial nitric oxide synthase: mutagenesis of palmitoylation sites, cysteines-15 and/or -26, argues against depalmitoylation-induced translocation of the enzyme. *Biochemistry* 1995;34:12333–12340. [PubMed: 7547976]
- Luckhoff A, Pohl U, Mulsch A, Busse R. Differential role of extra- and intracellular calcium in the release of EDRF and prostacyclin from cultured endothelial cells. *Br J Pharmacol* 1988;95:189–196. [PubMed: 3064851]
- Luhrs H, Papadopoulos T, Schmidt HH, Menzel T. Type I nitric oxide synthase in the human lung is predominantly expressed in capillary endothelial cells. *Respir Physiol* 2002;129:367–374. [PubMed: 11788139]
- Luksha L, Agewall S, Kublickiene K. Endothelium-derived hyperpolarizing factor in vascular physiology and cardiovascular disease. *Atherosclerosis* 2009;202:330–344. [PubMed: 18656197]
- Lung CC, Jagels MA, Daffern PJ, Tan EM, Zuraw BL. Induction of human B2 bradykinin receptor mRNA and membrane receptors by IFN-gamma. *Immunopharmacology* 1998;39:243–253. [PubMed: 9754910]
- Ma QP, Heavens R. Basal expression of bradykinin B(1) receptor in the spinal cord in humans and rats. *Neuroreport* 2001;12:2311–2314. [PubMed: 11496101]

- Marceau F, Hess JF, Bachvarov DR. The B1 receptors for kinins. *Pharmacol Rev* 1998;50:357–386. [PubMed: 9755287]
- Marceau F, Sabourin T, Houle S, Fortin JP, Petitclerc E, Molinaro G, Adam A. Kinin receptors: functional aspects. *Int Immunopharmacol* 2002;2:1729–1739. [PubMed: 12489786]
- Masters BS, McMillan K, Sheta EA, Nishimura JS, Roman LJ, Martasek P. Neuronal nitric oxide synthase, a modular enzyme formed by convergent evolution: structure studies of a cysteine thiolate-ligated heme protein that hydroxylates L-arginine to produce NO. as a cellular signal. *FASEB J* 1996;10:552–558. [PubMed: 8621055]
- Matsuda H, Iyanagi T. Calmodulin activates intramolecular electron transfer between the two flavins of neuronal nitric oxide synthase flavin domain. *Biochim Biophys Acta* 1999;1473:345–355. [PubMed: 10594372]
- McClintock DE, Ware LB, Eisner MD, Wickersham N, Thompson BT, Matthay MA, the National Heart, L. a. B. I. A. N. Higher Urine Nitric Oxide Is Associated with Improved Outcomes in Patients with Acute Lung Injury. *Am J Respir Crit Care Med* 2007;175:256–262. [PubMed: 17082495]
- McLean PG, Perretti M, Ahluwalia A. Inducible expression of the kinin B1 receptor in the endotoxemic heart: mechanisms of des-Arg9bradykinin-induced coronary vasodilation. *Br J Pharmacol* 1999;128:275–282. [PubMed: 10510436]
- McMurray JJ, Pfeffer MA, Swedberg K, Dzau VJ. Which inhibitor of the renin-angiotensin system should be used in chronic heart failure and acute myocardial infarction? *Circulation* 2004;110:3281–3288. [PubMed: 15545527]
- Merino VF, Todiras M, Campos LA, Saul V, Popova E, Baltatu OC, Pesquero JB, Bader M. Increased susceptibility to endotoxic shock in transgenic rats with endothelial overexpression of kinin B(1) receptors. *J Mol Med*. 2008
- Michel JB, Feron O, Sase K, Prabhakar P, Michel T. Caveolin versus calmodulin. Counterbalancing allosteric modulators of endothelial nitric oxide synthase. *J Biol Chem* 1997;272:25907–25912. [PubMed: 9325323]
- Michel T, Li GK, Busconi L. Phosphorylation and subcellular translocation of endothelial nitric oxide synthase. *Proc Natl Acad Sci U S A* 1993;90:6252–6256. [PubMed: 7687064]
- Michell BJ, Griffiths JE, Mitchelhill KI, Rodriguez-Crespo I, Tiganis T, Bozinovski S, de Montellano PR, Kemp BE, Pearson RB. The Akt kinase signals directly to endothelial nitric oxide synthase. *Curr Biol* 1999;9:845–848. [PubMed: 10469573]
- Michell BJ, Harris MB, Chen ZP, Ju H, Venema VJ, Blackstone MA, Huang W, Venema RC, Kemp BE. Identification of regulatory sites of phosphorylation of the bovine endothelial nitric-oxide synthase at serine 617 and serine 635. *J Biol Chem* 2002;277:42344–42351. [PubMed: 12171920]
- Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 1991;43:109–142. [PubMed: 1852778]
- Morgan-Boyd R, Stewart JM, Vavrek RJ, Hassid A. Effects of bradykinin and angiotensin II on intracellular Ca²⁺ dynamics in endothelial cells. *Am J Physiol* 1987;253:C588–598. [PubMed: 3661696]
- Nardone J, Hogan PG. Delineation of a region in the B2 bradykinin receptor that is essential for high-affinity agonist binding. *Proc Natl Acad Sci U S A* 1994;91:4417–4421. [PubMed: 8183923]
- Newton R, Eddleston J, Haddad el B, Hawisa S, Mak J, Lim S, Fox AJ, Donnelly LE, Chung KF. Regulation of kinin receptors in airway epithelial cells by inflammatory cytokines and dexamethasone. *Eur J Pharmacol* 2002;441:193–202. [PubMed: 12063092]
- Ni A, Yin H, Agata J, Yang Z, Chao L, Chao J. Overexpression of Kinin B1 Receptors Induces Hypertensive Response to Des-Arg9-bradykinin and Susceptibility to Inflammation. *J Biol Chem* 2003;278:219–225. [PubMed: 12411434]
- Papapetropoulos A, Rudic RD, Sessa WC. Molecular control of nitric oxide synthases in the cardiovascular system. *Cardiovasc Res* 1999;43:509–520. [PubMed: 10690323]
- Pesquero JB, Araujo RC, Heppenstall PA, Stucky CL, Silva JA Jr, Walther T, Oliveira SM, Pesquero JL, Paiva AC, Calixto JB, Lewin GR, Bader M. Hypoalgesia and altered inflammatory responses in mice lacking kinin B1 receptors. *Proc Natl Acad Sci U S A* 2000;97:8140–8145. [PubMed: 10859349]

- Pesquero JB, Bader M. Genetically altered animal models in the kallikrein-kinin system. *Biol Chem* 2006;387:119–126. [PubMed: 16497143]
- Pietrovski EF, Otuki MF, Regoli D, Bader M, Pesquero JB, Cabrini DA, Zampronio AR. The non-peptide kinin receptor antagonists FR 173657 and SSR 240612: preclinical evidence for the treatment of skin inflammation. *Regulatory peptides* 2009;152:67–72. [PubMed: 18977249]
- Prabhakar P, Cheng V, Michel T. A chimeric transmembrane domain directs endothelial nitric-oxide synthase palmitoylation and targeting to plasmalemmal caveolae. *J Biol Chem* 2000;275:19416–19421. [PubMed: 10787410]
- Prado GN, Taylor L, Zhou X, Ricupero D, Mierke DF, Polgar P. Mechanisms regulating the expression, self-maintenance, and signaling-function of the bradykinin B2 and B1 receptors. *J Cell Physiol* 2002;193:275–286. [PubMed: 12384980]
- Predescu D, Predescu S, Shimizu J, Miyawaki-Shimizu K, Malik AB. Constitutive eNOS-derived nitric oxide is a determinant of endothelial junctional integrity. *Am J Physiol Lung Cell Mol Physiol* 2005;289:L371–381. [PubMed: 16093363]
- Regoli D, Barabe J. Pharmacology of bradykinin and related kinins. *Pharmacol Rev* 1980;32:1–46. [PubMed: 7015371]
- Regoli D, Jukic D, Gobeil F, Rhaleb NE. Receptors for bradykinin and related kinins: a critical analysis. *Can J Physiol Pharmacol* 1993;71:556–567. [PubMed: 8306193]
- Reverter D, Maskos K, Tan F, Skidgel RA, Bode W. Crystal structure of human carboxypeptidase M, a membrane-bound enzyme that regulates peptide hormone activity. *J Mol Biol* 2004;338:257–269. [PubMed: 15066430]
- Robinson LJ, Michel T. Mutagenesis of palmitoylation sites in endothelial nitric oxide synthase identifies a novel motif for dual acylation and subcellular targeting. *Proc Natl Acad Sci U S A* 1995;92:11776–11780. [PubMed: 8524847]
- Roman LJ, Masters BS. Electron transfer by neuronal nitric-oxide synthase is regulated by concerted interaction of calmodulin and two intrinsic regulatory elements. *J Biol Chem* 2006;281:23111–23118. [PubMed: 16782703]
- Rosenkranz B, Bork K, Frank J, Kreuz W, Dong L, Knolle J. Proof-of-concept study of icatibant (JE 049), a bradykinin B2 receptor antagonist in treatment of hereditary angioedema. *Clin Pharmacol Ther* 2005;77:P14–P14.
- Sabatini RA, Guimaraes PB, Fernandes L, Reis FC, Bersanetti PA, Mori MA, Navarro A, Hilzendeger AM, Santos EL, Andrade MC, Chagas JR, Pesquero JL, Casarini DE, Bader M, Carmona AK, Pesquero JB. ACE activity is modulated by kinin B2 receptor. *Hypertension* 2008;51:689–695. [PubMed: 18212275]
- Sakamoto T, Sun J, Barnes PJ, Chung KF. Effect of a bradykinin receptor antagonist, HOE 140, against bradykinin- and vagal stimulation-induced airway responses in the guinea-pig. *Eur J Pharmacol* 1994;251:137–142. [PubMed: 8149970]
- Samadifam R, Teixeira C, Bkaily G, Sirois P, de Brum-Fernandes A, D'Orleans-Juste P. Contribution of B(2) receptors for bradykinin in arthus reaction-induced plasma extravasation in wild-type or B(2) transgenic knockout mice. *Br J Pharmacol* 2000;129:1732–1738. [PubMed: 10780980]
- Schilling WP, Rajan L, Strobl-Jager E. Characterization of the bradykinin-stimulated calcium influx pathway of cultured vascular endothelial cells. Saturability, selectivity, and kinetics. *J Biol Chem* 1989;264:12838–12848. [PubMed: 2546937]
- Schmidt K, Gibraeil HD, Mayer B. Lack of involvement of extracellular signal-regulated kinase (ERK) in the agonist-induced endothelial nitric oxide synthesis. *Biochem Pharmacol* 2002;63:1137–1142. [PubMed: 11931846]
- Schulze-Toppf U, Prat A, Prozorovski T, Siffrin V, Paterka M, Herz J, Bendix I, Ifergan I, Schadock I, Mori MA, Van Horssen J, Schroter F, Smorodchenko A, Han MH, Bader M, Steinman L, Aktas O, Zipp F. Activation of kinin receptor B1 limits encephalitogenic T lymphocyte recruitment to the central nervous system. *Nat Med* 2009;15:788–793. [PubMed: 19561616]
- Shaul PW. Regulation of endothelial nitric oxide synthase: location, location, location. *Annu Rev Physiol* 2002;64:749–774. [PubMed: 11826287]

- Shaul PW, Smart EJ, Robinson LJ, German Z, Yuhanna IS, Ying Y, Anderson RG, Michel T. Acylation targets endothelial nitric-oxide synthase to plasmalemmal caveolae. *J Biol Chem* 1996;271:6518–6522. [PubMed: 8626455]
- Shesely EG, Maeda N, Kim HS, Desai KM, Krege JH, Laubach VE, Sherman PA, Sessa WC, Smithies O. Elevated blood pressures in mice lacking endothelial nitric oxide synthase. *Proc Natl Acad Sci U S A* 1996;93:13176–13181. [PubMed: 8917564]
- Shughrue PJ, Ky B, Austin CP. Localization of B1 bradykinin receptor mRNA in the primate brain and spinal cord: an in situ hybridization study. *J Comp Neurol* 2003;465:372–384. [PubMed: 12966562]
- Sittipunt C, Steinberg KP, Ruzinski JT, Myles C, Zhu SHA, Goodman RB, Hudson LD, Matalon S, Martin TR. Nitric Oxide and Nitrotyrosine in the Lungs of Patients with Acute Respiratory Distress Syndrome. *Am J Respir Crit Care Med* 2001;163:503–510. [PubMed: 11179131]
- Skidgel RA. Proliferation of regulatory mechanisms for eNOS: an emerging role for the cytoskeleton. *Am J Physiol Lung Cell Mol Physiol* 2002;282:L1179–1182. [PubMed: 12003771]
- Skidgel RA, Erdös EG. Cellular carboxypeptidases. *Immunol Rev* 1998;161:129–141. [PubMed: 9553770]
- Skidgel, RA.; Erdös, EG.; Said, SI. Pro-inflammatory and Anti-inflammatory Peptides. Marcel Dekker; New York: 1998. Enzymatic breakdown of bradykinin; p. 459-476.
- Skidgel RA, Stanisavljevic S, Erdos EG. Kinin- and angiotensin-converting enzyme (ACE) inhibitor-mediated nitric oxide production in endothelial cells. *Biol Chem* 2006;387:159–165. [PubMed: 16497147]
- Smith JA, Webb C, Holford J, Burgess GM. Signal transduction pathways for B1 and B2 bradykinin receptors in bovine pulmonary artery endothelial cells. *Mol Pharmacol* 1995;47:525–534. [PubMed: 7700250]
- Stanisavljevic S, Ignjatovic T, Deddish PA, Brovkovich V, Zhang K, Erdos EG, Skidgel RA. Angiotensin I-converting enzyme inhibitors block protein kinase C epsilon by activating bradykinin B1 receptors in human endothelial cells. *J Pharmacol Exp Ther* 2006;316:1153–1158. [PubMed: 16282523]
- Stuehr DJ. Structure-function aspects in the nitric oxide synthases. *Annu Rev Pharmacol Toxicol* 1997;37:339–359. [PubMed: 9131257]
- Tasaka S, Hasegawa N, Ishizaka A. Pharmacology of acute lung injury. *Pulm Pharmacol Ther* 2002;15:83–95. [PubMed: 12090781]
- Tirapelli CR, Bonaventura D, de Oliveira AM. Functional characterization of the mechanisms underlying bradykinin-induced relaxation in the isolated rat carotid artery. *Life Sci* 2007;80:1799–1805. [PubMed: 17367816]
- Tropea MM, Gummelt D, Herzig MS, Leeb-Lundberg LM. B1 and B2 kinin receptors on cultured rabbit superior mesenteric artery smooth muscle cells: receptor-specific stimulation of inositol phosphate formation and arachidonic acid release by des-Arg9-bradykinin and bradykinin. *J Pharmacol Exp Ther* 1993;264:930–937. [PubMed: 8382284]
- Tsutsui M, Onoue H, Iida Y, Smith L, O'Brien T, Katusic ZS. B(1) and B(2) bradykinin receptors on adventitial fibroblasts of cerebral arteries are coupled to recombinant eNOS. *Am J Physiol Heart Circ Physiol* 2000;278:H367–372. [PubMed: 10666066]
- Velarde V, Ullian ME, Morinelli TA, Mayfield RK, Jaffa AA. Mechanisms of MAPK activation by bradykinin in vascular smooth muscle cells. *Am J Physiol* 1999;277:C253–261. [PubMed: 10444401]
- Venema RC. Post-translational mechanisms of endothelial nitric oxide synthase regulation by bradykinin. *Int Immunopharmacol* 2002;2:1755–1762. [PubMed: 12489789]
- Voyno-Yasensetskaya TA, Tkachuk VA, Cheknyova EG, Panchenko MP, Grigorian GY, Vavrek RJ, Stewart JM, Ryan US. Guanine nucleotide-dependent, pertussis toxin-insensitive regulation of phosphoinositide turnover by bradykinin in bovine pulmonary artery endothelial cells. *Faseb J* 1989;3:44–51. [PubMed: 2535990]
- Walker K, Perkins M, Dray A. Kinins and kinin receptors in the nervous system. *Neurochem Int* 1995;26:1–16. discussion 17–26. [PubMed: 7787759]
- Wang PH, Campanholle G, Cenedeze MA, Feitoza CQ, Goncalves GM, Landgraf RG, Jancar S, Pesquero JB, Pacheco-Silva A, Camara NO. Bradykinin B1 receptor antagonism is beneficial in renal ischemia-reperfusion injury. *PLoS ONE* 2008;3:e3050. [PubMed: 18725957]

- Whalley ET, Solomon JA, Modafferi DM, Bonham KA, Cheronis JC. CP0127, a novel potent bradykinin antagonist, increases survival in rat and rabbit models of endotoxin shock. *Agents and actions* 1992;38(Pt 3):413–420. [PubMed: 1462875]
- Wiemer G, Wirth K. Production of cyclic GMP via activation of B1 and B2 kinin receptors in cultured bovine aortic endothelial cells. *J Pharmacol Exp Ther* 1992;262:729–733. [PubMed: 1323660]
- Wink DA, Miranda KM, Espey MG, Pluta RM, Hewett SJ, Colton C, Vitek M, Feelisch M, Grisham MB. Mechanisms of the antioxidant effects of nitric oxide. *Antioxid Redox Signal* 2001;3:203–213. [PubMed: 11396476]
- Wotherspoon G, Winter J. Bradykinin B1 receptor is constitutively expressed in the rat sensory nervous system. *Neurosci Lett* 2000;294:175–178. [PubMed: 11072143]
- Xu J, Carretero OA, Shesely EG, Rhaleb NE, Yang JJ, Bader M, Yang XP. The kinin B1 receptor contributes to the cardioprotective effect of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers in mice. *Exp Physiol* 2009;94:322–329. [PubMed: 19060116]
- Yang X, Taylor L, Polgar P. Effect of the G-protein, G alpha(i2), and G alpha(i3) subunit knockdown on bradykinin-induced signal transduction in rat-1 cells. *Mol Cell Biol Res Commun* 1999;1:227–236. [PubMed: 10425231]
- Yang XP, Liu YH, Mehta D, Cavasin MA, Shesely E, Xu J, Liu F, Carretero OA. Diminished cardioprotective response to inhibition of angiotensin-converting enzyme and angiotensin II type 1 receptor in B(2) kinin receptor gene knockout mice. *Circ Res* 2001;88:1072–1079. [PubMed: 11375278]
- Yang XP, Liu YH, Scicli GM, Webb CR, Carretero OA. Role of kinins in the cardioprotective effect of preconditioning: study of myocardial ischemia/reperfusion injury in B2 kinin receptor knockout mice and kininogen-deficient rats. *Hypertension* 1997;30:735–740. [PubMed: 9323015]
- Zhang X, Tan F, Zhang Y, Skidgel RA. Carboxypeptidase M and kinin B1 receptors interact to facilitate efficient b1 signaling from B2 agonists. *J Biol Chem* 2008;283:7994–8004. [PubMed: 18187413]
- Zhang Y, Brovkovich V, Brovkovich S, Tan F, Lee BS, Sharma T, Skidgel RA. Dynamic receptor-dependent activation of inducible nitric-oxide synthase by ERK-mediated phosphorylation of Ser745. *J Biol Chem* 2007;282:32453–32461. [PubMed: 17804409]
- Zhou L, Zhu DY. Neuronal nitric oxide synthase: structure, subcellular localization, regulation, and clinical implications. *Nitric Oxide* 2009;20:223–230. [PubMed: 19298861]
- Zhu SHA, Ware LB, Geiser T, Matthay MA, Matalon S. Increased Levels of Nitrate and Surfactant Protein A Nitration in the Pulmonary Edema Fluid of Patients with Acute Lung Injury. *Am J Respir Crit Care Med* 2001;163:166–172. [PubMed: 11208643]

Fig. 1. Schematic diagram showing the generation of kinin peptide agonists for the B2R and B1R and downstream signaling

Bradykinin and kallidin, generated by the action of plasma or tissue kallikrein on precursor high-molecular-weight (HMW) or low-molecular-weight (LMW) kininogen, are ligands of the B2R. They are converted to corresponding agonists of the B1R by removal of the C-terminal Arg by membrane-bound carboxypeptidase M (CPM), which interacts with the B1R, or soluble plasma carboxypeptidase N (CPN). The B2R is constitutively expressed whereas B1R expression is induced by injury or inflammatory conditions. Both the B2R and B1R can couple through either Gαq/11 or Gαi/o to release downstream mediators such as intracellular Ca²⁺, NO and arachidonic acid, which leads to generation of prostaglandins and other metabolites such as epoxyeicosatrienoic acids (which can act as endothelial derived hyperpolarizing factor). On endothelial cells, activation of B2Rs results in Gαq/11 and Ca²⁺-calmodulin-dependent activation of eNOS as well as Akt activation and phosphorylation of Ser¹¹⁷⁷ (as well as other sites not shown), dephosphorylation of Thr⁴⁹⁵ and generation of NO. However, in endothelial cells under inflammatory conditions, B1R stimulation results in much higher and prolonged NO production via Gαi, Gβγ and Src-dependent activation of the ERK/MAP kinase pathway leading to activation of iNOS via phosphorylation at Ser⁷⁴⁵. See text for further details.

Fig. 2. Comparison of B2R and B1R - mediated NO generation in control and cytokine-treated HLMVECs
HLMVECs were pre-incubated in normal medium (**A**; Control) or with IL-1 β (5 ng/ml) and IFN- γ (100 U/ml) for 16–24 h (**B**; Cytokine-treated). **A**. eNOS was activated with Ca²⁺ ionophore A23187 (10 μ M), BK (100 nM) or 10 μ M A23187 followed by 100 nM BK as indicated by the arrows. Measurements were made in the continuous presence of agonists. **B**. Cytokine-treated HLMVECs were treated with 100 nM des-Arg¹⁰-kallidin (DAKD) alone or after preincubation (20 min) with 1 μ M B1R antagonist des-Arg¹⁰-Leu⁸-KD (DALKD) or iNOS inhibitor 1400W (4 μ M). In both **A** & **B**, NO production was measured in real time with a porphyrinic electrode in the continuous presence of agonist. Data shown in **B** are from (Zhang et al., 2007).