

# International Union of Pharmacology. XLV. Classification of the Kinin Receptor Family: from Molecular Mechanisms to Pathophysiological Consequences

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**Abstract**—Kinins are proinflammatory peptides that mediate numerous vascular and pain responses to tissue injury. Two pharmacologically distinct kinin receptor subtypes have been identified and characterized for these peptides, which are named B<sub>1</sub> and B<sub>2</sub> and belong to the rhodopsin family of G protein-coupled receptors. The B<sub>2</sub> receptor mediates the action of bradykinin (BK) and lysyl-bradykinin (Lys-BK), the first set of bioactive kinins formed in response to injury from kininogen precursors through the actions of plasma and tissue kallikreins, whereas the B<sub>1</sub> receptor mediates the action of des-Arg<sup>9</sup>-BK and Lys-des-Arg<sup>9</sup>-BK, the second set of bioactive kinins formed through the actions of carboxypeptidases on BK and Lys-BK, respectively. The B<sub>2</sub> receptor is ubiquitous and constitutively expressed, whereas the B<sub>1</sub> receptor is expressed at a very low level in healthy tissues but in-

duced following injury by various proinflammatory cytokines such as interleukin-1 $\beta$ . Both receptors act through G $\alpha_q$  to stimulate phospholipase C $\beta$  followed by phosphoinositide hydrolysis and intracellular free Ca<sup>2+</sup> mobilization and through G $\alpha_i$  to inhibit adenylate cyclase and stimulate the mitogen-activated protein kinase pathways. The use of mice lacking each receptor gene and various specific peptidic and non-peptidic antagonists have implicated both B<sub>1</sub> and B<sub>2</sub> receptors as potential therapeutic targets in several pathophysiological events related to inflammation such as pain, sepsis, allergic asthma, rhinitis, and edema, as well as diabetes and cancer. This review is a comprehensive presentation of our current understanding of these receptors in terms of molecular and cell biology, physiology, pharmacology, and involvement in human disease and drug development.

## I. A Short History of Kinins and Their Receptors

Kinin receptors have come a long way since Rocha e Silva made some speculations about their dual nature in his visionary book on "Kinin Hormones" (Rocha e Silva, 1970). He concluded in 1970 that "... it is perhaps a little early to deduce any convincing configuration for the bradykinin receptors from the data obtained by studying the biological actions of bradykinin analogs and derivatives" (Rocha e Silva, 1970). Indeed, it took almost a quarter of a century before the first kinin receptor cDNA was cloned (McEachern et al., 1991), and more than a dozen years later the three-dimensional structure of a kinin receptor is still elusive.

Tracing back the history of kinin receptors inevitably leads to the early work of the 1930's when Werle and Frey described kallikrein, the kinin-producing enzyme from pancreas (Kraut et al., 1930), and identified the biological actions of lysyl-bradykinin (Lys-BK<sup>1</sup>) or kalli-

<sup>1</sup>Abbreviations: Lys-BK, kallidin; BK, bradykinin; GPCR, G protein-coupled receptor; NO, nitric oxide; eNOS, endothelial nitric-oxide synthase; LPS, lipopolysaccharide; ACE, angiotensin-converting enzyme; CHO, Chinese hamster ovary; HEK, human embryonic kidney; TM, transmembrane domain; PI, phosphoinositide; PKC, protein kinase C; MAPK, mitogen-activated protein kinase; STAT, signal transducer and activator of transcription; EGF, epidermal growth factor; Igl,  $\alpha$ -2-indanyl-glycine; Hyp, hydroxyproline; Oic, octahydroindole-2 acetic acid; Thi,  $\beta$ -thienyl-alanine; Tic, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid;

din, then called substance DK (Werle and Grunz, 1939). In the 1940's, the kinins came into the limelight with the identification of bradykinin (BK) by Rocha e Silva and his colleagues (Rocha e Silva et al., 1949). At the end of the 1950's, BK had been isolated in significant quantities from plasma (Elliott et al., 1959), and finally its primary structure was solved in a dramatic race between the groups of Boissonnas and Elliott (Boissonnas et al., 1960; Elliott et al., 1960a,b). Interestingly, BK was the first biologically active peptide to be assembled by the then newly developed solid phase peptide synthesis (Merrifield, 1964). By the end of the 1960's, hundreds of different kinin derivatives had been synthesized and analyzed for their biological effects in vitro and in vivo (Frey et al., 1967).

In the 1970's, Regoli and coworkers set the early landmarks in the molecular characterization of kinin receptors by pointing to the existence of two types of kinin receptors, B<sub>1</sub> and B<sub>2</sub>, which differ in their pharmacological profiles as well as in their expression patterns (Regoli et al., 1977, 1978; Drouin et al., 1979) (Table 1 and Fig. 1). The development of specific agonists and antagonists to the B<sub>1</sub> receptor by the same group was instrumental in establishing the dualism of kinin receptors in mammals (Regoli et al., 1977; Regoli and Barabé, 1980). At the same time, the first steps toward the solubilization and purification of the receptors were made by Goodfriend and coworkers (Ody and Goodfriend, 1979). In the 1980's, a breakthrough came from the development of the first bradykinin analogs with antagonist activity at the B<sub>2</sub> receptor (Vavrek and Stewart, 1985).

Hoe 140, icatibant, D-Arg[Hyp<sup>3</sup>, Thi<sup>5</sup>, D-Tic<sup>7</sup>, Oic<sup>8</sup>]-BK; NPC17731, D-Arg[Hyp<sup>3</sup>, D-HypE(transpropyl)<sup>7</sup>, Oic<sup>8</sup>]-BK; B9430, D-Arg-[Hyp<sup>3</sup>, Igl<sup>5</sup>, D-Igl<sup>7</sup>, Oic<sup>8</sup>]-BK; B9858, Lys-Lys-[Hyp<sup>3</sup>, Igl<sup>5</sup>, D-Igl<sup>7</sup>, Oic<sup>8</sup>]-des-Arg<sup>9</sup>-BK; CP-0127, delatibant; FR190997, 8-[2,6-dichloro-3-[N-(E)-4-(N-methyl-carbamoyl)cinnamidoacetyl]-N-methylamino]benzyloxy]-2-methyl-4-(2-pyridylmethoxy)quinoline; FR173657, (E)-3-(6-acetamido-3-pyridyl)-N-[N-[2,4-dichloro-3-[(2-methyl-8-quinolinyl)oxymethyl]phenyl]-N-methylaminocarbonylmethyl]acrylamide; WIN64338, [(S)-4-[2-bis(cyclohexylamino)methyleneamino]-3-(2-naphthalenyl)-1-oxopropylamino]benzyl tributyl phosphonium chloride hydrochloride; LF 16-0687, 1-[[2,4-dichloro-3-[(2,4-dimethylquinolin-8-yl)oxy]methyl]phenyl]sulfonyl]-N-[3-[[4-(aminoiminomethyl)phenyl]carbonylamino]propyl]-2(S)-pyrrolidine carboxamide; SSR240612, (2R)-2-[[[(3R)-3-(1,3-benzodioxol-5-yl)-3-[[6-methoxy-2-naphthyl]sulfonyl]amino]propanoyl]amino]-3-(4-[[2R,6S]-2,6-dimethylpiperidinyl]methyl]phenyl)-N-isopropyl-N-methylpropanamide hydrochloride; NF-κB, nuclear factor of the κ-enhancer in B cells; IL, interleukin; AP-1, activator protein-1; ERK, extracellular signal-regulated kinase; GRK, G protein-coupled receptor kinase; GFP, green fluorescent protein; PD 98059, 2-(2-amino-3-methoxyphenyl)-4H-1-benzopyran-4-one; SB 203580, 4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)-1H-imidazole; TNF, tumor necrosis factor; bp, base pair(s); COX, cyclooxygenase; B9340, D-Arg-[Hyp<sup>3</sup>, Thi<sup>5</sup>, D-Igl<sup>7</sup>, Oic<sup>8</sup>]-BK; SNP, single nucleotide polymorphism; LV, left ventricular; CNS, central nervous system; HAE, hereditary angioedema; FR184280, 8-[[2,6-dichloro-3-[N-methyl-N-(E)-3-[6-(E)-2-(4-pyridinyl)ethenyl]pyridin-3-yl]acryloyl]glycyl]amino]benzyl-oxy]-2-methylquinoline; LF 16-0335, 1-[[3-[(2,4-dimethylquinolin-8-yl)oxymethyl]-2,4-dichloro-phenyl]sulphonyl]-2(S)-[[4-[4-(aminoiminomethyl)phenyl]carbonyl]piperazin-1-yl]carbonyl]pyrrolidine; CP-0597, D-Arg<sup>0</sup>-[Hyp<sup>3</sup>, Thi<sup>5</sup>, D-Tic<sup>7</sup>-N-Chg<sup>8</sup>]-BK.

TABLE 1  
Human B<sub>1</sub> and B<sub>2</sub> receptor nomenclature

International Union of Pharmacology (IUPHAR) name	B <sub>1</sub>	B <sub>2</sub>
IUPHAR code	2.1:BK:1:B1: HUMAN:00	2.1:BK:2:B2: HUMAN:00
Alternate names	Bradykinin B <sub>1</sub>	Bradykinin B <sub>2</sub>
Amino acid composition	353	391 <sup>a</sup>
Gene/chromosome	<i>BDKRB1</i> /14q32.2	<i>BDKRB2</i> /14q32.2
Gene accession no.	U12512	M88714
SwissProt accession no.	P46663	P30411
Selective agonist	Lys-des-Arg <sup>9</sup> -BK	BK
Selective antagonist	Lys-[Leu <sup>8</sup> ]des-Arg <sup>9</sup> -BK	Icatibant
Primary G protein coupling	Gα <sub>q</sub> /Gα <sub>i</sub>	Gα <sub>q</sub> /Gα <sub>i</sub>
Primary regulation		
Expression	Induction	Constitutive
Signaling	Limited desensitization	Extensive desensitization

<sup>a</sup> Includes N-terminal sequence coded in exon 2 (27 residues; AbdAlla et al., 1996a).

The identification of the B<sub>2</sub> receptor as a G protein-coupled receptor (GPCR), which signals through the phospholipase C pathway leading to inositol 3-phosphate (IP<sub>3</sub>) formation and intracellular Ca<sup>2+</sup> mobilization and through the phospholipase A<sub>2</sub> pathway resulting in arachidonic acid release, was another landmark discovery that paved the way for the identification of the many intracellular signaling routes triggered by the kinin receptors (Higashida et al., 1986; Burch and Axelrod, 1987). At the same time, the role of BK as a potent stimulator of endothelial nitric-oxide synthase (eNOS) via the B<sub>2</sub> receptor signaling pathway was recognized (Palmer et al., 1987).

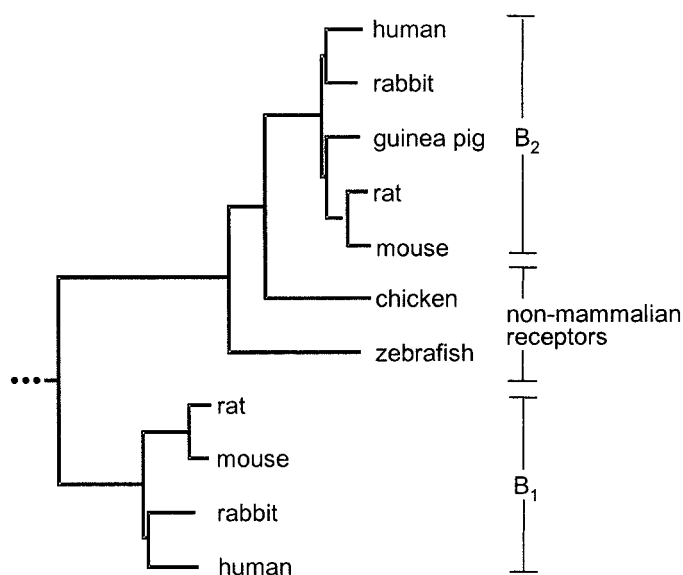


FIG. 1. Phylogenetic tree for the kinin receptor family. The dendrogram was constructed using PAUP and Clustalw1.7 software. Branch lengths are proportional to distances between sequences. Human AT<sub>2</sub> receptor (not shown) was used as an outgroup to root the tree (modified after Schroeder et al., 1997 and Dunér et al., 2002).

The search for kinin receptors culminated in the 1990s with the cloning of the rat B<sub>2</sub> receptor cDNA by Jarnagin and coworkers (McEachern et al., 1991). Soon after, Hess and coworkers succeeded in cloning the first B<sub>1</sub> receptor (Menke et al., 1994), and this was followed by the identification of the first nonmammalian kinin receptor, the chick ornithokinin receptor (Schroeder et al., 1997). More recently, the advent of the first nonpeptidic B<sub>2</sub> receptor antagonists has opened new possibilities for the application of kinin receptor blockers in various diseases (Asano et al., 1997). Eventually the targeted ablation of the genes for the B<sub>2</sub> (Borkowski et al., 1995) and B<sub>1</sub> receptor (Pesquero et al., 2000) in mice has helped to define more precisely the (patho)physiological roles of kinin receptors. This goal was further accomplished with the most recent advent of double knockout mice lacking both kinin receptor genes rendering these animals resistant to LPS-induced septic shock (Cayla et al., 2002b). Clearly, routine clinical applications of kinin receptor antagonists in various disease states are to be expected soon.

## II. Pharmacological Classification of Kinin Receptor Subtypes

### A. Peptide Agonists

Thousands of peptide kinin analogs have been synthesized and pharmacologically evaluated. Kininogens are defined as circulating proteins that contain the BK sequence, but Lys-BK is also a cleavage product of human kininogen (Fig. 2). These sequences and their metabolites without the C-terminal arginine residue (des-Arg<sup>9</sup>) assume particular importance as natural ligands of the two human receptor (R) subtypes (B<sub>1</sub>, B<sub>2</sub>; Table 2). The B<sub>2</sub> receptor has a high affinity for “native” kinins (those generated by either plasma or tissue kallikreins), BK and kallidin, in all mammalian species. Kallidin has itself a significant affinity at the human and rabbit B<sub>1</sub> receptors, but not at the mouse B<sub>1</sub> receptor (Table 2). The most discriminative structural determinant for high affinity at all mammalian B<sub>1</sub> receptors is the removal of the C-terminal arginine, which at the same time is detrimental for affinity toward the B<sub>2</sub> receptor. This holds true for Lys-BK and Lys-des-Arg<sup>9</sup>-BK, which have higher affinities for the human and rabbit B<sub>1</sub> receptors compared with BK and des-Arg<sup>9</sup>-BK, respectively (Table 2, Fig. 2). The only natural kinin sequence with a subnanomolar affinity for the human and rabbit B<sub>1</sub> receptors is Lys-des-Arg<sup>9</sup>-BK, a fact of particular significance that may suggest that the B<sub>1</sub> receptor belongs to the “tissue” kallikrein-kinin system, jointly with tissue kallikrein that generates Lys-BK from low molecular weight kininogen (L-kininogen). If one considers a more distantly related mammalian species, the pig, functional data (porcine renal vein contractility) show that the B<sub>1</sub> receptor is also preferentially stimulated by Lys-des-Arg<sup>9</sup>-BK, 16-fold more potent than des-Arg<sup>9</sup>-BK (Rizzi et al., 1997). Thus, an order of agonist potency widely valid

for mammalian kinin receptors could be: B<sub>1</sub>, Lys-des-Arg<sup>9</sup>-BK > Lys-BK ≈ des-Arg<sup>9</sup>-BK ≫ BK; B<sub>2</sub>, BK ≈ Lys-BK ≫ des-Arg<sup>9</sup>-BK and Lys-des-Arg<sup>9</sup>-BK.

However, the pharmacological profile varies significantly for this set of four natural peptides in the Muridae rodents (the laboratory rat and mouse; Ni et al., 1998a; Table 2). Lys-des-Arg<sup>9</sup>-BK has no decisive advantage over des-Arg<sup>9</sup>-BK in these species. Indeed, a form of coevolution of the B<sub>1</sub> receptor with the kininogen genes may have occurred to maintain the B<sub>1</sub> receptor function by conferring a subnanomolar affinity to des-Arg<sup>9</sup>-BK since the kininogen genes in rodents code for an arginine instead of a lysine residue N-terminal to the BK sequence or Ile-Ser in T-kininogen (Hess et al., 1996; Takano et al., 1997). The high potency of des-Arg<sup>9</sup>-BK is specific to these rodents (superior or equal to Lys-des-Arg<sup>9</sup>-BK in functional assays; as reviewed by Marceau et al., 1998).

Thus, the B<sub>1</sub> receptor is obviously specialized across species to respond to different kinin metabolites, either des-Arg<sup>9</sup>-BK or Lys-des-Arg<sup>9</sup>-BK, generated by arginine carboxypeptidases, such as carboxypeptidase N and M. No other kinin fragment seems to retain pharmacological activity. For instance, des-Arg<sup>1</sup>-BK (generated by aminopeptidase P) or des(Phe<sup>8</sup>, Arg<sup>9</sup>)-BK [the primary metabolite generated by kininase II or angiotensin-converting enzyme (ACE)] do not retain significant activity on either known receptor type (Regoli and Barabé, 1980). The presence of arginine carboxypeptidases in functional systems frequently distorts the potency estimates for BK or Lys-BK on the B<sub>1</sub> receptor, as these ligands are transformed into their respective des-Arg<sup>9</sup> metabolites (discussed by Marceau et al., 1998). Thus, intact BK may exhibit a very high selectivity toward B<sub>2</sub>, and this makes [<sup>3</sup>H]BK an indisputably selective B<sub>2</sub> receptor ligand.

Synthetic peptides incorporate modifications to improve resistance to metabolism. In labradimil (RMP-7, [Hyp<sup>3</sup>, Thi<sup>5</sup>, 4-Me-Tyr<sup>8</sup> Ψ(CH<sub>2</sub>-NH)Arg<sup>9</sup>]-BK), the peptide bond between the two C-terminal residues has been replaced by a nonhydrolyzable bond, resulting in complete resistance to kininases I (arginine carboxypeptidases) and II (ACE) (Table 2; Doctrow et al., 1994). Labradimil is a selective B<sub>2</sub> receptor agonist developed to transiently open the blood-brain barrier to optimize the chemotherapy of intracranial tumors (Emerich et al., 2001). This compound has been used in clinical trials. An earlier version of a kininase-resistant BK analog with a similar design, [Phe<sup>8</sup>ψ(CH<sub>2</sub>-NH)Arg<sup>9</sup>]BK, has been described (Drapeau et al., 1988). It permanently promotes the endocytosis of a fluorescent form of the rabbit B<sub>2</sub> receptor in live cells, whereas the effect of BK is transient due to its degradation in serum-containing medium (Marceau et al., 2002). Sar-[D-Phe<sup>8</sup>]des-Arg<sup>9</sup>-BK is a B<sub>1</sub> receptor agonist of high selectivity that is completely resistant to blood aminopeptidase, kininases I and II (ACE), and kidney neutral endopeptidase (Table 2; Drapeau et al., 1991, 1993). It does not exhibit a very high affinity (intermediate between des-Arg<sup>9</sup>-BK and



Structures of Peptide B<sub>1</sub> and B<sub>2</sub> Receptor Modulators

Arg-Pro-Gly-Phe-Ser-Pro-Phe-Arg  
BK (B<sub>2</sub> agonist)

Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg  
Lys-BK (B<sub>1</sub>/B<sub>2</sub> agonist)

Arg-Pro-Gly-Phe-Ser-Pro-Phe  
des-Arg<sup>9</sup>-BK (B<sub>1</sub> agonist)

Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe  
Lys-des-Arg<sup>9</sup>-BK (B<sub>1</sub> agonist)

Arg-Pro-Gly-Phe-Ser-Pro-Leu  
[Leu<sup>8</sup>]des-Arg<sup>9</sup>-BK (B<sub>1</sub> antagonist)

D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg  
Icatibant (Hoe 140; B<sub>2</sub> antagonist)

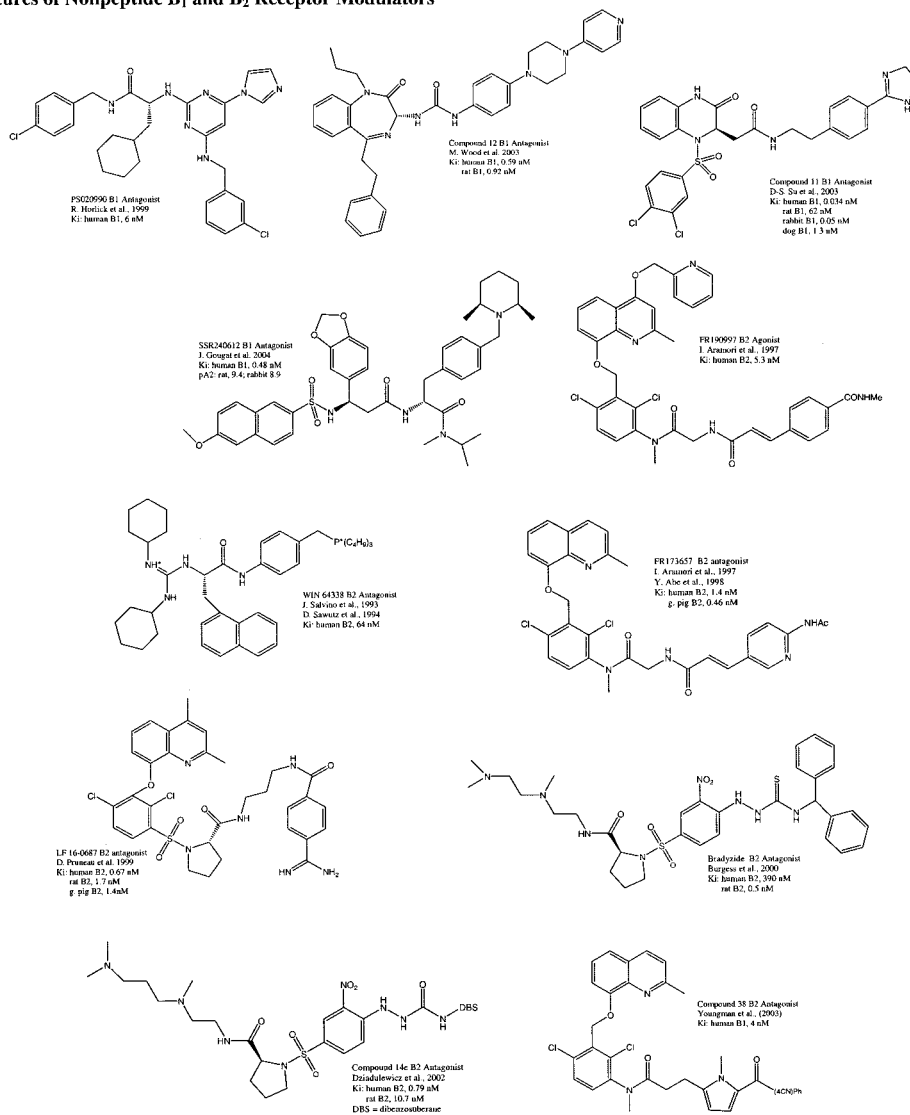
Structures of Nonpeptide B<sub>1</sub> and B<sub>2</sub> Receptor Modulators

FIG. 2. Structures of peptidic and nonpeptidic B<sub>1</sub> and B<sub>2</sub> receptor modulators.

Lys-des-Arg<sup>9</sup>-BK) but its prolonged duration of action is proven by its hypotensive effect in endotoxin-pretreated rabbits (Drapeau et al., 1991) and its efficacy as an angiogenesis-promoting agent in mice with an arterial occlusion (Emanuelli et al., 2002).

### B. Peptide Antagonists

Efforts to develop receptor antagonists by modifying the BK sequence date back to the 1970's (see Stewart, 1995 for review). The first family of compounds capable

of antagonizing BK and des-Arg<sup>9</sup>-BK with specificity was based on the prototype [Leu<sup>8</sup>]des-Arg<sup>9</sup>-BK (Fig. 2 and Table 2; Regoli et al., 1977). The same group of investigators had previously developed angiotensin II antagonists by replacing the aromatic and C-terminal Phe residue with an aliphatic residue (Regoli et al., 1974). The B<sub>1</sub> receptor nomenclature was later applied to the rabbit aortic preparation in which the kinin receptors were defined for the first time. This was based on both a typical order of agonist potency, with des-Arg<sup>9</sup>

TABLE 2  
Affinities of selected peptide ligands obtained using radioligand binding assays for recombinant kinin receptors  
The reference sequence is that of BK: H-Arg<sup>1</sup>-Pro<sup>2</sup>-Phe<sup>3</sup>-Ser<sup>4</sup>-Pro<sup>5</sup>-Phe<sup>6</sup>-Arg<sup>7</sup>-OH.

Peptide	Affinity Estimates						Notes
	Human Receptors		Mouse Receptors		Rabbit Receptors		
	B <sub>1</sub>	B <sub>2</sub>	B <sub>1</sub>	B <sub>2</sub>	B <sub>1</sub>	B <sub>2</sub>	
<b>B<sub>2</sub> agonists</b>							
BK							
Labradimil (RMP-7, [Hyp <sup>3</sup> , Thi <sup>5</sup> , 4-Me-Tyr <sup>8</sup> ] ψ(CH <sub>2</sub> -NH)Arg <sup>9</sup> -BK)	>10,000 (K <sub>i</sub> ) <sup>a</sup>	0.54 (K <sub>i</sub> ) <sup>b</sup>	200 (K <sub>i</sub> ) <sup>c</sup>	0.48 (IC <sub>50</sub> ) <sup>b</sup>	>5000 (K <sub>i</sub> ) <sup>d</sup>	4.5 (IC <sub>50</sub> ) <sup>e</sup>	IC <sub>50</sub> 10 nM for wild-type rat B <sub>2</sub> ; Resistant to carboxypeptidases.
<b>Promiscuous agonist</b>							
Kallidin (Lys-BK)	2.54 (K <sub>i</sub> ) <sup>a</sup>	0.63 (K <sub>i</sub> ) <sup>b</sup>	510 (K <sub>i</sub> ) <sup>c</sup>	0.52 (IC <sub>50</sub> ) <sup>b</sup>	19 (K <sub>i</sub> ) <sup>d</sup>	2 (IC <sub>50</sub> ) <sup>e</sup>	
<b>B<sub>1</sub> agonists</b>							
Des-Arg <sup>9</sup> -BK	1930 (K <sub>i</sub> ) <sup>a</sup>	8100 (K <sub>i</sub> ) <sup>b</sup>	0.7 (K <sub>i</sub> ) <sup>c</sup>	6400 (IC <sub>50</sub> ) <sup>b</sup>	32 (K <sub>i</sub> ) <sup>d</sup>	>1000 (IC <sub>50</sub> ) <sup>e</sup>	
Des-Arg <sup>10</sup> -kallidin (Lys-des-Arg <sup>9</sup> -BK)	0.12 (K <sub>i</sub> ) <sup>a</sup>	>30,000 (K <sub>i</sub> ) <sup>b</sup>	1.7 (K <sub>i</sub> ) <sup>c</sup>	25,000 (IC <sub>50</sub> ) <sup>b</sup>	0.23 (K <sub>i</sub> ) <sup>d</sup>	>1000 (IC <sub>50</sub> ) <sup>e</sup>	
Sar-[D-Phe <sup>8</sup> ]des-Arg <sup>9</sup> -BK	60 (IC <sub>50</sub> ) <sup>a</sup>				100 (IC <sub>50</sub> ) <sup>b</sup>		Resistant to multiple kininases.
<b>B<sub>2</sub> antagonists</b>							
Icatibant (Hoe 140; D-Arg-[Hyp <sup>3</sup> , Thi <sup>5</sup> , D-Tic <sup>7</sup> , Oic <sup>8</sup> ]BK)	437 (K <sub>i</sub> ) <sup>a</sup>	0.41 (IC <sub>50</sub> ) <sup>b</sup>	>10,000 (K <sub>i</sub> ) <sup>c</sup>	0.23 (IC <sub>50</sub> ) <sup>b</sup>	>5000 (IC <sub>50</sub> ) <sup>d</sup>	2 (IC <sub>50</sub> ) <sup>e</sup>	
<b>B<sub>1</sub> antagonists</b>							
[Leu <sup>8</sup> ]des-Arg <sup>9</sup> -BK	276 (K <sub>i</sub> ) <sup>a</sup>	>30,000 (IC <sub>50</sub> ) <sup>b</sup>	4.1 (K <sub>i</sub> ) <sup>c</sup>	>30,000 (IC <sub>50</sub> ) <sup>b</sup>	80 (K <sub>i</sub> ) <sup>d</sup>	374 (IC <sub>50</sub> ) <sup>b</sup>	
[Leu <sup>8</sup> ]des-Arg <sup>10</sup> -kallidin (Lys-[Leu <sup>8</sup> ]des-Arg <sup>9</sup> -BK)	0.58 (K <sub>i</sub> ) <sup>a</sup>	>30,000 (IC <sub>50</sub> ) <sup>b</sup>	7.5 <sup>c</sup>	>30,000 (IC <sub>50</sub> ) <sup>b</sup>	0.43 (K <sub>i</sub> ) <sup>d</sup>	>1000 (IC <sub>50</sub> ) <sup>e</sup>	
B-9858 (Lys-Lys-[Hyp <sup>3</sup> , Igl <sup>5</sup> , D-Igl <sup>7</sup> , Oic <sup>8</sup> ]des-Arg <sup>9</sup> -BK)	0.04 (K <sub>i</sub> ) <sup>a</sup>	146 (K <sub>i</sub> ) <sup>b</sup>	5.4 (K <sub>i</sub> ) <sup>c</sup>	44 (K <sub>i</sub> ) <sup>d</sup>	15 (IC <sub>50</sub> ) <sup>b</sup>		
<b>Promiscuous antagonists</b>							
B-9430 (D-Arg-[Hyp <sup>3</sup> , Igl <sup>5</sup> , D-Igl <sup>7</sup> , Oic <sup>8</sup> ]BK)	12.6	0.25	248 (K <sub>i</sub> ) <sup>d</sup>	0.33 (K <sub>i</sub> ) <sup>e</sup>			Combined blockade of B <sub>1</sub> and B <sub>2</sub> in vivo. <sup>f</sup>
des-Arg <sup>10</sup> -Hoe 140 (D-Arg-[Hyp <sup>3</sup> , Thi <sup>5</sup> , D-Tic <sup>7</sup> , Oic <sup>8</sup> ]-des-Arg <sup>9</sup> -BK)	174 (K <sub>i</sub> ) <sup>a</sup>		24 (K <sub>i</sub> ) <sup>c</sup>				

<sup>a</sup> Bastian et al., 1997.

<sup>b</sup> Hess et al., 1994.

<sup>c</sup> Hess et al., 1996.

<sup>d</sup> MacNeil et al., 1995.

<sup>e</sup> Bachvarov et al., 1995.

<sup>f</sup> Doctrow et al., 1994.

<sup>g</sup> G. Morissette and F. Marceau, unpublished data.

<sup>h</sup> Sabourin et al., 2002a (binding to rabbit B<sub>1</sub> conjugated to yellow fluorescent protein).

<sup>i</sup> MacNeil et al., 1997.

<sup>j</sup> Stewart et al., 1996.

fragment more potent than the native sequence, and by the affinity of antagonists modeled on [Leu<sup>8</sup>]des-Arg<sup>9</sup>-BK, thus satisfying the two first Schild criteria for receptor classification (Schild, 1973). However, these antagonists were not active in established bioassays for BK such as contraction of the rat uterus and guinea pig ileum *in vitro* or production of hypotension *in vivo* (Regoli and Barabé, 1980). Early compounds such as [Leu<sup>8</sup>]des-Arg<sup>9</sup>-BK and Lys-[Leu<sup>8</sup>]des-Arg<sup>9</sup>-BK had a complete selectivity, being devoid of affinity for the B<sub>2</sub> receptor (Table 2). Structure-activity relationships for peptide antagonists of the B<sub>1</sub> receptor are discussed elsewhere (Regoli and Barabé, 1980; Marceau et al., 1998; Gobeil et al., 1999). [Leu<sup>8</sup>]des-Arg<sup>9</sup>-BK, the prototype B<sub>1</sub> receptor antagonist, may exhibit fairly high partial agonist behavior in some species, especially in the rats and mice (reviewed elsewhere, Marceau et al., 1998). This has practical implications because one of the emerging therapeutic applications of B<sub>1</sub> receptor antagonists, analgesia, is commonly evaluated using behavioral models involving these species.

The B<sub>2</sub> receptor was not well defined until 1985 when the first generation of antagonists based on [D-Phe<sup>7</sup>]BK were produced (Vavrek and Stewart, 1985). In these early compounds (e.g., [Thi<sup>5,8</sup>, D-Phe<sup>7</sup>]BK), the transition to an antagonist/partial agonist was caused by a structural constraint on the peptide backbone introduced by the non-natural conformation in D-Phe<sup>7</sup>. The added rigidity at this position in subsequent peptide antagonists such as icatibant (Hoe 140, D-Arg-[Hyp<sup>3</sup>, Thi<sup>5</sup>, D-Tic<sup>7</sup>, Oic<sup>8</sup>]-BK; Fig. 2 and Table 2; Hock et al., 1991) and NPC17731 (D-Arg[Hyp<sup>3</sup>, D-HypE(trans-propyl)<sup>7</sup>, Oic<sup>8</sup>]-BK) (Trifilieff et al., 1993) showed that the spatial orientation of the C-terminal region of the peptide molecule is critical for antagonism. Icatibant exhibits a high affinity, no residual agonist activity in most mammalian species and an impressive resistance to peptidases; the combinations of these properties contribute to its prolonged duration of action (several hours) in animal models. In practical terms, this peptide exhibits a high selectivity toward the B<sub>2</sub> receptor, although its affinity for the human B<sub>1</sub> receptor is measurable (Table 2). Icatibant has been used extensively (about 700 publications indexed in Medline) to assess the role of kinins in various models of physiological adaptation or experimental pathology and occasionally in humans (Groves et al., 1995; Akbary et al., 1996; Dear et al., 1996; Gainer et al., 1998; Squire et al., 2000; Witherow et al., 2001; Hornig et al., 2003).

Optimal peptide B<sub>2</sub> receptor antagonists retain both Arg<sup>1</sup> and Arg<sup>9</sup> residues, and B<sub>1</sub> receptor agonists or antagonists typically lack Arg<sup>9</sup>. However, more recent antagonists with backbones constrained by non-natural residues are somewhat more promiscuous in their selectivity. The des-Arg fragment of icatibant (des-Arg<sup>10</sup>-Hoe 140) has been promoted as a B<sub>1</sub> receptor antagonist by some investigators, although it is a mixed antagonist

(Table 2). This peptide exhibits increased affinity toward the B<sub>1</sub> receptor relative to icatibant in all species studied, but retains fairly high residual antagonistic effects on the B<sub>2</sub> receptor in functional studies (Rhaleb et al., 1992; Lagneux and Ribouot, 1997). Recently produced antagonists retaining Arg<sup>9</sup>, such as B9430 (D-Arg-[Hyp<sup>3</sup>, Igl<sup>5</sup>, D-Igl<sup>7</sup>, Oic<sup>8</sup>]BK) represent combined B<sub>1</sub> and B<sub>2</sub> receptor antagonists, although the corresponding des-Arg<sup>9</sup> fragment has substantial selectivity toward the B<sub>1</sub> receptor (Burkard et al., 1996). B9430 has been deliberately exploited as a dual kinin receptor antagonist in the blood pressure assay of the dog where it can abolish, at certain doses, hypotensive responses to either BK or des-Arg<sup>9</sup>-BK without affecting the responses to several other unrelated agonists (Stewart et al., 1996). The development of this compound not only demonstrates that a “polypharmaceutic” approach covering both receptor types is possible, but also that the structures of the B<sub>1</sub> and B<sub>2</sub> receptor molecules are sufficiently compatible to accommodate a universal antagonist “pharmacophore”.

An example of a peptidic B<sub>1</sub> receptor antagonist of a modern design is B9858 (Lys-Lys-[Hyp<sup>3</sup>, Igl<sup>5</sup>, D-Igl<sup>7</sup>, Oic<sup>8</sup>]des-Arg<sup>9</sup>-BK); it exhibits a fairly high selectivity for the B<sub>1</sub> receptor (Table 2; Gera et al., 1996; Gobeil et al., 1999). This peptide retains the Lys<sup>0</sup> residue, a favorable feature for binding to the human B<sub>1</sub> receptor (Table 2). In binding assays, B9858 is more potent at the human B<sub>1</sub> receptor than at its murine ortholog; it is highly active as a competitive antagonist against Lys-des-Arg<sup>9</sup>-BK-induced contractility of the human umbilical vein (pA<sub>2</sub> 9.2) and is potent, but insurmountable, at the rabbit B<sub>1</sub> receptor (Larrivée et al., 2000). In the anesthetized dog representing a hemodynamic system with mixed B<sub>1</sub> and B<sub>2</sub> receptor responses (see below), B9858 is reported to be 20 times more potent than Lys-[Leu<sup>8</sup>]des-Arg<sup>9</sup>-BK in antagonizing des-Arg<sup>9</sup>-BK-induced hypotension, and the antagonist effect lasts for more than 4 h (versus about 15 min for Lys-[Leu<sup>8</sup>]des-Arg<sup>9</sup>-BK; Stewart et al., 1996).

Numerous structural experiments have been conducted with peptide kinin antagonists, such as producing pseudopeptides that retain limited structural elements of the parent peptide (Chakravarty et al., 1995; Galoppini et al., 1999; Bedos et al., 2000), or the production of dimers of antagonists. An early implementation of the latter idea was deltibant (CP-0127), the homodimer of D-Arg<sup>0</sup>[Hyp<sup>3</sup>, Thi<sup>5</sup>, Cys<sup>6</sup>, D-Phe<sup>7</sup>, Leu<sup>8</sup>]BK coupled through the side chains of Cys<sup>6</sup> by the linker bis-succinimidohexane. This B<sub>2</sub> receptor antagonist of rather low potency had nevertheless a good metabolic stability and reached clinical trials for sepsis and head trauma but with mixed results (Fein et al., 1997; Marmarou et al., 1999). The production of such antagonists of molecular weight (~2.5 kDa) higher than that of the antagonist peptide and of high solubility in water goes against common drug design wisdom unless special effects are sought. A recent avatar of the dimer approach

is B9870 (also called CU201, two molecules of D-Arg-[Hyp<sup>3</sup>, Igl<sup>5</sup>, D-Igl<sup>7</sup>, Oic<sup>8</sup>]BK linked at their N terminus by the suberimidyl moiety, Stewart et al., 1997). In tumor-derived cell lines, B9870 antagonized BK-induced calcium signaling but also directly blocked cell proliferation and activated c-Jun kinase and caspase-3 (Chan et al., 2002a). The monomeric component of B9870 was essentially devoid of the “biased agonist” properties of the dimer. The B9870 dimer has been called a biased agonist since it activates only a subset of the signal transduction pathways activated by the reference agonist. Whether all effects of B9870 are truly B<sub>2</sub> receptor-mediated is currently unknown.

### C. Nonpeptide Ligands

The peptide analogs of BK described above have been extremely important in developing an understanding of the roles of kinins and their receptors in physiology and pathophysiology. Recently, that understanding has led to the conjecture that B<sub>1</sub> or B<sub>2</sub> receptor modulators could represent novel therapeutic agents across a wide range of human conditions and diseases such as pain, inflammation, stroke, asthma, rhinitis, endotoxic shock, and cancer. Development of nonpeptide agonists and antagonists offers the greatest opportunity for new medicines since peptides are generally poor for oral bioavailability and brain penetration. Numerous laboratories are now engaged in research activities to develop and clinically test novel B<sub>1</sub> and B<sub>2</sub> receptor modulators (Altamura et al., 1999; Heitsch, 2002; Sharma and Al-Dhalmawi, 2003). A key issue that has faced development of such compounds for either B<sub>1</sub> or B<sub>2</sub> receptors has been species selectivity of their pharmacology. In some cases, the human specificity of certain structural classes has limited the preclinical evaluation of efficacy, and conversely, rodent specific compounds are not useful for therapeutic development (Hess et al., 1994, 2002; Jones et al., 1999; Burgess et al., 2000; Marceau et al., 2003). Many of these efforts have yet to result in published articles describing the compounds, but the patent literature bears ample evidence for these activities (Bock and Longmore, 2000; Heitsch, 2002; Bock et al., 2003). No doubt the therapeutic potential of these approaches will become evident in the near future.

**1. B<sub>2</sub> Receptor Agonists.** FR190997 is a very interesting example of a nonpeptidyl structure exhibiting agonist activity at the B<sub>2</sub> receptor (Fig. 2; Aramori et al., 1997a). FR190997 was derived chemically from, and bears close structural similarity to, a series of nonpeptide B<sub>2</sub> receptor antagonists (see FR173657, Fig. 2). Other examples of GPCR ligands exist where very slight structural modifications, such as addition or deletion of a simple methylene group, change antagonists to agonists at cholecystokinin (Aquino et al., 1996) and AT<sub>1</sub> receptors (Perlman et al., 1995), notwithstanding the well known close structural similarity of the opiate ligands morphine and naloxone. In the case of FR190997,

potent B<sub>2</sub> receptor agonist activity is demonstrated in vitro with a level of efficacy approaching BK itself, and in vivo FR190997 produces hypotension in rats (Aramori et al., 1997a). Although compounds like FR190997 may have therapeutic utility as antihypertensive or as cardioprotective agents, appropriate clinical testing remains. Concerns over tachyphylaxis (Mathis et al., 1996; Cuthbert, 1999) or potential adverse effects of a BK mimic such as proinflammatory responses demonstrated with FR190997 in animals (Hayashi et al., 2001) may limit considerations for therapeutic development.

**2. B<sub>2</sub> Receptor Antagonists.** The earliest example of the successful synthesis of a nonpeptide B<sub>2</sub> receptor antagonist is WIN64338 (Fig. 2) (Salvino et al., 1993). As a prototype, WIN64338 had somewhat limited affinity for the human B<sub>2</sub> receptor ( $K_i$ , 64 nM), and in vivo studies have not been published (Sawutz et al., 1994). WIN64338, however, was clearly an important advance for the field and an early pharmacological tool.

Subsequently, researchers at Fujisawa described new structurally distinct nonpeptide B<sub>2</sub> receptor antagonists (Aramori et al., 1997b; Abe et al., 1998b), such as FR173657 (Fig. 2) with substantially higher B<sub>2</sub> receptor affinity and demonstrated selectivity versus the B<sub>1</sub> receptor. Moreover, these compounds including FR173657 and FR184280 (Abe et al., 1998a) were the first to demonstrate oral activity in vivo, in a guinea pig model of bronchoconstriction and various models of edema, inflammation, and pain (Asano et al., 1999). FR173657 in particular has proven to be a valuable tool to investigate the physiology and pathophysiology of BK and has been studied by a number of laboratories.

Another series of selective nonpeptide B<sub>2</sub> receptor antagonists have emerged from the Fournier laboratories exemplified by LF 16-0687 (Fig. 2; Pruneau et al., 1999) as a high affinity antagonist across species and a potent antagonist against BK-induced hypotension and edema. LF 16-0687 also was shown to reduce cerebral edema and improve neurological outcome in a rodent model of head trauma (Kaplanski et al., 2002) implicating BK in this pathology.

A striking example of the species selectivity that can emerge during the development of B<sub>2</sub> receptor antagonists is with bradyzide (Fig. 2; Burgess et al., 2000), a potent antagonist that displays >500 times higher affinity for the rat versus human B<sub>2</sub> receptor. Bradyzide is highly selective for the B<sub>2</sub> receptor over the B<sub>1</sub> receptor and other receptors, is orally active, and shows antihyperalgesic effects in a rat model of inflammatory pain. Interestingly, working from the thiosemicarbazide scaffold of bradyzide, Dziadulewicz et al. (2002) succeeded in obtaining subnanomolar affinity for the human receptor while maintaining affinity for the rat receptor in Compound 14e (Fig. 2) and other analogs. Like bradyzide, Compound 14e exhibited oral activity to block inflammatory hyperalgesia in rats.



Researchers from Johnson and Johnson have recently published their early efforts in the development of B<sub>2</sub> receptor antagonists (Lee et al., 2003) as exemplified by Compound 38 (Fig. 2; Youngman et al., 2003). FR173657 served as a starting template for this chemical series. Compound 38 is a high affinity antagonist ( $K_i$ , 4 nM) at the human B<sub>2</sub> receptor. These compounds are analgesic after oral dosing in a rodent model of chemical-induced irritation.

It is clear that the development of nonpeptide B<sub>1</sub> and B<sub>2</sub> receptor modulators as potential new therapeutics, particularly antagonists, is currently an area of considerable effort. At present, no articles have been published that disclose clinical results with nonpeptide BK antagonists, but it is likely that compounds will emerge soon that are suitable for human clinical trials.

**3. B<sub>1</sub> Receptor Antagonists.** Recently, the results of research into the development of B<sub>1</sub> receptor antagonists as novel therapeutic agents have begun to bear fruit. In 1999, researchers from Pharmacopeia published on a compound PS020990 as a potent and competitive B<sub>1</sub> receptor antagonist (Horlick et al., 1999). PS020990 was derived from compounds discovered from the screening of a combinatorial chemistry library and is the first nonpeptide B<sub>1</sub> receptor antagonist described in the literature. Although the structure was not initially disclosed, subsequent publications have identified PS020990 as shown in Fig. 2 (Anonymous, 2001). PS020990 exhibits high affinity for the human B<sub>1</sub> receptor ( $K_i$ , 6 nM) and is an appropriately potent B<sub>1</sub> receptor antagonist in vitro. To date, no results have been published on the in vivo properties of PS020990.

More recently, researchers from Merck Research Laboratories have disclosed two additional nonpeptide antagonists with divergent structures. Wood et al. (2003) described the in vitro and in vivo properties of a series of benzodiazepine-based compounds. The preferred structure from this series, Compound 12 (Fig. 2), exhibited high affinity for the human B<sub>1</sub> receptor ( $K_i$ , 0.6 nM) and appropriate functional antagonist potency in vitro. Species differences are well known for both B<sub>1</sub> and B<sub>2</sub> receptors (Hess et al., 1994, 2002; Jones et al., 1999), so it was of interest that Compound 12 showed equally high affinity ( $K_i$ , 0.9 nM) and antagonist potency at the rat B<sub>1</sub> receptor in vitro. This affinity allowed for its testing in a rat model of carrageenan-induced hyperalgesia where Compound 12 exhibited significant analgesic activity. A second series, the so-called dihydroquinoxalinone series, is represented by Compound 11 (Fig. 2) (Su et al., 2003). Compound 11 was derived from a micromolar affinity lead detected in a high throughput screening effort, and optimization efforts led to extraordinary affinity for the human B<sub>1</sub> receptor ( $K_i$ , 34 pM) producing a highly potent antagonist. Unlike the benzodiazepine-based Compound 12 described above, Compound 11 shows considerably lower affinity for the rat B<sub>1</sub> receptor ( $K_i$ , 62 nM), but

high affinity for the rabbit B<sub>1</sub> receptor ( $K_i$ , 50 pM) allowing for demonstration of robust antihyperalgesic activity in this species. Like the benzodiazepine Compound 12, the dihydroquinoxalinone antagonist exhibited high B<sub>1</sub> receptor selectivity versus the B<sub>2</sub> receptor and limited to no activity at a host of other enzyme, GPCR, and transporter targets. These representatives of both structural series, however, had suboptimal pharmacokinetic profiles that precluded further development. However, both Compounds 11 and 12 serve as structures for further optimization work, and their analgesic activity across rat and rabbit species further substantiate the role of B<sub>1</sub> receptor mechanisms to mediate pain (see below).

Researchers from Sanofi-Synthelabo recently published on a novel nonpeptidyl B<sub>1</sub> receptor antagonist, SSR240612 (Fig. 2) (Gougat et al., 2004). SSR240612 is a high affinity, competitive B<sub>1</sub> receptor antagonist with considerable selectivity against the B<sub>2</sub> receptor and a variety of other receptor and enzyme targets. Of interest, SSR240612 is a potent B<sub>1</sub> receptor antagonist across several species and exhibits oral antihyperalgesic and anti-inflammatory activities in mice and rats and is currently in preclinical development for inflammatory and neurogenic pain. Other efforts to develop nonpeptide B<sub>1</sub> receptor antagonists are evident at Novartis, Bayer, and Fournier based on their patent publications (Bock et al., 2003), but the status of any clinical progress is not known.

### III. Structural Aspects of Kinin Receptors and Their Genes

#### A. Organization and Structure of the Receptor Genes

A BK receptor was first cloned from rat uterus, using a *Xenopus* oocyte expression assay and shown to have the pharmacological profile of a B<sub>2</sub> receptor subtype (McEachern et al., 1991). Subsequently, orthologs have been isolated and sequenced in several mammalian species, including the human (Table 3; Hess et al., 1992). The B<sub>2</sub> receptor protein structure is typical of that of a GPCR consisting of a single polypeptide chain that spans the membrane seven times, with the amino terminus (N-terminal domain) being extracellular and the carboxy terminus (C-terminal domain) being intracellular, and with three extracellular loops (EL-1–3) and three intracellular loops (IL-1–3) (Fig. 3). Three consensus sites for N-linked glycosylation are found in extracellular domains. Furthermore, the protein contains motifs such as DRY and NPXXY partially embedded in cytosolic receptor domains that are common to most rhodopsin family GPCR (Fredriksson et al., 2003), and the C-terminal tail contains serines and threonines that are putative phosphorylation sites and cysteines that are putative sites for acylation.

The three-exon structure of the gene (*BDKRB2*) has been determined, as well as its localization on the hu-

TABLE 3  
GenBank accession numbers for kinin receptor genes of various species

Zoological Group	Species	B <sub>1</sub>	B <sub>2</sub>
Primates	Human	U12512	M88714
	Macaca mulatta	AY045568; AF540785	
	Two species of Cercopithecus	AY045569, AF540784	
Prosimians	Tupaia	AF540786	
Lagomorphs	Rabbit	U20507	U33334
Rodents	Mouse	U47281	L26047
	Rat	U66107	M59967
	Guinea pig		AJ003243
	Pig	AF540788	AB051422
Other mammals	Dog	AF0334947	AF33498

man chromosome 14 (Fig. 4; Powell et al., 1993; Kammerer et al., 1995). The coding sequence of the human B<sub>2</sub> receptor was initially believed to be intronless in the gene exon 3 where a plausible initiation codon was found; the recombinant receptor based on this initiation codon is fully functional. However, another methionine codon in exon 2 is the likely initiation codon and extends the amino terminal sequence (first extracellular domain) by 27 residues (proved notably by sequencing the immunoprecipitated receptor from human HF15 cells; AbdAlla et al., 1996a). However, the exon 3 methionine has generally been used in the numbering of residues in the B<sub>2</sub> receptor. In Fig. 3, upstream residues are labeled with negative numbers.

The expression cloning of the human B<sub>1</sub> receptor was achieved later (Menke et al., 1994), and its orthologs have been sequenced in several mammalian species (Ta-

ble 3). The B<sub>1</sub> receptor is homologous to the B<sub>2</sub> receptor (36% identity at the amino acid sequence level) and also possesses three consensus sites for N-linked glycosylation in extracellular domains, DRY and NPXXY motifs, and putative sites for phosphorylation and acylation (Fig. 3). The three-exon structure of the human B<sub>1</sub> receptor gene (*BDKRB1*) has been determined with the protein sequence being encoded by exon 3 exclusively (Fig. 4; Bachvarov et al., 1996; Yang and Polgar, 1996).

The two genes corresponding to the kinin receptors are clustered in tandem in a compact locus, the B<sub>2</sub> receptor gene being located 5' relative to the B<sub>1</sub> receptor gene separated by only 12 kb (Cayla et al., 2002a; <http://www.ncbi.nlm.nih.gov/mapview/>, Fig. 4). Species-specific alterations of this scheme, such as the deletion of B<sub>1</sub> receptor exon 2 in the rat and mouse genome and the possible existence of an alternatively spliced exon 2b in

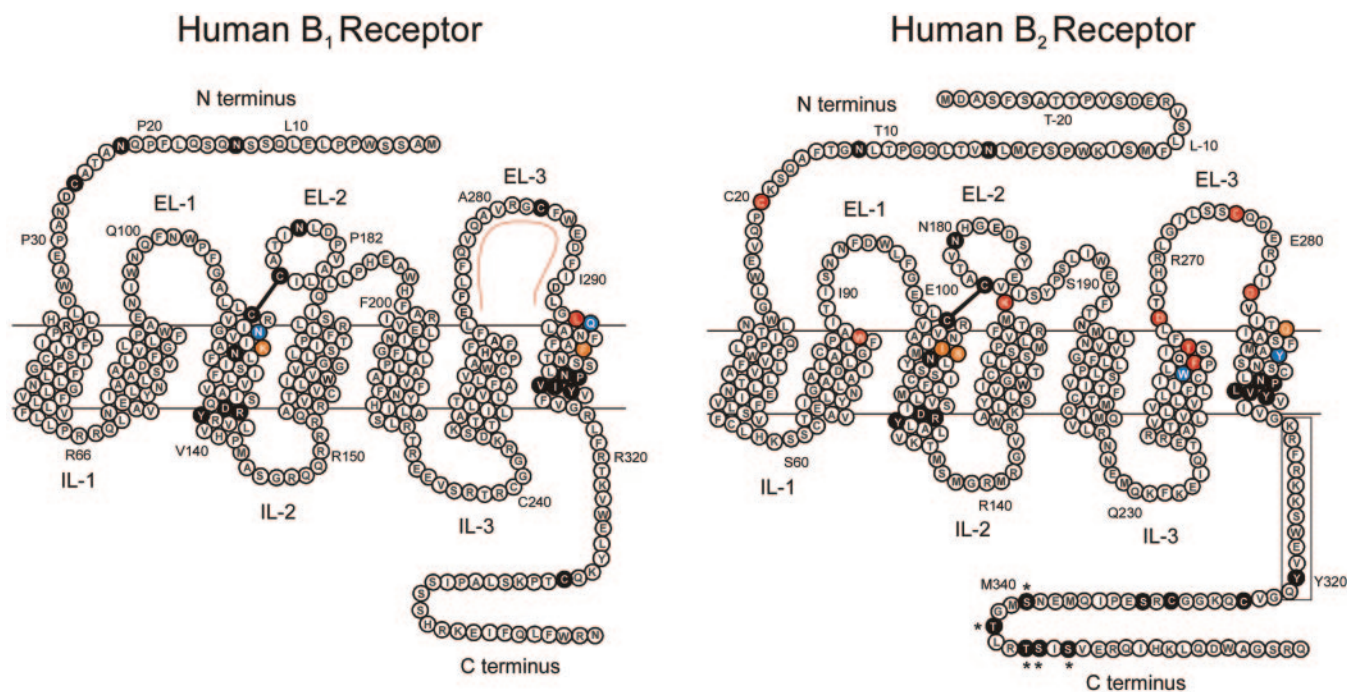


FIG. 3. Schematic representations of the human B<sub>1</sub> and B<sub>2</sub> amino acid sequences. In B<sub>2</sub>, amino acid residues (single letter code) are numbered from the most 3' putative translational start site with negative numbers for the 27 residues N-terminal to that methionine. EL, extracellular loops; IL, intracellular loops. Single amino acid residues or sequence segments discussed in the text are highlighted with potential residues facing specifically the binding pockets filled with red or red line (agonist), blue (antagonist), and orange (agonist and antagonist). In the B<sub>2</sub> receptor, the boxed area indicates the putative helix 8, and the stars indicate the cluster of serines and threonines phosphorylated by GRK or PKC and important for desensitization.

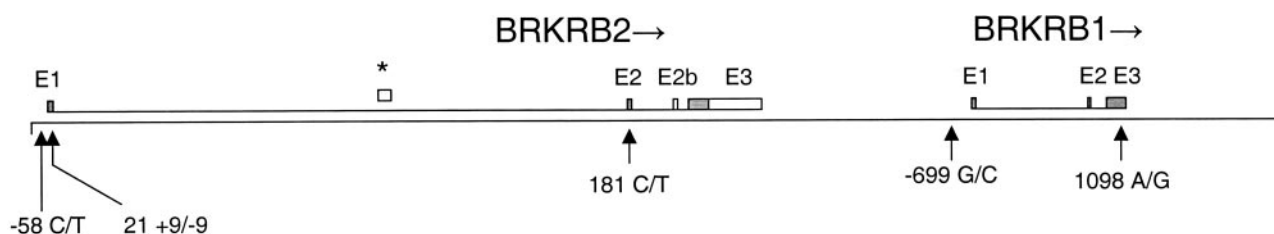


FIG. 4. Schematic diagram of the human kinin receptor gene locus and polymorphisms associated with a clinical phenotype. The area shown (chromosome 14q32, positions 94.66 to 94.73 Mb, total of 70 kb) contains the two kinin receptor genes organized in tandem. The three major exons (E) of each gene are shown. The open box in  $B_2$  receptor exon 3 represents the large 3'-untranslated sequence. Single nucleotide polymorphisms are indicated below the gene structure. The alternatively spliced exon 2b of the  $B_2$  receptor is indicated (Cayla et al., 2002a). \*, *CKS1BP* pseudogene.

the  $B_2$  receptor, have been discussed (Cayla et al., 2002a). This locus is on chromosome 14 in the human (14q32). The mRNA coding for the  $B_2$  receptor is large compared with that of the  $B_1$  receptor (approximately 4 and 1.4 kb, respectively, depending on the species). The large 3'-untranslated region of the  $B_2$  receptor accounts for most of the difference (Fig. 4).

Boels and Schaller (2003) claimed that a human orphan GPCR, GPR100 (GenBank accession number AY170828), is an additional receptor for BK, only distantly related to the known kinin receptors. The cloned receptor was said to confer calcium transients to cells stimulated with BK only if the promiscuous  $G\alpha_{16}$  protein is cotransfected. The pharmacological profile was that of a  $B_2$  receptor because the stimulation was common to BK and Lys-BK, whereas the des-Arg<sup>9</sup> fragments of these peptides were inactive. No antagonists were tested in this study. These findings were provocative since the  $B_2$  receptor gene knockout mouse has lost all responses to exogenous BK (Borkowski et al., 1995), failing to support the concept of additional  $B_2$  receptor subtypes. GPR100 (also called GPCR142 or relaxin-3 receptor-2) has also been shown to bind relaxin-3 and to inhibit adenylate cyclase upon stimulation with this peptide (Liu et al., 2003). In a subsequent study by Meini et al. (2004a), human GPR100 was compared with human  $B_2$  receptor in CHO cells in the absence of  $G\alpha_{16}$  cotransfection. It was shown that GPR100 is a high affinity relaxin-3 receptor, which is coupled to  $G\alpha_i$ -mediated inhibition of adenylate cyclase but not to  $G\alpha_q$ -mediated stimulation of phospholipase C and intracellular calcium mobilization. Interestingly, BK stimulates this receptor with relatively lower potency, whereas the non-peptide  $B_2$  receptor agonist FR190997 was completely devoid of effect. The BK response is antagonized by icatibant supporting an evolutionary relationship between GPR100 and  $B_2$  receptor binding sites.

Two receptors cloned from the chicken and the zebrafish are more related to the mammalian  $B_2$  receptor than to the  $B_1$  receptor and are stimulated by sequences related to BK (ornithokinin = RPPGFSPLR, trout BK = RRPPGWSPLR), but not by BK itself (Schroeder et al., 1997; Dunér et al., 2002). The peptide icatibant, which acts as an antagonist of BK at mammalian  $B_2$  receptors, acts as a full agonist at the ornithokinin receptor (Table

2). The fish receptor gene is located in a chromosomal region syntenic to the human kinin receptor gene region with implications for the evolution of the kallikrein-kinin system.

The targeted disruption ("knockout") of each kinin receptor gene by homologous recombination has been reported. The  $B_2$  receptor knockout mice fail to respond to BK using assays such as smooth muscle contraction and afferent nerve stimulation (Borkowski et al., 1995). These animals are fertile and apparently healthy, although they may not develop and age in an entirely normal manner (see below). The  $B_1$  receptor knockout mice have been produced more recently (Pesquero et al., 2000). These animals develop normally, are normotensive, but fail to respond to des-Arg<sup>9</sup>-BK (e.g., contractility of the mouse-isolated stomach). The creation of a double  $B_1/B_2$  receptor knockout strain is not a trivial crossing experiment since both genes lie in the same chromosomal locus (see above), and the probability of recombination is very low at such a small genetic distance. Numerous physiological and pathological analyses based on either knockout strain will be reported below.

### B. Receptors and Their Post-Translational Modifications

Kinin receptors undergo multiple post-translational modifications including glycosylation and disulfide bridge (cystine) formation in their extracellular domains as well as acylation and phosphorylation of their intracellular domains. Most of these modifications, including phosphorylation, can occur in a ligand-independent manner, although phosphorylation and possibly even acylation (palmitoylation) appear to be primarily regulated through receptor activation.

1. *Glycosylation.* All known mammalian and non-mammalian kinin receptors expose several (two to four) consensus sites of the Asn-Xaa-Ser/Thr type for *N*-glycosylation on their predicted extracellular domains, and most of them are clustered on their N-terminal domains (Fig. 3). For mammalian  $B_2$  receptors, conclusive evidence has been presented that they are sialoglycoproteins, and it appears likely that nonmammalian  $B_2$  receptors as well as mammalian  $B_1$  receptors fall into the same category. In general, glycosylation is thought to



increase the hydrophilic nature of the extracellular portions of the receptors, however, glycosylation may also affect ligand affinity, receptor oligomerization, efficient G protein coupling, receptor degradation, maturation (folding, stabilization), and/or intracellular trafficking.

Human and rabbit B<sub>1</sub> receptors expose three consensus sites for *N*-glycosylation in their extracellular domains (Fig. 3; Menke et al., 1994; MacNeil et al., 1995). The presence of multiple bands of the recombinantly expressed human B<sub>1</sub> receptor that is strongly reminiscent of the patterns of the partially glycosylated B<sub>2</sub> receptor has pointed to the possibility that the B<sub>1</sub> receptor is also prone to *N*-glycosylation (Blaukat et al., 1999a). Treatment of the HA-tagged human B<sub>1</sub> receptor expressed in HEK293 cells with *N*-glycosidase F resulted in the conversion of the receptor from a heterogeneous species migrating at 35 to 45 kDa to a relatively homogeneous species migrating at 37 kDa. Furthermore, a receptor in which the N-terminal domain had been truncated to remove putative *N*-glycosylation sites also migrated as a homogeneous species (Kang et al., 2005). In addition, studies using the *N*-glycosylation inhibitor tunicamycin have revealed a profound influence on des-Arg<sup>9</sup>-BK-induced responses in the isolated human umbilical vein (Sardi et al., 1999). Likewise tunicamycin caused a significant, although incomplete, reduction of des-Arg<sup>9</sup>-BK responses in the isolated rabbit aorta (Audet et al., 1994). The precise molecular mechanisms underlying the observed effects remain to be elucidated.

The protein sequences of the rat (McEachern et al., 1991) and human B<sub>2</sub> receptors (Hess et al., 1992) predict three potential *N*-glycosylation sites for each of these orthologs. Two of the sites are present in the N-terminal domain of the receptors, whereas a single site is present in the second extracellular loop, EL-2 (Fig. 3). Nonmammalian B<sub>2</sub> receptors differ from most mammalian B<sub>2</sub> receptors in that they lack a predicted *N*-glycosylation site in EL-2 (zebrafish B<sub>2</sub> receptor) (Dunér et al., 2002) or have two additional sites in their N-terminal domain (ornithokinin receptor) (Schroeder et al., 1997).

A careful study of the rat B<sub>2</sub> receptor by Yaqoob et al. (1995) provided conclusive evidence that all three predicted sites are indeed glycosylated, most likely through complex-type oligosaccharide side chains containing terminal sialic acid residues. The same authors were unable to find evidence for *O*-glycosylation of the rat B<sub>2</sub> receptor. Removal of the N-linked carbohydrates from the native receptor (81 kDa) by endo- $\beta$ -*N*-acetylglucosaminidase yielded a core protein of 42 to 44 kDa, indicating that the difference in the apparent molecular sizes is not due to dimerization of the unmodified receptors but truly reflects receptor glycosylation (Yaqoob et al., 1995). This notion is further corroborated by the findings from overexpression systems where multiple bands for the human B<sub>2</sub> receptor were observed that were sensitive to *N*-glycosidase F treatment (Blaukat et

al., 1999a). The complex carbohydrate chains with their terminal sialic acid residues may also contribute to the apparent isoelectric point of 4.5 to 4.7 for the mature rat B<sub>2</sub> receptor, which is significantly lower than the predicted pI of 7.0 calculated from its primary structure. Importantly, glycosylation does not appear to affect ligand-receptor interactions, as deglycosylation failed to change the affinity of the rat B<sub>2</sub> receptor for the agonist BK as well as for the antagonist icatibant (Yaqoob et al., 1995).

**2. Disulfide Bridging.** The predicted protein structures of mammalian kinin receptors identify four cysteine residues in the extracellular domains of both B<sub>1</sub> and B<sub>2</sub> receptors, one each being located in the N-terminal domain, EL-1, EL-2, and EL-3, respectively (Fig. 3). Of the nonmammalian kinin receptors, the chick receptor shows the same distribution pattern of cysteine residues, whereas the zebrafish B<sub>2</sub> receptor has a single cysteine each in EL-1 and EL-2 and lacks cysteines in the N-terminal domain and EL-3, respectively. Notably, cysteine in EL-2 and EL-3 are conserved in most members of the GPCR superfamily, and the crystal structure of rhodopsin has revealed that they form a disulfide loop which most likely stabilizes the correctly folded conformation of the receptor (Palczewski et al., 2000).

To date, no experimental data are available for the kinin receptors that would prove the presence of disulfide bonds in their extracellular domains. However, a careful study by Leeb-Lundberg and coworkers addressing the availability of free thiol residues in wild-type and mutant B<sub>2</sub> receptors has provided indirect evidence for the presence of a disulfide bond between Cys<sup>103</sup> (EL-1) and Cys<sup>184</sup> (EL-2) (Herzig and Leeb-Lundberg, 1995; Herzig et al., 1996). They showed that Cys<sup>20</sup> (N-terminal domain) and Cys<sup>277</sup> (EL-3) of the wild-type B<sub>2</sub> receptor were accessible for heterobifunctional cross-linkers that bind to free thiol groups and to the amino group of BK, whereas Cys<sup>103</sup> (EL-1 domain) and Cys<sup>184</sup> (EL-2) were not. The authors concluded that residues Cys<sup>103</sup> and Cys<sup>184</sup> likely form a disulfide bridge, whereas the former two residues, i.e., Cys<sup>20</sup> and Cys<sup>277</sup>, do not (Herzig et al., 1996). Of note, application of dithiothreitol had little or no effect on the binding of BK to the B<sub>2</sub> receptor in both bovine myometrial membranes and intact rat myometrial cells suggesting that, even though disulfide bond formation between extracellular cysteines in the B<sub>2</sub> receptor may be crucial during insertion of the receptor into the membrane to form a proper binding site, reducing some of these bonds in receptors already expressed in the membrane does not destroy their agonist binding site (Herzig et al., 1996).

**3. Acylation.** As with many other GPCRs, the kinin receptors may expose cysteine residue(s) in their C-terminal portion facing the cytosol (Fig. 3). These cysteines are often modified by palmitoylation, and due to the insertion of the fatty acid chain into the membrane, an extra loop is created which may modulate G protein



coupling, internalization, resensitization, and/or intracellular trafficking of the receptors. All the known mammalian B<sub>2</sub> receptors contain two cysteine residues in their tail region, separated by four amino acid residues (CxxxGC). The nonmammalian B<sub>2</sub>-like receptors from chick and zebrafish have a single cysteine residue in their cytosolic tail region. Mammalian B<sub>1</sub> receptors fall into two main groups regarding cysteines for putative palmitoylation (Marceau et al., 1998); the rodent B<sub>1</sub> receptor with an unusually short C-terminal tail of 11 residues is devoid of cysteines and the nonrodent receptors contain one or two cysteines in their C-terminal tail.

Mass spectrometric analysis of the rat B<sub>2</sub> receptor has revealed that the proximal Cys<sup>326</sup> residue present in the hexapeptide CRKGGC is indeed palmitoylated (Soskic et al., 1999). Site-directed mutagenesis studies of the human B<sub>2</sub> receptor demonstrated that both the proximal (Cys<sup>324</sup>) and the distal (Cys<sup>329</sup>) cysteines present in the linear sequence of CQKGGC are subject to palmitoylation based on serine mutants, indicating that the two cysteines may be used alternatively by the corresponding acyl transferase (Pizard et al., 2001). At present, the acylation status of the B<sub>1</sub> receptor has not yet been addressed experimentally; however, given the fact that the rodent B<sub>1</sub> receptor lacks a cysteine residue in their tail region, it is conceivable that palmitoylation is dispensable for proper function of B<sub>1</sub> receptors in general.

**4. Phosphorylation.** On repeated or prolonged agonist stimulation, GPCR signaling is attenuated by negative feedback loops. Agonist-induced reversible phosphorylation, typically of serine and threonine residues located in the C-terminal domains, plays an important role in the desensitization of GPCRs (Fig. 3). Phosphorylation may also affect endocytosis, recycling, trafficking, or even G protein coupling of the receptors. Both mammalian and nonmammalian B<sub>2</sub> receptors expose clusters of Ser/Thr residues in the tail region, and their ligand-dependent phosphorylation has been extensively studied for human and rat B<sub>2</sub> receptors (Blaukat, 2002). Mammalian B<sub>1</sub> receptors fall into two categories regarding putative serines and threonines for phosphorylation. The rodent receptors with a truncated tail region contain only two threonine residues but not a single serine residue, and the nonrodent B<sub>1</sub> receptors contain a cluster of at least six Ser/Thr residues in their C terminus (Marceau et al., 1998; Hess et al., 2002).

The correlation between agonist-induced receptor phosphorylation and desensitization has been firmly established both for endogenous and recombinant B<sub>2</sub> receptors (Blaukat et al., 1996, 2001). The precise mapping of the relevant phosphorylation sites revealed three major sites, i.e., Ser<sup>339</sup>, Ser<sup>346</sup>, and Ser<sup>348</sup> and two minor sites, i.e., Thr<sup>342</sup> and Thr<sup>345</sup>, forming a cluster in the C-terminal region of the human B<sub>2</sub> receptor which spans over 10 residues (S<sup>339</sup>MGTLRTSIS<sup>348</sup>) (Figs. 3 and 5; see Section IV. for the functional consequences of mutating these residues in the B<sub>2</sub> receptor and B<sub>1</sub>/B<sub>2</sub> re-

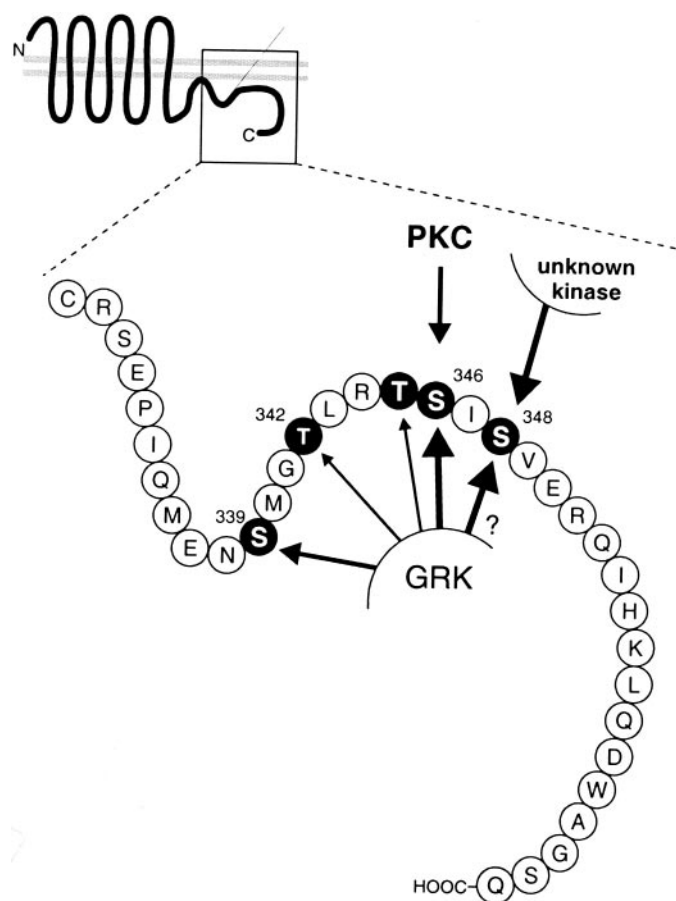


FIG. 5. In vivo phosphorylation sites present in the human B<sub>2</sub> receptor tail region. A schematic representation of the amino acid sequence (*single letter code*) is shown starting from the distal palmitoylation site (Cys<sup>329</sup>) and ending at the receptor C terminus. Identified phosphoacceptor sites are numbered and highlighted, and candidate kinases are indicated (GRK, PKC). The thickness of the arrows reflects the relative quantity of phosphate incorporation (modified after Blaukat et al., 2001).

ceptor chimeras). These studies revealed that tandem phosphorylation of two serine residues, i.e., Ser<sup>346</sup>/Ser<sup>348</sup> or Ser<sup>339</sup>/Ser<sup>346</sup> in the human B<sub>2</sub> receptor is necessary and sufficient for desensitization of BK-triggered phospholipase C activation (Blaukat et al., 2001). Mass spectrometry of the unstimulated rat B<sub>2</sub> receptor had also demonstrated tandem phosphorylation, although with varying combinations, e.g., Ser<sup>346</sup>/Ser<sup>348</sup>, Thr<sup>342</sup>/Ser<sup>348</sup>, and Ser<sup>331</sup>/Ser<sup>339</sup> (numbering according to the human B<sub>2</sub> receptor) (Soskic et al., 1999).

Tyrosine phosphorylation has also been reported for human and rat B<sub>2</sub> receptors. Early studies claimed the tyrosine phosphorylation of the human B<sub>2</sub> receptor (Jong et al., 1993), and mass spectrometric analysis of unstimulated rat B<sub>2</sub> receptors demonstrated phosphorylation of Tyr<sup>131</sup> (human residue Tyr<sup>129</sup>) present in the second intracellular loop (IL-2) and of Tyr<sup>322</sup> (human residue Tyr<sup>320</sup>) in proximity to the identified palmitoylation site in the C-terminal domain (Soskic et al., 1999). However, ligand-dependent tyrosine phosphorylation of the B<sub>2</sub> receptor has not yet been shown. Therefore, it is possible that the receptor becomes phosphorylated on

TABLE 4

Summary of potential residues facing the B<sub>2</sub> and B<sub>1</sub> agonist and antagonist binding pockets as determined by mutagenesis, cross-linking, and competition with domain-specific antibodies<sup>a</sup>

Domain	B <sub>2</sub>					B <sub>1</sub>			
	Agonists		Antagonists			Agonist		Antagonist	
	BK	FR190997	NPC/Icatib. <sup>b</sup>	LF16-0335	FR173657	LDBK <sup>b</sup>	LDLBK <sup>b</sup>	NPC18565	Compound 11
N	Cys <sup>20</sup>								
TM-I									
TM-II		Trp <sup>86</sup>							
EL-II									
TM-III	Ser <sup>111</sup>	Ile <sup>110</sup>	Ser <sup>111</sup>	Ile <sup>110</sup>	Ile <sup>110</sup>	Lys <sup>118</sup>	(Lys <sup>118</sup> ) <sup>c</sup>	Lys <sup>118</sup>	Asn <sup>114</sup>
TM-IV	Lys <sup>172</sup>								
EL-III	Yes <sup>d</sup>			Yes <sup>d</sup>					
TM-V									
TM-VI	Phe <sup>259</sup> , Thr <sup>263</sup>	Phe <sup>259</sup>		Trp <sup>256</sup>					
EC-IV	Cys <sup>277</sup> , Asp <sup>266</sup> , Asp <sup>284</sup>						Yes <sup>d</sup>		
TMVII	Gln <sup>288</sup>				Gln <sup>288</sup> , Tyr <sup>295</sup>	Tyr <sup>295</sup>	Leu <sup>294</sup> , Phe <sup>302</sup>	Phe <sup>302</sup>	Gln <sup>295</sup>

<sup>a</sup> Discussion of individual residues and references are in the main text.

<sup>b</sup> NPC, NPC17731; Icatib., Icatibant; LDBK, Lys-des-Arg<sup>9</sup>-BK; LDLBK, Lys-[Leu<sup>8</sup>]des-Arg<sup>9</sup>-BK.

<sup>c</sup> Inferred as an important residue by extrapolating data for NPC18565.

<sup>d</sup> Domain is important but no specific residues have been identified.

tyrosine residues through homologous B<sub>2</sub> receptor-mediated or through nonreceptor-regulated heterologous mechanisms (Blaukat et al., 2000). Accordingly, the effects seen for mutations of Tyr<sup>131</sup> and Tyr<sup>322</sup> of the rat B<sub>2</sub> receptor on the signaling properties of the receptor apparently do not correlate with phosphorylation of these residues (Prado et al., 1997).

Unlike the phosphorylation of B<sub>2</sub> receptors, ligand-induced phosphorylation of B<sub>1</sub> receptors was undetectable in the absence or presence of an agonist, even by the most sensitive radiolabeling techniques (Blaukat et al., 1999a). In line with these findings, the signaling patterns of the two types of kinin receptors differ with respect to their duration; B<sub>2</sub> receptor-mediated signaling is transient, whereas B<sub>1</sub> receptor-induced signaling is sustained (Mathis et al., 1996; Faussner et al., 1998) (see Section IV.D.).

### C. Agonist and Antagonist Binding Sites in the Receptors

**1. B<sub>2</sub> Receptor Agonists.** Several methods have been used to map the agonist and antagonist ligand binding sites in B<sub>2</sub> and B<sub>1</sub> receptors including site-directed and chimeric B<sub>2</sub> and B<sub>1</sub> receptor mutagenesis, ligand receptor cross-linking, and probing with antipeptide antibodies against extracellular receptor domains and anti-idiotypic antibodies. These studies have led to the identification of several residues that appear to face the ligand binding sites of the B<sub>2</sub> and B<sub>1</sub> receptors (Table 4). The binding energy for BK and related peptide agonists in the B<sub>2</sub> receptor seems to be provided by nonionic interactions contributed by residues located throughout the peptide as well as by ionic interactions provided by the side chain of Arg<sup>1</sup> or the N terminus (Regoli and Barabé, 1980; Tancredi et al., 1997). An early model of BK bound to the rat B<sub>2</sub> receptor based on structural homology modeling with bacteriorhodopsin, molecular

modeling, and systematic conformational searching methods led to the identification of two candidate residues, Asp<sup>268</sup> and Asp<sup>286</sup> (human residues Asp<sup>266</sup> and Asp<sup>284</sup>) in EL-3 near the extracellular ends of TM-6 and TM-7, respectively, with which BK may interact (Fig. 3) (Kyle et al., 1994). It was proposed that these aspartates interact electrostatically with the N-terminal amino group, the guanidinyll side chain, or both on Arg<sup>1</sup> in BK, a residue absolutely critical for function (Regoli and Barabé, 1980). Alanine mutation of these aspartate residues in the rat receptor confirmed their critical role in BK binding (Novotny et al., 1994, Jarnagin et al., 1996). Conclusive evidence for this orientation of the receptor-bound BK was gained from chemical cross-linking of the N termini of bound [<sup>3</sup>H]BK and [<sup>125</sup>I-Tyr<sup>8</sup>]BK to Cys<sup>277</sup> in EL-3 (Herzig and Leeb-Lundberg, 1995; Herzig et al., 1996) and Lys<sup>172</sup> near TM-4 (AbdAlla et al., 1996b), respectively, in the human B<sub>2</sub> receptor (Fig. 3). Indeed, the close proximity of the BK N terminus (3 Å) to both Cys<sup>277</sup> in EL-3 and Cys<sup>20</sup> in the receptor N terminus provided direct support for the helical bundle GPCR structure (Herzig et al., 1996). Inhibition of BK binding by antipeptide antibodies specific for the N-terminal halves of EL-3 and EL-2 provided further evidence for this orientation (AbdAlla et al., 1996c). The sensitivity of the human B<sub>1</sub> receptor to the presence and absence of L-Lys at the N-terminal end of the peptide ligand was also used to orient the bound peptide agonists in the human B<sub>2</sub> receptor (Fathy et al., 2000). To do so, a chimeric construct was made in which B<sub>1</sub> receptor EL-3 was substituted in the B<sub>2</sub> receptor. This substitution resulted in a drop in the affinity of BK to that for the B<sub>1</sub> receptor. Addition of an L-Lys at the N terminus of the peptide to make Lys-BK, which is critical for high affinity binding to B<sub>1</sub> receptors, fully restored the affinity of the peptide.

Early mutagenesis studies in the rat B<sub>2</sub> receptor also identified Thr<sup>265</sup> and Phe<sup>261</sup> (human residues Thr<sup>263</sup> and Phe<sup>259</sup>), located one and two helical turns proximal to Asp<sup>266</sup> in TM-6, as critical BK binding residues (Fig. 3; Nardone and Hogan, 1994; Jarnagin et al., 1996). Thr<sup>265</sup> → Ala and Phe<sup>261</sup> → Val mutations yielded approximately 2000- and 1000-fold drops, respectively, in BK binding affinity to this receptor. Critical roles for these residues in the human B<sub>2</sub> receptor were again confirmed by a chimeric approach in which human B<sub>1</sub> receptor TM-6 was substituted in the B<sub>2</sub> receptor. Substitution of B<sub>1</sub> receptor TM-6, which is only 36% identical to B<sub>2</sub> receptor TM-6, yielded alanine and tyrosine at the corresponding positions and resulted in a 76-fold drop in BK binding affinity (Leeb et al., 1997). Replacement with the original B<sub>2</sub> receptor residues, threonine and phenylalanine, at these respective positions completely restored BK binding affinity.

Successive substitution of individual human B<sub>1</sub> receptor TM and extracellular domains in the human B<sub>2</sub> receptor resulted in the identification of a residue, Ser<sup>111</sup>, two helical turns into TM-3, which is thought to be adjacent to the C-terminal end of BK (Fig. 3) (Fathy et al., 1998, 2000). Substitution of B<sub>1</sub> receptor TM-3, which again is only 36% identical to B<sub>2</sub> receptor TM-3, yielded lysine at the corresponding position and resulted in a complete loss of BK binding (Fathy et al., 1998). Replacement with the original B<sub>2</sub> receptor residue, serine, at this position completely restored BK binding affinity. The minimal effect of alanine mutation and the inhibitory effect of lysine mutation of Ser<sup>111</sup> on BK binding in the wild-type B<sub>2</sub> receptor argues that it is the presence of a positively charged residue rather than the absence of serine at this position that inhibits binding, possibly by repelling the positively charged C-terminal arginine. Thus, the C terminus of bound BK seems to be adjacent to but not interacting with Ser<sup>111</sup> in TM-3. In summary, it is concluded that BK reaches from the extracellular surface of the receptor adjacent to EL-3, where the BK N terminus is located, down two helical turns along the interior face of TM-VI and residues Thr<sup>263</sup> and Phe<sup>259</sup>, and across into a pocket bordered by the interior face of TM-3 and presumably TM-4, -5, and -6, adjacent to Ser<sup>111</sup> in TM-III and where the C terminus is located.

The binding of the nonpeptide B<sub>2</sub> receptor agonist FR190997 only partially overlaps with that of BK (Bellucci et al., 2003). Mutation of Trp<sup>86</sup> → Ala in TM-2 and Phe<sup>259</sup> → Ala in TM-6 of the human B<sub>2</sub> receptor caused significant drops in the affinities of both FR190997 and BK (Fig. 3). On the other hand, alanine mutation of other residues including Ile<sup>110</sup> and Tyr<sup>295</sup> interfered only with FR190997. Phe<sup>259</sup> and Tyr<sup>295</sup> are interesting since the former residue is, therefore, a common critical agonist binding epitope, whereas the latter is also a critical nonpeptide antagonist binding epitope (Marie et

al., 2001; Meini et al., 2004b) and, consequently, may be specific for some nonpeptide ligands.

**2. B<sub>2</sub> Receptor Antagonists.** B<sub>2</sub> receptor binding of the peptide antagonists NPC17731 and icatibant is insensitive to most mutations that perturb BK binding (Jarnagin et al., 1996, Fathy et al., 2000; Marie et al., 2001), indicating that the peptide agonist and antagonist binding sites in the B<sub>2</sub> receptor are not identical. The only binding site overlap seems to be by Ser<sup>111</sup> (Fig. 3). Analogous to BK binding (see above), substitution of B<sub>1</sub> receptor TM-3 in the B<sub>2</sub> receptor led to a loss of NPC17731 binding, which was fully restored upon replacing the lysine at position 111 in the chimera with the original B<sub>2</sub> receptor residue, serine (Fathy et al., 1998). Furthermore, Ser<sup>111</sup> → Lys mutation in the B<sub>2</sub> receptor yielded a small but significant drop in NPC17731 binding, which was not observed with Ser<sup>111</sup> → Glu or Ala (Fathy et al., 1998; Marie et al., 2001). That the C termini of BK, NPC17731, and icatibant overlap may not be surprising since they all depend on a β-turn in the C-terminal end for high affinity binding. Using the B<sub>2</sub> receptor Ser<sup>111</sup> → Lys mutant and a series of icatibant analogs with either an amidated C terminus or Arg<sup>10</sup> replaced with alanine or glutamate, it was confirmed that the peptide C-terminal arginine residue is adjacent to Ser<sup>111</sup> and that high affinity binding is dependent on the positive charge of the guanidinyll side chain being compensated by the negative charge of the C terminus (Cucchi et al., 2002).

Alanine scanning mutagenesis of a large number of residues in the human B<sub>2</sub> receptor revealed that the binding of the nonpeptide antagonist ligand LF 16-0335 involves contacts with I<sup>110</sup>, Trp<sup>256</sup>, Tyr<sup>295</sup>, and Gln<sup>288</sup>, with the ligand quinoline moiety positioned in a lipophilic environment between Trp<sup>256</sup> and Tyr<sup>295</sup> (Fig. 3) (Marie et al., 2001; Meini et al., 2004b). Although the binding of FR173657, a structurally different nonpeptide antagonist ligand containing the same critical quinoline pharmacophore, overlapped with LF 16-0335 at I<sup>110</sup> and Tyr<sup>295</sup>, it did not overlap at Trp<sup>256</sup> (Meini et al., 2002). Thus, it was proposed that the quinoline moiety of FR173657 is positioned between I<sup>110</sup> and Tyr<sup>295</sup>. Interestingly, mutations affecting nonpeptide antagonist binding affected peptide antagonist binding only minimally, if at all. Thus, antagonism may be caused by ligands interacting with widely different binding epitopes, as described for other GPCRs.

**3. B<sub>1</sub> Receptor Agonists.** Relatively few binding site mapping studies have been done in the B<sub>1</sub> receptor. Considering that the endogenous ligands for the B<sub>2</sub> and B<sub>1</sub> receptors are structurally very similar differing only by the absence and presence of a C-terminal arginine and that both peptide agonists (e.g., Lys-BK) and antagonists (e.g., B9430) with low selectivity exist, the peptide binding sites in the two receptors are probably similar. Unlike BK, the binding energy for Lys-des-Arg<sup>9</sup>-BK in the B<sub>1</sub> receptor seems to be provided primarily by ionic



interactions contributed by the positive charges of Lys<sup>1</sup> and/or Arg<sup>2</sup> and the negatively charged C terminus, whereas positions 3–6 serve primarily a structural role (Regoli and Barabé, 1980; Tancredi et al., 1997). Furthermore, the dependence on an N-terminal L-lysine for binding to the B<sub>1</sub> receptor also varies with the species. Whereas B<sub>1</sub> receptors in most species have a much lower affinity for des-Arg<sup>9</sup>-BK compared with Lys-des-Arg<sup>9</sup>-BK, rodent receptors bind the two peptides with almost equal high affinity (nanomolar) (see *Section II.A.*). As described above, this phenotype may be traced to the fact that rodent kininogen contains an arginine rather than a lysine N-terminal of the BK sequence (Hess et al., 1996; Takano et al., 1997); arginine cannot replace lysine for high affinity binding to the human B<sub>1</sub> receptor (Fathy et al., 2000).

The B<sub>1</sub> receptor contains a conserved glutamate (human residue Glu<sup>273</sup>) and aspartate (human residue Asp<sup>291</sup>) at the positions corresponding to the two aspartates in EL-3 in the human B<sub>2</sub> receptor, which are assumed to interact with Arg<sup>1</sup> in des-Arg<sup>9</sup>-BK and Lys-des-Arg<sup>9</sup>-BK, a residue critical for peptide B<sub>1</sub> receptor binding (Regoli and Barabé, 1980), even though this needs to be tested (Fig. 3). Likewise, Lys<sup>1</sup> in Lys-des-Arg<sup>9</sup>-BK is thought to interact specifically with a residue(s) in EL-3 (Fathy et al., 2000). For the C-terminal end of the peptide, serine mutation of Lys<sup>118</sup> in TM-3 of the human B<sub>1</sub> receptor (Ser<sup>111</sup> in the human B<sub>2</sub> receptor) resulted in a complete loss of Lys-des-Arg<sup>9</sup>-BK binding showing that Lys<sup>118</sup> provides a counterion for the C terminus of peptide ligands lacking the C-terminal arginine. Indeed, the acceptance by the receptor of a C-terminal arginine in the peptide ligand, the most important peptide ligand determinant for receptor subtype specificity, may be determined by the absence and presence of a lysine at this position.

The human B<sub>1</sub> receptor expressed in HEK293 cells was reported to be activated directly by enalaprilat and other ACE inhibitors (Ignjatovic et al., 2002). This activation was found to be dependent on the Zn<sup>2+</sup>-binding consensus motif HEXXH at positions 195–199 at the top of TM-5 in EL-2 in the receptor (Fig. 3). Indeed, His<sup>195</sup> → Ala mutation or incubation with a peptide spanning positions 192–202 blocked activation of the receptor by these inhibitors. The direct effect of ACE inhibitors on the B<sub>1</sub> receptor was subsequently re-evaluated by Fortin et al. (2003b), who were completely unsuccessful in showing any agonism by these inhibitors at the human or rabbit receptor in either contractility assays or cell-based functional assays. The conserved Zn<sup>2+</sup>-binding consensus motif in the B<sub>1</sub> receptor, which is not present in the B<sub>2</sub> receptor, may participate in diverse functions such as the formation of heterodimers with other molecular partners that remain to be identified, some enzymatic activity, or the direct binding of other kallikrein-kinin system components.

*4. B<sub>1</sub> Receptor Antagonists.* Peptide antagonists are also sensitive to the type of residue at position 118 in the B<sub>1</sub> receptor (Fig. 3). The B<sub>2</sub> receptor affinity of NPC18565, the des-Arg<sup>10</sup> analog of NPC17731, increased upon replacement of Ser<sup>111</sup> with lysine. On the other hand, alanine had no effect and glutamate decreased binding further supporting the counter-ion role of a basic residue at this position (Fathy et al., 1998). Another discriminating region for peptide antagonists in B<sub>1</sub> and B<sub>2</sub> receptors is located in the N-terminal half of TM-7. Substitution of the B<sub>2</sub> receptor TM-VII into the B<sub>1</sub> receptor yielded 7- and 20-fold decreases in the affinities of Lys-des-Arg<sup>9</sup>-BK and Lys-[Leu<sup>8</sup>]des-Arg<sup>9</sup>-BK, respectively. Interestingly, the affinity of icatibant was increased 13-fold (Bastian et al., 2000). Replacement of Thr<sup>287</sup> in this chimera with the corresponding B<sub>1</sub> receptor residue, leucine, led to a complete recovery of Lys-des-Arg<sup>9</sup>-BK affinity, and replacement of Tyr<sup>295</sup> with the B<sub>1</sub> receptor residue phenylalanine restored both peptide agonist and antagonist binding properties. Individual substitution of Leu<sup>294</sup> and Phe<sup>302</sup> in the B<sub>1</sub> receptor with the B<sub>2</sub> receptor counterparts increased icatibant affinity 6- and 69-fold, respectively. Thus, these residues may make up part of the binding pocket for peptide antagonists in both the B<sub>1</sub> and B<sub>2</sub> receptors. Interestingly, these residues are close to Gln<sup>290</sup> in the rat B<sub>2</sub> receptor (human B<sub>2</sub> residue Gln<sup>288</sup>; human B<sub>1</sub> residue Gln<sup>295</sup>), which upon alanine mutation caused an 11-fold reduction in BK affinity (Jarnagin et al., 1996).

B<sub>1</sub> receptor binding of Compound 11, a recently developed selective B<sub>1</sub> receptor nonpeptide antagonist (Fig. 2; Su et al., 2003), was insensitive to alanine mutation of Lys<sup>118</sup> in TM-III (Fig. 3). On the other hand, a 15-fold drop in affinity was observed upon alanine mutation of Asn<sup>114</sup> located one turn above Lys<sup>118</sup> in TM-3. Furthermore, Gln<sup>295</sup> → Ala mutation in TM-VII decreased the affinity of Compound 11 by 26-fold without perturbing peptide agonist binding. Whereas these mutations suggest that the binding site for this nonpeptide antagonist may be proximal to the peptide agonist binding site in this receptor, the two sites are distinct.

#### D. Evolutionary Aspects of Kinin Receptors

To date, our knowledge on the evolution of kinin receptors is rather limited. For B<sub>1</sub> receptors, orthologs have been identified in human (Menke et al., 1994), rat (Ni et al., 1998a), mouse (Pesquero et al., 1996b), rabbit (MacNeil et al., 1995), dog, African green monkey, rhesus monkey (Hess et al., 2001), and *Tupaia minor* (lesser treeshrew). For B<sub>2</sub> receptors, orthologs have been described in human (Hess et al., 1992), rat (McEachern et al., 1991), mouse (McIntyre et al., 1993; Hess et al., 1994), guinea pig (Farmer et al., 1998), rabbit (Bachvarov et al., 1995), dog (Hess et al., 2001), *T. minor*, and pig. Only two nonmammalian kinin receptors are known: the chick ornithokinin receptor (Schroeder et al., 1997) which responds to ornithokinin, [Thr<sup>6</sup>, Leu<sup>8</sup>]-BK,



and the zebrafish kinin receptor (Dunér et al., 2002) triggered by piscine kinin, [Arg<sup>0</sup>,Trp<sup>5</sup>,Leu<sup>8</sup>]-BK.

As described above, genomic sequencing of the human chromosomal regions encoding the B<sub>1</sub> and B<sub>2</sub> receptors (chromosome 14) revealed that the two genes are located in tandem orientation on chromosome 14q32.1-q32.2 with the B<sub>2</sub> receptor gene being proximal to the B<sub>1</sub> receptor gene, separated by an intergenic region of only 12 kb (Fig. 4; Cayla et al., 2002a). Likewise, the two kinin receptor genes are juxtaposed in a single locus of the mouse (7.8 kb apart on chromosome 12) and rat (9.5 kb apart in chromosome 6) genomes. The close proximity of the two genes clearly suggests that they evolved from a common ancestor by a gene duplication event. The three exon organization of the B<sub>1</sub> and B<sub>2</sub> receptor genes is similar in human, mouse, and rat, except that the human and rat, but not the mouse, B<sub>2</sub> receptor genes carry an alternatively spliced exon 2b interspersed between exons 2 and 3 (Cayla et al., 2002a). These findings exemplify the principle that human evolution may have progressed without a change in the total gene number but by increasing the diversity of the gene products due to the insertion of alternatively spliced exons (Cayla et al., 2002a). Phylogenetic analyses comparing the amino acid sequences of mammalian kinin receptors demonstrated only 80% identity among human, mouse, rat, and rabbit B<sub>2</sub> receptors suggesting that the underlying gene has evolved fairly rapidly. In a neighbor-joining tree, the nonmammalian kinin receptors from chicken and zebrafish form a cluster with mammalian B<sub>2</sub> receptors (Dunér et al., 2002) indicating that the latter may indeed represent the primordial kinin receptor gene (Fig. 1). This notion is reinforced by the fact that B<sub>1</sub> receptor-like genes have not been found in chicken or zebrafish to date.

Although sequence information on kinin receptors from other vertebrate or nonvertebrate species, including reptiles or insects, is still lacking, there is at least circumstantial evidence that kinin receptors may exist in many more species where kinins have been unequivocally identified. For instance, reptiles such as crocodiles, turtles, and tortoises generate [Thr<sup>6</sup>]-BK, whereas snakes produce [Ala<sup>1</sup>, Thr<sup>6</sup>]-BK (python) or [Val<sup>1</sup>, Thr<sup>6</sup>]-BK (colubrid snakes), whereas fish release [Trp<sup>5</sup>]-BK (bowfish, gar), [Met<sup>1</sup>, Met<sup>5</sup>]-BK (sturgeon), or extended versions of BK, i.e., [Arg<sup>0</sup>,Trp<sup>5</sup>,Leu<sup>8</sup>]-BK (trout, cod, eel) (Conlon, 1999). Amphibia, in particular frogs of the genus *Rana*, produce massive amounts of “authentic” BK in their skin, and insects such as wasp perfectly “mimic” the human kinin and produce high amounts of [Hyp<sup>3</sup>]-BK in their venoms. At this time, it is unclear whether these species produce kinins as components of their own signaling cascades involving homologous kinin receptors, or whether they have developed the capacity, due to convergent evolution, to produce kinins in venoms or in skin to target their kinin-sensitive prey. With a rapidly growing number of genomes from diverse species in the

pipeline, we will likely learn more about nonmammalian and nonvertebrate kinin receptors soon.

#### IV. Molecular and Cellular Aspects of Kinin Receptor Signaling and Regulation

##### A. Agonist-Dependent and -Independent Mechanisms of Receptor Activation

As GPCRs, the B<sub>1</sub> and B<sub>2</sub> receptors are thought to spontaneously isomerize between inactive and active conformational states. Evidence for agonist-independent, spontaneous B<sub>2</sub> receptor activity was first discovered with inverse agonists in primary cultures of late-pregnant rat myometrial cells (Leeb-Lundberg et al., 1994b). In this system, the BK antagonists NPC17731, icatibant, and NPC567 inhibited basal cellular phosphoinositide (PI) hydrolysis in a dose-dependent, saturable, and BK-independent manner and with an order of potency typical of a B<sub>2</sub> receptor. The presence of a limited but significant amount of spontaneous human B<sub>2</sub> receptor activity has subsequently been confirmed in HEK293 cells transfected with the receptor (Fathy et al., 1999; Marie et al., 1999). The B<sub>2</sub> receptor can be constitutively activated by mutating specific residues in the transmembrane helices that presumably participate in interhelical contacts that stabilize the inactive conformation(s) of the receptor. Asp<sup>76</sup> → Asn mutation in TM-2 of the rat receptor (human residue Asp<sup>78</sup>) was the first such mutation and led to a modest 3-fold increase in the agonist-independent receptor activity (Quitterer et al., 1996). In that study, agonist-independent activity was also elevated by a reduction in the Na<sup>+</sup> ion concentration, and the salt effect was lost upon Asp<sup>76</sup> mutation. Therefore, it was proposed that Na<sup>+</sup> ions stabilize the inactive states of the receptor by interacting with Asp<sup>76</sup>. Mutating the corresponding residue, Asp<sup>78</sup>, in the human B<sub>2</sub> receptor did not confirm this role (Marie et al., 1999). On the other hand, Asn<sup>113</sup> → Ala mutation in TM-3 was found to increase the human B<sub>2</sub> receptor activity by 40-fold (Fig. 3). A smaller but significant increase was also observed by Trp<sup>256</sup> → Ala mutation in TM-6. Both peptide antagonist icatibant and nonpeptide antagonist LF 16-0335 behaved as inverse agonists on this constitutive activity.

The above observations led to a model of B<sub>2</sub> receptor activation in which the inactive state is stabilized by an interaction between Asn<sup>113</sup> and Trp<sup>256</sup> (Fig. 3) (Marie et al., 2001). This interaction was directly confirmed by the ability of Zn<sup>2+</sup> to coordinate histidine residues substituted at these receptor positions. It was further proposed that the quinoline moiety of the nonpeptide inverse agonist LF 16-0335 is positioned between Tyr<sup>295</sup> in TM-7 and Trp<sup>256</sup> TM-6, two critical binding epitopes for this ligand, to favor the interaction of Trp<sup>256</sup> with Asn<sup>113</sup>. In the active receptor state, Trp<sup>256</sup> was instead proposed to interact with Phe<sup>259</sup> (Marie et al., 2001). This interaction may be preferred or favored by agonists

since Phe<sup>259</sup> is a specific critical epitope for high affinity binding of both BK (Nardone and Hogan, 1994; Jarnagin et al., 1996; Leeb et al., 1997) and nonpeptide agonist FR190997 (Bellucci et al., 2003). However, the agonist-receptor interaction is probably more complex, since B<sub>2</sub> receptor activation seems to involve more than one agonist-binding mode (Leeb-Lundberg and Mathis, 1990).

Critical receptor epitopes for G protein binding and activation are often located on multiple intracellular domains, which are thought to act in concert to form a binding site. Some efforts have been made to identify epitopes in the B<sub>2</sub> and B<sub>1</sub> receptors that are important for G protein signaling to PI hydrolysis and arachidonic acid release. Truncation of the human B<sub>2</sub> receptor C-terminal tail up to and including Ser<sup>316</sup> attenuated but did not inhibit receptor coupling (Fig. 3; Prado et al., 1997; Pizard et al., 1999; Kang and Leeb-Lundberg, 2002). On the other hand, truncation beyond this residue completely abolished activity (Kang and Leeb-Lundberg, 2002). This effect may be due to the disruption of an amphipathic  $\alpha$  helix, helix 8, that is presumed to span positions 310 to 321 in the human receptor and which may be required for effective receptor coupling to G $\alpha_q$  (Kang and Leeb-Lundberg, 2002). Two residues in this helix have been found to influence signal coupling. Alanine mutation of Arg<sup>311</sup> yielded a dramatic increase in the coupling to PI hydrolysis suggesting that this residue has a structural role in this helix (Kang and Leeb-Lundberg, 2002). Alanine mutation of Tyr<sup>322</sup> in the rat receptor (human residue Tyr<sup>320</sup>) had no direct effect on signal coupling. On the other hand, this mutation completely recovered the deleterious effect of mutating Tyr<sup>131</sup> (human residue Tyr<sup>129</sup>) in IC-II and part of the DRY motif. Several additional mutations led to the conclusion that these two tyrosines are involved in an interaction, which is controlled by Thr<sup>137</sup> (human residue Tyr<sup>135</sup>) and important for G protein activation and receptor internalization (Prado et al., 1998). Truncation of the B<sub>1</sub> receptor C-terminal tail revealed that this domain is not necessary for efficient coupling to PI hydrolysis, even though residues in the membrane distal portion of the tail greatly augment both constitutive and agonist-promoted signaling (Kang and Leeb-Lundberg, 2002).

A potentially novel mode of B<sub>2</sub> receptor activation involving proteases was recently proposed (Hecquet et al., 2000). It was reported that tissue and plasma kallikrein, trypsin, and cathepsin G triggered receptor-dependent intracellular Ca<sup>2+</sup> mobilization that was inhibited by the BK antagonist icatibant. That tissue kallikrein activates the B<sub>2</sub> receptor directly has been challenged since it was found that at low tissue kallikrein concentrations a local generation of kinins was observed that could mediate the response, whereas at high concentrations, tissue kallikrein cleaved and degraded the receptor and exerted nonreceptor-mediated effects on Ca<sup>2+</sup> signaling (Houle et al., 2003). That the

B<sub>2</sub> receptor is a typical protease-activated receptor seems unlikely since BK and Lys-BK are natural ligands for this receptor, and BK-like sequences are conspicuously absent from the known primary structures of kinin receptors.

The B<sub>1</sub> receptor is constitutively active in transfected HEK293 cells (Leeb-Lundberg et al., 2001). Indeed, this activity is almost as high as that of the agonist-stimulated B<sub>2</sub> receptor. Nevertheless, agonists further stimulate B<sub>1</sub> receptor activity. Constitutive B<sub>1</sub> receptor activity has not been confirmed in a native system since at present time such activity can only be detected using inverse agonists, and no such agents are currently available for this receptor. Interestingly, alanine mutation of Asn<sup>121</sup> in the human B<sub>1</sub> receptor, which corresponds to Asn<sup>113</sup> in the human B<sub>2</sub> receptor, caused a further large increase in constitutive B<sub>1</sub> receptor activity suggesting that this activity has a molecular basis that is different from the basal constitutive activity (Fig. 3) (Leeb-Lundberg et al., 2001; Ni et al., 2003).

### B. Receptor Cellular Signaling Pathways

The B<sub>2</sub> receptor is generally described as signaling through G $\alpha_q$  (Gutowski et al., 1991; LaMorte et al., 1993; Liao and Homcy, 1993; Wilk-Blaszczak et al., 1994; Jones et al., 1995), even though this receptor interacts with several other G proteins as well including G $\alpha_i$  (Ewald et al., 1989; Linder et al., 1990), G $\alpha_s$  (Liebmann et al., 1996), and G $\alpha_{12/13}$  (Gohla et al., 1999). BK-stimulated G $\alpha_q$ -sensitive phospholipase C $\beta$  yielding PI hydrolysis and an increase in intracellular free Ca<sup>2+</sup> has been observed in many cell types (e.g., Yano et al., 1985; Tilly et al., 1987; Fasolato et al., 1988; Voyno-Yasenetskaya et al., 1989; Byron et al., 1992; Derian and Moskowitz, 1986). Furthermore, BK translocation of the protein kinase C (PKC) isozymes  $\alpha$ ,  $\epsilon$ , and  $\zeta$  has been shown in both fibroblasts (Tippmer et al., 1994) and endothelial cells (Ross and Joyner, 1997). BK stimulation of phospholipase A<sub>2</sub> activity appears to occur via G protein-mediated mechanisms (Burch and Axelrod, 1987; Yanaga et al., 1991) through Ca<sup>2+</sup>- and phosphorylation-dependent activation of the cytosolic isoform of phospholipase A<sub>2</sub> (Xing et al., 1997; Lal et al., 1998), whereas phospholipase D activation can be mediated via Ca<sup>2+</sup> influx, PKC activation, and the monomeric GTPase RhoA (Pyne and Pyne, 1995; Meacci et al., 1999; Han et al., 2003).

BK is an efficacious stimulator of eNOS and NO production through Ca<sup>2+</sup>-mediated mechanisms in endothelial cells (Busse and Fleming, 1995; see below). In these cells, BK also transiently promotes tyrosine phosphorylation of MAPK (Fleming et al., 1995), phospholipase C $\gamma$  (Fleming et al., 1996), Hsp90 (Harris et al., 2000), and the B<sub>2</sub> receptor (Marrero et al., 1999) and induces B<sub>2</sub> receptor association with phospholipase C $\gamma$  (Venema et al., 1998). Furthermore, BK activates a caveolae-associated Janus-activated kinase/STAT path-

way in these cells. This involves tyrosine phosphorylation of both the Janus-activated kinase family tyrosine kinase Tyk2 and STAT3 followed by STAT3 nuclear translocation. Following BK stimulation, the B<sub>2</sub> receptor and STAT3 are translocated out of caveolae (Ju et al., 2000).

Depending on the cell type, BK induces proliferative (Owen and Villereal, 1983; Goldstein and Wall, 1984) or antiproliferative (Patel and Schrey, 1992; Dixon and Dennis, 1997; Alric et al., 2000) responses. The proliferative response involves many of the typical growth factor-promoted pathways. BK stimulation of MAPK, which is independent of receptor endocytosis (Blaukat et al., 1999b), appears to involve a synergistic coupling of the receptor to both G $\alpha_q$  and G $\alpha_i$  (Blaukat et al., 2000) proceeding through PKC and calcium-dependent pathways involving transactivation of the epidermal growth factor (EGF) receptor or the combined actions of the protein tyrosine kinases Pyk2 and Src (Dikic et al., 1996; Zwick et al., 1997; Adomeit et al., 1999; Velarde et al., 1999). In fibroblasts, BK also stimulates transient tyrosine phosphorylation and activation of focal adhesion kinase (Leeb-Lundberg et al., 1994a) as well as several other focal adhesion-associated phosphotyrosine substrates (Leeb-Lundberg and Song, 1991) including paxillin and p130<sup>Cas</sup> (Leeb-Lundberg et al., 1994a), events that have been linked to regulation of proliferation, adhesion, migration, and apoptosis. In these cells, BK also stimulates peripheral actin microspikes and membrane ruffling via activation of Cdc42 and Rac1 (Kozma et al., 1995). The antiproliferative actions of BK may be prostaglandin-mediated (Patel and Schrey, 1992), involve activation of a tyrosine phosphatase (Alric et al., 2000; Duchene et al., 2002; see *Section IV.C.*), or occur via further downstream mechanisms (Dixon and Dennis, 1997).

BK activates multiple transcription factors that regulate the induction of several cytokines involved in tissue injury and inflammation as well as B<sub>1</sub> receptor induction. BK stimulates nuclear factor- $\kappa$ B (NF- $\kappa$ B) activation leading to interleukin (IL)-1 $\beta$  gene expression in cultured fibroblasts (Pan et al., 1996). This activation was inhibited by pertussis toxin indicating the participation of G $\alpha_i$  and/or G $\beta\gamma$ . Subsequent studies have shown that BK stimulation of NF- $\kappa$ B activation proceeds through both G $\beta\gamma$  and G $\alpha_q$  to PI-3K and Akt (Xie et al., 2000) as well as RhoA (Pan et al., 1998). BK also stimulates expression of IL-6 and IL-8 (Phagoo et al., 1999; Hayashi et al., 2000). In cultured smooth muscle cells, BK-stimulated expression of IL-8 was dependent on both a prostanoid-independent and -dependent mechanism (Zhu et al., 2003). The prostanoid-independent mechanism involved activation of NF- $\kappa$ B, whereas the prostanoid-dependent mechanism involved activation of the transcription factors AP-1 and NF-IL-6. BK-stimulated c-fos expression and AP-1 formation in smooth muscle has been reported to occur through a pathway

involving ERK and Elk-1 (El-Dahr et al., 1998) and reactive oxygen species (Greene et al., 2000).

The B<sub>1</sub> receptor also directly interacts with G $_q$  and G $_i$  (Austin et al., 1997) through which it mediates agonist stimulation of many of the same signaling pathways as the B<sub>2</sub> receptor (reviewed by Marceau et al., 1998) including PI hydrolysis (Tropea et al., 1993), elevation of intracellular Ca<sup>2+</sup> (Bascands et al., 1993; Marsh and Hill, 1994; Smith et al., 1995; Mathis et al., 1996), arachidonic acid release (Tropea et al., 1993), eicosanoid production (Levesque et al., 1995), and eNOS activation and NO production (Tsutsui et al., 2000). Furthermore, the B<sub>1</sub> receptor is also either proliferative (Marceau and Tremblay, 1986) or antiproliferative (Dixon et al., 2002). The proliferative response probably involves MAPK activation (Christopher et al., 2001a), although prolonged activation of MAPK leading to increased p27<sup>Kip1</sup> activity has been proposed to be responsible for the antiproliferative effect of B<sub>1</sub> receptor stimulation in arterial smooth muscle cells (Dixon et al., 2002).

Although the B<sub>1</sub> and B<sub>2</sub> receptors seem to couple to similar cellular signal transduction pathways, the patterns of signaling are different. In vascular smooth muscle cells, B<sub>2</sub> receptor stimulation led to a transient increase in PI hydrolysis that exhibited little dependence on extracellular Ca<sup>2+</sup>, whereas B<sub>1</sub> receptor stimulation was more sustained and significantly dependent on extracellular Ca<sup>2+</sup>. Furthermore, the B<sub>2</sub> receptor elicited a transient increase in intracellular free Ca<sup>2+</sup>, whereas the B<sub>1</sub> receptor elicited a sustained signal which was characterized by a plateau of elevated Ca<sup>2+</sup> or baseline oscillation that were dependent on extracellular Ca<sup>2+</sup> influx (Bascands et al., 1993; Tropea et al., 1993; Smith et al., 1995; Mathis et al., 1996). The distinct patterns of signaling are probably consequences of the rather different degrees of short-term regulation that these receptors are subject to, including both receptor desensitization and internalization (see *Section IV.D.*).

### C. Protein-Protein Interactions in Receptor Signaling

BK binding kinetics to the B<sub>2</sub> receptor expressed in CHO cells at different levels of receptor occupancy followed the model of negative cooperativity (Pizard et al., 1998). This result is directly indicative of receptor-receptor interactions in the membrane. Indeed, BK binding to the B<sub>2</sub> receptor on PC-12 cells followed by cross-linking of the agonist to the receptor led to the identification of monomeric as well as dimeric and possibly higher order homo-oligomeric receptor structures (AbdAlla et al., 1999). No oligomeric structures were detected following cross-linking of the B<sub>2</sub> receptor antagonist icatibant. Incubation of the cells with a peptide corresponding to the receptor N-terminal domain or deletion of this domain in receptors expressed in HEK293 cells disrupted the BK-promoted structures. Neither peptide competition nor deletion mutagenesis affected receptor-mediated stimulation of cellular PI hydrolysis and intracel-



lular  $\text{Ca}^{2+}$  mobilization. On the other hand, a decrease in receptor desensitization and internalization was observed. Although these results suggest that  $\text{B}_2$  receptor homo-oligomers exist and participate in receptor regulation, our understanding of receptor oligomerization is still in its infancy.

Recently, Kang et al. (2005) provided evidence consistent with  $\text{B}_1$  receptor homo-oligomerization by using coimmunoprecipitation of differentially tagged receptors and immunoelectron microscopy. Truncation of the receptor indicated that the epitope for oligomerization appears to be located between Leu<sup>26</sup> and Val<sup>71</sup> (Fig. 3). Furthermore, receptor fragments acted as dominant-negative mutants by competitively preventing formation of intact receptor homo-oligomers and redistributing the receptors from the cell surface to a common intracellular compartment. It was concluded that receptor homo-oligomerization is required for expression of receptors on the cell surface and subsequent constitutive and agonist-stimulated cell signaling.

Evidence for a heterologous  $\text{B}_2$  and  $\text{B}_1$  receptor complex has also been presented (Kang et al., 2004). Human  $\text{B}_2$  and  $\text{B}_1$  receptors were found to spontaneously heterodimerize when coexpressed in transfected HEK293, as determined by both coimmunoprecipitation and identification of plasma membrane colocalization by immunoelectron microscopy. Heterodimerization was associated with a proteolytic degradation specifically of the participating  $\text{B}_2$  receptor and an increase in both agonist-dependent and -independent signaling of the heterologous receptor complex. This cell-specific regulatory mechanism was suggested to participate in converting a kinin signal from a  $\text{B}_2$ -type to a  $\text{B}_1$ -type during persistent insult and  $\text{B}_1$  receptor induction. Direct support for an interaction between  $\text{B}_2$  and  $\text{B}_1$  receptors was provided by studying kinin-stimulated proliferative signaling in PC3 prostate cancer cells, where the pharmacological profile of kinin signaling through each receptor subtype suggested the coengagement of the other subtype in the response (Barki-Harrington et al., 2003).

A heterodimeric complex of the  $\text{B}_2$  and  $\text{AT}_1$  receptors has also been observed in both A10 vascular smooth muscle cells and in transfected HEK293 cells (AbdAlla et al., 2000). The formation of this complex, as detected using agonist cross-linked receptors and immunoaffinity chromatography, was independent of agonist binding. On the other hand, receptor coexpression increased the potency and efficacy of angiotensin II, whereas it decreased the potency and efficacy of BK. Furthermore,  $\text{B}_2$  and  $\text{AT}_1$  receptors expressed individually sequestered through a dynamin I-independent mechanism, whereas coexpression yielded dynamin I-dependent receptor sequestration. The enhancement of angiotensin II signaling was independent of BK  $\text{B}_2$  receptor binding but dependent on an intact  $\text{B}_2$  receptor G protein coupling mechanism. The existence of a  $\text{B}_2/\text{AT}_1$  heterodimeric complex may have implications for blood pressure con-

trol since these two receptors are functional antagonists in cardiovascular regulation with BK being a vasodepressor and angiotensin II being a vasoconstrictor. Furthermore, the levels of these two agonists are oppositely regulated by ACE, underlying part of the beneficial effects of ACE inhibitors. Pre-eclampsia involves a hypersensitivity to angiotensin II. Interestingly, evaluation of platelets and omental vessels from a group of pre-eclamptic women revealed an increased level of  $\text{B}_2/\text{AT}_1$  heterodimers on these cellular structures (AbdAlla et al., 2001). The increase was due to an elevated expression of the  $\text{B}_2$  receptor. As predicted, this was associated with an increased sensitivity to angiotensin II. Furthermore, the dimerization conferred an increased resistance in the  $\text{AT}_1$  signaling to reactive oxygen species. Thus,  $\text{B}_2/\text{AT}_1$  receptor heterodimers may in part underlie the increased sensitivity to angiotensin II observed in pre-eclampsia.

In addition to physically interacting with effector G proteins (Mathis and Leeb-Lundberg, 1991), the  $\text{B}_2$  receptor has also been found to directly interact with other effectors in a G protein-independent manner. BK stimulation of endothelial NO production involves both a  $\text{Ca}^{2+}$ - and phosphorylation-dependent activation of eNOS (Busse and Fleming, 1995; Venema, 2002). In addition,  $\text{B}_2$  receptors physically interact with eNOS via an epitope in the membrane-proximal portion of the receptor C-terminal domain, as shown by both coimmunoprecipitation in endothelial cells and enrichment of eNOS using a glutathione *S*-transferase fusion protein containing the C-terminal tail of the receptor (Ju et al., 1998; Golser et al., 2000). Since a C-terminal peptide containing the epitope inhibited purified eNOS activity, and BK binding to the  $\text{B}_2$  receptor caused a dissociation of the receptor eNOS interaction in the endothelial cells; a possible regulatory scenario is that this interaction contributes to BK activation of the enzyme by disinhibiting it. A more detailed description was recently published elsewhere (Venema, 2002).

The antiproliferative action of BK in rat mesangial cells involves a direct physical interaction of the  $\text{B}_2$  receptor with the protein tyrosine phosphatase SHP-2 (Duchene et al., 2002). Interaction via an ITIM domain located at the bottom of TM-7 was proven both by  $\text{B}_2$ -SHP-2 coimmunoprecipitation, receptor mutagenesis, and by surface plasmon resonance with SHP-2 using synthetic wild-type and mutated receptor peptides. The involvement of this interaction in the antiproliferative action of BK was shown in stable CHO cells expressing receptor mutated in the ITIM sequence as well as in rat mesangial cells transfected with a dominant-negative SHP-2 mutant.

The  $\text{B}_2$  receptor is also thought to interact with ACE. Inhibitors of ACE exert many of their beneficial cardiovascular effects via the  $\text{B}_2$  receptor (Linz et al., 1995). In addition to preventing BK degradation, evidence has accumulated pointing toward the fact that ACE inhibi-



tors potentiate BK actions via a more direct effect on the B<sub>2</sub> receptor and its regulation and that the B<sub>2</sub> receptor and ACE communicate directly in the membrane (Hecker et al., 1994; Minshall et al., 1997a,b). For example, ACE inhibitors enhance the effect of ACE-resistant analogs of BK (Hecker et al., 1994). Furthermore, they reactivate B<sub>2</sub> receptors following BK-promoted receptor desensitization (Benzing et al., 1999; Marcic et al., 1999), which is absolutely dependent on ACE and B<sub>2</sub> coexpression (Marcic et al., 1999). ACE inhibitors also prevent the sequestration of the B<sub>2</sub> receptor in caveolae (Benzing et al., 1999). Indeed, the B<sub>2</sub> receptor and ACE apparently coimmunoprecipitate in stable CHO cells coexpressing the proteins (Marcic et al., 2000). Furthermore, following addition of a glycosylphosphatidylinositol moiety to ACE, which targets the enzyme to cholesterol-rich plasma membrane lipid rafts, ACE inhibitors were unable to reactivate the desensitized B<sub>2</sub> receptor (Marcic et al., 2000). On the other hand, treatment with filipin to disrupt the microdomains recovered the effect of the inhibitors. Furthermore, BK enhanced the activity of ACE-associated casein kinase 2 and the phosphorylation of ACE residue Ser<sup>1270</sup> in human endothelial cells, and this is linked to c-Jun NH<sub>2</sub>-terminal kinase association of ACE and nuclear accumulation of phosphorylated c-Jun (Kohlstedt et al., 2004).

#### D. Receptor Desensitization

At the cellular level, BK stimulation of the B<sub>2</sub> receptor leads to a rapid desensitization of the receptor response as determined both by PI hydrolysis and the increase in intracellular Ca<sup>2+</sup> in a number of native systems including NG108–15 cells (Wolsing and Rosenbaum, 1991), rat mesangial cells (Bascands et al., 1993), vascular endothelial cells (Smith et al., 1995), vascular smooth muscle cells (Mathis et al., 1996), and various cell systems transfected with the B<sub>2</sub> receptor (Blaukat et al., 1996, 2001; Fathy and Leeb-Lundberg, 1999). The mechanism of B<sub>2</sub> receptor desensitization involves phosphorylation of specific serines and threonines in the receptor C-terminal tail (see *Section III.B.* and Fig. 5). Coexpression of the B<sub>2</sub> receptor in HEK293 cells with individual G protein-coupled receptor kinases (GRKs) revealed that GRK2, GRK3, GRK5, or GRK6 did not significantly change basal or BK-mediated B<sub>2</sub> receptor phosphorylation, whereas GRK4 $\alpha$  drastically increased the basal level of <sup>32</sup>P-incorporation into the B<sub>2</sub> receptor (Blaukat et al., 2001). Mapping studies showed quantitative changes in the distribution pattern of phosphopeptides, e.g., phosphorylation of Ser<sup>346</sup>/Ser<sup>348</sup> was enhanced 1.5- to 3-fold over mock-transfected cells in the order GRK3>GRK4 $\alpha$ >GRK2>GRK5>GRK6. Most prominently, GRK4 $\alpha$  elevated the basal phosphorylation of Ser<sup>339</sup> and Ser<sup>346</sup>/Ser<sup>348</sup> 15- and 24-fold, respectively. Thus, various endogenous GRKs may phosphorylate the B<sub>2</sub> receptor and induce distinct phosphorylation patterns, even without apparent effect on total B<sub>2</sub> receptor

phosphorylation, with possible functional consequences for receptor desensitization and internalization.

Among the non-GRKs, PKC induced a ligand-independent phosphorylation of the B<sub>2</sub> receptor (Blaukat et al., 1996). Pretreatment of cells with phorbol 12-myristate 13-acetate significantly reduced BK-triggered phospholipase C stimulation indicating that heterologous receptor phosphorylation may negatively affect the signaling cascade of the B<sub>2</sub> receptor. 2-D phosphopeptide mapping of <sup>32</sup>P-labeled cells pretreated with phorbol 12-myristate 13-acetate identified Ser<sup>346</sup> as the major PKC phosphorylation site. PKC-mediated phosphorylation of Ser<sup>346</sup> was also found in the absence of exogenous PKC activators. Of note, PKC stimulation did not produce tandem phosphorylation of Ser<sup>346</sup>/Ser<sup>348</sup> indicating that basal phosphorylation at Ser<sup>348</sup> and PKC-mediated phosphorylation at Ser<sup>346</sup> is mutually exclusive (Blaukat et al., 2001).

Reversible post-translational modifications such as phosphorylation and palmitoylation of the B<sub>2</sub> receptor may be dynamic and physiological processes that influence both receptor coupling efficacy and agonist behavior. Alanine mutation of the cluster of serines and threonines in the B<sub>2</sub> receptor C-terminal tail that are targets for GRK phosphorylation elevated both agonist-dependent and -independent receptor activity in transfected HEK293 cells (Fathy et al., 1999). This observation suggests that the B<sub>2</sub> receptor is subject to not only agonist-promoted desensitization but also a form of basal desensitization, possibly caused in response to the agonist-independent activity leading to a basal level of phosphate in the receptor (Blaukat et al., 2001). Interestingly, the increase in the basal receptor activity also changed the behavior of the peptide antagonists NPC17731 and icatibant from weak partial agonists to inverse agonists, classifying them as protean agonists. Alanine mutation of two cysteines (Cys<sup>324</sup> and Cys<sup>329</sup>) in the C-terminal domain, which are targets for palmitoylation, had no direct effect on spontaneous or BK-stimulated receptor activity (Fig. 3). On the other hand, these mutations caused the peptide antagonist icatibant to go from a weak partial agonist to an agonist with an efficacy similar to that of BK (Pizard et al., 2001). Consequently, one should keep in mind that the clinical use of B<sub>2</sub> receptor antagonists may produce adverse effects, depending on the status of receptor phosphorylation and palmitoylation (Fathy et al., 1999; Blaukat, 2002).

Another mechanism that may participate in B<sub>2</sub> receptor desensitization involves BK-promoted transient association of G $\alpha_q$  and G $\alpha_i$  with caveolae, which occurred with a time course more or less parallel to that of receptor desensitization (de Weerd and Leeb-Lundberg, 1997). Additionally, BK affinity and stimulation of G protein activity in endothelial cells was attenuated by NO donors through a mechanism dependent on cGMP suggesting that B<sub>2</sub> receptor coupling is feed-back inhib-

ited by cGMP-dependent protein kinase (Miyamoto et al., 1997).

The B<sub>1</sub> receptor differs from the B<sub>2</sub> receptor in that it is desensitized only to a very limited degree (Bascands et al., 1993; Smith et al., 1995; Mathis et al., 1996). This receptor lacks any serines and threonines in the C-terminal tails that are conserved across species. Indeed, rodent B<sub>1</sub> receptors have a severely truncated C-terminal tail, which lacks any consensus phosphorylation motifs. Furthermore, human B<sub>1</sub> receptors are not phosphorylated to any significant degree either in the absence or presence of agonist (Blaukat et al., 1999a). The difference in the extent of desensitization of B<sub>2</sub> and B<sub>1</sub> receptors may contribute to the rather distinct patterns of receptor signaling through common effector pathways in different cells (Bascands et al., 1993; Tropea et al., 1993; Marsh and Hill, 1994; Mathis et al., 1996). Substitution of the human B<sub>2</sub> receptor tail in the human B<sub>1</sub> receptor drastically reduced all B<sub>1</sub> receptor activities including the basal and Asn<sup>121</sup> → Ala-induced constitutive activities and the agonist-promoted activity (Leeb-Lundberg et al., 2001). Furthermore, mutation to alanine of the cluster of serines and threonines in the C-terminal tail of this chimera, which are important for phosphorylation and desensitization of the B<sub>2</sub> receptor, partially restored the B<sub>1</sub>-like activity. Considering that the wild-type B<sub>1</sub> receptor is neither phosphorylated (Blaukat et al., 1999a) nor desensitized (Mathis et al., 1996), these results suggest that it is the lack of this regulation that contributes to the constitutive activity of the receptor.

### E. Cellular Distribution and Trafficking of Receptors

Direct identification of the B<sub>2</sub> receptor on the surface of naive cells has been determined both immunocytochemically in A431 cells (Haasemann et al., 1998) and in tissues (Figuroa et al., 2001) and by fluorescence of receptors fused with green fluorescent protein (GFP) in transfected HEK293 cells (Bachvarov et al., 2001; Lamb et al., 2001). Even though microscopy indicated a relatively even distribution of the receptors in the plasma membrane, specific enrichment of caveolae-like lipid rafts indicated some constitutive targeting of the B<sub>2</sub> receptor to these domains in some cells (Lamb et al., 2002). Agonist-independent nuclear localization of a B<sub>2</sub> receptor-GFP construct in HEK293 cells was recently reported (Lee et al., 2004). The significance of this localization is presently not known.

BK treatment leads to a rapid (minutes) loss of cell surface B<sub>2</sub> receptors and BK internalization (Roscher et al., 1984, 1990; Roberts and Gullick, 1989; Munoz et al., 1993). BK and B<sub>2</sub> receptor internalization in HEK293 cells was largely arrestin- and dynamin-independent (Lamb et al., 2001) suggesting a clathrin-independent mechanism of endocytosis. Mutagenesis revealed that BK-promoted internalization of the human B<sub>2</sub> receptor is dependent on the cluster of serines and threonines

located in the receptor C-terminal tail (see *Section III.B.* and Fig. 5) and upon which receptor desensitization also is dependent (Pizard et al., 1999). Detailed analysis of the effect of different combinations of mutations on BK internalization revealed that internalization could be initiated with a relatively low level of phosphorylation and without any residue specificity. Interestingly, a small but significant fraction of BK internalization remained insensitive to mutation of these residues suggesting that alternative epitopes and mechanisms exist for this regulatory process. Also, mutation of both Cys<sup>324</sup> and Cys<sup>329</sup> to alanines to create a nonpalmitoylated receptor causes only a minimal (20%) reduction in internalization (Pizard et al., 2001).

Subcellular fractionation of cultured smooth muscle cells showed that the internalized BK was directed to both microsomal and plasma membrane-associated compartments (Munoz and Leeb-Lundberg, 1992). Further fractionation combined with immunoelectron microscopy revealed that BK binding triggers a rapid redistribution of both BK and B<sub>2</sub> receptors to plasma membrane caveolae (de Weerd and Leeb-Lundberg, 1997; Haasemann et al., 1998). A B<sub>2</sub> receptor-GFP conjugate colocalized with transferrin following BK stimulation supporting endocytosis of the receptor to a recycling compartment (Bachvarov et al., 2001). Indeed, B<sub>2</sub> receptors rapidly recycle to the plasma membrane as determined both by fluorescence microscopy and by the reappearance of receptor binding sites following removal of extracellular BK (Munoz et al., 1993; Bachvarov et al., 2001). Indeed, careful stoichiometric analysis suggests that the B<sub>2</sub> receptor rapidly recycles in the process of internalizing BK (Lamb et al., 2002). Thus, BK-promoted B<sub>2</sub> receptor trafficking appears to proceed at least in part initially through a caveolae compartment, which may merge with an endocytic compartment in the cell and involves recycling of the receptor to the cell surface.

The B<sub>2</sub> receptor down-regulates to a very limited extent even after prolonged agonist exposure (days) (Bachvarov et al., 2001; Blaukat et al., 2003). B<sub>2</sub> receptor down-regulation may be artificially induced by inhibiting PI-3 kinase, which interferes with the recycling pathway and results in the formation of giant endosomes (Houle and Marceau, 2003). Degradation of plasma membrane B<sub>2</sub> receptors by extracellular proteases released in various pathological states may be an alternative mechanism of receptor down-regulation (Bachvarov et al., 2001; Houle et al., 2003). A slow sequestration of the rabbit B<sub>2</sub> receptor-GFP construct in HEK293 cells in response to the peptide antagonists NPC17731 and icatibant, but not the nonpeptide antagonist LF 16-0687 has been observed, and this effect has been used as an explanation for the insurmountable antagonism observed with the peptides on BK responses in rabbit (Houle et al., 2000b).

Cell surface B<sub>1</sub> receptors have been identified using heterologous cell systems expressing receptors fused to

yellow fluorescent protein by confocal fluorescence microscopy (Sabourin et al., 2002a) and HA-tagged receptors using immunoelectron microscopy (Kang et al., 2005). The B<sub>1</sub> receptor differs from the B<sub>2</sub> receptor in its distribution in that it is not enriched in caveolae-related lipid rafts under naive conditions (Lamb et al., 2002). Furthermore, the B<sub>1</sub> receptor is not internalized to any appreciable extent in response to agonist exposure (Faussner et al., 1998; Zhou et al., 2000; Lamb et al., 2001). On the other hand, the B<sub>1</sub> receptor seems to be subject to a considerably faster agonist-independent clearance than the B<sub>2</sub> receptor (Fortin et al., 2003a). One study reported that agonist promoted aggregation of a rabbit B<sub>1</sub> receptor-yellow fluorescent protein conjugate within the plasma membrane of HEK293 cells and colocalization with caveolin-1 as determined by confocal fluorescence microscopy suggesting caveolae as the targeted compartment (Sabourin et al., 2002a). A comparative immunological and biochemical analysis of the wild-type B<sub>1</sub> and B<sub>2</sub> receptors in caveolae-enriched fractions of the same type of cells reported a relatively low association of the B<sub>1</sub> receptor in this compartment regardless of whether the agonist Lys-des-Arg<sup>9</sup>-BK was present or not (Lamb et al., 2002). Although some agonist became associated with the caveolae-like rafts, acid washing of cells prior to cell fractionation and raft enrichment revealed that it was completely exposed to the extracellular environment. This is in contrast to B<sub>2</sub> receptor-bound BK, which was completely sequestered in the raft. Thus, the precise role of caveolae in B<sub>1</sub> receptor trafficking remains to be fully clarified. As with agonist-promoted B<sub>1</sub> receptor desensitization, the lower level of agonist-promoted receptor internalization relative to the B<sub>2</sub> receptor is to a large extent due to different complements of regulatory epitopes, such as phosphorylation sites in the C-terminal tails, since substituting the human B<sub>2</sub> receptor C-terminal tail in the B<sub>1</sub> receptor dramatically increased the internalization of the chimera in transfected CHO cells (Faussner et al., 1998).

## V. Long-Term Regulation of Kinin Receptors by Proinflammatory Factors

### A. Postisolation Induction of the B<sub>1</sub> Receptor

Smooth muscle preparations utilized in contractility assays have played a particular historical role in the discovery of the B<sub>1</sub> receptor, which was first pharmacologically defined in the isolated rabbit aorta (Regoli et al., 1977). In this preparation, exogenous kinins exert a contractile effect that increases with *in vitro* incubation time (Bouthillier et al., 1987). In such preparations, the *de novo* synthesis of B<sub>1</sub> receptors is relatively specific; responses mediated by other receptor types (including B<sub>2</sub> receptors in some preparations) are much more stable as a function of time after harvesting the tissue. Inhibition of RNA synthesis (with actinomycin D), protein synthesis (with cycloheximide), or protein maturation

along the secretory pathway (with brefeldin A) specifically prevent the development of a responsiveness to exogenous B<sub>1</sub> receptor agonists without displaying toxic effects on other types of responses. The isolated rabbit aorta is a model for many other smooth muscle preparations (e.g., freshly isolated umbilical vein, coronary arteries) and used to illustrate the phenomenon of post-isolation B<sub>1</sub> receptor induction (Marceau et al., 1998; Trevisani et al., 1999). Nonmuscle cellular elements can also exhibit the postisolation induction of B<sub>1</sub> receptors: vascular endothelial and colonic epithelial cells acquire such a responsiveness *in vitro* from no response or small initial response in the isolated bovine or porcine coronary artery and rat colon, respectively (Drummond and Cocks, 1995; Pruneau et al., 1996; Teater and Cuthbert, 1997).

In animal models, noxious treatments applied *in vivo* render subsequently isolated smooth muscle preparations immediately responsive to B<sub>1</sub> receptor agonists (e.g., the chemical inflammation of the rat urinary bladder, the injection of a sublethal dose of LPS in the rabbit; Marceau et al., 1998). Stress signaling has been studied following isolation and incubation of tissue preparations in nutrient-poor media, such as the Krebs' solution. The sustained activation of mitogen-activated protein kinase/c-Jun NH<sub>2</sub>-terminal kinase pathways has been observed in freshly isolated rabbit aortic tissue relative to untreated cultured cells of various types (Larrivée et al., 1998). Furthermore, pharmacological inhibitors of some of these MAP kinases (PD 98059 for ERK-1, SB 203580 for p38 $\alpha$  and - $\beta$ ) reduced with specificity the time-dependent functional up-regulation of the B<sub>1</sub> receptor, as assessed by contractility. Regulation of the B<sub>1</sub> receptor expression by the p38 and ERK MAP kinases has been subsequently observed in other experimental systems (hyperalgesia in rats, Ganju et al., 2001; B<sub>1</sub> receptor expression in cultured rat smooth muscle cells, Lagneux et al., 2001). Pharmacological inhibition of NF- $\kappa$ B, although relatively toxic for the preparation, also prevents the postisolation development of responses mediated by B<sub>1</sub> receptors in the rabbit aorta (Sabourin et al., 2002b). Indeed, the antiapoptotic action of this form of signaling may be necessary for the preparation viability over several hours.

### B. Proinflammatory Cytokines and Growth Factors

The early connection between the cytokine network and the induction of the B<sub>1</sub> receptor was again made using the isolated rabbit aorta, in which several inflammatory cytokines and growth factors increase the rate of sensitization to B<sub>1</sub> receptor agonists in rabbit aortic rings. IL-1, IL-2, EGF, and oncostatin M have a similar effect on the rabbit aorta (Bouthillier et al., 1987; Deblouis et al., 1988; Marceau et al., 1998) as interferon- $\gamma$  does on the bovine isolated mesenteric artery (de Kimpe et al., 1994). Glucocorticoids and protein synthesis in-



hibitors suppressed the up-regulation of the B<sub>1</sub> receptor-mediated responses in the rabbit aorta, either under the spontaneous or cytokine-stimulated conditions (Deblois et al., 1988). Radioligand-based techniques applied to cultured rabbit vascular smooth muscle cells essentially showed that cytokines such as IL-1, EGF, and tumor necrosis factor- $\alpha$ , but also fetal bovine serum and LPS, up-regulate B<sub>1</sub> receptor abundance ( $B_{\max}$ ) without changing the affinity ( $K_D$ ) of the radioligand (Schneck et al., 1994; Galizzi et al., 1994; Haddad et al., 2000; Sabourin et al., 2002b) and confirmed the inhibitory effect of glucocorticoids. These findings are suggestive for a gene regulated by the cytokine network and immunopathology. However, the cytokines, growth factors, and other substances such as LPS (activator of toll-like receptor-4 signaling) that regulate the process are very diverse as far as their receptor structure and signal transduction system pathways are involved. NF- $\kappa$ B is a transcription factor (or more precisely, a family of factors) controlling the expression of numerous genes associated with immunity and inflammation under the effect of receptors for inflammatory cytokines and LPS (Baldwin, 2001). Furthermore, glucocorticoid antagonism of NF- $\kappa$ B is well documented and may involve induction of the inhibitory subunit I $\kappa$ B $\alpha$ , direct protein-protein interaction between the activated glucocorticoid receptor and the p65 subunit of NF- $\kappa$ B, or competition for coactivators common to the glucocorticoid receptor and NF- $\kappa$ B (Yamamoto and Gaynor, 2001). Surgical or chemical adrenalectomy, with the resulting ablation of the endogenous glucocorticoids, produced both systemic NF- $\kappa$ B activation and B<sub>1</sub> receptor up-regulation in rats (Cabrini et al., 2001). There is now a strong agreement that B<sub>1</sub> receptor induction in various systems is correlated to NF- $\kappa$ B stimulation (i.e., nuclear translocation) and that pharmacologic interventions that inhibit that process prevent the receptor up-regulation (Ni et al., 1998b; Schanstra et al., 1998; Cabrini et al., 2001; Sabourin et al., 2002b). In cultured rabbit aortic smooth muscle cells, NF- $\kappa$ B also mediates induction of B<sub>1</sub> receptor expression by fetal bovine serum and EGF, showing that these stimuli are somehow distantly coupled to NF- $\kappa$ B and providing a unified mechanism for receptor up-regulation in this particular system.

The up-regulation of B<sub>2</sub> receptor mRNA and protein by inflammatory cytokines is also documented: for instance, interferon- $\gamma$  is active in T24 human urinary bladder transitional carcinoma cells (Lung et al., 1998) and IL-1 $\beta$  and TNF- $\alpha$  induce B<sub>2</sub> up-regulation in human embryonic lung fibroblasts HEL299 (Haddad et al., 2000). In the latter system, the induction of B<sub>2</sub> receptors is parallel to that of B<sub>1</sub> receptors, but apparently mechanistically different (protein kinase A is claimed to have a role). As the B<sub>2</sub> receptor population is preformed in many tissues, it appears to be down-regulated in some chronic inflammatory models (see below), but the functional consequences of the up-regulation have not been

investigated as much as those of the B<sub>1</sub> receptor. Functional and molecular approaches show that estrogens are permissive for the expression of B<sub>2</sub> receptors in rats (Madeddu et al., 1997). Culture medium with high glucose or stimulation with angiotensin II also up-regulate B<sub>2</sub> receptor expression in specific experimental systems (Christopher et al., 2001b; Kintsurashvili et al., 2001) as does stimulation with nerve growth factor (Lee et al., 2002).

### C. Agonists

The sustained activation of the B<sub>2</sub> receptor is expected to lead to functional desensitization, which is associated with receptor phosphorylation/dephosphorylation and endocytosis/surface re-expression cycles (Blaukat et al., 1996). In contrast, true B<sub>2</sub> receptor down-regulation is observed in some forms of intense, chronic inflammation (e.g., loss of B<sub>2</sub> receptor immunoreactivity in renal grafts undergoing acute rejection in humans, Naidoo et al., 1996; specific loss of BK functional effect in rat colonic mucosa submitted to persistent inflammation, Kachur et al., 1986). The mechanism of this hypothetical down-regulation is unknown, as is the obligatory role of the autologous agonist in this phenomenon. A 3-h stimulation with BK does not lead to the detectable degradation of a green fluorescent protein conjugate of the rabbit B<sub>2</sub> receptor, although persistent endocytosis is observed (Bachvarov et al., 2001). This and other observations suggest that ligand-receptor complexes are not targeted to the lysosomes to any measurable extent for this particular GPCR. Endogenously expressed B<sub>2</sub> receptors are down-regulated by prolonged ( $\geq 12$  h) exposure to BK in cultured human fibroblasts (Blaukat et al., 2003); a mechanism involving the combined transcriptional repression and accelerated receptor degradation has been proposed to account for these observations. An additional agonist-independent mechanism for inflammatory B<sub>2</sub> receptor down-regulation has been proposed; several extracellular proteases, including tissue kallikrein and proteases secreted by activated neutrophils, have been shown to cleave a B<sub>2</sub> receptor-GFP in minutes in intact cells (Marceau et al., 2002; Houle et al., 2003).

The induction of B<sub>1</sub> receptor expression has been observed in human IMR-90 cells following B<sub>1</sub> or B<sub>2</sub> receptor stimulation (Schanstra et al., 1998; Phagoo et al., 1999). Phagoo et al. (1999) proposed a model of kinin receptor "autoregulation" based on this system. Addition of the B<sub>2</sub> receptor agonist BK (100 nM) reduced by 89% the amount of the surface B<sub>2</sub> receptors in a few minutes and up-regulated B<sub>1</sub> receptors (2- to 3-fold) in a few hours. Treatment with the B<sub>1</sub> receptor agonist Lys-des-Arg<sup>9</sup>-BK did not influence the population of B<sub>2</sub> receptors, but also up-regulated the B<sub>1</sub> receptors. Both agonists increased the expression of IL-1 $\beta$  in the cells, and the natural IL-1 receptor antagonist, IRA, decreased the induction of B<sub>1</sub> receptors by BK (Phagoo et al., 1999). Therefore, this autoregulation model integrates the au-

tologous desensitization of the B<sub>2</sub> receptor and the involvement of cytokines in B<sub>1</sub> receptor gene transcriptional control. This model has been tested in the cardiovascular system of the rabbit. Activation of the contact system (also known as the kallikrein/kinin system or the intrinsic pathway of coagulation) following intravenous dextran sulfate injection consumed kininogen and generated a prolonged hypotensive state mediated by the B<sub>2</sub> receptor, but failed to induce the B<sub>1</sub> receptor (vascular function, mRNA concentration in several organs) 5 h after treatment. For comparison, bacterial LPS treatment was active in this respect (Sabourin et al., 2001). Furthermore, kinin receptor antagonists did not modify LPS-induced B<sub>1</sub> receptor induction in the live rabbit (Sabourin et al., 2001). Exogenous kinins acting on either receptor types were unable to translocate NF- $\kappa$ B to the nucleus of rabbit aortic smooth muscle cells or to up-regulate the B<sub>1</sub> receptor expression (binding sites, mRNA; Sabourin et al., 2001; Marceau et al., 2002); by contrast, IL-1 $\beta$  was active in both respect. Therefore, the autoregulation model, a positive feedback between the kinin receptor stimulation and delayed B<sub>1</sub> receptor expression, is apparently not universally applicable or may be species-specific. Furthermore, the model was first developed in a cell type (IMR-90) in which IL-1 $\beta$  expression is activated by various GPCR (including the receptor for IL-8, Bastian et al., 1998). However, chronic pathologies in animal models may support kinin-induced kinin receptor up-regulation, perhaps because the chronic inflammatory tissue is composed of several cell types including leukocytes that allow different types of cross-talk involving local cytokine production. Thus, B<sub>2</sub> receptor antagonism or B<sub>2</sub> receptor gene deletion inhibit the enhanced B<sub>1</sub> receptor expression induced by ischemia-reperfusion in the mouse intestine (Souza et al., 2004).

#### D. Ras and B<sub>2</sub> Receptors

Ras GTPases have essential physiologic roles in regulating cell growth, differentiation, and apoptosis (Bokoch and Der, 1993). *Ras* genes were initially discovered, however, in the context of them being retroviral oncogenes "stolen" from the host genome by the Kirsten (Ki) and Harvey (Ha) rat sarcoma viruses. Tumor- or retrovirus-derived forms of the *ras* genes were found to encode proteins that were constitutively active because of point mutations in their coding sequences (Barbacid, 1987). Approximately 20% of all human tumors were found to express mutated Ras proteins (Bos, 1989).

In 1987, Ha-ras- or Ki-ras-transformed NIH 3T3 cells were shown to exhibit significantly enhanced sensitivity to BK, associated with an elevated level of high-affinity [<sup>3</sup>H]BK binding (Parries et al., 1987). Bradykinin was also found to act as a potent mitogen for the mutant Ha-ras-transfected cell line, Rat 13, compared with the parent nontransfected cell line, Rat 1 (Roberts and Gullick, 1989). Further analysis showed that the ras-trans-

ected Rat 13 cells expressed more than 10-fold higher numbers of B<sub>2</sub> receptors on their surface than did Rat 1 cells. Interestingly, dbl-transformed NIH 3T3 cells also exhibited increased inositol lipid turnover in response to BK as well as a significantly higher number of B<sub>2</sub> receptors compared with control NIH 3T3 cells (Ruggiero et al., 1989).

The effect of Ha-ras oncogene transformation in NIH 3T3 fibroblasts was subsequently studied in greater detail (Hembree and Leeb-Lundberg, 1996). Expression of Ha-ras was found to increase B<sub>2</sub> receptor mRNA and protein levels as well as increased [<sup>3</sup>H]BK binding. Transformed NIH 3T3 cells also showed an increased mitogenic response to BK stimulation at high but not low levels of Ha-ras expression. Thus, ras transformation appears to regulate the sensitivity of NIH 3T3 cells to BK through at least two different mechanisms. Consistent with these findings, coexpression of a ras oncogene also up-regulated B<sub>2</sub> receptor promoter activity in transfected NG108–15 neuroglioma cells (Pesquero et al., 1996a).

#### E. Regulatory Elements in the Gene Promoters

Pesquero et al. (1994) identified the genomic structure of the B<sub>2</sub> receptor gene. They found no evidence of a classical transcription initiation motif, but reported that a 1143-bp region of the putative rat B<sub>2</sub> receptor promoter was sufficient to drive expression of a fused luciferase reporter gene in NG108–15 cells. This region was found to contain a cAMP-response element which mediated significant transcriptional activity when stimulated with 8-bromo-cAMP (Pesquero et al., 1996a). BK itself as well as phorbol esters also stimulated promoter activity. More recently, the same group identified a series of regulatory elements in a 4.6-kb fragment of the 5'-flanking sequence of the rat B<sub>2</sub> receptor promoter (Baptista et al., 2002). Computer analysis of potential binding sites revealed multiple sites for a number of factors including: GATA-1, CCAAT displacement protein, E2F, Egr-2, interleukin-6 activator protein, SP-1, ras, NF- $\kappa$ B, p53, SEF-1, cAMP-response element binding protein, sterol regulatory element binding protein, serum, glucocorticoid, interferon, and estrogen. By transfecting deletion mutants into either NG108–15 cells or rat aorta vascular smooth muscle cells, they identified a series of positive and negative regulatory elements in the B<sub>2</sub> receptor promoter. A classical silencing element was found at position –1086 to –1142, whereas the core promoter activity was present within the first 287 bp of the regulatory region.

Transcription of the rat B<sub>2</sub> receptor gene was shown to be regulated by the tumor suppressor p53 (Saifudeen et al., 2000, 2002), a sequence-specific DNA-binding protein. Both p53-activating and -repressing elements were identified in the rat B<sub>2</sub> receptor promoter (Marks et al., 2003). Specific deletion of the p53-activating element abrogated p53-induced activation, whereas deletion of

the p53-repressing element enhanced p53-mediated activation.

The B<sub>1</sub> receptor gene structure also revealed three exons with two intervening introns. Unlike the B<sub>2</sub> receptor gene, the human B<sub>1</sub> receptor gene contains a consensus TATA box (Bachvarov et al., 1996). Multiple potential transcription factor binding sites and putative enhancer and silencer elements were identified within a 1369-bp sequence in the 5'-flanking region. Of particular interest were the presence of NF- $\kappa$ B, H-APF-1, and multiple AP-1 binding motifs within the promoter region, consistent with the B<sub>1</sub> receptor gene being inducible. Yang and Polgar identified two promoter regions in the human B<sub>1</sub> receptor gene, one located in the 5'-flanking region and the other located within intron II (Yang and Polgar, 1996). Both of these regions demonstrated activity in driving expression of a fused luciferase gene in transfected cells, although the 5'-flanking region was by far the more potent promoter. A series of deletion mutants, generated from a 1.8-kb fragment of the 5'-flanking region of the human B<sub>1</sub> receptor gene, revealed evidence for several positive and negative regulatory regions and allowed definition of a minimal enhancer element between -548 and -448 (Yang et al., 1998). The activity of this minimal enhancer element was shown to be dependent on intact AP-1- and NF-1-like binding sites and to bind AP-1 but not NF-1. The B<sub>1</sub> receptor promoter activity was also shown to be cell type specific.

Although LPS and TNF- $\alpha$  have been shown to strongly up-regulate B<sub>1</sub> receptor expression in multiple animal models, neither significantly increased the promoter activity of the 1.8-kb human 5'-flanking region fragment described above. An approximately 2.6-kb fragment (-2582 to +34) of the 5'-flanking region of the human B<sub>1</sub> receptor gene, however, displayed promoter activity in vascular smooth muscle cells following stimulation with LPS, TNF- $\alpha$ , or IL-1 $\beta$  (Ni et al., 1998b). The first 111 bp of the 5'-flanking region were found to be sufficient for transcriptional induction, and mutagenesis of a  $\kappa$ B site at -64 to -55 abolished most of the cytokine effect. Mutagenesis of a cAMP response element at -50 to -43 markedly reduced basal promoter activity as well as inducibility. Furthermore, Schanstra et al. (1998) showed that IL-1 $\beta$  and Lys-des-Arg-BK both induced up-regulation of the B<sub>1</sub> receptor in IMR 90 (lung fibroblast) cells through an NF- $\kappa$ B-mediated mechanism. The  $\kappa$ B site identified by Schanstra et al. lies approximately 1100 bp upstream of the site described by Ni et al., suggesting that there is a negative regulatory site between these  $\kappa$ B sites. The importance of the  $\kappa$ B site in the regulation of B<sub>1</sub> receptor expression was demonstrated by pharmacologic inhibition of NF- $\kappa$ B activation which prevented postisolation and cytokine-induced B<sub>1</sub> receptor up-regulation in fresh rabbit aortic and cultured smooth muscle cells (Sabourin et al., 2002b), human umbilical vein (Sardi et al., 1999, 2002), as well as

cytokine and Lys-des-Arg<sup>9</sup>-BK-induced B<sub>1</sub> receptor up-regulation in IMR 90 cells (Schanstra et al., 1998).

Other evidence suggests that a second genomic site downstream from the 5' promoter may be involved in LPS and Lys-des-Arg-BK inducible promoter activity. Transfection of a B<sub>1</sub> receptor minigene (containing a 1.8-kb promoter, exon 1, 1.5 kb of intron 1, exon 2, intron 2, and the luciferase gene) into IMR 90 cells resulted in promoter activity which was activated by LPS and Lys-des-Arg-BK; however, transfection of the 1.8-kb promoter fused to the luciferase gene did not show increased promoter activity in response to LPS or Lys-des-Arg-BK (Yang et al., 2001a). Activation of this minigene was further shown to require c-Jun, suggesting a critical role for AP-1 sites. p53 was also shown to strongly suppress luciferase reporter activity driven by the 1.8-kb B<sub>1</sub> receptor promoter as well as its minigene, although mutagenesis of a putative p53 binding site in the promoter did not abrogate the ability of p53 to down-regulate the promoter activity (Yang et al., 2001a).

#### F. mRNA Stability

Although not extensively studied, mRNA stability may play a role in the regulation of kinin receptor expression. Up-regulation of B<sub>1</sub> receptor mRNA levels following IL-1 $\beta$  stimulation in IMR 90 cells was shown to be mediated, in part, by stabilization of the mRNA (Zhou et al., 1998), potentially through a destabilizing sequence in the 3'UTR region (Zhou et al., 1999). IL-1 $\beta$  has also been shown to induce a rapid but transient increase in B<sub>2</sub> receptor expression in cultured human bronchial smooth muscle cells (Schmidlin et al., 1998). IL-1 $\beta$  increased both the number of B<sub>2</sub> receptors as well as the level of B<sub>2</sub> receptor mRNA. The increase in B<sub>2</sub> receptor expression correlated with enhancement of BK-stimulated inositol phosphate formation and involved both an enhancement of the transcriptional rate and a lengthened mRNA half-life.

## VI. Distribution and Pathophysiological Function of Kinin Receptors

Both receptor subtypes for kinins can be expressed by the same cell types: vascular cells (endothelial, smooth muscle; Figueroa et al., 2001), nonvascular smooth muscle (e.g., uterine), fibroblasts (Ricupero et al., 2000), epithelial cells (Bhoola et al., 1997), nervous (afferent sensory) cells, and various tumor cells (Wu et al., 2002; Taub et al., 2003). There is an increasing interest in central nervous system localization of the kinin receptors and their regulation (Ongali et al., 2003). The expression and role of kinin receptors in leukocytes is a relatively neglected research area of possible relevance (Prat et al., 1999; Bockmann and Paegelow, 2000; Aliberti et al., 2003).



### A. Circulation and Renal Function

Kinins are very potent vasodilators. Thus, infused BK more than doubled the flow of blood in the human forearm, and the effect was abrogated by icatibant treatment, supporting a role for preformed  $B_2$  receptors (Cockcroft et al., 1994a). Most circulatory effects of BK-related peptides are determined by the stimulation of endothelial cells from which secondary mediators are released to affect the vascular smooth muscle (Fig. 6). The discovery of endothelium-derived relaxing factor was soon followed by the demonstration of its release by a variety of agonists, including BK (Furchgott, 1983). Endothelium-derived relaxing factor, now identified as nitric oxide (NO), is metabolically derived from L-arginine by eNOS (see above). NO-dependent relaxation is a prominent mode for BK action in the human forearm vasculature because an eNOS inhibitor reduces approximately by half the effect of BK infusion on blood flow (Cockcroft et al., 1994b). NO diffuses from the endothelium to the smooth muscle where it activates guanylate cyclase. Prostaglandin  $I_2$  (prostacyclin) is another secondary mediator frequently released by kinins from the endothelium. Prostacyclin stimulates cyclic AMP production in the smooth muscle cells (Ignarro et al., 1987; Fig. 6). Prostanoid formation probably occurs in many cell types possessing receptors for kinins, probably via the cytosolic  $Ca^{2+}$ -sensitive isoforms of phospholipase  $A_2$  (such as cPLA<sub>2</sub>). Other mechanisms of endothelial-dependent vasorelaxation are also suspected, such as the activation of a number of NO-independent ion channels located in the smooth muscle cells that may account for “endothelium-dependent hyperpolarization” (see ap-

plication to the BK-induced relaxation of human isolated coronary microarteries; Batenburg et al., 2004). The physiological correlate of the vasodilator effect of kinins is hypotension when BK is administered systemically or when kinin formation is artificially promoted in a massive manner, as in hypertensive animals treated with a vector for activated tissue kallikrein (Xiong et al., 1995) or following activation of the contact system with intravenous dextran sulfate (Sabourin et al., 2001). Transgenic mice overexpressing the human  $B_2$  receptor are also hypotensive and hyper-responsive to exogenous BK (Wang et al., 1997). BK-induced hypotension results from a decreased peripheral resistance, not from a drop of cardiac output; in fact, reflex tachycardia may compensate in part the peripheral response during an acute episode of BK-induced hypotension. Relevant to vasodilation, icatibant abrogates flow-dependent vasodilation (induced by papaverine) in the human myocardium (Groves et al., 1995), showing that the endogenous kallikrein-kinin system can be rapidly recruited in the autoregulation of peripheral circulation in physiological conditions. However, icatibant treatment, unlike eNOS inhibition, has very modest to no hypertensive effect in normal humans or animals, failing to support a tonic vasodilator effect of the kallikrein-kinin system on the peripheral vasculature.

Most laboratories report that mice lacking the  $B_2$  receptor are normotensive, but a relatively large dietary sodium intake provokes a malignant form of hypertension in these animals (Alfie et al., 1997; Cervenka et al., 1999; Trabold et al., 2002), indicating a renal role of the kallikrein-kinin system in handling such salt excesses.

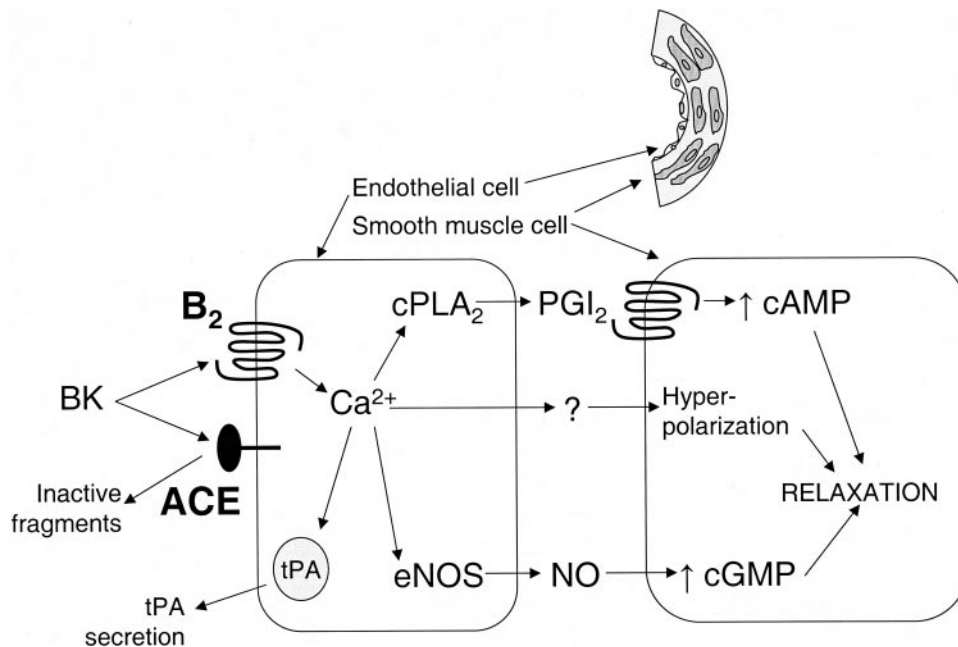


FIG. 6. The vascular responses to kinins involve secondarily released mediators from the endothelial cells and their subsequent action on vascular smooth muscle cells. The preformed  $B_2$  receptor is illustrated, but the  $B_1$  receptor has been documented to recruit the same pathways if expressed by endothelial cells.

These knockout mice have been extensively studied in several laboratories and exhibit a number of physiological alterations relevant for cardiovascular function: enhanced effects of endogenous and exogenous arginine-vasopressin (Alfie et al., 1999) and exogenous angiotensin II (Cervenka et al., 2001), distorted renal development in the fetus with abnormal distal nephrons (El-Dahr et al., 2000), reduced glomerular capillary surface area (Schanstra et al., 2003), decreased renin and cyclooxygenase (COX)-2 expression in the kidney (Imig et al., 2003), a state of insulin resistance (Duka et al., 2001b), and increased renal fibrosis in response to unilateral ureteral obstruction (Schanstra et al., 2002). Mild degenerative and fibrotic changes are observed in the myocardium of aging B<sub>2</sub> receptor knockout mice (Maestri et al., 2003). All these findings support a protective role of the endogenous kallikrein-kinin system on various aspects of cardiovascular and renal function. Extensive blockade of the B<sub>2</sub> receptor with icatibant for 48 h does not up-regulate B<sub>1</sub> receptor mRNA and function in the rabbit (Marceau et al., 1999), failing to support a physiological retroaction loop between B<sub>2</sub> receptor stimulation and the repression of B<sub>1</sub> receptor expression in adults. Mice with genetic deletion of the B<sub>2</sub> receptor gene exhibit a consistent up-regulation and expression of the B<sub>1</sub> receptor at the cardiovascular level (mRNA, function), which mediate hypotension via endogenous kinins or following exogenous administration of des-Arg<sup>9</sup>-BK (Duka et al., 2001a, 2003). This suggests a form of compensation opposite to the autoregulation hypothesis (see above). Another unexplained humoral disturbance suggestive of an inflammatory phenotype in the B<sub>2</sub> receptor knockout animals includes the tonic vasoconstrictor effect of a thromboxane-like substance (Duka et al., 2003).

Few cardiovascular drug classes have been more successful than ACE inhibitors in the last two decades. Drugs such as enalapril, ramipril, and several others have been clinically shown to exert beneficial effects in patients with high blood pressure, cardiac hypertrophy, congestive heart failure, and diabetic nephropathy. Yet the mode of action of ACE inhibitors is not totally clear. ACE (kininase II) is a metallopeptidase, which can accept with high affinity a variety of substrates in addition to angiotensin I, including BK and des-Arg<sup>9</sup>-BK, which are inactivated by this enzyme (Blais et al., 2000). There is an enthusiastic and persistent suggestion in the literature that at least a part of the beneficial effects or side effects of ACE inhibitors are derived from the potentiation of endogenous BK (Linz et al., 1995). The rationale is that increasing the tissue concentration of BK through decreased degradation could stimulate endogenous vasodilator and other beneficial mechanisms. The approach for studying this problem has been mainly the pharmacological inhibition of kinin receptors in animals and humans because analytical biochemistry does not show conclusively that kinins are elevated in the blood

or urine of patients under ACE blockade (Blais et al., 2000). Among the vast amount of published material, clinical studies based on acute treatments with the B<sub>2</sub> receptor antagonist icatibant used to reverse at least in part the therapeutic effect of ACE inhibitors are probably most revealing. Icatibant decreased by about a third the acute hypotensive effect of captopril in human subjects but not the renal hemodynamic response to the ACE inhibitor (Gainer et al., 1998; Squire et al., 2000). Also, treatment with ACE inhibitors reduces postinfarction myocardial fibrosis and improves hemodynamics in animal models; the deletion of the B<sub>2</sub> receptor in mice shows that kinins are necessary to achieve both types of benefits (Yang et al., 2001b).

In intact humans or laboratory animals (with the exception of the dog), cardiovascular actions of kinins are mediated by preformed B<sub>2</sub> receptors and the contribution of the B<sub>1</sub> receptor is not detectable (Marceau et al., 1998). On the other hand, tissue injury changes the situation as the B<sub>1</sub> receptor expression is induced, whereas the B<sub>2</sub> receptor expression usually remains the same. For instance, following a sublethal LPS dose, Lys-des-Arg<sup>9</sup>-BK or des-Arg<sup>9</sup>-BK elicit hypotensive and vasodilator effects in the rabbit and the rat (Marceau et al., 1998; McLean et al., 1999), hypotension or edema in a nonhuman primate (deBlois and Horlick, 2001), and B<sub>1</sub> receptors are up-regulated in the rat glomerular afferent and efferent arterioles (where they mediate vasoconstriction; Schanstra et al., 2000). Both B<sub>1</sub> and B<sub>2</sub> receptor mRNA are up-regulated in the chronic stages of cardiac remodeling induced by pressure-overload or by experimental diabetes in the rat (Spillmann et al., 2002). Complete blockade of ACE with enalaprilat during 48 h did not up-regulate the B<sub>1</sub> receptor in the cardiovascular system of the healthy rabbit (mRNA, function; Marceau et al., 1999). However, a discrepant result has been obtained in intact rats and mice treated for 6 weeks with ramipril; the B<sub>1</sub> receptor was induced in the kidneys and the vasculature and this induction was reduced by a B<sub>1</sub> receptor antagonist, but not by icatibant or in B<sub>2</sub> receptor knockout mice, showing that some kinin-induced B<sub>1</sub> receptor induction may occur in this situation (Marin-Castano et al., 2002). The hypotensive effect of ramipril was mediated by both B<sub>1</sub> and B<sub>2</sub> receptors, as shown with antagonist drugs. Whether the B<sub>1</sub> receptor induction is relevant to the therapeutic effect of ACE inhibitors in humans or whether it is a side effect due to chronic electrolyte imbalance remains to be established (note that rabbits submitted to chronic ACE inhibition exhibit a form of nephrotoxicity that can be entirely prevented with saline cotreatment; Minsker et al., 1990). Congestive heart failure represents another indication of ACE inhibitors; in human patients with this condition, infusion of a kinin receptor antagonist decreases the forearm blood flow, an effect absent in patients without ACE inhibition or in normal volunteers (Wetherow et al., 2001). The identity of the antagonists

utilized is of interest for investigating the kinin receptor subtype involved; icatibant, a selective B<sub>2</sub> receptor antagonist, was ineffective, whereas B9340 supported the demonstration. The latter peptide (D-Arg-[Hyp<sup>3</sup>, Thi<sup>5</sup>, D-Igl<sup>7</sup>, Oic<sup>8</sup>]BK) is a promiscuous B<sub>1</sub> and B<sub>2</sub> receptor antagonist chemically and pharmacologically similar to B9430 (Table 2; Stewart et al., 1997). Thus, it was concluded that the vasodilator effect of ACE inhibition was probably mediated by the B<sub>1</sub> receptor in patients with congestive heart failure (Witherow et al., 2001), although this has been addressed only indirectly. Angioedema is a rare but potentially life-threatening side effect of therapy with inhibitors of ACE inhibitors; a decreased capacity to catabolize immunoreactive des-Arg<sup>9</sup>-BK has been observed in the plasma of hypertensive patients that have experienced this side effect (Molinaro et al., 2002), suggesting that the accidental expression of B<sub>1</sub> receptors in such patients could precipitate the reaction. Thus, the therapeutic effects and side effects of ACE inhibition may be mediated by the B<sub>1</sub> receptor under some circumstances, but the evidence is presently limited.

Further suspected beneficial effects of ACE inhibitors include an antiproliferative effect on smooth muscle (arterial lesions) and a stimulation of the fibrinolytic system through the release of tissue-type plasminogen activator. Kinins exert antiproliferative effects on vascular smooth muscle (notably via B<sub>1</sub> receptors, Dixon et al., 2002) and reduce the reactive proliferative response of the arterial intima following angioplasty if endogenous kinins are allowed to act via ACE inhibition or kallikrein gene delivery (Linz et al., 1995; Agata et al., 2000). However, it is not clear that ACE inhibition can achieve the inhibition of postangioplasty proliferation in human coronary arteries (Hermans et al., 1994), and the current approach to this problem is the clinical use of stents eluting antimitotic drugs. The release of tissue-type plasminogen activator has been observed in response to either BK or ACE inhibition in the forearm circulation of normal volunteers (Pretorius et al., 2003); the effect of the ACE inhibitor enalaprilat was abrogated by the B<sub>2</sub> receptor antagonist icatibant. The possible benefit of this kinin-mediated effect is the improvement of the fibrinolytic balance with decreased incidence of thromboembolic accidents.

The role of kinins in the side effects of ACE inhibitors (nonproductive cough, rare but life-threatening angioedema, hypotension in patients subjected to extracorporeal circulation) may be significant and has been discussed elsewhere (Fox et al., 1996; Blais et al., 2000; Mukae et al., 2000; Scholzen et al., 2003). The idea that an increased local level of kinins is beneficial but that an excessive level will trigger inflammatory reactions such as increased vascular permeability and the irritation of airway nerve terminals is implicit to this debate. More recent antihypertensive drugs not yet in clinical use combine ACE and neutral endopeptidase inhibition (e.g.,

omapatrilat). They raise a particular safety concern about kinin-mediated angioedema, as neutral endopeptidase is also a kininase (Campbell, 2003). Omapatrilat administration to healthy rats increases vascular permeability to a greater extent than do ACE inhibitors and in a manner partly dependent on the B<sub>2</sub> receptor (Sulpizio et al., 2004). Another recent debate related to kinins and cardiovascular drugs is the role of bradykinin peptides in the therapeutic and side effects of angiotensin AT<sub>1</sub> receptor antagonists (Hornig et al., 2003; Tschöpe et al., 2004). The mechanism of such a cross-talk has not yet been fully elucidated. High angiotensin II concentrations observed under AT<sub>1</sub> receptor blockade have been proposed to stimulate angiotensin AT<sub>2</sub> receptors, the latter being responsible for a hypothetical kallikrein activation and kinin release (Katada and Majima, 2002). Intact tissue kallikrein and AT<sub>2</sub> receptor genes are both required in flow-dependent dilation of mice carotid arteries, a physiological adaptative circulatory response mediated in part by endogenous kinins (Bergaya et al., 2004).

Ischemia is one of the basic pathological processes, and there is ample evidence that it activates the kallikrein-kinin system. This has found two general applications: the generally protective role of kinins against the tissue damage that follows ischemia and reperfusion (this includes preconditioning) and ischemia-driven angiogenesis. Although analytical biochemistry has been used to study the role of kinin in ischemia-reperfusion systems (Blais et al., 2000), most of the reported experiments were based on a pharmacological approach. The endothelial B<sub>2</sub> receptor and its secondarily released mediators (Fig. 6) largely mediate the beneficial effects of ACE in such systems (reviewed by Baxter and Ebrahim, 2002). B<sub>2</sub> receptor knockout mice or kininogen-deficient rats (Brown-Norway Katholiek strain) are refractory to ischemic preconditioning in vivo (Yang et al., 1997). Kinins are not the only studied mediators of ischemic preconditioning; adenosine and opioid peptides are other mediators of interest in this field of investigation (Mikhail et al., 2003). Ischemia induces endothelial B<sub>1</sub> receptor expression (Mazenot et al., 2001), and there is mounting evidence, partly based on B<sub>1</sub> receptor knockout mice, that the B<sub>1</sub> receptor may play a detrimental role opposed to that of the B<sub>2</sub> receptor in ischemia-reperfusion systems (heart: Lagneux et al., 2002; small intestine: Souza et al., 2004). A prominent physiological response to persistent ischemia is angiogenesis. Kinins appear to be important in angiogenesis driven by chronic vascular occlusion in rodents. ACE inhibition exerts a certain proangiogenic effect in mice with femoral artery occlusion; that effect is absent in B<sub>2</sub> receptor gene knockout animals (Silvestre et al., 2001). Another laboratory has developed the idea that the B<sub>1</sub> receptor, but not the B<sub>2</sub> receptor, accelerates spontaneous angiogenesis due to arterial occlusion (based on agonist and antagonist peptide drugs and genetic B<sub>1</sub> receptor deletion; Eman-



ueli and Madeddu, 2001, Emanuelli et al., 2002); however, both receptors participate in the increased angiogenesis induced by delivery of human kallikrein (Emanuelli et al., 2001). The endothelial effect mediated by the B<sub>1</sub> receptor relevant for angiogenesis has been addressed; anti-FGF-2 antibodies abrogate the angiogenic effect of Lys-des-Arg<sup>9</sup>-BK administered as an implant in the rabbit cornea (a B<sub>2</sub> receptor agonist is ineffective in this system; Parenti et al., 2001).

Given the generally protective B<sub>2</sub> receptor-mediated actions in the cardiovascular and renal systems and because a large body of animal data suggests that such drugs may precipitate cardiovascular accidents in the predisposed individual, the development of B<sub>2</sub> receptor antagonists as anti-inflammatory and analgesic drugs may be a potential concern, even though clinical evidence for this notion is lacking. There have been speculations about the use of B<sub>2</sub> receptor agonists for clinical cardiovascular applications, although it is recognized that inflammatory side effect cannot be dissociated from the vasodilator effects (Heitsch, 2003). The case for B<sub>1</sub> receptor antagonist safety is both less documented and more balanced, as several counterproductive circulatory changes mediated by this receptor have been detected in inflammatory or ischemic conditions (see above).

### B. Inflammation

Synthetic BK injected in human or animal tissues reproduces the four classical cardinal signs of inflammation: redness, local heat, swelling, and pain (Elliott et al., 1960a). Exudation of protein-rich fluid from the circulation is largely determined by vascular mechanisms involving the physical separation of endothelial cells, particularly at the level of postcapillary venules. Recent investigations show that the small G proteins Rho and Rac antagonistically regulate the endothelial barrier function. Rho stimulation by BK or other inflammatory agents facilitates the breakdown of intercellular junctions, whereas Rac stabilizes them (Wojciak-Stothard and Ridley, 2002). Local vasodilation produced by kinins (inflammatory hyperemia) further facilitates exudation.

Consistent with the regulatory patterns of kinin receptor subtypes, kinins stimulate B<sub>2</sub> receptors when injected in normal tissues. Thus, specific B<sub>2</sub> receptor antagonists or inactivation of the B<sub>2</sub> receptor in knockout mice prevent specific aspects of the inflammatory response in specific models, e.g., tissue swelling and systemic signs of inflammation in peptidoglycan-induced arthritis in the Lewis rat (Uknis et al., 2001) and plasma extravasation in the Arthus reaction (Samadfam et al., 2000). B<sub>2</sub> receptor antagonism may have a special interest in some forms of vasogenic edema, the brain edema that follows head trauma or stroke and hereditary angioedema. This first clinical application is supported by encouraging results obtained with the nonpeptide antagonist LF 16-0687 in animal models (Kaplanski et al., 2002; Zausinger et al., 2002) and may represent a

situation physiologically symmetrical to the deliberate use of the B<sub>2</sub> receptor agonist labradimil to open the blood-brain barrier (Table 2). Hereditary angioedema is modeled by the C1 inhibitor gene deletion in mice; the increased vascular permeability observed in these animals was reversed by treatment with the B<sub>2</sub> receptor antagonist icatibant or by genetic ablation of the B<sub>2</sub> receptor gene (Han et al., 2002).

Not surprisingly, models of persistent inflammation in animals, especially those involving cytokine or microbial product administration, involve the B<sub>1</sub> receptor. Several such systems are reviewed elsewhere, including the mediation of fever, of the systemic complications of sepsis, and of changes in the hepatic expression of systemic inflammatory markers (Marceau et al., 1998; Couture et al., 2001). The recent literature illustrates that IL-1 $\beta$  injection in the rat paw sensitizes the rat paw to the edema-forming effect of des-Arg<sup>9</sup>-BK (Campos et al., 2002). The analysis of this system shows the sequential mediation of platelet-activation factor, of local TNF- $\alpha$  production, and of neutrophil extravasation in this response and the obligatory role of the latter process in B<sub>1</sub> receptor expression (mRNA). LPS-induced induction of B<sub>1</sub> receptor expression in the rat paw follows the same lines and provides evidence for a role of local NF- $\kappa$ B activation and IL-1 $\beta$  formation in addition to that of TNF- $\alpha$  (Passos et al., 2004). The role of infiltrating leukocytes is interesting to note in these localized models, as they may supply the cytokine levels required for B<sub>1</sub> receptor induction; however, circulating neutrophils are not required to induce cardiovascular responsiveness to B<sub>1</sub> receptor agonists in rabbits treated with LPS (Bouthillier et al., 1987). The cytokines may be supplied by fixed macrophages, such as those in the reticuloendothelial system, in this form of blood-borne endotoxin stimulation. Clinical samples of chronic inflammatory tissues (nasal tissue samples from subjects with allergic rhinitis, psoriatic skin) show the up-regulation of the B<sub>1</sub> receptor (mRNA in both systems, downstream MAP kinase signaling in nasal samples) without the down-regulation of the preformed B<sub>2</sub> receptor (Schremmer-Danninger et al., 1999; Christiansen et al., 2002). Whether each kinin receptor subtype mediates different aspects of the inflammatory response is an issue of considerable interest. B<sub>1</sub> receptor antagonists reduce the influx of eosinophils but not the airway hyper-reactivity to carbachol in antigen-induced pulmonary inflammation in mice (Eric et al., 2003b). The B<sub>2</sub> receptor antagonist icatibant exerted a wider effect, inhibiting the influx of both eosinophils and mononuclear cells while also preventing the hypersensitivity of the airways. Although allergen-induced bronchial hyper-responsiveness parallels B<sub>1</sub> receptor mRNA up-regulation and a certain therapeutic effect of des-Arg<sup>10</sup>-Hoe 140 has been observed in rats (Huang et al., 1999), clinical experiments demonstrated that atopic patients exhibit airway hypersensitivity to aerosolized BK, but not to Lys-des-Arg<sup>9</sup>-BK

(Reynolds et al., 1999). Icatibant has shown a certain anti-inflammatory potential in a clinical trial in asthmatic patients (Akbari et al., 1996). However, the bronchoalveolar lavage cells from ovalbumin-sensitized and -challenged mice contained eosinophils that responded strongly ( $[Ca^{2+}]_i$ ) to des-Arg<sup>9</sup>-BK in a B<sub>1</sub> receptor-dependent manner (Eric et al., 2003a), supporting a role of B<sub>1</sub> receptors in specific aspects of lung inflammation.

It was noted early on that the B<sub>1</sub> receptor gene knockout mouse is resistant to some forms of inflammatory end points, such as neutrophil accumulation in tissues (Pesquero et al., 2000). This deficit in leukocyte migration is intriguing and its mechanism has not been fully elucidated. In the mouse mesentery examined using intravital microscopy, des-Arg<sup>9</sup>-BK has no effect on leukocytes, but caused neutrophil rolling, adhesion, and emigration in IL-1 $\beta$ -treated mesenteries (McLean et al., 2000a). The effect is inhibited by capsaicin, neurokinin antagonists, histamine H<sub>1</sub> receptor antagonists, and mast cell degranulation, suggesting that an axon reflex elicited by the kinin recruits mast cells that ultimately mediate the leukocyte emigration (perhaps by an action of released mediators on vascular cells). Thus, a neurohumoral form of inflammation may be involved in this system.

### C. Pain and Neurology

BK is one of the few mediators of inflammation that directly stimulates afferent nerves. This is caused by the presence of preformed B<sub>2</sub> receptors on neural elements, notably in the nonmyelinated nerve terminals, sensory ganglions, and dorsal layers of the spinal cord (Dray et al., 1988; Steranka et al., 1988). Thus, B<sub>2</sub> receptor antagonists exhibit potential as analgesics in situations where tissue injury promotes local activation of the kallikrein-kinin system (for recent use of nonpeptide B<sub>2</sub> receptor antagonists in such situations, see Bélichard et al., 2000; Burgess et al., 2000). Mediation by vanilloid receptors and 12-lipoxygenase products of arachidonate has been proposed for B<sub>2</sub> receptor-induced inflammatory hyperalgesia (Shin et al., 2002). Perkins et al. (1993) first described the antinociceptive effect of the B<sub>1</sub> receptor antagonist [Leu<sup>8</sup>]des-Arg<sup>9</sup>-BK in rats submitted to Freund's adjuvant-induced hyperalgesia of the knee or ultraviolet ray-induced hyperalgesia of the paw. The time course of these experimental pathologies revealed that the B<sub>2</sub> receptor antagonist icatibant was effective at the beginning, but that the B<sub>1</sub> receptor ligand progressively became the best analgesic in a few days after lesion initiation. These kinetics are consistent with the B<sub>1</sub> receptor up-regulation by tissue injury; in addition, exogenous des-Arg<sup>9</sup>-BK exacerbated the pain. These observations were confirmed and extended in several other laboratories (Bélichard et al., 2000; Couture et al., 2001) and species (nociceptive spinal reflex mediated by inducible B<sub>1</sub> receptors in the rabbit, Mason et al., 2002). The early behavioral assessments based on water-soluble

peptide drugs showed that the kinin B<sub>1</sub> receptor antagonists possess a spectrum of tolerance-free analgesia similar to that of COX inhibitors (nonsteroidal anti-inflammatory drugs), raising the possibility of a peripheral mode of action involving the inhibition of the kinin-controlled formation of hyperalgesic prostanoids. However, the neural B<sub>1</sub> receptor expression is now well documented. A chronic constriction lesion of the rat sciatic nerve up-regulated B<sub>2</sub> receptors and induced B<sub>1</sub> receptor expression (mRNA) at a later stage in the ipsilateral lumbar root ganglia (Levy and Zochodne, 2000); these neuronal receptor variations were predictive of the capacity of peptide antagonists specific for each receptor type to reverse the thermal hypersensitivity in this model of neuropathic pain (COX inhibitors are rather ineffective for neuropathic pain providing an opportunity of application for kinin receptor antagonists). Furthermore, the B<sub>1</sub> receptor knockout mouse is resistant to chemical and thermal nociception, functional B<sub>1</sub> receptors are present in the normal spinal cord, and their activation can facilitate a nociceptive reflex (Pesquero et al., 2000). Indeed, activity-dependent facilitation (wind-up) of nociceptive reflexes is absent in B<sub>1</sub> receptor-deficient animals. These observations have stimulated interest in the role of the central (spinal, cerebral) expression of kinin receptors in pain perception. Whereas des-Arg<sup>9</sup>-BK is dependent on the induction of peripheral inflammation to produce hyperalgesia when injected into rat paws, the intrathecal administration of the B<sub>1</sub> receptor agonist induced some mechanical hyperalgesia even in naive animals (Fox et al., 2003). In these studies, B<sub>1</sub> receptors were localized in dorsal root ganglion primary afferent terminations (immunofluorescence) and somewhat up-regulated by peripheral inflammation. Both human and rat spinal cords were shown to express a basal level of B<sub>1</sub> receptor mRNA (specific lamina of the dorsal horns; Ma and Heavens, 2001) or protein (Wotherspoon and Winter, 2000). Furthermore, *in situ* hybridization showed that B<sub>1</sub> receptor mRNA is expressed throughout the rostral-caudal extent of the brain and spinal cord of the monkey (hippocampus, thalamus, hypothalamus, spinal cord, dorsal root ganglions, etc.; Shughrue et al., 2003). In the rat brain, a radioligand-based approach indicated a relatively low expression of the B<sub>1</sub> receptor, but a wide distribution of B<sub>2</sub> receptor (Ongali et al., 2003). Kindling-induced epilepsy was associated with B<sub>1</sub> receptor up-regulation in various brain areas in these studies, consistent with functional evidence of neuronal responsiveness to a B<sub>1</sub> receptor agonist in hippocampal and cortical regions of animals with experimental epilepsy (potentiation of electrically evoked glutamate overflow; Bregola et al., 1999). Comparative radioligand binding of B<sub>1</sub> and B<sub>2</sub> receptor ligands applied to postmortem human medulla also demonstrated the expression of the B<sub>2</sub> receptor in many of the studied nuclei, but showed a more restricted expression of the B<sub>1</sub> receptor (de Sousa Buck et al., 2002).

Thus, the possible differential therapeutic effects of kinin receptor antagonists permeant to the blood-brain barrier are intriguing in pain, epilepsy, and other diseases.

Visceral innervation is responsive to the inflammatory environment. The majority of ganglion cells express B<sub>2</sub> receptors (often coexpressed with neuropeptide Y and substance P) in the submucosal plexus of the guinea pig small intestine (Hu et al., 2004). Those peptides mediate the excitatory action of BK on submucosal plexus neurons. Chemical inflammation of the rat urinary bladder is associated with a decrease of micturition threshold when the bladder is progressively inflated; a viscerovisceral hyper-reflexia is the cause of this phenomenon. Kinin receptor antagonists were shown to inhibit this hyper-reflexia; icatibant prevents or reverses it, whereas [Leu<sup>8</sup>]des-Arg<sup>9</sup>-BK only reverses the hyper-reflexia when administered at least 5 h after the induction of local inflammation (Jaggar et al., 1998). This suggests that endogenous kinins are mediators of the afferent part of the reflex with a temporal shift of mediation from B<sub>2</sub> to B<sub>1</sub>.

The extravascular administration of BK, one of the most potent hypotensive agents, paradoxically elicits hypertension (e.g., application to the peritoneal serosa, Beauchamp et al., 1991). This is explained by the stimulation of sensory afferents (response inhibited by capsaicin, lidocaine, or spinalization) and the reflex activation of the sympathetic nervous system with resulting vasoconstriction and tachycardia. Corticosteroid release dependent on sympathetic postganglionic nerves also occurs in BK-stimulated rats (Green et al., 1997). The spinal administration of kinins causes a similar reflex mediated by the B<sub>2</sub> receptor and increased in the spontaneously hypertensive rats (Cloutier et al., 2002). This type of hypertensive response is extended to the B<sub>1</sub> receptor agonist des-Arg<sup>9</sup>-BK under pathological conditions that induce the expression of the corresponding gene (e.g., streptozotocin-induced diabetes, see below, Cloutier and Couture, 2000). Spontaneously (genetically) hypertensive rats overexpress both kinin receptor subtypes in the hypothalamus, the B<sub>1</sub> receptor in a delayed manner in the course of the pathology (Qadri et al., 2002). Centrally injected B<sub>1</sub> receptor antagonists or antisense oligonucleotides targeted to the rat B<sub>1</sub> receptor decreased the blood pressure in these rats, but not in control, normotensive rats (Emanueli et al., 1999a). Transgenic mice overexpressing the rat B<sub>1</sub> receptor in all tissues exhibited a hypertensive response to des-Arg<sup>9</sup>-BK injected by the intravenous route (Ni et al., 2003), a response that may be linked to neurological mechanisms.

#### D. Diabetes

Streptozotocin-injected rodents eventually develop an insulin-dependent form of diabetes mellitus; [Leu<sup>8</sup>]des-Arg<sup>9</sup>-BK, but not icatibant, could reduce the insulinitis, thus preventing the diabetes in mice treated daily (Zuccollo et al., 1999). These observations are of great poten-

tial significance, as a peptide B<sub>1</sub> receptor antagonist, presumably via an anti-inflammatory effect, prevented the progression of the insulin-dependent diabetes. Established streptozotocin-induced diabetes in rodents has been repeatedly shown to be associated with strong B<sub>1</sub> receptor expression in peripheral tissues with various functional consequences when exogenous des-Arg<sup>9</sup>-BK (Cloutier and Couture, 2000; Campos et al., 2001; Abdouh et al., 2003; Vianna et al., 2003) or an ACE inhibitor (Mage et al., 2002) are administered. Interestingly, this type of diabetes in mice is associated with a state of hyperalgesia that is reversed by peptide antagonists of the B<sub>1</sub> receptor (Gabra and Sirois, 2002), suggesting some activation of the endogenous kallikrein-kinin system. The modeled pathology is insulin-dependent diabetes without insulin treatment, a wasting disease with high blood cytokine levels and a particularly low life expectancy in humans in the preinsulin era. Insulin treatment of diabetic rats reversed the up-regulation of the B<sub>1</sub> receptor in streptozotocin-treated rats (radioligand binding, function, Vianna et al., 2003). The forms of diabetes that the physicians treat today are no longer wasting diseases, and many observations based on the streptozotocin models have an uncertain significance for the complications of diabetes, but remain interesting as models of systemic inflammatory responses. The 3-fold up-regulation of the B<sub>2</sub> receptor by high glucose in vascular smooth muscle cells (Christopher et al., 2001b) has not been shown for the B<sub>1</sub> receptor.

### VII. Kinin Receptors and Human Disease

Considerable progress has been made in understanding the biology and function of kinin receptors. A large body of animal studies suggests that kinin receptors may influence pathophysiological responses. Little is directly known, however, about the clinically relevant consequences of kinin receptor activation in humans. This section will review the available information concerning the potential role of kinin receptors in human diseases. Inferences about the clinical relevance of kinin receptors are derived from studies examining kinin receptor polymorphisms (Fig. 4 and Table 5), measurement of differences in kinin receptor expression level, and detection of differences in receptor function.

Studies reporting associations between single nucleotide polymorphisms (SNPs) in kinin receptor genes and complex diseases require particular care in interpretation. The vast majority of these studies involve a case control association design in which allele frequencies are contrasted in cases versus controls. Although simple, this design has frequently resulted in detection of spurious associations due principally to confounding factors (particularly population stratification) as well as a low a priori probability that the SNP is related to the disease being studied (Risch, 2000). In addition, most case-control studies have inadequate statistical power to detect



TABLE 5  
*Kinin receptor polymorphisms associated with clinical phenotype<sup>a</sup>*

Gene Location	Position	Polymorphism	Comment	Reference
B <sub>2</sub>				
Promoter	-58	-58C/T		Braun et al., 1996
Exon 1	21	9 nt insertion/deletion	-9 allele linked to higher transcript levels	Lung et al., 1997; Braun et al., 1995
Exon 2	181	181C/T	R14C; T allele associated with increased potency of B <sub>2</sub> agonist	Braun et al., 1995
B <sub>1</sub>				
Promoter	-699	G/C	ActI RFLP; C allele linked to higher transcription	Bachvarov et al., 1998
Exon 3	1098	A/G	TaqI RFLP	Bachvarov et al., 1998

RFLP, restriction fragment length polymorphism.

<sup>a</sup> Eight additional polymorphisms in the BDKRB2 promoter region as well as one missense mutation (T21M) were found by Erdmann et al. (1998); however, these polymorphisms appear to be present at too low a frequency to be of value for association studies. Eight additional polymorphisms in the BDKBR1 coding region, including two coding for substitution and one coding for premature truncation, were described by Hess et al. (2002). Three of these were found at a frequency >2%; however, none of these polymorphisms have been reported yet to be associated with a clinical phenotype.

associations conferred by low-penetrance alleles or fail to correct for multiple comparisons (Houlston and Peto, 2004). Even if a relationship is statistically valid, the possibility exists that the association represents linkage disequilibrium with a nearby gene rather than the gene of interest. Alternatively, failure to detect an association between a given SNP and the disease of interest does not exclude the possibility of a true relationship since the SNP may not be in linkage disequilibrium with the disease allele. The likelihood that a reported association represents a true causal relationship is difficult to assess but will be increased to the extent that confounding factors are controlled or if there is functional evidence suggesting plausible involvement of the kinin receptor. The robustness and power of association studies to detect causal relationships can also be increased by using appropriate family-based designs or by case-control designs analyzing haplotypes rather than single SNPs (Akey, 2001; Pe'er and Beckmann, 2004). Ultimately, however, clear delineation of true causal relationships between kinin receptors and a given human disease must await future pharmacologic studies demonstrating the predicted response.

### A. Cardiovascular Disease

**1. Left Ventricular Hypertrophy and Cardiomyopathy.** Substantial and consistent data has been generated that links kinin receptors to left ventricular (LV) hypertrophy and cardiomyopathy. Using a prospective cohort design, Brull et al. (2001) studied LV mass in army recruits who underwent a vigorous 10-week physical training program. The increase in LV mass during this 10-week period was found to be strongly associated with the B<sub>2</sub> receptor 21 +9/-9 Exon 1 genotype (Table 5), with increased LV growth correlating with increased gene dosage of the +9 allele (Brull et al., 2001). Subjects with the greatest increase in LV mass had the highest levels of ACE (DD genotype) and the lowest levels of B<sub>2</sub> receptors (+9/+9 genotype). A subsequent study examined the relationship between the B<sub>2</sub> receptor 21 +9/-9 polymorphism and pathologic LV hypertrophy (Hallberg et al., 2003). Regression of LV mass was monitored during antihypertensive therapy in patients with essential hypertension and echo-

cardiographically diagnosed LV hypertrophy. Subjects with the B<sub>2</sub> receptor 21 +9/+9 genotype showed significantly less LV mass regression compared with other genotypes. Interestingly, the B<sub>2</sub> receptor 21 +9/-9 polymorphism was also shown to be significantly associated with the efficiency of muscular contraction (Williams et al., 2004), an effect that showed evidence for interaction with the ACE insertion/deletion polymorphism. End-stage cardiac disease was found to be associated with decreased expression of cardiac B<sub>2</sub> receptors. Human heart tissue samples from excised hearts of patients undergoing cardiac transplantation for idiopathic dilated cardiomyopathy or coronary heart disease expressed significantly lower levels of B<sub>2</sub> receptor mRNA and B<sub>2</sub> receptor protein than did heart tissue from normal hearts unsuitable for donation (Kuoppala et al., 2002). Consistent with these results, disruption of the B<sub>2</sub> receptor leads to hypertension, LV remodeling, and functional impairment (Emanueli et al., 1999b), whereas overexpression of human tissue kallikrein in transgenic rats reduced cardiac hypertrophy (Silva et al., 2000).

**2. Vascular Tone.** ACE inhibitors have been shown to mediate important therapeutic benefits in heart failure (Garg and Yusuf, 1995), and kinins have been proposed to contribute to the vasodilatation associated with ACE inhibitor therapy. Indeed, the cardioprotective effect of ACE inhibitors was lost in B<sub>2</sub> receptor knockout mice (Yang et al., 2001b). Acting through the B<sub>2</sub> receptor, BK was shown to directly cause vasodilation and increased blood flow in the human forearm (Cockcroft et al., 1994a). BK was also shown to contribute to the short-term effects of ACE inhibition on blood pressure in normotensive and hypertensive subjects (Gainer et al., 1998; Squire et al., 2000). A recent report, however, found that the combined B<sub>1</sub>/B<sub>2</sub> receptor antagonist B9340 produced dose-dependent vasoconstriction in subjects with heart failure taking ACE inhibitors, whereas the closely related B<sub>2</sub> receptor antagonist icatibant did not produce vasoconstriction, suggesting that the B<sub>1</sub> receptor may mediate the kinin vasodilation effect (Witherow et al., 2001).

BK is also known to regulate resting and flow-mediated epicardial tone. Infusion of the B<sub>2</sub> receptor antag-

onist icatibant into left main coronary arteries of patients with significant coronary stenosis resulted in reduction in arterial luminal area, increased coronary vascular resistance, and decreased coronary blood flow (Groves et al., 1995). Patients with significant coronary artery disease demonstrate reduced coronary artery vasodilation in response to BK (Kuga et al., 1995). In patients with mild atherosclerosis or its risk factors, BK-induced coronary artery vasomotion was near normal; however, BK-induced vasodilation was depressed following epicardial stress induced with pacing (Prasad et al., 2000). A study comparing expression of kinin receptors on normal and atheromatous blood vessels most strikingly revealed increased B<sub>1</sub> receptor expression on multiple cells within the atheromatous vessels (Raidoo et al., 1997). The cells showing increased B<sub>1</sub> receptor immunostaining included endothelial cells, foamy macrophages, inflammatory cells, proliferating fibroblasts, and smooth muscle cells.

Houle et al. (2000a) examined the association between B<sub>2</sub> receptor polymorphisms and the contractile response of human umbilical veins to stimulation with kinins. Presence of the nonsynonymous coding region B<sub>2</sub> receptor 181 T allele was associated with increased contractile potency in response to stimulation with BK consistent with the enhanced *in vitro* potency of BK at the Arg<sup>14</sup> → Cys mutant receptor. In contrast, the B<sub>2</sub> receptor 21 -9/-9 genotype was associated with increased contractile potency to a B<sub>1</sub> receptor agonist, but not a B<sub>2</sub> receptor agonist, suggesting that the B<sub>2</sub> receptor 21 locus may be in linkage disequilibrium with the B<sub>1</sub> receptor gene. The B<sub>2</sub> receptor -58 C/T polymorphism as well as the B<sub>1</sub> receptor 1098 A/G and -699 G/C polymorphisms did not correlate with any functional changes.

**3. Hypertension.** Several studies have detected a significant association between the B<sub>2</sub> receptor -58 C/T polymorphism and hypertension. The C allele was associated with hypertension in African-American (Gainer et al., 2000), Japanese (Aoki et al., 2001), and Chinese subjects (Wang et al., 2001). In addition, the B<sub>2</sub> receptor -58 C allele was strongly associated with hypertension in a population of patients with primary aldosteronism (Mulatero et al., 2002). In contrast, the risk of significant arterial hypertension developing in a large cohort of previously healthy men was significantly associated with both the B<sub>1</sub> receptor -699 G/G genotype and the B<sub>2</sub> receptor 21 +9/+9 genotype (Dhamrait et al., 2003). This prospective study is particularly important in that it utilized a cohort rather than a case-control design. Postmortem human medulla samples obtained from normotensive, hypertensive, and diabetic subjects and studied by *in vitro* receptor autoradiography revealed increased B<sub>2</sub> receptor expression in the hypertensive patients, suggesting a possible role for kinin receptors in central cardiovascular regulation (de Sousa Buck et al., 2002).

## B. Renal Disease

Genetic associations between B<sub>1</sub> receptor polymorphisms and end-stage renal failure have been reported. Bachvarov et al. (1998b) looked for associations between the B<sub>1</sub> receptor 1098 A/G, B<sub>1</sub> receptor -699 G/C, and B<sub>2</sub> receptor 181 C/T polymorphisms and end-stage renal failure. Although the B<sub>1</sub> receptor 1098 and B<sub>2</sub> receptor 181 polymorphisms were neutral, a significant underrepresentation of the B<sub>1</sub> receptor -699 C allele was found in end-stage renal patients compared with normal controls, suggesting a protective role for the B<sub>1</sub> receptor since the C allele leads to lower B<sub>1</sub> receptor expression. Zychma et al. (1999) also described underrepresentation of the B<sub>1</sub> receptor -699 C allele in patients with end-stage renal failure; however, they also found less frequent transmission of the B<sub>2</sub> receptor 181 T allele. A third study found no evidence of an association between renal failure and the B<sub>1</sub> receptor -699 genotype (Knigge et al., 2000).

In contrast, the B<sub>2</sub> receptor 21 +9/-9 polymorphism (but not the B<sub>1</sub> receptor -699, B<sub>2</sub> receptor -58, or B<sub>2</sub> receptor 181 polymorphisms) was significantly associated with diabetic nephropathy (Maltais et al., 2002). A highly significant decrease in the albumin/creatinine ratio was seen in diabetic patients who were homozygous for the +9 allele. Another study involving diabetic nephropathy failed to show any association with the B<sub>1</sub> receptor -699 or B<sub>2</sub> receptor 181 polymorphisms although the B<sub>2</sub> receptor 181 T allele was associated with lower blood pressure (Zychma et al., 2003).

The expression of kallikrein-kinin components in kidney tissue from patients with renal disease has also been reported (Naicker et al., 1999). Tissue kallikrein excretion was significantly decreased in patients with mild renal disease compared with normal controls, and this effect was more pronounced in patients with severe renal disease. B<sub>2</sub> receptor immunoreactivity in nephrons was also decreased in the renal disease patients (Naicker et al., 1999) as well as in patients undergoing acute renal transplant rejection (Naidoo et al., 1996). Immunoreactive B<sub>1</sub> receptor was observed only in the distal tubules and collecting ducts in patients with renal disease and not in normal controls.

Patients experiencing hypersensitivity to dialysis membranes have been shown to have increased plasma levels of BK (Schaefer et al., 1993; Verresen et al., 1994). Lower serum aminopeptidase P activity differentiated dialysis patients who had experienced a hypersensitivity reaction to the negatively charged membrane from patients who had not experienced a reaction (Blais et al., 1999). Interestingly, serum aminopeptidase P activity was shown to correlate with the plasma half-life of the B<sub>1</sub> receptor agonist des-Arg<sup>9</sup>-BK and not BK, suggesting that the B<sub>1</sub> receptor may also play a role in these reactions.

### C. Airway Disease

The B<sub>2</sub> receptor is thought to be the major kinin receptor involved in airway responses to kinins, and radioligand binding studies have provided independent confirmation of the presence of B<sub>2</sub> receptors in healthy and carcinomatous lung samples (Trifilieff et al., 1994). Inhalation of the B<sub>2</sub> receptor agonists BK or Lys-BK caused bronchospasm in asthmatic but not normal subjects (Polosa and Holgate, 1990; Polosa et al., 1992). Inhalation of the weak B<sub>1</sub> receptor agonist des-Arg<sup>9</sup>-BK failed to cause bronchospasm in either asthmatic or normal subjects. Nasal challenge with BK but not des-Arg<sup>9</sup>-BK similarly caused rhinitis symptoms (Churchill et al., 1991). Nasal airflow resistance in response to BK or house dust mite challenge could be inhibited by B<sub>2</sub> receptor but not B<sub>1</sub> receptor antagonists (Austin and Foreman, 1994; Austin et al., 1994). Intranasal challenge with platelet-activating factor also caused nasal hyper-responsiveness to histamine, and this was abolished by pretreatment with icatibant (Turner et al., 2000). BK itself failed to cause histamine hyper-responsiveness.

A potentially important role for the B<sub>2</sub> receptor in asthma was demonstrated by a clinical trial of nebulized icatibant in patients with persistent asthma (Akbary et al., 1996). After 1 month of therapy, patients given the higher dose of icatibant had a mean increase of 10% in forced expiratory volume in 1 s. No effect on acute bronchodilation was seen suggesting an anti-inflammatory effect of the B<sub>2</sub> receptor antagonist although the B<sub>2</sub> receptor antagonist NPC17731 was able to inhibit the early antigen-induced airway response to aerosolized allergen challenge in a non-human primate model of asthma (Hogan et al., 1997). Intranasal insufflation of icatibant prior to nasal allergen challenge had no effect on the immediate allergic nasal response but blocked histamine hyper-responsiveness at 24 h as well as allergen-induced increases in eosinophils, eosinophil cationic protein, kinin, and IL-8 in 6-h nasal lavage fluid (Turner et al., 2001). An association study examining B<sub>2</sub> receptor polymorphisms and pediatric asthma failed to find a significant association between a genotype and the presence or absence of asthma; however, a significant association between the B<sub>2</sub> receptor 21 -9/-9 genotype and onset of asthma prior to the age of 4 was found (Kusser et al., 2001).

A potential role for the B<sub>1</sub> receptor in airway disease has been difficult to establish. The challenge studies described above used des-Arg<sup>9</sup>-BK as a B<sub>1</sub> receptor agonist, however, des-Arg<sup>9</sup>-BK is a relatively weak ligand for the human B<sub>1</sub> receptor. Reynolds et al. (1999) challenged perennial allergic rhinitis or asthma patients with BK or the potent B<sub>1</sub> receptor agonist Lys-des-Arg<sup>9</sup>-BK. BK challenges again resulted in acute symptoms, and repeated challenges resulted in tachyphylaxis. Lys-des-Arg<sup>9</sup>-BK had no acute effect on either the upper or lower airway. In a rat model of asthma, however, a B<sub>1</sub> receptor antagonist inhibited allergen-induced bron-

chial hyper-responsiveness suggesting that the B<sub>1</sub> receptor may be involved in allergic airway inflammation (Huang et al., 1999). A recent human study lends further support to this concept. Allergic rhinitis subjects, but not normal subjects, were shown to express B<sub>1</sub> receptors in nasal tissue, and allergen challenge further increased nasal expression of B<sub>1</sub> receptor mRNA (Christiansen et al., 2002). Nasal challenge with the B<sub>1</sub> receptor agonist Lys-des-Arg<sup>9</sup>-BK activated the ERK MAPK in nasal samples from subjects with allergic rhinitis but not in normal subjects, suggesting that the B<sub>1</sub> receptor expressed in nasal tissue is functional and may play a role in human airway inflammation. Expression of immunoreactive B<sub>1</sub> receptor was found in transbronchial biopsies from patients with interstitial lung disease associated with progressive systemic sclerosis but not in normal lung tissue (Nadar et al., 1996).

ACE inhibitors are well known to cause cough in a subset of individuals receiving them. BK, acting in part through stimulation of prostaglandin generation, has been suggested as a possible mediator of the ACE inhibitor-induced cough (Choudry et al., 1989; Semple, 1995). The B<sub>2</sub> receptor promoter -58 T/C polymorphism was shown to have a significant association with ACE inhibitor cough in a Japanese population (Mukae et al., 2000, 2002). Patients with cough were more likely to have the T allele (Mukae et al., 2000), which is associated with a higher B<sub>2</sub> receptor transcription rate than the C allele. This study did not find an association between cough and the B<sub>2</sub> receptor 21 +9/-9 polymorphism; however, as previously reported (Lung et al., 1997), the +9 allele is not represented within the Asian population. Zee et al. (1998), however, failed to find an association between either the ACE I/D polymorphism or the B<sub>2</sub> receptor 21 +9/-9 polymorphism in a European population. Another study involving Chinese females with noninsulin-dependent diabetes mellitus who were being treated with ACE inhibitors found an association between cough and the ACE I/D polymorphism but not the B<sub>2</sub> receptor -58 T/C polymorphism (Lee and Tsai, 2001).

### D. Neurological Disease

A significant role for kinin receptors has been proposed in a variety of central nervous system diseases. The widespread expression of B<sub>2</sub> receptors within the CNS vasculature has led to intense interest in using kinin agonists to increase permeability and delivery of chemotherapeutic agents across the blood-brain barrier (Borlongan and Emerich, 2003). Early clinical trials of this approach for gliomas have demonstrated some efficacy (Emerich et al., 2001).

Kinin receptors may also play an important role in the regulation of post-traumatic cerebral edema. BK has been widely appreciated to increase blood-brain barrier permeability (Wahl et al., 1996; Sarker et al., 2000). Several trials have now reported the effects of using a B<sub>2</sub> receptor antagonist in the treatment of human traumatic brain



injury. In the first study, the B<sub>2</sub> receptor antagonist CP-0127 was infused for 7 days in patients with severe focal cerebral contusions presenting 24 to 96 h after injury (Narotam et al., 1998). Mean peak increase in intracranial pressure as well as the mean increase in severity scores was significantly less in the treated group. In another study, CP-0127 was infused continuously for 5 days in severely brain-injured patients with a Glasgow Coma Score of 3 to 8 (Marmarou et al., 1999). A trend toward improvement in intracranial pressure was seen, and there was a significant lowering of the time that the intracranial pressure was above 15 mm Hg on days 4 and 5. A trend toward fewer deaths and better outcomes was also seen at 3 and 6 months.

Functional differences in kinin receptors have been linked to Alzheimer's disease. Studying inositol phosphate production in fibroblasts, Huang et al. (1995) showed that skin fibroblasts derived from patients with familial Alzheimer's disease generated increased amounts of IP<sub>3</sub> in response to stimulation with BK compared with fibroblasts derived from normal subjects. They further showed that Alzheimer disease fibroblasts expressed increased numbers of B<sub>2</sub> receptors on their surface. These findings could not be reproduced in a subsequent study where fibroblasts derived from sporadic Alzheimer patients or normal subjects generated the same amounts of IP<sub>3</sub> and released the same amount of soluble amyloid precursor protein following stimulation with BK (Racchi et al., 1998). More recently, however, Zhao et al. (2002) reported enhanced and prolonged IP<sub>3</sub>-sensitive Erk1/2 phosphorylation in response to BK stimulation in fibroblasts derived from both familial and sporadic Alzheimer's disease compared with age-matched normal and Huntington's disease controls. Furthermore, B<sub>2</sub> receptor activation was shown to result in selective serine phosphorylation of tau in skin fibroblasts from persons who have or will develop Alzheimer's disease due to presenilin-1 mutations or Trisomy 21, but not in skin fibroblasts from normal individuals at any age (Jong et al., 2003).

### E. Cancer

The potential role of kinin receptors in human cancer has not been well studied, however, the ability of BK to stimulate growth and increase vascular permeability may contribute to the biological behavior of tumors. Evidence for increased generation of kinins in several types of cancer has been reported (Matsumura et al., 1988, 1991), and the B<sub>2</sub> receptor has been detected in a number of different cancer tissues (Wu et al., 2002).

Kinin receptor antagonists have been proposed for the treatment of various cancers, particularly lung and prostate cancers (Stewart, 2003). The novel BK antagonist dimer, CU201, was shown to induce apoptosis and growth inhibition in various lung cancer and other cancer cell lines (Chan et al., 2002a,b). BK antagonists also inhibited the growth of prostate cancer in xenografts in nude mice (Stewart et al., 2002a).

Tissue kallikrein and the B<sub>2</sub> receptor were expressed in endometrial and prostate cancers (Clements and Mukhtar, 1997). Compared with normal or inflamed vulvar tissue, human vulvar cancer tissue showed decreased levels of B<sub>2</sub> receptor mRNA (Olejek et al., 2001). In contrast, cervical cancer tissue as well as cervical cancer metastatic lesions displayed higher expression of both B<sub>1</sub> and B<sub>2</sub> receptors than did normal cervical tissue and the levels normalized following brachytherapy (Olejek et al., 2000). Prostate cancer tissue was found to express increased levels of B<sub>1</sub> receptors compared with normal prostate tissue (Taub et al., 2003). Potential consequences of up-regulation of B<sub>1</sub> receptor expression were suggested by the ability of B<sub>1</sub> receptor ligands to promote cell growth and stimulate migration and invasion in a prostate cell line (Stewart et al., 2002a; Barki-Harrington et al., 2003; Taub et al., 2003). Normal brain tissue expresses B<sub>2</sub> receptors on cortical neurons but not on glial cells (Raidoo et al., 1996). Brain tissue obtained from patients with astrocytoma, however, showed B<sub>2</sub> receptors on the astrocytic cells as well as variable expression of B<sub>1</sub> receptors (Raidoo et al., 1999).

### F. Other Disease States

Potential associations between kinin receptors and a variety of other diseases have been reported, and several of these are briefly reviewed here. Kinin receptors may play a role in inflammatory arthritis. B<sub>2</sub> receptor expression was reported to be increased in rheumatoid arthritis synovial tissue compared with osteoarthritis tissue (Bathon et al., 1992). Increased expression of B<sub>2</sub> receptors have also been reported on synovial neutrophils and synovial lining cells from rheumatoid arthritis subjects, however, no evidence for synovial B<sub>1</sub> receptor expression was found (Cassim et al., 1996, 1997).

Expression of the B<sub>2</sub> receptor as well as tissue kallikrein in the human uterus varied during the phases of gestation, possibly contributing to the local regulation necessary to support the developing fetus (Valdes et al., 2001). A remarkable increase in B<sub>2</sub> receptor expression in platelets and omental vessels was found in pre-eclamptic women compared with normotensive pregnant women (AbdAlla et al., 2001). There was also evidence of increased heterodimerization between B<sub>2</sub> and AT<sub>1</sub> receptors in the pre-eclamptic women associated with increased responsiveness to angiotensin-II despite no difference in the expression of AT<sub>1</sub> receptors.

Hereditary angioedema (HAE) is a disease known to be associated with increased generation of BK. Recent studies in mice genetically engineered to be deficient in C1 inhibitor have shown that the B<sub>2</sub> receptor antagonist icatibant can abrogate the permeability defect seen in C1 inhibitor deficiency (Han et al., 2002). One study reported that HAE patients with the B<sub>2</sub> receptor 21 +9/+9 genotype had less swelling symptoms than patients with the -9 allele (Lung et al., 1997); however, the B<sub>2</sub> receptor 21 polymorphism had no association

with HAE severity in a different Czech cohort (Freiberger et al., 2002). Of note, the B<sub>2</sub> receptor antagonist icatibant is currently in trials for the treatment of attacks of angioedema in HAE patients.

Several reports have implicated kinin receptors in gastrointestinal diseases. Comparing gastritis to normal gastric tissue, Bhoola et al. (1997) found decreased B<sub>2</sub> receptors and appearance of B<sub>1</sub> receptors in the gastritis tissue. Inflammatory bowel disease was found to be significantly associated with a decreased presence of the C allele at the B<sub>1</sub> receptor -699 locus compared with normal controls (Bachvarov et al., 1998a). Expression of B<sub>2</sub> receptor mRNA as well as binding of [<sup>3</sup>H]BK was assessed in human gallbladders (Trevisani et al., 2003). Gallbladders removed for acute cholecystitis expressed significantly increased levels of B<sub>2</sub> receptors than control gallbladders removed for gallstones or during elective gastroenteropancreatic surgery. The cholecystitis gallbladders also demonstrated increased contractility to stimulation with exogenous BK.

### G. Caveats

Evidence is slowly accumulating linking kinin receptors to a variety of clinical diseases. Interpretation of the data are hampered, however, by the paucity of human studies directly testing the impact of kinin receptors on disease. A significant amount of inference has been drawn from examining kinin receptor SNPs or short genetic deletions/insertions. It is important to bear in mind that such variations are merely genetic markers and may not always be of significance. Although some of the polymorphisms of the kinin receptor locus have been proposed to determine alterations of the expression of one of the receptors, the evidence is limited and not always reproducible (Lung et al., 1997; Bachvarov et al., 1998b; Houle et al., 2000a). Very few known polymorphisms affect the kinin receptor amino acid sequences. Three such mutations have been described for the coding region of the B<sub>1</sub> receptor, one of which determines a premature stop codon, but they are likely to be very infrequent alleles (Hess et al., 2002). When larger or more diverse populations are examined, predictions based on SNPs do not always hold true. An avenue for the future would be to define full haplotypes for the kinin receptor locus (i.e., phased SNPs determined by sequencing full DNA strands). This approach has produced more predictive data for the β<sub>2</sub>-adrenergic receptor gene (Drysdale et al., 2000). However, this is complicated by the fact that the two kinin receptor genes are so close to each other. Thus, a genetic marker in one gene could be in linkage disequilibrium with a functional alteration of the other (see Houle et al., 2000a) because there is little probability of recombination within zones of 60 kb in the human genome (Reich et al., 2001).

## VIII. Kinin Receptors and Drug Development

Over the past two decades it has become clear that the various kinins and their receptors are involved in an in-

creasing list of physiological functions. Prominent among these processes from the standpoint of therapeutic intervention is the well known proinflammatory effects of BK and its metabolites that are mediated through B<sub>1</sub> and B<sub>2</sub> receptors (Table 6; Ahluwalia and Perretti, 1999; McLean et al., 2000b; Couture et al., 2001; Marceau and Regoli, 2004). Important disease states such as chronic inflammatory pain, sepsis, edema, head trauma, and asthma all have their basis in the inflammatory response. As such, the development of B<sub>1</sub> and B<sub>2</sub> receptor antagonists could provide novel therapeutics according to the importance of the specific roles of the receptors in the pathology. These therapeutic targets have been the subject of conjecture for some time now (Fujie et al., 1993; Perkins et al., 1993; Sharma, 1993; Fink, 1998; Stewart et al., 1999; Heitsch, 2003; Howl and Payne, 2003; Zausinger, 2003), and a limited number of published human clinical studies have been conducted in the area of pain, asthma/rhinitis, and septic shock (see Whalley et al., 1987; Proud et al., 1995; Akbary et al., 1996; Fein et al., 1997; Turner et al., 2001) showing some level of efficacy with BK antagonists. However, being peptide-based structures, the properties of these early compounds were likely not ideal, perhaps limiting potential efficacy. A number of pharmaceutical efforts are currently ongoing to discover and optimize novel nonpeptidyl chemical structures acting through B<sub>1</sub> or B<sub>2</sub> receptor mechanisms (see *Section II.*). A key question that must await appropriate clinical trials is to what extent the results from animal studies translate to humans. The therapeutic areas currently of most interest are driven by medical need and include pain, cardiovascular function, asthma, and cancer.

### A. Pain

Perhaps the most promising area where kinin receptor antagonists could prove useful is in the treatment of pain. The role of kinins in mediating pain responses in animals has been known for almost two decades and exemplified by the observation that injection of BK into the knee of rats elicited a hyperalgesic response (Davis and Perkins, 1994a). B<sub>1</sub> and B<sub>2</sub> receptors share an overlapping distribution among tissue and cell types, albeit, at different expression levels, and their activation can elicit similar intracellular effector mechanisms and physiological responses. The specific role of B<sub>2</sub> and B<sub>1</sub> receptors to underlie pain, however, relates to the magnitude and kinetics of their expression. B<sub>2</sub> receptors are generally constitutively expressed on a number of cell types including primary sensory neurons, and BK activates these nociceptors to contribute to the acute pain response (Dray et al., 1992). Such a response is evident when applying low concentrations of BK to a blister base in humans (Whalley et al., 1987). It appears that the acute blister base pain response to BK is mediated by the B<sub>2</sub> receptor because the B<sub>1</sub> receptor-selective agonist des-Arg<sup>9</sup>-BK was not as active at high concentrations. However, a role of the B<sub>1</sub> receptor should not be ruled

TABLE 6  
*Potential clinical applications of kinin receptor ligands*

Type of Agent	Application	Investigational Status	References
B <sub>2</sub> receptor agonists Labradimil	Transient opening of the blood-brain barrier (adjuvant to chemotherapy of brain tumors)	Extensive animal studies performed	Emerich et al., 2001
Lys-BK	Additive to vaccines to skew the immune response from humoral (T <sub>H2</sub> ) to cellular responses (T <sub>H1</sub> )	Hypothesis supported by animal data	Aliberti et al., 2003
FR190997, others	Renal, cardiac protection, and hypertension, via endothelial mediator release	Speculative; unfavorable risk to benefit ratio probable	Heitsch, 2002
B <sub>1</sub> receptor agonists Sar-[d-Phe <sup>6</sup> ]-des-Arg <sup>9</sup> -BK	Angiogenesis, vasculogenesis in severe ischemic disease	Speculative; supported by animal data	Emanueli et al., 2002
B <sub>2</sub> receptor antagonists Icatibant, several others	Inflammatory pain (hyperalgesia)	Abundantly supported by animal studies	Steranka et al., 1988; Perkins et al., 1993; Burgess et al., 2000
Icatibant, others LF16-0687, deltibant	Inflammatory edema Brain edema (post-traumatic or secondary to stroke)	Supported by many animal studies Mixed results of a limited clinical trial of deltibant for head trauma; extensive animal data supporting LF16-0687; the later drug is being clinically evaluated for head trauma	Uknis et al., 2002; Samadifam et al., 2000 Narotam et al., 1998; Kaplanski et al., 2002; Zausigner et al., 2002
Icatibant	Hereditary angioedema	Active in a mouse model (C1 inhibitor gene knockout); Icatibant being clinically evaluated for this indication, as well as for ascites secondary to liver cirrhosis (Jerini AG)	Han et al., 2002
Icatibant	Allergic asthma, rhinitis	Objective and protracted anti-inflammatory effects shown in humans following local application to mucosa	Akbary et al., 1996; Turner et al., 2001
Deltibant	Sepsis	Disappointing results of a clinical trial	Fein et al., 1997
B <sub>1</sub> receptor antagonists [Leu <sup>8</sup> ]-des-Arg <sup>9</sup> -BK, others	Inflammatory pain (hyperalgesia); effect may extend to neuropathic pain, wind-up, and viscerovisceral hyper-reflexia	Abundantly supported by animal studies; may be effective during a retarded time window, relative to B <sub>2</sub> receptor antagonists	Perkins et al., 1993; Bélichard et al., 2000; Levy and Zochodne, 2000; Pesquero et al., 2000; Jaggar et al., 1998
Lys-[Leu <sup>8</sup> ]-des-Arg <sup>9</sup> -BK, others Unspecified	Inflammation Epilepsy	Effects in animal models Speculative; based on the epileptogenic effect of the agonist in animal models	Blais et al., 2000; deBlois and Horlick, 2001 Bregola et al., 1999
Des-Arg <sup>10</sup> -Hoe 140 Unspecified	Airway allergy Sepsis	Benefits in a rat model; speculative in humans Speculative; based on the counterproductive hemodynamic effect of agonist in LPS-pretreated rabbits	Huang et al., 1999; Christiansen et al., 2002 Audet et al., 1997
Promiscuous ligands CU201 = B9870	Cancer	Whereas both antagonists of the B <sub>1</sub> and B <sub>2</sub> receptors have been proposed or used in a limited manner as experimental antineoplastic agents, CU201 may be more effective in specific tumorigenic cell lines than conventional antagonists	Chan et al., 2002a,b; Stewart et al., 2003



out because it is now known that des-Arg<sup>9</sup>-BK exhibits low affinity for the human B<sub>1</sub> receptor and was thus not an appropriate tool to test the hypothesis (see Marceau et al., 1998). Consistent with these observations, intradermal administration of BK is well known to produce an acute, painful response accompanied by swelling and redness (Kindgen-Milles and Klement, 1992; Fadel et al., 2000). Of interest, the intranasal administration of BK or its topical application to the back of the throat produces an irritating sore throat (Proud et al., 1988; Rees and Eccles, 1994), providing the mechanism behind the so-called “bradykinin cough” that sometimes accompanies the reduced metabolism of BK after treatment of high blood pressure with ACE inhibitors.

The constitutive expression of B<sub>2</sub> receptors on vascular endothelial cells underlies the initial inflammatory response leading to edema, plasma extravasation, and the ensuing generalized inflammatory response, which includes local tissue generation of high levels of kinins (see Ahluwalia and Perretti, 1999; McLean et al., 2000b). The generalized inflammation including enhanced release of local inflammatory cytokine mediators (e.g., TNF- $\alpha$ , IL-1, prostaglandins, etc.) induces the local expression of B<sub>1</sub> receptors on various cell types, thereby propagating the inflammatory response, including the associated activation of nociceptors by a variety of mediators (Ni et al., 1998b; Poole et al., 1999; Cabrini et al., 2001; Sardi et al., 2002). Thus, this cascade of events has led to the view that B<sub>2</sub> receptors are involved in the initial inflammatory response followed by the appropriate induction of B<sub>1</sub> receptors to potentiate and mediate longer term inflammation and pain, as initially proposed by Perkins and colleagues (Perkins and Kelly, 1993; Perkins et al., 1993; Davis and Perkins, 1994a,b) to explain their findings in rats. Contributing to this sequence is the kinetics of receptor down-regulation whereby B<sub>2</sub> receptors appear to be readily phosphorylated and internalized, whereas B<sub>1</sub> receptors have a longer functional half-life on the surface of the cell (Austin et al., 1997; Blaukat et al., 2001; Lamb et al., 2001; Prado et al., 2002). It should be noted, however, that the conversion of B<sub>2</sub> to B<sub>1</sub> receptor-dominant responses over the course of an inflammatory insult can be species-dependent. The i.v. administration of LPS to rats, rabbits, and monkeys (Regoli et al., 1981; Drapeau et al., 1991; Nicolau et al., 1996; deBlois and Horlick, 2001) elicits a B<sub>1</sub> receptor-dependent depressor response that was absent before the LPS treatment, whereas in dogs such a depressor response is constitutive (Nakhostine et al., 1993).

Based on these data then, the prevailing picture that has emerged is that B<sub>2</sub> receptor antagonists would target the early phases of inflammatory pain, whereas B<sub>1</sub> receptor antagonists would be most likely to treat chronic inflammatory pain. This view, along with a peripheral site of action, is supported by pain studies in animals whereby local injections of peptidyl B<sub>2</sub> and B<sub>1</sub> receptor agonists produce time-dependent algescic responses that are in turn inhibited by receptor specific

kinin antagonists (Perkins et al., 1993; Davis and Perkins, 1994a; Tonussi and Ferreira, 1997; Poole et al., 1999). However, over the past few years an expanded view has developed particularly for the potential role of B<sub>1</sub> receptor antagonists for the broad treatment of the various types of pain. The phenotypic profile of the B<sub>1</sub> receptor knockout mouse has contributed to this expanded view (Pesquero et al., 2000; Ferreira et al., 2001). Although appearing normal upon observation, as expected, certain responses to inflammation are absent or markedly reduced in the B<sub>1</sub> receptor knockout mouse including thermal hyperalgesic responses to Freund's adjuvant and carrageenan, LPS-induced hypotension, and carrageenan-induced pleurisy. In addition, responses to painful stimuli in the absence of inflammation are also reduced in the B<sub>1</sub> receptor knockout mouse, suggesting a potential role of B<sub>1</sub> receptors in acute pain. Significantly, a role for a CNS component in pain perception is suggested by *in vitro* studies showing that the ventral root potential evoked by electrical stimulation of the dorsal root is enhanced by the application of des-Arg<sup>9</sup>-BK to a spinal cord preparation from wild-type but not B<sub>1</sub> receptor knockout mice. The phenomenon of “wind-up” to repeated dorsal root stimulation *in vitro* is also reduced by about 50% in the B<sub>1</sub> receptor knockout mice (Pesquero et al., 2000). Wind-up is a response observed after peripheral nerve damage whereby continuous primary afferent nociceptive input causes neuronal plasticity in the spinal cord leading to hyperalgesia and allodynia. Many drugs that are effective in treating neuropathic pain are also effective in reducing wind-up (Boyce et al., 1999). A potential role for B<sub>1</sub> receptor mechanisms to underlie neuropathic pain has recently been demonstrated in animals after nerve damage (Levy and Zochodne, 2000; Rashid et al., 2004) or induced by diabetes (Gabra and Sirois, 2002), whereby B<sub>1</sub> receptor antagonists are analgesic. In some cases, the analgesic response of B<sub>1</sub> receptor antagonists was demonstrated to parallel the induction of B<sub>1</sub> receptors in primary afferents and spinal cord (Levy and Zochodne, 2000). Indeed, it has also been shown that peripheral nerve injury induces a switch from the expression of B<sub>2</sub> to B<sub>1</sub> receptors in large diameter peripheral neurons as well as a switch from a B<sub>2</sub> to B<sub>1</sub> receptor-dependent functional and nociceptive response (Rashid et al., 2004). A contribution of a spinal cord B<sub>1</sub> receptor mechanism to mediate inflammatory pain responses induced by intraplantar injection of complete Freund's adjuvant is demonstrated by the antihyperalgesic response of intrathecally delivered B<sub>1</sub> receptor antagonist (Fox et al., 2003). In agreement with these findings, a hyperalgesic response to intrathecal des-Arg<sup>9</sup>-BK is markedly reduced in the B<sub>1</sub> receptor knockout mouse (Ferreira et al., 2002). For a CNS-based mechanism, B<sub>1</sub> receptor antagonists could be acting at B<sub>1</sub> receptors expressed on nerve terminals in the dorsal horn or on intrinsic spinal cord neurons to interrupt pain signals. Importantly, all of the

components of the kinin system have been demonstrated in CNS to subserve such a mechanism (Lopes and Couture, 1997; Raidoo and Bhoola, 1997; Couture and Lindsey, 2000; Ma et al., 2000; Shughrue et al., 2003).

Based on the results from animal studies generated over the past decade, it would appear that antagonists of both the B<sub>2</sub> and B<sub>1</sub> receptors are legitimate targets as novel analgesic agents. To date, no clinical studies have been published testing this hypothesis. Overall, the analgesic profile of a B<sub>2</sub> receptor antagonist may be different from a B<sub>1</sub> receptor antagonist by being most effective during acute phases of inflammation. B<sub>1</sub> receptor mechanisms both in the periphery and the CNS appear to be involved in inflammatory and neuropathic pain responses and in certain circumstances, pain responses in the absence of inflammation. The analgesic profile of B<sub>1</sub> receptor antagonists differs from that of nonsteroidal anti-inflammatory drugs by their activity in the models of neuropathic pain and some instances in the absence of inflammation and from opiates by the absence of sedation and lack of disruption of sensorimotor function. The development of a centrally active compound may provide the greatest potential for maximal efficacy across different clinical pain states. Of course, this conjecture must await the results of appropriate clinical trials with the novel antagonists currently being optimized and developed (see Section II., Fig. 2).

### B. Cardiovascular Function

BK is well known for its multiple effects on the cardiovascular system particularly vasodilation and plasma extravasation (Ahluwalia and Perretti, 1999; Heitsch, 2003; Howl and Payne, 2003), key activities leading to the inflammatory response. In animals and humans, the vasodilation under normal conditions is mediated by the B<sub>2</sub> receptor (Cockcroft et al., 1994a,b; Groves et al., 1995; Weldon et al., 1995), but it is known that under inflammatory conditions, newly expressed B<sub>1</sub> receptors can also mediate kinin-induced vasodilation and hypotension (Regoli et al., 1981; Drapeau et al., 1991; deBlois and Horlick, 2001). As such, B<sub>2</sub> receptor agonists have been considered as potential antihypertensive or cardioprotective agents (see Heitsch, 2003; FR190997, Section II.). At least part of the beneficial effects of ACE inhibitors, and possibly AT<sub>1</sub> antagonists, to reduce blood pressure or impart cardioprotection have been linked to the local generation of kinins and resulting B<sub>2</sub> receptor stimulation (Linz et al., 1997; Gainer et al., 1998; Zhu et al., 1999; Witherow et al., 2001). Although B<sub>2</sub> receptors have been primarily implicated in the preconditioning and cardioprotective effects of BK (Goto et al., 1995; Baxter and Ebrahim, 2002), it is known that cardiac ischemia can induce B<sub>1</sub> receptors locally (Tschöpe et al., 2000; Mazenot et al., 2001; Lagneux et al., 2002) providing a basis that B<sub>1</sub> receptor activity may also promote preconditioning and cardioprotection (Chahine et al., 1993; Bouchard et al., 1998).

On the other hand, B<sub>1</sub> receptor activation has been shown to exert *protective* effects after cardiac ischemia in mice (Lagneux et al., 2002). Clearly, there is potential for kinin agonists, particularly B<sub>2</sub> receptor agonists, as novel cardioprotective agents; however, it is unclear whether proinflammatory effects would preclude their development (Aramori et al., 1997a; Heitsch, 2003).

BK has been implicated in the pathogenesis of septic shock syndrome either directly or indirectly, along with a number of other potential mediators, by virtue of its increased production after infection and its well known effects to produce hypotension and plasma extravasation (Fink, 1998). B<sub>2</sub> receptor antagonists, however, have not proven particularly effective in animal models (Otterbein et al., 1993; Feletou et al., 1996) or in an initial clinical trial (Fein et al., 1997). It will be of interest to study a potent and long-acting B<sub>1</sub> receptor antagonist, knowing that B<sub>1</sub> receptors are readily induced in the vasculature under conditions of inflammation (Regoli et al., 1981; Drapeau et al., 1991; Nicolau et al., 1996; deBlois and Horlick, 2001).

Angioedema is a condition characterized by swelling of the extremities and abdomen leading to pain, nausea, and vomiting (see Section VII.F.). A hereditary form of the disease is characterized by activation of the kallikrein-kinin system or decreased kinin metabolism (Adam et al., 2002; Molinaro et al., 2002) resulting in increased kinin levels in the circulation (Nussberger et al., 1999), which presumably is the cause of angioedema arising in a small portion of patients taking ACE inhibitors (Warner et al., 2000). Of interest, a recent communication from researchers at Jerini AG suggests efficacy of icatibant or JE049 to treat the symptoms of angioedema in a Phase II clinical trial.

The vasodilatory, edema-inducing, and overall proinflammatory activity of BK in the brain after trauma provides the basis for the current interest in the development of B<sub>2</sub> receptor antagonists in the treatment of stroke and ischemia-related neurological problems (Sobey, 2003; Zausinger, 2003). Both CP-0597 and LF 16-0687 (see Section II.; Fig. 2) have shown activity to reduce infarct size, brain edema, and improve neurological outcome in rats (Relton et al., 1997; Kaplanski et al., 2002; Zausinger et al., 2003). LF 16-0687 is currently in clinical trials for the treatment of head trauma.

### C. Airway Function

The role of BK in the inflammatory processes leading to conditions such as asthma and rhinitis gathered the earliest interest in B<sub>2</sub> receptor antagonists as potential novel therapeutics. Increased kinin formation has been demonstrated in pulmonary exudates during allergic reactions in humans (Proud et al., 1983; Naclerio et al., 1988). In addition, BK administration to airways has been shown to induce bronchoconstriction in asthmatics, but not normals (Fuller et al., 1987; Polosa and Holgate, 1990), perhaps due to enhanced B<sub>2</sub> receptor expression

in this sensitized patient population (Schmidlin et al., 2000). BK challenge also produces sore throat and rhinitis (Proud et al., 1988), and these symptoms are blocked by the potent and selective B<sub>2</sub> receptor antagonist icatibant (Proud et al., 1995). In allergic individuals exposed to grass pollen, prior intranasal administration of icatibant suppressed some of the allergic responses (increase in eosinophil number, IL-8 in lavage fluid, and histamine reactivity), but not others (nasal obstruction and albumin extravasation) (Turner et al., 2001). Icatibant, on the other hand, reduced nasal blockade in allergic individuals exposed to house mite dust (Austin et al., 1994). Icatibant has also been studied in patients with moderately severe asthma in a double-blind, placebo controlled, 4-week clinical trial (Akbariy et al., 1996). Compared with placebo, icatibant produced a relatively modest improvement in pulmonary performance but did not cause bronchodilation or impact on global symptom score or the frequency of rescue medication use in this patient population. Although icatibant was eventually not developed for pulmonary disorders, there does appear to be a significant contribution of B<sub>2</sub> receptor mechanisms in human pulmonary disorders such as allergic rhinitis. It may be that the therapeutic potential of B<sub>2</sub> receptor antagonists will be revisited with newer compounds under development.

B<sub>1</sub> receptor mechanisms as they relate to pulmonary function have been less studied than B<sub>2</sub> receptor mechanisms. B<sub>1</sub> receptor activation has been shown to relax mouse tracheal smooth muscle (Trevisani et al., 1999), and the B<sub>1</sub> receptor has been shown to be functionally up-regulated in tissue taken from allergic patients with rhinitis (Christiansen et al., 2002), but not normal subjects. A significant role for the B<sub>1</sub> receptor pathway, however, is not supported by a study where nasal administration of the B<sub>1</sub> receptor agonist, Lys-des-Arg<sup>9</sup>-BK, did not elicit pulmonary responses in asthmatic or allergic patients with rhinitis (Reynolds et al., 1999).

#### D. Cancer

Recently, some interest has emerged in the potential chemotherapeutic effects of BK antagonists. Small-cell lung carcinoma cells and other cancer cells are known to express B<sub>1</sub> receptors, and activation of B<sub>2</sub> receptor stimulates growth of these cells (Bunn et al., 1992). Synthesis of dimeric peptide antagonists of BK such as CU201 (Chan et al., 2002a,b) has provided novel compounds that have shown anticancer effects in different cell lines (see Stewart et al., 1999; Worden and Kalemkerian, 2000; Howl and Payne, 2003) and in vivo (Stewart et al., 2002b). Although the cytotoxic effects of these compounds are clear, their mechanism of action is less clear and appears to not necessarily be related to BK antagonist activity (Stewart et al., 1999, 2002b). More work to elucidate the mechanism of action is needed.

Another strategy to approach a novel chemotherapeutic agent via a BK-based mechanism could be derived

from recent findings that BK promotes angiogenesis and neovascularization in response to conditions of ischemia (Emanueli and Madeddu, 2001). Both B<sub>1</sub> and B<sub>2</sub> receptors have been implicated in the BK-mediated angiogenic response to ischemia (Emanueli and Madeddu, 2001; Silvestre et al., 2001). Therefore, the therapeutic evaluation of novel B<sub>1</sub> or B<sub>2</sub> receptor antagonists to impair vascularization-supported tumor growth may be warranted.

### IX. Epilogue

Much progress has been made in our understanding of the molecular biology, cell biology, physiology, and pharmacology of peptides belonging to the kinin family since their initial discovery in the 1930's. It is now well established that these peptides act through two receptors, B<sub>1</sub> and B<sub>2</sub>. These receptors are linked physiologically through their natural stimuli and their common participation in a variety of inflammatory responses. The functional difference between these two receptors seems to lie primarily in the mechanism by which they are regulated; the B<sub>2</sub> receptor is more or less constitutively expressed and subject to rapid and pronounced desensitization in response to agonist stimulation, whereas the B<sub>1</sub> receptor is induced by the insult and subject to considerably less agonist desensitization. This differential regulation is consistent with the thought that in general the B<sub>2</sub> receptor is involved in the short-term inflammatory and pain responses to insult, whereas the B<sub>1</sub> receptor is involved in the long-term responses. The availability of mice lacking each receptor gene has provided much detailed insight into the specific roles of each receptor in the inflammatory response. The development and use of a number of peptidic and nonpeptidic antagonists in various animal models has implicated both B<sub>1</sub> and B<sub>2</sub> receptors as potential drug targets in several pathophysiological events related to inflammation such as inflammatory pain, sepsis, allergic asthma, rhinitis, inflammatory edema, etc. Currently, the most promising area for drug therapy targeting kinin receptors is in pain, where B<sub>1</sub> receptor antagonism may target both inflammatory as well as certain types of acute pain. The importance of kinins in several of the beneficial effects of ACE inhibitors is also now appreciated. However, this therapeutic avenue also presents the potential "double-edged" nature of kinins, since these peptides may also participate in the angioedema that sometimes appears as a result of ACE inhibitor treatment. Nevertheless, this unwanted side effect is now also being targeted with certain kinin antagonists. The future use of recently generated double knockout mice lacking both receptor genes is bound to offer even further insights into the roles of these receptors, which undoubtedly will open up new therapeutic opportunities.

#### References

- Abe Y, Kayakiri H, Satoh S, Inoue T, Sawada Y, Inamura N, Asano M, Aramori I, Hatori C, Sawai H, et al. (1998a) A novel class of orally active non-peptide



- bradykinin B<sub>2</sub> receptor antagonists. 3. Discovering bioisosteres of the imidazo[1,2-*a*]pyridine moiety. *J Med Chem* **41**:4062–4079.
- Abe Y, Kayakiri H, Satoh S, Inoue T, Sawada Y, Inamura N, Asano M, Hatori C, Sawai H, Oku T, et al. (1998b) A novel class of orally active non-peptide bradykinin B<sub>2</sub> receptor antagonists. 2. Overcoming the species difference between guinea pig and man. *J Med Chem* **41**:4053–4061.
- AbdAlla S, Godovac-Zimmermann J, Braun A, Roscher AA, Müller-Esterl W, and Quitterer U (1996a) Structure of the bradykinin B<sub>2</sub> receptors' amino terminus. *Biochemistry* **35**:7514–7519.
- AbdAlla S, Jarnagin K, Müller-Esterl W, and Quitterer U (1996b) The N-terminal amino group of [Tyr<sup>8</sup>]bradykinin is bound adjacent to analogous amino acids of the human and rat B<sub>2</sub> receptor. *Biochemistry* **35**:7514–7519.
- AbdAlla S, Lother H, el Massiery A, and Quitterer U (2001) Increased AT<sub>1</sub> receptor heterodimers in preeclampsia mediate enhanced angiotensin II responsiveness. *Nat Med* **7**:1003–1009.
- AbdAlla S, Lother H, and Quitterer U (2000) AT<sub>1</sub>-receptor heterodimers show enhanced G-protein activation and altered receptor sequestration. *Nature (Lond)* **407**:94–98.
- AbdAlla S, Quitterer U, Grigoriev S, Maidhof A, Haasemann M, Jarnagin K, and Müller-Esterl W (1996c) Extracellular domains of the bradykinin B<sub>2</sub> receptor involved in ligand binding and agonist sensing defined by anti-peptide antibodies. *J Biol Chem* **271**:1748–1755.
- AbdAlla S, Zaki E, Lother H, and Quitterer U (1999) Involvement of the amino terminus of the B<sub>2</sub> receptor in agonist-induced receptor dimerization. *J Biol Chem* **274**:26079–26084.
- Abdoh M, Khanjari A, Abdelazziz N, Ongali B, Couture R, and Hasséssian HM (2003) Early upregulation of kinin B<sub>1</sub> receptors in retinal microvessels of the streptozotocin-diabetic rat. *Br J Pharmacol* **140**:33–40.
- Adam A, Cugno M, Molinaro G, Perez M, Lepage Y, and Agostoni A (2002) Aminopeptidase P in individuals with a history of angio-oedema on ACE inhibitors. *Lancet* **359**:2088–2089.
- Adomeit A, Graness A, Gross S, Seedorf K, Wetzker R, and Liebmann C (1999) Bradykinin B<sub>2</sub> 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* **19**:5289–5297.
- Agata J, Miao RQ, Yayama K, Chao L, and Chao J (2000) Bradykinin B<sub>1</sub> receptor mediates inhibition of neointima formation in rat artery after balloon angioplasty. *Hypertension* **36**:364–370.
- Ahluwalia A and Perretti M (1999) B<sub>1</sub> receptors as a new inflammatory target. Could this be the 1? *Trends Pharmacol Sci* **20**:100–104.
- Akbary A, Wirth KJ, and Schölkens BA (1996) Efficacy and tolerability of icatibant (Hoe 140) in patients with moderately severe chronic bronchial asthma. *Immunopharmacology* **33**:238–242.
- Akey J (2001) Haplotypes vs single marker linkage disequilibrium tests: what do we gain? *Eur J Hum Genet* **9**:291–300.
- Alfie ME, Alim S, Mehta D, Shesely EG, and Carretero OA (1999) An enhanced effect of arginine vasopressin in bradykinin B<sub>2</sub> receptor null mutant mice. *Hypertension* **33**:1436–1440.
- Alfie ME, Sigmon DH, Pomposiello SI, and Carretero OA (1997) Effect of high salt intake in mutant mice lacking bradykinin-B<sub>2</sub> receptors. *Hypertension* **29**:483–487.
- Aliberti J, Viola JP, Vieira-de-Abreu A, Bozza PT, Sher A, and Scharfstein J (2003) Cutting edge: bradykinin induces IL-12 production by dendritic cells: a danger signal that drives Th1 polarization. *J Immunol* **170**:5349–5353.
- Alric C, Pecher C, Schanstra JP, Bascands JL, and Girolami JP (2000) Bradykinin-induced inhibition of cell proliferation and tyrosine kinase activity in rat mesangial cells. *Int J Mol Med* **5**:85–93.
- Altamura M, Meini S, Quartara L, and Maggi CA (1999) Nonpeptide antagonists for kinin receptors. *Regul Pept* **80**:13–26.
- Anonymous (2001) Bradykinin B<sub>1</sub> receptor antagonists. Pharmacocepeia Inc.: WO0105783. *Expert Opin Ther Patents* **11**:1479–1482.
- Aoki S, Mukae S, Itoh S, Sato R, Nishio K, Iwata T, and Katagiri T (2001) The genetic factor in acute myocardial infarction with hypertension. *Jpn Circ J* **65**:621–626.
- Aquino CJ, Armour DR, Berman JM, Birkemo LS, Carr RAE, Croom DK, Dezube M, Dougherty RW, Ervin GN, Grizzle MK, et al. (1996) Discovery of 1,5-benzodiazepines with peripheral cholecystokinin (CCK-A) receptor agonist activity. 1. Optimization of the agonist "trigger". *J Med Chem* **39**:562–569.
- Aramori I, Zenkoh J, Morikawa N, Asano M, Hatori C, Sawai H, Kayakiri H, Satoh S, Inoue T, Abe Y, et al. (1997a) Nonpeptide mimic of bradykinin with long-acting properties at the bradykinin B<sub>2</sub> receptor. *Mol Pharmacol* **52**:16–20.
- Aramori I, Zenkoh J, Morikawa N, O'Donnell N, Asano M, Nakamura K, Iwami M, Kojo H, and Notsu Y (1997b) Novel subtype-selective nonpeptide bradykinin receptor antagonists FR167344 and FR173657. *Mol Pharmacol* **51**:171–176.
- Asano M, Inamura N, Hatori C, Sawai H, Fujiwara T, Abe Y, Kayakiri H, Satoh S, Oku T, and Nakahara K (1999) Discovery of orally active nonpeptide bradykinin B<sub>2</sub> receptor antagonists. *Immunopharmacology* **43**:163–168.
- Asano M, Inamura N, Hatori C, Sawai H, Fujiwara T, Katayama A, Kayakiri H, Satoh S, Abe Y, Inoue T, et al. (1997) The identification of an orally active, nonpeptide bradykinin B<sub>2</sub> receptor antagonist, FR173657. *Br J Pharmacol* **120**:617–624.
- Audet R, Pétitclerc E, Drapeau G, Rioux F, and Marceau F (1994) Further analysis of the upregulation of bradykinin B<sub>1</sub> receptors in isolated rabbit aorta by using metabolic inhibitors. *Eur J Pharmacol* **271**:551–555.
- Audet R, Rioux F, Drapeau G, and Marceau F (1997) Cardiovascular effects of Sar-[D-Phe<sup>8</sup>]des-Arg<sup>9</sup>-bradykinin, a metabolically protected agonist of the B<sub>1</sub> receptor for kinins, in the anesthetized rabbit pretreated with a sublethal dose of bacterial lipopolysaccharide. *J Pharmacol Exp Ther* **280**:6–15.
- Austin CE, Faussner A, Robinson HE, Chakravarty S, Kyle DJ, Bathon JM, and Proud D (1997) Stable expression of the human kinin B<sub>1</sub> receptor in Chinese hamster ovary cells: characterization of ligand binding and effector pathways. *J Biol Chem* **272**:11420–11425.
- Austin CE and Foreman JC (1994) A study of the action of bradykinin and bradykinin analogues in the human nasal airway. *J Physiol* **478**:351–356.
- Austin CE, Foreman JC, and Scadding GK (1994) Reduction by Hoe 140, the B<sub>2</sub> kinin receptor antagonist, of antigen-induced nasal blockage. *Br J Pharmacol* **111**:969–971.
- Bachvarov D, Hess JF, Menke JG, Larrivé J-F, and Marceau F (1996) Structure and genomic organization of the human B<sub>1</sub> receptor gene for kinins (BDKRB1). *Genomics* **33**:374–381.
- Bachvarov DR, Houle S, Bachvarova M, Bouthillier J, Adam A, and Marceau F (2001) Bradykinin B<sub>2</sub> receptor endocytosis, recycling and down-regulation assessed using green fluorescent protein conjugates. *J Pharmacol Exp Ther* **297**:19–26.
- Bachvarov DR, Landry M, Houle S, Pare P, and Marceau F (1998a) Altered frequency of a promoter polymorphic allele of the kinin B<sub>1</sub> receptor gene in inflammatory bowel disease. *Gastroenterology* **115**:1045–1048.
- Bachvarov DR, Landry M, Pelletier I, Chevrette M, Betard C, Houle I, Bergeron J, Lebel M, and Marceau F (1998b) Characterization of two polymorphic sites in the human kinin B<sub>1</sub> receptor gene: altered frequency of an allele in patients with a history of end-stage renal failure. *J Am Soc Nephrol* **9**:598–604.
- Bachvarov DR, Saint-Jacques E, Larrivé JF, Levesque L, Rioux F, Drapeau G, and Marceau F (1995) Cloning and pharmacological characterization of the rabbit bradykinin B<sub>2</sub> receptor. *J Pharmacol Exp Ther* **275**:1623–1630.
- Baldwin AS (2001) The transcription factor NF- $\kappa$ B and human disease. *J Clin Invest* **107**:3–6.
- Baptista HA, Avellar MC, Araujo RC, Pesquero JL, Schanstra JP, Bascands JL, Esteve JP, Paiva AC, Bader M, and Pesquero JB (2002) Transcriptional regulation of the rat bradykinin B<sub>2</sub> receptor gene: identification of a silencer element. *Mol Pharmacol* **62**:1344–1355.
- Barbacid M (1987) *ras* genes. *Annu Rev Biochem* **56**:779–827.
- Barki-Harrington L, Bookout AL, Wang G, Lamb ME, Leeb-Lundberg LM, and Daaka Y (2003) Requirement for direct cross-talk between B<sub>1</sub> and B<sub>2</sub> kinin receptors for the proliferation of androgen-insensitive prostate cancer PC3 cells. *Biochem J* **371**:581–587.
- Bascands JL, Pecher C, Rouaud S, Emond C, Tack JL, Bastie MJ, Burch R, Regoli D, and Girolami JP (1993) Evidence for existence of two distinct bradykinin receptors on rat mesangial cells. *Am J Physiol* **264**:F548–F556.
- Bastian S, Loillier B, Paquet JL, and Pruneau D (1997) Stable expression of human kinin B<sub>1</sub> receptor in 293 cells: pharmacological and functional characterization. *Br J Pharmacol* **122**:393–399.
- Bastian S, Paquet JL, Robert C, Cremers B, Loillier B, Larrivé JF, Bachvarov DR, Marceau F, and Pruneau D (1998) Interleukin 8 (IL-8) induces the expression of kinin B<sub>1</sub> receptor in human lung fibroblasts. *Biochem Biophys Res Commun* **253**:750–755.
- Bastian S, Pruneau D, Loillier B, Robert C, Bonnafous J-C, and Paquet J-L (2000) Identification of a key region of kinin B<sub>1</sub> receptor for high affinity binding of peptide antagonists. *J Biol Chem* **275**:6107–6113.
- Batenburg WW, Garrelds IM, van Kats JP, Saxena PR, and Danser AH (2004) Mediators of bradykinin-induced vasorelaxation in human coronary microarteries. *Hypertension* **43**:488–492.
- Bathon JM, Manning DC, Goldman DW, Towns MC, and Proud D (1992) Characterization of kinin receptors on human synovial cells and upregulation of receptor number by interleukin-1. *J Pharmacol Exp Ther* **260**:384–392.
- Baxter GF and Ebrahim Z (2002) Role of bradykinin in preconditioning and protection of the ischaemic myocardium. *Br J Pharmacol* **135**:843–854.
- Beauchamp JF, Lemieux M, Drapeau G, and Marceau F (1991) Mechanism of the pressor response to intraperitoneal injection of bradykinin in guinea pigs. *Peptides* **12**:513–521.
- Bedos P, Amblard M, Subra G, Dodey P, Luccarini JM, Paquet JL, Pruneau D, Aumelas A, and Martinez J (2000) A rational approach to the design and synthesis of a new bradykinin B<sub>1</sub> receptor antagonist. *J Med Chem* **43**:2387–2394.
- Bélichard P, Landry M, Faye P, Bachvarov DB, Bouthillier J, Pruneau D, and Marceau F (2000) Inflammatory hyperalgesia induced by zymosan in the plantar tissue of the rat: effect of kinin receptor antagonists. *Immunopharmacology* **46**:139–147.
- Bellucci F, Meini S, Cucchi P, Catalani P, Reichert W, Zappitelli S, Rotondaro L, Quartara L, Giolitti A, and Maggi CA (2003) A different molecular interaction of bradykinin and the synthetic agonist FR190997 with the human B<sub>2</sub> receptor: evidence from mutational analysis. *Br J Pharmacol* **140**:500–506.
- Benzing T, Fleming I, Blaukat A, Müller-Esterl W, and Busse R (1999) Angiotensin-converting enzyme inhibitor ramiprilat interferes with the sequestration of the B<sub>2</sub> kinin receptor within the plasma membrane of native endothelial cells. *Circulation* **99**:2034–2040.
- Bergaya S, Hilgers RH, Meneton P, Dong Y, Bloch-Faure M, Inagami T, Alhenc-Gelas F, Levy BI, and Boulanger CM (2004) Flow-dependent dilation mediated by endogenous kinins requires angiotensin AT<sub>2</sub> receptors. *Circ Res* **94**:1623–1629.
- Bhoola R, Ramsaroop R, Naidoo S, Müller-Esterl W, and Bhoola KD (1997) Kinin receptor status in normal and inflamed gastric mucosa. *Immunopharmacology* **36**:161–165.
- Blais C Jr, Marc-Aurele J, Simmons WH, Loute G, Thibault P, Skidgel RA, and Adam A (1999) Des-Arg<sup>9</sup>-bradykinin metabolism in patients who presented hypersensitivity reactions during hemodialysis: role of serum ACE and aminopeptidase P. *Peptides* **20**:421–430.
- Blais C Jr, Marceau F, Rouleau JL, and Adam A (2000) The kallikrein-kininogen-kinin system: lessons from the quantification of endogenous kinins. *Peptides* **21**:1903–1940.
- Blaukat A (2002) Structure and signalling pathways of kinin receptors. *Andrologia* **35**:17–23.
- Blaukat A, AbdAlla S, Lohse MJ, and Müller-Esterl W (1996) Ligand-induced phosphorylation/dephosphorylation of the endogenous bradykinin B<sub>2</sub> receptor from human fibroblasts. *J Biol Chem* **271**:32366–32374.
- Blaukat A, Barac A, Cross MJ, Offermanns S, and Dikic I (2000) G protein-coupled

- receptor-mediated mitogen-activated protein kinase activation through cooperation of  $G_{\alpha_o}$  and  $G_{\alpha_i}$  signals. *Mol Cell Biol* **20**:6837–6848.
- Blaukat A, Herzer K, Schroeder C, Bachmann M, Nash N, and Müller-Esterl W (1999a) Overexpression and functional characterization of kinin receptors reveal subtype-specific phosphorylation. *Biochemistry* **38**:1300–1309.
- Blaukat A, Micke P, Kalatskaya I, Faussner A, and Müller-Esterl W (2003) Down-regulation of bradykinin B2 receptor in human fibroblasts during prolonged agonist exposure. *Am J Physiol* **284**:H1909–H1916.
- Blaukat A, Pizard A, Breit A, Wernstedt C, Alhenc-Gelas F, Müller-Esterl W, and Dikic I (2001) Determination of bradykinin B2 receptor in vivo phosphorylation sites and their role in receptor function. *J Biol Chem* **276**:40431–40440.
- Blaukat A, Pizard A, Rajerison RM, Alhenc-Gelas F, Müller-Esterl W, and Dikic I (1999b) Activation of mitogen-activated protein kinase by the bradykinin B2 receptor is independent of receptor phosphorylation and phosphorylation-triggered internalization. *FEBS Lett* **451**:337–341.
- Bock MG, Hess JF, and Pettibone DJ (2003) Section III. Inflammatory, pulmonary and gastrointestinal diseases, in *Annual Reports in Medicinal Chemistry* (Doherty A ed), vol 38, pp 111–120, Academic Press, New York.
- Bock MG and Longmore J (2000) Bradykinin antagonists: new opportunities. *Curr Opin Chem Biol* **4**:401–406.
- Bockmann S and Paegelow I (2000) Kinins and kinin receptors: importance for the activation of leukocytes. *J Leukoc Biol* **68**:587–592.
- Boels K and Schaller HC (2003) Identification and characterisation of GPR100 as a novel human G-protein-coupled bradykinin receptor. *Br J Pharmacol* **140**:932–938.
- Bokoch GM and Der CJ (1993) Emerging concepts in the Ras superfamily of GTP-binding proteins. *FASEB J* **7**:750–759.
- Boissonnas RA, Guttman A, and Jaquenoud PA (1960) Synthèse de la L-arginyl-L-propyl-L-propyl-glycyl-L-phenylalanyl-L-seryl-L-propyl-L-phenylalanyl-L-arginine, un nonapeptide présentant le propriétés de la bradykinine. *Helv Chim Acta* **43**:1349–1355.
- Borkowski JA, Ransom RW, Seabrook GR, Trumbauer M, Chen H, Hill RG, Strader CD, and Hess JF (1995) Targeted disruption of a B2 bradykinin receptor gene in mice eliminates bradykinin action in smooth muscle and neurons. *J Biol Chem* **270**:13706–13710.
- Borlongan CV and Emerich DF (2003) Facilitation of drug entry into the CNS via transient permeation of blood brain barrier: laboratory and preliminary clinical evidence from bradykinin receptor agonist, Cereport. *Brain Res Bull* **60**:297–306.
- Bos JL (1989) ras oncogenes in human cancer: a review. *Cancer Res* **49**:4682–4689.
- Bouchard J-F, Chouinard J, and Lamontagne D (1998) Role of kinins in the endothelial protective effect of ischaemic preconditioning. *Br J Pharmacol* **123**:413–420.
- Bouthillier J, Deblois D, and Marceau F (1987) Studies on the induction of pharmacological responses to des-Arg<sup>9</sup>-bradykinin in vitro and in vivo. *Br J Pharmacol* **92**:257–264.
- Boyce S, Wyatt A, Webb JK, O'Donnell R, Mason G, Rigby M, Sirinathsinghi D, Hill RG, and Rupniak NMJ (1999) Selective NMDA NR2B antagonists induce antinociception without motor dysfunction: correlation with restricted localisation of NR2B subunit in dorsal horn. *Neuropharmacology* **38**:611–623.
- Braun A, Kammerer S, Bohme E, Muller B, and Roscher AA (1995) Identification of polymorphic sites of the human bradykinin B2 receptor gene. *Biochem Biophys Res Commun* **211**:234–240.
- Braun A, Maier E, Kammerer S, Muller B, and Roscher AA (1996) A novel sequence polymorphism in the promoter region of the human B2-bradykinin receptor gene. *Hum Genet* **97**:688–689.
- Bregola G, Varani K, Gessi S, Beani L, Binachi C, Borea PA, Regoli D, and Simonato M (1999) Changes in hippocampal and cortical B<sub>1</sub> bradykinin receptor biological activity in two experimental models of epilepsy. *Neuroscience* **92**:1043–1049.
- Brull D, Dhamrait S, Myerson S, Erdmann J, Woods D, World M, Pennell D, Humphries S, Regitz-Zagrosek V, and Montgomery H (2001) Bradykinin B2BKR receptor polymorphism and left-ventricular growth response. *Lancet* **358**:1155–1156.
- Bunn PA Jr, Chan D, Dienhart DG, Tolley R, Tagawa M, and Jewett P (1992) Neuropeptide signal transduction in lung cancer: clinical implications of bradykinin sensitivity and overall heterogeneity. *Cancer Res* **52**:24–31.
- Burch RM and Axelrod J (1987) Dissociation of bradykinin-induced prostaglandin formation from phosphatidylinositol turnover in Swiss 3T3 fibroblasts: evidence for G protein regulation of phospholipase A2. *Proc Natl Acad Sci USA* **84**:6374–6378.
- Burgess GM, Perkins MN, Rang HP, Campbell EA, Brown MC, McIntyre P, Urban L, Dziadulewicz EK, Ritchie TJ, Hallet A, et al. (2000) Bradyzide, a potent non-peptide B<sub>2</sub> receptor antagonist with long-lasting oral activity in animal models of inflammatory hyperalgesia. *Br J Pharmacol* **129**:77–86.
- Burkard M, Zuzack JS, Jones S, Francis M, Whalley ET, Stewart JM, and Gera L (1996) Comparative profile of novel potent bradykinin antagonists at human B<sub>1</sub> and B<sub>2</sub> receptors. *Immunopharmacology* **33**:186–190.
- Busse R and Fleming I (1995) Regulation and functional consequences of endothelial nitric oxide formation. *Ann Med* **27**:331–340.
- Byron KL, Babnigg G, and Villereal ML (1992) Bradykinin-induced Ca<sup>2+</sup> entry, release and refilling of intracellular Ca<sup>2+</sup> stores. Relationships revealed by image analysis of individual human fibroblasts. *J Biol Chem* **267**:108–118.
- Cabrini DA, Campos MM, Tratsk KS, Merino VF, Silva JA Jr, Souza GE, Avellar MC, Pesquero JB, and Calixto JB (2001) Molecular and pharmacological evidence for modulation of kinin B<sub>1</sub> receptor expression by endogenous glucocorticoids hormones in rats. *Br J Pharmacol* **132**:567–577.
- Campbell DJ (2003) Vasopeptidase inhibition: a double-edged sword? *Hypertension* **41**:383–389.
- Campos MM, Cabrini DA, Cardozo AH, Rae GA, Huidobro Toro J, and Calixto JB (2001) Changes in paw oedema triggered via bradykinin B<sub>1</sub> and B<sub>2</sub> receptors in streptozotocin-diabetic rats. *Eur J Pharmacol* **416**:169–177.
- Campos MM, de Souza GEP, Ricci ND, Pesquero JL, Teixeira MM, and Calixto JB (2002) The role of migrating leukocytes in IL-1 $\beta$ -induced up-regulation of kinin B<sub>1</sub> receptors in rats. *Br J Pharmacol* **135**:1107–1114.
- Cassim B, Naidoo S, Naidoo Y, Williams R, and Bhoola KD (1996) Immunolocalisation of the kinin moiety and bradykinin (B<sub>2</sub>) receptors on synovial fluid neutrophils in rheumatoid arthritis. *Immunopharmacology* **33**:321–324.
- Cassim B, Naidoo S, Ramsaroop R, and Bhoola KD (1997) Immunolocalization of bradykinin receptors on human synovial tissue. *Immunopharmacology* **36**:121–125.
- Cayla C, Merino VF, Cabrini DA, Silva JA Jr, Pesquero JB, and Bader M (2002a) Structure of the mammalian kinin receptor gene locus. *Int Immunopharmacol* **2**:1721–1727.
- Cayla C, Todiras M, Iliescu R, Chai G, Pesquero JB, Baltatu O, and Bader M (2002b) Resistance against septic shock in B<sub>1</sub> and B<sub>2</sub> receptor deficient mice (Abstract). *Hypertension* **40**:391–392.
- Cervenka L, Harrison-Bernard LM, Dipp S, Primrose G, Imig JD, and El-Dahr SS (1999) Early onset salt-sensitive hypertension in bradykinin B<sub>2</sub> receptor null mice. *Hypertension* **34**:176–180.
- Cervenka L, Maly J, Karasova L, Simova M, Vitko S, Hellerova S, Heller J, and El-Dahr SS (2001) Angiotensin II-induced hypertension in bradykinin B<sub>2</sub> receptor knockout mice. *Hypertension* **37**:967–973.
- Chahine R, Adam A, Yamaguchi N, Gaspo R, Regoli D, and Nadeau R (1993) Protective effects of bradykinin on the ischaemic heart: implication of the B<sub>1</sub> receptor. *Br J Pharmacol* **108**:318–322.
- Chakravarty S, Connolly M, and Kyle DJ (1995) Novel pseudopeptides with high affinities for the human bradykinin B<sub>2</sub> receptor. *Pept Res* **8**:16–19.
- Chan D, Gera L, Stewart J, Helfrich B, Verella-Garcia M, Johnson G, Baron A, Yang J, Puck T, and Bunn P Jr (2002a) Bradykinin antagonist dimer, CU201, inhibits the growth of human lung cancer cell lines by a “biased agonist” mechanism. *Proc Natl Acad Sci USA* **99**:4608–4613.
- Chan DC, Gera L, Stewart JM, Helfrich B, Zhao TL, Feng WY, Chan KK, Covey JM, and Bunn PA Jr (2002b) Bradykinin antagonist dimer, CU201, inhibits the growth of human lung cancer cell lines in vitro and in vivo and produces synergistic growth inhibition in combination with other antitumor agents. *Clin Cancer Res* **8**:1280–1287.
- Choudry NB, Fuller RW, and Pride NB (1989) Sensitivity of the human cough reflex: Effect of inflammatory mediators prostaglandin E<sub>2</sub>, bradykinin and histamine. *Am Rev Resp Dis* **140**:137–141.
- Christiansen SC, Eddleston J, Woessner KM, Chambers SS, Ye R, Pan ZK, and Zuraw BL (2002) Up-regulation of functional kinin B1 receptors in allergic airway inflammation. *J Immunol* **169**:2054–2060.
- Christopher J, Velarde V, and Jaffa AA (2001a) Induction of B(1)-kinin receptors in vascular smooth muscle cells: cellular mechanisms of map kinase activation. *Hypertension* **38**:602–605.
- Christopher J, Velarde V, Zhang D, Mayfield D, Mayfield RK, and Jaffa AA (2001b) Regulation of B<sub>2</sub>-kinin receptors by glucose in vascular smooth muscle cells. *Am J Physiol* **280**:H1537–H1546.
- Churchill L, Pongracic JA, Reynolds CJ, Naclerio RM, and Proud D (1991) Pharmacology of nasal provocation with bradykinin: studies of tachyphylaxis, cyclooxygenase inhibition, alpha-adrenergic stimulation and receptor subtype. *Int Arch Allergy Appl Immunol* **95**:322–331.
- Clements J and Mukhtar A (1997) Tissue kallikrein and the bradykinin B2 receptor are expressed in endometrial and prostate cancers. *Immunopharmacology* **36**:217–220.
- Cloutier F and Couture R (2000) Pharmacological characterization of the cardiovascular responses elicited by kinin B<sub>1</sub> and B<sub>2</sub> receptor agonists in the spinal cord of streptozotocin-diabetic rats. *Br J Pharmacol* **130**:375–385.
- Cloutier F, de Souza Buck H, Ongali B, and Couture R (2002) Pharmacologic and autoradiographic evidence for an up-regulation of kinin B<sub>2</sub> receptors in the spinal cord of spontaneously hypertensive rats. *Br J Pharmacol* **135**:1641–1654.
- Cockcroft JR, Chowieńczyk PJ, Brett SE, Bender N, and Ritter JM (1994a) Inhibition of bradykinin-induced vasodilation in human forearm vasculature by icatibant, a potent B2-receptor antagonist. *Br J Clin Pharmacol* **38**:317–322.
- Cockcroft JR, Chowieńczyk PJ, Brett SE, and Ritter JM (1994b) Effect of N<sup>G</sup>-monomethyl-L-arginine on kinin-induced vasodilation in the human forearm. *Br J Clin Pharmacol* **38**:307–310.
- Conlon JM (1999) Bradykinin and its receptors in non-mammalian vertebrates. *Regul Pept* **79**:71–81.
- Couture R, Harrisson M, Vianna RM, and Cloutier F (2001) Kinin receptors in pain and inflammation. *Eur J Pharmacol* **429**:161–176.
- Couture R and Lindsey CJ (2000) Brain kallikrein-kinin system: from receptors to neuronal pathways and physiological functions, in *Handbook of Chemical Neuroanatomy* (Quirion R, Björklund A, and Hökfelt T eds) vol 16, p 241, Elsevier Science B. V., Amsterdam.
- Cucchi P, Meini S, Quartara L, Giolitti A, Zappitelli S, Rotondaro L, and Maggi CA (2002) Interaction of linear and cyclic peptide antagonists at the human B<sub>2</sub> kinin receptor. *Peptides* **23**:1457–1463.
- Cuthbert AW (1999) Anion secretory effects of a non-peptide mimic of bradykinin (FR190997) on mouse colon epithelium. *Immunopharmacology* **45**:191–198.
- Davis AJ and Perkins MN (1994a) Induction of B1 receptors in vivo in a model of persistent inflammatory mechanical hyperalgesia in the rat. *Neuropharmacology* **33**:127–133.
- Davis AJ and Perkins MN (1994b) The involvement of bradykinin B<sub>1</sub> and B<sub>2</sub> receptor mechanisms in cytokine-induced mechanical hyperalgesia in the rat. *Br J Pharmacol* **113**:63–68.
- Dear JW, Wirth K, Scadding GK, and Foreman JC (1996) Characterization of the bradykinin receptor in the human nasal airway using the binding of [<sup>125</sup>I]-Hoe 140. *Br J Pharmacol* **119**:1054–1062.
- Deblois D, Bouthillier J, and Marceau F (1988) Effect of glucocorticoids, monokines and growth factors on the spontaneously developing responses of the rabbit aorta to des-Arg<sup>9</sup>-bradykinin. *Br J Pharmacol* **93**:969–977.
- deBlois D and Horlick RA (2001) Endotoxin sensitization to kinin B1 receptor agonist



- in a non-human primate model: haemodynamic and pro-inflammatory effects. *Br J Pharmacol* **132**:327–335.
- de Kimpse SJ, Tieleman W, Van Heuven-Nolsen D, and Nijkamp FP (1994) Reversal of bradykinin-induced relaxation to contraction after interferon- $\gamma$  in bovine isolated mesenteric arteries. *Eur J Pharmacol* **261**:111–120.
- Derian CK and Moskowitz MA (1986) Polyphosphoinositide hydrolysis in endothelial cells and carotid artery segments. Bradykinin-2 receptor stimulation is calcium-independent. *J Biol Chem* **261**:3831–3837.
- de Sousa Buck H, Ongali B, Thibault G, Lindsey CJ, and Couture R (2002) Autoradiographic detection of kinin receptors in the human medulla of control, hypertensive and diabetic donors. *Can J Physiol Pharmacol* **80**:249–257.
- de Weerd WFC and Leeb-Lundberg LMF (1997) Bradykinin sequesters B2 bradykinin receptors and the receptor-coupled  $G\alpha$  subunits  $G\alpha_q$  and  $G\alpha_i$  in caveolae in DDT<sub>1</sub> MF-2 smooth muscle cells. *J Biol Chem* **272**:17858–17866.
- Dhamrait SS, Payne JR, Li P, Jones A, Toor IS, Cooper JA, Hawe E, Palmes JM, Wootton PT, Miller GJ, et al. (2003) Variation in bradykinin receptor genes increases the cardiovascular risk associated with hypertension. *Eur Heart J* **24**:1672–1680.
- Dikic I, Tokiwa G, Lev S, Courtneidge SA, and Schlessinger J (1996) A role for Pyk2 and Src in linking G-protein-coupled receptors with MAP kinase activation. *Nature (Lond)* **383**:547–550.
- Dixon BS and Dennis MJ (1997) Regulation of mitogenesis by kinins in arterial smooth muscle cells. *Am J Physiol* **273**:C7–C20.
- Dixon BS, Evanoff D, Fang WB, and Dennis MJ (2002) Bradykinin B1 receptor blocks PDGF-induced mitogenesis by prolonging ERK activation and increasing p27Kip1. *Am J Physiol* **283**:C193–C203.
- Doctrow SR, Abelleira SM, Curry LA, Heller-Harrison R, Kozarich JW, Malfroy B, McCarroll LA, Morgan KG, Morrow AR, and Musso GF (1994) The bradykinin analog RMP-7 increases intracellular free calcium levels in rat brain microvascular endothelial cells. *J Pharmacol Exp Ther* **271**:229–237.
- Drapeau G, Audet R, Levesque L, Godin D, and Marceau F (1993) Development and *in vivo* evaluation of metabolically resistant antagonists of B1 receptors for kinins. *J Pharmacol Exp Ther* **266**:192–199.
- Drapeau G, Deblois D, and Marceau F (1991) Hypotensive effects of Lys-des-Arg<sup>9</sup>-bradykinin and metabolically protected agonists of B<sub>1</sub> receptors for kinins. *J Pharmacol Exp Ther* **259**:997–1003.
- Drapeau G, Rhaleb NE, Dion S, Jukic D, and Regoli D (1988) [Phe<sup>8</sup>Ψ(CH<sub>2</sub>-NH)Arg<sup>9</sup>]bradykinin, a B<sub>2</sub> receptor selective agonist which is not broken down by either kininase I or kininase II. *Eur J Pharmacol* **155**:193–195.
- Dray A, Bettaney J, Forster P, and Perkins MN (1988) Activation of a bradykinin receptor in peripheral and spinal cord in the neonatal rat *in vitro*. *Br J Pharmacol* **95**:1008–1010.
- Dray A, Patel IA, Perkins MN, and Rueff A (1992) Bradykinin-induced activation of nociceptors: mechanistic studies on the neonatal rat spinal cord-tail preparation. *Br J Pharmacol* **107**:1129–1134.
- Drouin JN, St. Pierre SA, and Regoli D (1979) Receptors for bradykinin and kallidin. *Can J Physiol Pharmacol* **57**:375–379.
- Drummond GR and Cocks TM (1995) Endothelium-dependent relaxation mediated by inducible B<sub>1</sub> and constitutive B<sub>2</sub> kinin receptors in the bovine isolated coronary artery. *Br J Pharmacol* **116**:2473–2481.
- Drysdale CM, McGraw DW, Stack CB, Stephens JC, Judson RS, Nandabalan K, Arnold K, Ruano G, and Liggett SB (2000) Complex promoter and coding region beta 2-adrenergic receptor haplotypes alter receptor expression and predict *in vivo* responsiveness. *Proc Natl Acad Sci USA* **97**:10483–10488.
- Duchene J, Schanstra JP, Pecher C, Pizard A, Susini C, Esteve JP, Bascands JL, and Girolami JP (2002) A novel protein-protein interaction between a G protein-coupled receptor and the phosphatase SHP-2 is involved in bradykinin-induced inhibition of cell proliferation. *J Biol Chem* **277**:40375–40383.
- Duka I, Duka A, Kintsurashvili E, Johns C, Gavras I, and Gavras H (2003) Mechanisms mediating the vasoactive effects of the B1 receptors of bradykinin. *Hypertension* **42**:1021–1025.
- Duka I, Kintsurashvili E, Gavras I, Johns C, Bresnahan M, and Gavras H (2001a) Vasoactive potential of the B<sub>1</sub> bradykinin receptor in normotension and hypertension. *Circ Res* **88**:275–281.
- Duka I, Shenouda S, Johns C, Kintsurashvili E, Gavras I, and Gavras H (2001b) Role of the B<sub>2</sub> receptor of bradykinin in insulin sensitivity. *Hypertension* **38**:1355–1360.
- Dunér T, Conlon JM, Kukkonen JP, Akerman KE, Yan YL, Postlethwait JH, and Larhammar D (2002) Cloning, structural characterization and functional expression of a zebrafish bradykinin B2-related receptor. *Biochem J* **364**:817–824.
- Dziadulewicz EK, Ritchie TJ, Hallett A, Snell CR, Davies JW, Wrigglesworth R, Dunstan AR, Bloomfield GC, Drake GS, McIntyre P, et al. (2002) Nonpeptide bradykinin B<sub>2</sub> receptor antagonists: conversion of rodent-selective bradykinin analogues into potent, orally-active human bradykinin B<sub>2</sub> receptor antagonists. *J Med Chem* **45**:2160–2172.
- El-Dahr SS, Dipp S, and Baricos WH (1998) Bradykinin stimulates the ERK- $\rightarrow$ Elk-1- $\rightarrow$ Fos/AP-1 pathway in mesangial cells. *Am J Physiol* **275**:F343–F352.
- El-Dahr SS, Harrison-Bernard LM, Dipp S, Yosipiv IV, and Meleg-Smith S (2000) Bradykinin B<sub>2</sub> null mice are prone to renal dysplasia: gene-environment interactions in kidney development. *Physiol Genomics* **3**:121–131.
- Elliott DF, Horton EW, and Lewis GP (1960a) Actions of pure bradykinin. *J Physiol (Lond)* **153**:473–480.
- Elliott DF, Lewis GP, and Horton EW (1959) The isolation of bradykinin. A plasma kinin from ox blood. *Biochem J* **74**:15P.
- Elliott DF, Lewis GP, and Horton EW (1960b) The structure of bradykinin—a plasma kinin from ox blood. *Biochem Biophys Res Commun* **3**:87–91.
- Emanueli C, Bonaria Salis M, Stacca T, Pintus G, Kirchmair R, Isner JM, Pinna A, Gaspa L, Regoli D, Cayla C, et al. (2002) Targeting kinin B<sub>1</sub> receptor for therapeutic neovascularization. *Circulation* **105**:360–366.
- Emanueli C, Chao J, Regoli D, Chao L, Ni A, and Madeddu P (1999a) The bradykinin B<sub>1</sub> receptor and the central regulation of blood pressure in spontaneously hypertensive rats. *Br J Pharmacol* **126**:1769–1776.
- Emanueli C and Madeddu P (2001) Targeting kinin receptors for the treatment of tissue ischaemia. *Trends Pharmacol Sci* **22**:478–484.
- Emanueli C, Maestri R, Corradi D, Marchione R, Minasi A, Tozzi MG, Salis MB, Straino S, Capogrossi MC, Olivetti G, et al. (1999b) Dilated and failing cardiomyopathy in bradykinin B(2) receptor knockout mice. *Circulation* **100**:2359–2365.
- Emanueli C, Minasi A, Zacheo A, Chao J, Chao L, Salis MB, Straino S, Tozzi MG, Smith R, Gaspa L, et al. (2001) Local delivery of human tissue kallikrein gene accelerates spontaneous angiogenesis in mouse model of hindlimb ischemia. *Circulation* **103**:125–132.
- Emerich DF, Dean RL, Osborn C, and Bartus RT (2001) The development of the bradykinin agonist labradimil as a means to increase the permeability of the blood-brain barrier: from concept to clinical evaluation. *Clin Pharmacokinet* **40**:105–123.
- Erdmann J, Hegemann N, Weidemann A, Kallisch H, Hummel M, Hetzer R, Fleck E, and Regitz-Zagrosek V (1998) Screening the human bradykinin B2 receptor gene in patients with cardiovascular diseases: identification of a functional mutation in the promoter and a new coding variant (T21M). *Am J Med Genet* **80**:521–525.
- Eric J, Bkaily G, Bkaily GB, Volkov L, Gabra BH, and Sirois P (2003a) Des-Arg<sup>9</sup>-bradykinin increases intracellular Ca<sup>2+</sup> in bronchoalveolar eosinophils from ovalbumin-sensitized and -challenged mice. *Eur J Pharmacol* **475**:129–137.
- Eric J, Gabra BH, and Sirois P (2003b) Implication of the bradykinin receptors in antigen-induced pulmonary inflammation in mice. *Br J Pharmacol* **138**:1589–1597.
- Ewald DA, Pang IH, Sternweis PC, and Miller RJ (1989) Differential G protein-mediated coupling of neurotransmitter receptors to Ca<sup>2+</sup> channels in rat dorsal root ganglion neurons *in vitro*. *Neuron* **2**:1185–1193.
- Fadel R, Ramboer I, Chatterjee N, Rihoux J-P, and Derde M-P (2000) Cetirizine inhibits bradykinin-induced cutaneous wheal and flare in atopic and healthy subjects. *Allergy* **55**:888–891.
- Farmer SG, Powell SJ, Wilkins DE, and Graham A (1998) Cloning, sequencing and functional expression of a guinea pig lung bradykinin B2 receptor. *Eur J Pharmacol* **346**:291–298.
- Fasolato C, Pandiella A, Meldolesi J, and Pozzan T (1988) Generation of inositol phosphates, cytosolic Ca<sup>2+</sup> and ionic fluxes in PC12 cells treated with bradykinin. *J Biol Chem* **263**:17350–17359.
- Fathy DB, Kyle DJ, and Leeb-Lundberg LMF (2000) High affinity binding of peptide agonists to the human B1 bradykinin receptor depends on the interaction between the peptide N-terminal L-lysine and the fourth extracellular domain of the receptor. *Mol Pharmacol* **57**:171–179.
- Fathy DB, Leeb T, Mathis SA, and Leeb-Lundberg LMF (1999) Spontaneous human B2 bradykinin receptor activity determines the action of partial agonists as agonists or inverse agonists: Effect of basal desensitization. *J Biol Chem* **274**:29603–29606.
- Fathy DB, Mathis SA, Leeb T, and Leeb-Lundberg LMF (1998) A single position in the third transmembrane domains of the human B1 and B2 bradykinin receptors is adjacent to and discriminates between the C-terminal residues of subtype-selective ligands. *J Biol Chem* **273**:12210–12218.
- Faussner A, Proud D, Towns M, and Bathon JM (1998) Influence of the cytosolic carboxyl termini of human B1 and B2 kinin receptors on receptor sequestration, ligand internalization and signal transduction. *J Biol Chem* **273**:2617–2623.
- Fein AM, Bernard GR, Criner GJ, Fletcher EC, Good JT Jr, Knaus WA, Levy H, Matuschak GM, Shanie HM, Taylor RW, et al. (1997) Treatment of severe systemic inflammatory response syndrome and sepsis with a novel bradykinin antagonist, deltibant (CP-0127). Results of a randomized, double-blind, placebo-controlled trial. CP-0127 SIRS and Sepsis Study Group. *J Am Med Assoc* **277**:482–487.
- Feletou M, Jamonneau I, Germain M, Thureau C, Fauchere J-L, Villa P, Ghezzi P, and Canet E (1996) Bradykinin B2 receptor involvement in rabbit and murine models of septic shock. *J Cardiovasc Pharmacol* **27**:500–507.
- Ferreira J, Campos MM, Araújo R, Bader M, Pesquero JB, and Calixto JB (2002) The use of kinin B<sub>1</sub> and B<sub>2</sub> receptor knockout mice and selective antagonists to characterize the nociceptive responses caused by kinins at the spinal level. *Neuropharmacology* **43**:1188–1197.
- Ferreira J, Campos MM, Pesquero JB, Araújo RC, Bader M, and Calixto JB (2001) Evidence for the participation of kinins in Freund's adjuvant-induced inflammatory and nociceptive responses in kinin B<sub>1</sub> and B<sub>2</sub> receptor knockout mice. *Neuropharmacology* **41**:1006–1012.
- Figuerola CD, Marchant A, Nova U, Förstermann U, Jarnagin K, Schölkens B, and Müller-Esterl W (2001) Differential distribution of bradykinin B<sub>2</sub> receptors in the rat and human cardiovascular system. *Hypertension* **37**:110–120.
- Fink MP (1998) Therapeutic options directed against platelet activating factor, eicosanoids and bradykinin in sepsis. *J Antimicrob Chemother* **41**:81–94.
- Fleming J, Fisslhalter B, and Busse R (1995) Calcium signaling in endothelial cells involves activation of tyrosine kinases and leads to activation of mitogen-activated protein kinases. *Circ Res* **76**:522–529.
- Fleming J, Fisslhalter B, and Busse R (1996) Interdependence of calcium signaling and protein tyrosine phosphorylation in human endothelial cells. *J Biol Chem* **271**:11009–11015.
- Fortin J-P, Bouthillier J, and Marceau F (2003a) High agonist-independent clearance of rabbit B1 receptors on cultured cells. *Am J Physiol Heart Circ Physiol* **284**:H1647–H1654.
- Fortin JP, Gobeil F Jr, Adam A, Regoli D, and Marceau F (2003b) Do angiotensin-converting enzyme inhibitors directly stimulate the kinin B<sub>1</sub> receptor? *Am J Physiol Heart Circ Physiol* **285**:H277–H282.
- Fox A, Wotherspoon G, McNair K, Hudson L, Patel S, Gentry C, and Winter J (2003) Regulation and function of spinal and peripheral neuronal B<sub>1</sub> bradykinin receptors in inflammatory mechanical hyperalgesia. *Pain* **104**:683–691.
- Fox AJ, Lalloo UG, Belvisi MG, Bernareggi M, Chung KF, and Barnes PJ (1996) Bradykinin-evoked sensitization of airway sensory nerves: a mechanism for ACE-inhibitor cough. *Nat Med* **2**:814–817.
- Fredriksson C, Lagerström MC, Lundin LG, and Schiöth HB (2003) The G-protein-



- coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups and fingerprints. *Mol Pharmacol* **63**:1256–1272.
- Freiberger T, Vyskocilova M, Kolarova L, Kuklinek P, Krystufkova O, Lahodna M, Hanzlikova J, and Litzman J (2002) Exon 1 polymorphism of the B2BKR gene does not influence the clinical status of patients with hereditary angioedema. *Hum Immunol* **63**:492–494.
- Frey EK, Kraut H, Werle E, Vogel R, Zickgraf G, and Trautschold I (1967) *Das Kallikrein-Kinin-System und seine Inhibitoren*. Ferdinand Enke Verlag, Stuttgart, Germany.
- Fujie H, Hayashi I, and Oh-ishi S (1993) The kinin released from high molecular weight-kininogen is responsible for inflammatory exudation in rats: detection of kinin-free-kininogen in the exudate by immunoblot analysis. *Life Sci* **53**:1691–1701.
- Fuller RW, Dixon CMS, Cuss FMC, and Barnes PJ (1987) Bradykinin-induced bronchoconstriction in humans. Mode of action. *Am Rev Respir Dis* **135**:176–180.
- Furchgott RF (1983) Role of endothelium in responses of vascular smooth muscle. *Circ Res* **53**:557–573.
- Gabra BH and Sirois P (2002) Role of bradykinin B<sub>1</sub> receptors in diabetes-induced hyperalgesia in streptozotocin-treated mice. *Eur J Pharmacol* **457**:115–124.
- Gainer JV, Brown NJ, Bachvarova M, Bastien L, Maltais I, Marceau F, and Bachvarov DR (2000) Altered frequency of a promoter polymorphism of the kinin B<sub>2</sub> receptor gene in hypertensive African-Americans. *Am J Hypertens* **13**:1268–1273.
- Gainer JV, Morrow JD, Loveland A, King DJ, and Brown NJ (1998) Effect of bradykinin-receptor blockade on the response to angiotensin-converting-enzyme inhibitor in normotensive and hypertensive subjects. *N Engl J Med* **339**:1285–1292.
- Galizzi JP, Bodinier MC, Chapelain B, Ly SM, Coussy L, Gireaud S, Neliat G, and Jean T (1994) Up-regulation of [<sup>3</sup>H]-des-Arg<sup>10</sup>-kallidin binding to the bradykinin B<sub>1</sub> receptor by interleukin-1 in isolated smooth muscle cells: correlation with B<sub>1</sub> agonist-induced PGI<sub>2</sub> production. *Br J Pharmacol* **113**:389–394.
- Galoppini C, Meini S, Tancredi M, Di Fenza A, Quartara L, Maggi CA, Formaggio F, Toniolo C, Mazzucco S, et al. (1999) A new class of pseudopeptide antagonists of the kinin B<sub>1</sub> receptor containing alkyl spacers. *J Med Chem* **42**:409–414.
- Ganju P, Davis A, Patel S, Nunez X, and Fox A (2001) p38 stress-activated protein kinase inhibitor reverses bradykinin B<sub>1</sub> receptor-mediated component of inflammatory hyperalgesia. *Eur J Pharmacol* **421**:191–199.
- Garg R and Yusuf S (1995) Overview of randomized trials of angiotensin-converting enzyme inhibitors on mortality and morbidity in patients with heart failure. Collaborative Group on ACE Inhibitor Trials. *J Am Med Assoc* **273**:1450–1456.
- Gera L, Stewart JM, Whalley ET, Burkard M, and Zuzack JS (1996) New bradykinin antagonists having high potency at B<sub>1</sub> receptors. *Immunopharmacology* **33**:183–185.
- Gobeil F Jr, Charland S, Filteau C, Perron SI, Neugebauer W, and Regoli D (1999) Kinin B<sub>1</sub> receptor antagonists containing alpha-methyl-L-phenylalanine: in vitro and in vivo antagonistic activities. *Hypertension* **33**:823–829.
- Gohla AS, Offermanns S, Wilkie TM, and Schultz G (1999) Differential involvement of Galph<sub>12</sub> and Galph<sub>13</sub> in receptor-mediated stress fiber formation. *J Biol Chem* **274**:17901–17907.
- Goldstein RH and Wall M (1984) Activation of protein formation and cell division by bradykinin and des-Arg<sup>9</sup>-bradykinin. *J Biol Chem* **259**:9263–9268.
- Golser R, Gorren AC, Leber A, Andrew P, Habisch HJ, Werner ER, Schmidt K, Venema RC, and Mayer B (2000) Interaction of endothelial and neuronal nitric-oxide synthases with the bradykinin B<sub>2</sub> receptor. Binding of an inhibitory peptide to the oxygenase domain blocks uncoupled NADPH oxidation. *J Biol Chem* **275**:5291–5296.
- Goto M, Liu Y, Yang X-M, Ardell JL, Cohen MV, and Downey JM (1995) Role of bradykinin in protection of ischemic preconditioning in rabbit hearts. *Circ Res* **77**:611–621.
- Gougat J, Ferrari B, Sarrao L, Planchenault C, Poncelet M, Maruani J, Alonso R, Cudenneq A, Tiziano C, Guagnini F, et al. (2004) SSR240612, a new non-peptide antagonist of the bradykinin B<sub>1</sub> receptor, biochemical and pharmacological characterization. *J Pharmacol Exp Ther* **309**:661–669.
- Green PG, Jänig W, and Levine JD (1997) Negative feedback neuroendocrine control of inflammatory responses in the rat is dependent on the sympathetic postganglionic neuron. *J Neurosci* **17**:3234–3238.
- Greene EL, Velarde V, and Jaffa AA (2000) Role of reactive oxygen species in bradykinin-induced mitogen-activated protein kinase and c-fos induction in vascular cells. *Hypertension* **35**:942–947.
- Groves P, Kurz S, Just H, and Drexler H (1995) Role of endogenous bradykinin in human coronary vasomotor control. *Circulation* **92**:3424–3430.
- Gutowski S, Smrcka A, Nowak L, Wu DG, Simon M, and Sternweis PC (1991) Antibodies to the alpha q subfamily of guanine nucleotide-binding regulatory protein alpha subunits attenuate activation of phosphatidylinositol 4,5-bisphosphate hydrolysis by hormones. *J Biol Chem* **266**:20519–20524.
- Haasemann M, Cartaud J, Müller-Esterl W, and Dunia I (1998) Agonist-induced redistribution of bradykinin B<sub>2</sub> receptor in caveolae. *J Cell Sci* **111**:917–928.
- Haddad EB, Fox AJ, Roussel J, Burgess G, McIntyre P, Barnes PJ, and Chung KF (2000) Post-transcriptional regulation of bradykinin B<sub>1</sub> and B<sub>2</sub> receptor gene expression in human lung fibroblasts by tumor necrosis factor- $\alpha$ : modulation by dexamethasone. *Mol Pharmacol* **57**:1123–1131.
- Hallberg P, Lind L, Michaelsson K, Karlsson J, Kurland L, Kahan T, Malmqvist K, Ohman KP, Nyström F, and Melhus H (2003) B<sub>2</sub> bradykinin receptor (B2BKR) polymorphism and change in left ventricular mass in response to antihypertensive treatment: results from the Swedish Irbesartan Left Ventricular Hypertrophy Investigation versus Atenolol (SILVHIA) trial. *J Hypertens* **21**:621–624.
- Han ED, MacFarlane RC, Mulligan AN, Scafilei J, and Davis AE (2002) Increased vascular permeability in C1 inhibitor-deficient mice mediated by the bradykinin type 2 receptor. *J Clin Invest* **109**:1057–1063.
- Han JM, Kim JH, Lee BD, Lee SD, Kim Y, Jung YW, Lee S, Cho W, Ohba M, Kuroki T, et al. (2003) Phosphorylation-dependent regulation of phospholipase D2 by protein kinase C delta in rat pheochromocytoma PC12 cells. *J Biol Chem* **277**:8290–8297.
- Harris MB, Ju H, Venema VJ, Blackstone M, and Venema RC (2000) Role of heat shock protein 90 in bradykinin-stimulated endothelial nitric oxide release. *Gen Pharmacol* **35**:165–170.
- Hayashi I, Ishihara K, Kumagai Y, and Majima M (2001) Proinflammatory characteristics of a nonpeptide bradykinin mimic, FR1909977, in vivo. *Brit J Pharmacol* **133**:1296–1306.
- Hayashi R, Yamashita N, Matsui S, Fujita T, Araya J, Sassa K, Arai N, Yoshida Y, Kashii T, Maruyama M, et al. (2000) Bradykinin stimulates IL-6 and IL-8 production by human lung fibroblasts through ERK- and p38 MAPK-dependent mechanisms. *Eur Respir J* **16**:452–458.
- Hecker M, Porsti I, Bara AT, and Busse R (1994) Potentiation by ACE inhibitors of the dilator response to bradykinin in the coronary microcirculation: interaction at the receptor level. *Br J Pharmacol* **111**:238–244.
- Hecquet C, Tan F, Marcic BM, and Erdős EG (2000) Human bradykinin B<sub>2</sub> receptor is activated by kallikrein and other serine proteases. *Mol Pharmacol* **58**:828–836.
- Heitsch H (2002) Non-peptide antagonists and agonists of the bradykinin B<sub>2</sub> receptor. *Curr Med Chem* **9**:913–928.
- Heitsch H (2003) The therapeutic potential of bradykinin B<sub>2</sub> receptor agonists in the treatment of cardiovascular disease. *Expert Opin Investig Drugs* **12**:759–770.
- Hembree TN and Leeb-Lundberg LMF (1996) Regulation of bradykinin B<sub>2</sub> receptors by the ras oncogene: Evidence for multiple mechanisms. *J Cell Physiol* **169**:248–255.
- Hermans WR, Foley DP, Rensing BJ, and Serruys PW (1994) Morphologic changes during follow-up after successful percutaneous transluminal coronary balloon angioplasty: quantitative angiographic analysis in 778 lesions—further evidence for the restenosis paradox. MERCATOR Study Group (Multicenter European Research trial with Cilazapril after Angioplasty to prevent Transluminal Coronary Obstruction and Restenosis). *Am Heart J* **127**:483–494.
- Herzig MC and Leeb-Lundberg LM (1995) The agonist binding site on the bovine bradykinin B<sub>2</sub> receptor is adjacent to a sulfhydryl and is differentiated from the antagonist binding site by chemical crosslinking. *J Biol Chem* **270**:20591–20598.
- Herzig MCS, Nash N, Connolly M, Kyle DJ, and Leeb-Lundberg LMF (1996) The N terminus of bradykinin when bound to the human bradykinin B<sub>2</sub> receptors is adjacent to extracellular Cys<sup>20</sup> and Cys<sup>277</sup> in the receptor. *J Biol Chem* **271**:29746–29751.
- Hess JF, Borkowski JA, Macneil T, Stonesifer GY, Fraher J, Strader CD, and Ransom RW (1994) Differential pharmacology of cloned human and mouse B<sub>2</sub> bradykinin receptors. *Mol Pharmacol* **45**:1–8.
- Hess JF, Borkowski JA, Young GS, Strader CD, and Ransom RW (1992) Cloning and pharmacological characterization of a human bradykinin (BK-2) receptor. *Biochem Biophys Res Commun* **184**:260–268.
- Hess JF, Derrick AW, MacNeil T, and Borkowski JA (1996) The agonist selectivity of a mouse B<sub>1</sub> bradykinin receptor differs from human and rabbit B<sub>1</sub> receptors. *Immunopharmacology* **33**:1–8.
- Hess JF, Hey PJ, Chen T-B, O'Brien J, O'Malley SS, Pettibone DJ, and Chang RSL (2001) Molecular cloning and pharmacological characterization of the canine B<sub>1</sub> and B<sub>2</sub> bradykinin receptors. *Biol Chem* **382**:123–129.
- Hess JF, Hey PJ, Chen T-B, Pettibone DJ, and Chang RSL (2002) Molecular and pharmacological diversity of kinin B<sub>1</sub> receptor. *Intl Immunopharmacol* **2**:1747–1754.
- Higashida H, Streaty RA, Klee W, and Nirenberg M (1986) Bradykinin-activated transmembrane signals are coupled via No or Ni to production of inositol 1,4,5-trisphosphate, a second messenger in NG108–15 neuroblastoma-glioma hybrid cells. *Proc Natl Acad Sci USA* **83**:942–946.
- Hock FJ, Wirth K, Albus U, Linz W, Gerhards HJ, Wiemer G, Henke S, Breipohl G, König W, Knolle J, et al. (1991) Hoe 140 a new potent and long acting bradykinin-antagonist: in vitro studies. *Br J Pharmacol* **102**:769–773.
- Hogan MB, Harris KE, Protter AA, and Patterson R (1997) A bradykinin antagonist inhibits both bradykinin- and the allergen-induced airway response in primates. *Proc Assoc Am Physicians* **109**:269–274.
- Horlick RA, Ohlmeyer MH, Stroke IL, Strohl B, Pan G, Schilling AE, Paradkar V, Quintero JG, You M, Riviello C, et al. (1999) Small molecule antagonists of the bradykinin B<sub>1</sub> receptor. *Immunopharmacology* **43**:169–177.
- Hornig B, Kohler C, Schlink D, Tatge H, and Drexler H (2003) AT<sub>1</sub>-receptor antagonism improves endothelial function in coronary artery disease by a bradykinin/B<sub>2</sub>-receptor-dependent mechanism. *Hypertension* **41**:1092–1095.
- Houle S, Landry M, Audet R, Bouthillier J, Bachvarov DR, and Marceau F (2000a) Effect of allelic polymorphism of the B(1) and B(2) receptor genes on the contractile responses of the human umbilical vein to kinins. *J Pharmacol Exp Ther* **294**:45–51.
- Houle S, Larrivee J-F, Bachvarova M, Bouthillier J, Bachvarov D, and Marceau F (2000b) Antagonist-induced intracellular sequestration of rabbit bradykinin B<sub>2</sub> receptor. *Hypertension* **35**:1319–1325.
- Houle S and Marceau F (2003) Wortmannin alters the intracellular trafficking of the bradykinin B<sub>2</sub> receptor: role of phosphoinositide 3-kinase and Rab5. *Biochem J* **375**:151–158.
- Houle S, Molinaro G, Adam A, and Marceau F (2003) Tissue kallikrein actions at the rabbit natural or recombinant kinin B<sub>2</sub> receptors. *Hypertension* **41**:611–617.
- Houlston RS and Peto J (2004) The search for low-penetrance cancer susceptibility alleles. *Oncogene* **23**:6471–6476.
- Howl J and Payne SJ (2003) Bradykinin receptors as a therapeutic target. *Expert Opin Ther Targets* **7**:277–285.
- Hu HZ, Gao N, Liu S, Ren J, Wang X, Xia Y, and Wood JD (2004) Action of bradykinin in the submucosal plexus of guinea-pig small intestine. *J Pharmacol Exp Ther* **309**:320–327.
- Huang HM, Lin TA, Sun GY, and Gibson GE (1995) Increased inositol 1,4,5-trisphosphate accumulation correlates with an up-regulation of bradykinin receptors in Alzheimer's disease. *J Neurochem* **64**:761–766.
- Huang TJ, Haddad E, Fox AJ, Salmon M, Jones C, Burgess G, and Chung KF (1999)

- Contribution of bradykinin B<sub>1</sub> and B<sub>2</sub> receptors in allergen-induced bronchial hyperresponsiveness. *Am J Respir Crit Care Med* **160**:1717–1723.
- Ignarro LJ, Byrns RE, Buga GM, and Wood KS (1987) Mechanisms of endothelium dependent vascular smooth muscle relaxation elicited by bradykinin and VIP. *Am J Physiol* **253**:H1074–H1082.
- Ignjatovic T, Tan F, Brokovich V, Skidgel RA, and Erdös EG (2002) Novel mode of action of angiotensin I converting enzyme inhibitors: direct activation of bradykinin B<sub>1</sub> receptor. *J Biol Chem* **277**:16847–16852.
- Imig JD, Zhao X, Orengo SR, Dipp S, and El-Dahr SS (2003) The bradykinin B<sub>2</sub> receptor is required for full expression of renal COX-2 and renin. *Peptides* **24**:1141–1147.
- Jaggar SI, Habib S, and Rice ASC (1998) The modulatory effects of bradykinin B<sub>1</sub> and B<sub>2</sub> receptor antagonists upon viscerovisceral hyper-reflexia in a rat model of visceral hyperalgesia. *Pain* **75**:169–176.
- Jarnagin K, Bhakta S, Zuppan P, Yee C, Ho T, Phan T, Tahiramani R, Pease JHB, Miller A, and Freedman R (1996) Mutations in the B<sub>2</sub> Bradykinin Receptor Reveal a Different Pattern of Contacts for Peptidic Agonists and Peptidic Antagonists. *J Biol Chem* **271**:28277–28286.
- Jones C, Phillips E, Davis C, Arbuckle J, Yaqoob M, Burgess GM, Docherty RJ, Webb M, Bevan SJ, and McIntyre P (1999) Molecular characterization of cloned bradykinin B<sub>1</sub> receptors from rat and human. *Eur J Pharmacol* **374**:423–433.
- Jones S, Brown DA, Milligan G, Willer E, Buckley NJ, and Caulfield MP (1995) Bradykinin excites rat sympathetic neurons by inhibition of M current through a mechanism involving B<sub>2</sub> receptors and G alpha q/11. *Neuron* **14**:399–405.
- Jong YJ, Dalemar LR, Wilhelm B, and Baenziger NL (1993) Human bradykinin B<sub>2</sub> receptors isolated by receptor-specific monoclonal antibodies are tyrosine phosphorylated. *Proc Natl Acad Sci USA* **90**:10994–10998.
- Jong YJ, Ford SR, Seehra K, Malave VB, and Baenziger NL (2003) Alzheimer's disease skin fibroblasts selectively express a bradykinin signaling pathway mediating tau protein Ser phosphorylation. *FASEB J* **17**:2319–2321.
- Ju H, Venema VJ, Liang H, Harris MB, Zou R, and Venema RC (2000) Bradykinin activates the Janus-activated kinase/signal transducers and activators of transcription (JAK/STAT) pathway in vascular endothelial cells: localization of JAK/STAT signalling proteins in plasmalemmal caveolae. *Biochem J* **351**:257–264.
- Ju H, Venema VJ, Marrero MB, and Venema RC (1998) Inhibitory interactions of the bradykinin B<sub>2</sub> receptor with endothelial nitric-oxide synthase. *J Biol Chem* **273**:24025–24029.
- Kachur JF, Allbee W, and Gaginella TS (1986) Effect of bradykinin and des-Arg<sup>9</sup>-bradykinin on ion transport across normal and inflamed rat colonic mucosa (Abstract). *Gastroenterology* **90**:1481.
- Kammerer S, Braun A, Arnold N, and Roscher AA (1995) The human bradykinin B<sub>2</sub> receptor gene: full length cDNA, genomic organization and identification of the regulatory region. *Biochem Biophys Res Commun* **211**:226–233.
- Kang DS, Gustafsson C, Mörgelein M, and Leeb-Lundberg LMF (2005) B<sub>1</sub> bradykinin receptor homo-oligomers in receptor cell surface expression and signaling: effects of receptor fragments. *Mol Pharmacol* **67**:309–318.
- Kang DS and Leeb-Lundberg LMF (2002) Negative and positive regulatory epitopes in the C-terminal domains of the human B<sub>1</sub> and B<sub>2</sub> bradykinin receptor subtypes determine receptor coupling efficacy to G<sub>q</sub>-mediated phospholipase C $\beta$  activity. *Mol Pharmacol* **62**:281–288.
- Kang DS, Ryberg K, Mörgelein M, and Leeb-Lundberg LMF (2004) Spontaneous formation of a proteolytic B<sub>1</sub> and B<sub>2</sub> bradykinin receptor complex with enhanced signaling capacity. *J Biol Chem* **279**:22102–22107.
- Kaplanski J, Pruneau D, Asa I, Artru AA, Azez A, Ivashkova Y, Rudich Z, and Shapira Y (2002) LF 16-0687 Ms, a bradykinin B<sub>2</sub> receptor antagonist, reduces brain edema and improves long-term neurological function recovery after closed head trauma in rats. *J Neurotrauma* **19**:953–964.
- Katada J and Majima M (2002) AT<sub>2</sub> receptor-dependent vasodilation is mediated by activation of vascular kinin generation under flow conditions. *Br J Pharmacol* **136**:484–491.
- Kindgen-Milles D and Klement W (1992) Pain and inflammation evoked in human skin by bradykinin receptor antagonists. *Eur J Pharmacol* **218**:183–185.
- Kintsurashvili E, Duka I, Gavras I, Johns C, Farmakiotis D, and Gavras H (2001) Effects of ANG II on bradykinin receptor gene expression in cardiomyocytes and vascular smooth muscle cells. *Am J Physiol* **281**:H1778–H1783.
- Knigge H, Bluthner M, Bruntgens A, Sator H, and Ritz E (2000) G(-699)/C polymorphism in the bradykinin-1 receptor gene in patients with renal failure. *Nephrol Dial Transplant* **15**:586–588.
- Kohlstedt K, Brandes RP, Müller-Esterl W, Busse R, and Fleming I (2004) Angiotensin-converting enzyme is involved in outside-in signaling in endothelial cells. *Circ Res* **94**:60–67.
- Kozma R, Ahmed S, Best A, and Lim L (1995) The Ras-related protein Cdc42Hs and bradykinin promote the formation of peripheral actin microspikes and filopodia in Swiss 3T3 fibroblasts. *Mol Cell Biol* **15**:1942–1952.
- Kraut H, Frey EK, and Werle E (1930) Der nachweis eines krieslaufhormon in der pankreasdrüse. *Hoppe-Seyler's Z Physiol Chem* **189**:97–106.
- Kuga T, Egashira K, Mohri M, Tsutsui H, Harasawa Y, Urabe Y, Ando S, Shimokawa H, and Takeshita A (1995) Bradykinin-induced vasodilation is impaired at the atherosclerotic site but is preserved at the spastic site of human coronary arteries in vivo. *Circulation* **92**:183–189.
- Kuoppala A, Shiota N, Kokkonen JO, Liesmaa I, Kostner K, Mayranpaa M, Kovanen PT, and Lindstedt KA (2002) Down-regulation of cardioprotective bradykinin type-2 receptors in the left ventricle of patients with end-stage heart failure. *J Am Coll Cardiol* **40**:119–125.
- Kusser B, Braun A, Praun M, Illi S, von Mutius E, and Roscher AA (2001) Polymorphisms in the bradykinin B<sub>2</sub> receptor gene and childhood asthma. *Biol Chem* **382**:885–889.
- Kyle DJ, Chakravarty S, Sinsko JA, and Stormann TM (1994) A proposed model of bradykinin bound to the rat B<sub>2</sub> receptor and its utility for drug design. *J Med Chem* **37**:1347–1354.
- Lagneux C, Bader M, Pesquero JB, Demenge P, and Ribout C (2002) Detrimental implication of B<sub>1</sub> receptors in myocardial ischemia: evidence from pharmacological blockade and gene knockout mice. *Int Immunopharmacol* **2**:815–822.
- Lagneux C, Lebrin F, Demenge P, Godin-Ribuot D, and Ribout C (2001) MAP-kinase dependent activation of kinin B<sub>1</sub> receptor gene transcription after heat stress in rat vascular smooth muscle cells. *Int Immunopharmacol* **15**:533–538.
- Lagneux C and Ribout C (1997) In vivo evidence for B<sub>1</sub>-receptor synthesis by heat stress in the rat. *Br J Pharmacol* **121**:1045–1046.
- Lal MA, Proulx PR, and Hebert RL (1998) A role for PKC epsilon and MAP kinase in bradykinin-induced arachidonic acid release in rabbit CCD cells. *Am J Physiol* **274**:F728–F735.
- LaMorte VJ, Harootyan AT, Spiegel AM, Tsien RY, and Feramisco JR (1993) Mediation of growth factor induced DNA synthesis and calcium mobilization by Gq and Gi2. *J Cell Biol* **121**:91–99.
- Larriève JF, Bachvarov DR, Houle F, Landry J, Huot J, and Marceau F (1998) Role of the mitogen-activated protein kinases in the expression of the kinin B<sub>1</sub> receptors induced by tissue injury. *J Immunol* **160**:1419–1426.
- Larriève JF, Gera L, Houle S, Bouthillier J, Bachvarov DR, Stewart JM, and Marceau F (2000) Non-competitive pharmacological antagonism at the rabbit B<sub>1</sub> receptor. *Br J Pharmacol* **131**:885–892.
- Lamb ME, de Weerd WFC, and Leeb-Lundberg LMF (2001) Agonist-promoted trafficking of human bradykinin receptors: Arrestin- and dynamin-independent sequestration of the B<sub>2</sub> receptor in HEK293 cells. *Biochem J* **355**:741–750.
- Lamb ME, Zhang C, Shea T, Kyle DJ, and Leeb-Lundberg LMF (2002) Human B<sub>1</sub> and B<sub>2</sub> bradykinin receptors and their agonists target caveolae-related lipid rafts to different degrees in HEK293 cells. *Biochemistry* **41**:14340–14347.
- Lee DK, Lanca AJ, Cheng R, Nguyen T, Ji XD, Gobeil F Jr, Chemtob S, George SR, and O'Dowd BF (2004) Agonist-independent nuclear localization of the Apelin, angiotensin AT<sub>1</sub> and bradykinin B<sub>2</sub> receptors. *J Biol Chem* **279**:7901–7908.
- Lee J, Reynolds C, Jetter MC, Youngman MA, Hlasta DJ, Dax SL, Stone DJ, Zhang S-P, and Codd EE (2003) Design and synthesis of novel pyrrolidine-containing bradykinin antagonists. *Bioorg Med Chem Lett* **13**:1879–1882.
- Lee YJ and Tsai J C (2001) Angiotensin-converting enzyme gene insertion/deletion, not bradykinin B<sub>2</sub> receptor -58T/C gene polymorphism, associated with angiotensin-converting enzyme inhibitor-related cough in Chinese female patients with non-insulin-dependent diabetes mellitus. *Metabolism* **50**:1346–1350.
- Lee YJ, Zachrisson O, Tonge DA, and McNaughton PA (2002) Upregulation of bradykinin B<sub>2</sub> receptor expression by neurotrophic factors and nerve injury in mouse sensory neurons. *Mol Cell Neurosci* **19**:186–200.
- Leeb T, Mathis SA, and Leeb-Lundberg LMF (1997) The sixth transmembrane domains of the human B<sub>1</sub> and B<sub>2</sub> bradykinin receptors are structurally compatible and involved in discriminating between subtype-selective agonists. *J Biol Chem* **272**:311–317.
- Leeb-Lundberg LM, Song XH, and Mathis SA (1994a) Focal adhesion-associated proteins p125<sup>FAK</sup> and paxillin are substrates for bradykinin-stimulated tyrosine phosphorylation in Swiss 3T3 cells. *J Biol Chem* **269**:24328–24334.
- Leeb-Lundberg LMF, Kang DS, Lamb ME, Mathis S, and Fathy DB (2001) The human B<sub>1</sub> bradykinin receptor exhibits high ligand-independent, constitutive activity: roles of the third transmembrane and C-terminal domains. *J Biol Chem* **276**:8785–8792.
- Leeb-Lundberg LMF and Mathis SA (1990) Guanine nucleotide regulation of B<sub>2</sub> kinin receptors: time-dependent formation of a guanine nucleotide-sensitive receptor state from which [<sup>3</sup>H]bradykinin dissociates slowly. *J Biol Chem* **265**:9621–9627.
- Leeb-Lundberg LMF, Mathis SA, and Herzig MCS (1994b) Antagonists of bradykinin which stabilize a G-protein-uncoupled state of the B<sub>2</sub> receptor act as inverse agonists in rat myometrial cells. *J Biol Chem* **269**:25970–25973.
- Leeb-Lundberg LMF and Song X-H (1991) Bradykinin and bombesin rapidly stimulate tyrosine phosphorylation of a 120-kDa group of proteins in Swiss 3T3 cells. *J Biol Chem* **266**:7746–7749.
- Levesque L, Larriève JF, Bachvarov DR, Rioux F, Drapeau G, and Marceau F (1995) Regulation of kinin-induced contraction and DNA synthesis by inflammatory cytokines in the smooth muscle of the rabbit aorta. *Br J Pharmacol* **116**:1673–1679.
- Levy D and Zochodne DW (2000) Increased mRNA expression of the B<sub>1</sub> and B<sub>2</sub> bradykinin receptors and antinociceptive effects of their antagonists in an animal model of neuropathic pain. *Pain* **86**:265–271.
- Liao JK and Homcy CJ (1993) 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* **92**:2168–2172.
- Liebmann C, Graness A, Ludwig B, Adomeit A, Boehmer A, Boehmer FD, Nurnberg B, and Wetzker R (1996) Dual bradykinin B<sub>2</sub> receptor signalling in A431 human epidermoid carcinoma cells: activation of protein kinase C is counteracted by a G<sub>s</sub>-mediated stimulation of the cyclic AMP pathway. *Biochem J* **313**:109–118.
- Linder ME, Ewald DA, Miller RJ, and Gilman AG (1990) Purification and characterization of Go alpha and three types of Gi alpha after expression in Escherichia coli. *J Biol Chem* **265**:8243–8251.
- Linz W, Wiemer G, Gohlke P, Unger T, and Schölkens BA (1995) Contribution of kinins to the cardiovascular actions of angiotensin-converting enzyme inhibitors. *Pharmacol Rev* **47**:25–49.
- Linz W, Wiemer G, and Schölkens BA (1997) Beneficial effects of bradykinin on myocardial energy metabolism and infarct size. *Am J Cardiol* **80**:118A–123A.
- Liu C, Chen J, Sutton S, Roland B, Kuei C, Farmer N, Sillard R, and Lovenberg TW (2003) Identification of relaxin-3/INSL7 as a ligand for GPCR142. *J Biol Chem* **278**:50765–50770.
- Lopes P and Couture R (1997) Localization of bradykinin-like immunoreactivity in the rat spinal cord: effects of capsaicin, melittin, dorsal rhizotomy and peripheral axotomy. *Neuroscience* **78**:481–497.
- Lung CC, Chan EKL, and Zuraw BL (1997) Analysis of an exon 1 polymorphism of the B<sub>2</sub> bradykinin receptor gene and its transcript in normal subjects and C1 inhibitor deficient patients. *J Allergy Clin Immunol* **99**:134–146.
- Lung CC, Jagels MA, Daffern PJ, Tan EM, and Zuraw BL (1998) Induction of human



- B<sub>2</sub> bradykinin receptor mRNA and membrane receptors by IFN $\gamma$ . *Immunopharmacology* **39**:243–253.
- Ma QP and Heavens R (2001) Basal expression of bradykinin B<sub>1</sub> receptors in the spinal cord in humans and rats. *Neuroreport* **12**:2311–2314.
- Ma Q-P, Hill R, and Sirinathsinghji D (2000) Basal expression of bradykinin B<sub>1</sub> receptor in peripheral sensory ganglia in the rat. *Neuroreport* **11**:4003–4005.
- Madeddu P, Emanuelli C, Song Q, Varoni MV, Demontis MP, Anania V, Glorioso N, Chao J, and Gorioso N (1997) Regulation of bradykinin B<sub>2</sub>-receptor expression by oestrogen. *Br J Pharmacol* **121**:1763–1769.
- MacNeil T, Bierilo KK, Menke JG, and Hess JF (1995) Cloning and pharmacological characterization of a rabbit bradykinin B<sub>1</sub> receptor. *Biochim Biophys Acta* **1264**:223–228.
- MacNeil T, Feighner S, Hreniuk DL, Hess JF, and Van der Ploeg LH (1997) Partial agonists and full antagonists at the human and murine bradykinin B<sub>1</sub> receptors. *Can J Physiol Pharmacol* **75**:735–740.
- Maestri R, Milia AF, Salis MB, Graiani G, Lagrasta C, Monica M, Corradi D, Emanuelli C, and Madeddu P (2003) Cardiac hypertrophy and microvascular deficit in kinin B<sub>2</sub> receptor knockout mice. *Hypertension* **41**:1151–1155.
- Mage M, Pécher C, Neau E, Cellier E, Dos Reiss ML, Schanstra JP, Couture C, Bascands JL, and Girolami JP (2002) Induction of B<sub>1</sub> receptors in streptozotocin diabetic rats: possible involvement in the control of hyperglycemia-induced glomerular Erk 1 and 2 phosphorylation. *Can J Physiol Pharmacol* **80**:328–333.
- Maltais I, Bachvarova M, Maheux P, Perron P, Marceau F, and Bachvarov D (2002) Bradykinin B<sub>2</sub> receptor gene polymorphism is associated with altered urinary albumin/creatinine values in diabetic patients. *Can J Physiol Pharmacol* **80**:323–327.
- Marceau F, Fortin J-P, Morissette G, and Dzidulewicz EK (2003) A non-peptide antagonist unusually selective for the human form of the bradykinin B<sub>2</sub> receptor. *Int Immunopharmacol* **3**:1529–1536.
- Marceau F, Hess JF, and Bachvarov DR (1998) The B<sub>1</sub> receptors for kinins. *Pharmacol Rev* **50**:357–386.
- Marceau F, Larrivée JF, Bouthillier J, Bachvarova M, Houle S, and Bachvarov DR (1999) Effect of endogenous kinins, prostanoids and NO on kinin B<sub>1</sub> and B<sub>2</sub> receptor expression in the rabbit. *Am J Physiol* **277**:R1568–R1578.
- Marceau F and Regoli D (2004) Bradykinin receptor ligands: therapeutic perspectives. *Nat Rev Drug Discov* **3**:845–852.
- Marceau F, Sabourin T, Houle S, Fortin JP, Petitclerc E, Molinaro G, and Adam A (2002) Kinin receptors: functional aspects. *Int Immunopharmacol* **2**:1729–1739.
- Marceau F and Tremblay B (1986) Mitogenic effect of bradykinin and of des-Arg<sup>9</sup>-bradykinin on cultured fibroblasts. *Life Sci* **39**:2351–2358.
- Marcic B, Deddish PA, Jackman HL, and Erdős EG (1999) Enhancement of bradykinin and resensitization of its B<sub>2</sub> receptor. *Hypertension* **33**:835–843.
- Marcic B, Deddish PA, Skidgel RA, Erdős EG, Minshall RD, and Tan F (2000) Replacement of the transmembrane anchor in angiotensin I-converting enzyme (ACE) with a glycosylphosphatidylinositol tail affects activation of the B<sub>2</sub> receptor bradykinin receptor by ACE inhibitors. *J Biol Chem* **275**:16110–16118.
- Marie J, Koch C, Pruneau D, Paquet J-L, Groblewski T, Languier R, Lombart C, Deslauriers B, Maignet B, and Bonnafant J-L (1999) Constitutive activation of the human bradykinin B<sub>2</sub> receptor induced by mutations in transmembrane helices III and VI. *Mol Pharmacol* **55**:92–101.
- Marie J, Richard E, Pruneau D, Paquet J-L, Siatka C, Languier R, Ponce C, Vassault P, Groblewski T, Maignet B, et al. (2001) Control of conformational equilibria in the human B<sub>2</sub> bradykinin receptor. *J Biol Chem* **276**:41100–41111.
- Marin-Castano ME, Schanstra JP, Neau E, Pradauda F, Pécher C, Ader JL, Girolami JP, and Bascands JL (2002) Induction of functional bradykinin B<sub>1</sub>-receptors in normotensive rats and mice under chronic angiotensin-converting enzyme inhibitor treatment. *Circulation* **105**:627–632.
- Marks J, Saifudeen Z, Dipp S, and El-Dahr SS (2003) Two functionally divergent p53-responsive elements in the rat bradykinin B<sub>2</sub> receptor promoter. *J Biol Chem* **278**:34158–34166.
- Marmarou A, Nichols J, Burgess J, Newell D, Troha J, Burnham D, and Pitts L (1999) Effects of the bradykinin antagonist Bradycor (deltibant, CP-1027) in severe traumatic brain injury: results of a multi-center, randomized, placebo-controlled trial. American Brain Injury Consortium Study Group. *J Neurotrauma* **16**:431–444.
- Marrero MB, Venema VJ, Ju H, He H, Liang H, Caldwell RB, and Venema RC (1999) Endothelial nitric oxide synthase interactions with G-protein-coupled receptors. *Biochem J* **343**:335–340.
- Marsh KA and Hill SJ (1994) Des-Arg<sup>9</sup>-bradykinin-induced increases in intracellular calcium ion concentration in single bovine tracheal smooth muscle cells. *Br J Pharmacol* **112**:934–938.
- Mason GS, Cumberbatch MJ, Hill RG, and Rupniak NMJ (2002) The bradykinin B<sub>1</sub> receptor antagonist B9858 inhibits a nociceptive spinal reflex in rabbits. *Can J Physiol Pharmacol* **80**:264–268.
- Mathis SA, Criscimagna NL, and Leeb-Lundberg LMF (1996) B<sub>1</sub> and B<sub>2</sub> kinin receptors mediate distinct patterns of intracellular Ca<sup>2+</sup> signaling in single cultured vascular smooth muscle cells. *Mol Pharmacol* **50**:128–139.
- Mathis SA and Leeb-Lundberg LMF (1991) Bradykinin recognizes different molecular forms of the B<sub>2</sub> kinin receptor in the presence and absence of guanine nucleotides. *Biochem J* **276**:141–147.
- Matsumura Y, Kimura M, Yamamoto T, and Maeda H (1988) Involvement of the kinin-generating cascade in enhanced vascular permeability in tumor tissue. *Jpn J Cancer Res* **79**:1327–1334.
- Matsumura Y, Maruo K, Kimura M, Yamamoto T, Konno T, and Maeda H (1991) Kinin-generating cascade in advanced cancer patients and in vitro study. *Jpn J Cancer Res* **82**:732–741.
- Mazenot C, Loufrani L, Henrion D, Ribouot C, Müller-Esterl W, and Godin-Ribuot D (2001) Endothelial kinin B<sub>1</sub>-receptors are induced by myocardial ischaemia-reperfusion in the rabbit. *J Physiol* **530**:69–78.
- McEachern AE, Shelton ER, Bhakta S, Obernolte R, Bach C, Zuppan P, Fujisaki J, Aldrich RW, and Jarnagin K (1991) Expression cloning of a rat B<sub>2</sub> bradykinin receptor. *Proc Natl Acad Sci USA* **88**:7724–7728.
- McIntyre P, Phillips E, Skidmore E, Brown M, and Webb M (1993) Cloned murine bradykinin receptor exhibits a mixed B<sub>1</sub> and B<sub>2</sub> pharmacological selectivity. *Mol Pharmacol* **44**:346–355.
- McLean PG, Ahluwalia A, and Perretti M (2000a) Association between kinin B<sub>1</sub> receptor expression and leukocyte trafficking across mouse mesenteric postcapillary venules. *J Exp Med* **192**:367–380.
- McLean PG, Perretti M, and Ahluwalia A (1999) Inducible expression of the kinin B<sub>1</sub> receptor in the endotoxemic heart: mechanisms of des-Arg<sup>9</sup>bradykinin-induced coronary vasodilation. *Br J Pharmacol* **128**:275–282.
- McLean PG, Perretti M, and Ahluwalia A (2000b) Kinin B<sub>1</sub> receptors as novel anti-inflammatory targets. *Emerg Ther Targets* **4**:127–141.
- Meacci E, Vasta V, Moorman JP, Bobak DA, Bruni P, Moss J, and Vaughan M (1999) Effect of Rho and ADP-ribosylation factor GTPases on phospholipase D activity in intact human adenocarcinoma A549 cells. *J Biol Chem* **274**:18605–18612.
- Meini S, Bellucci F, Cucchi P, Giuliani S, Quartara L, Giolitti A, Zappitelli S, Rotondaro L, Boels K, and Maggi CA (2004a) Bradykinin B<sub>2</sub> and GPR100 receptors: a paradigm for receptor signal transduction pharmacology. *Br J Pharmacol* **143**:938–941.
- Meini S, Cucchi P, Bellucci F, Catalani C, Faiella A, Rotondaro L, Quartara L, Giolitti A, and Maggi CA (2004b) Site-directed mutagenesis at the human B<sub>2</sub> receptor and molecular modelling to define the pharmacophore of non-peptide bradykinin receptor antagonists. *Biochem Pharmacol* **67**:601–609.
- Meini S, Cucchi P, Zappitelli S, Rotondaro L, Quartara L, Giolitti A, and Maggi CA (2002) Preliminary mutational analysis of the human kinin B<sub>2</sub> receptor for non-peptide antagonist ligands recognition. *Can J Physiol Pharmacol* **80**:303–309.
- Menke JG, Borkowski JA, Bierilo KK, MacNeil T, Derrick AW, Schneck KA, Ransom RW, Strader CD, Linemeyer DL, and Hess JF (1994) Expression cloning of a human B<sub>1</sub> bradykinin receptor. *J Biol Chem* **269**:21583–21586.
- Merrifield RB (1964) Solid phase synthesis. III. An improved synthesis of bradykinin. *Biochemistry* **3**:1385–1390.
- Mikhailov V, Verma S, Fedak PW, Weisel RD, and Li RK (2003) Does ischemic preconditioning afford clinically relevant cardioprotection? *Am J Cardiovasc Drugs* **3**:1–11.
- Minshall RD, Erdős EG, and Vogel SM (1997a) Angiotensin I-converting enzyme inhibitors potentiate bradykinin's inotropic effects independently of blocking its inactivation. *Am J Cardiol* **80**:132A–136A.
- Minshall RD, Tan F, Nakamura F, Rabito SF, Becker RP, Marcic B, and Erdős EG (1997b) Potentiation of the actions of bradykinin by angiotensin I-converting enzyme inhibitors. The role of expressed human bradykinin B<sub>2</sub> receptors and angiotensin I-converting enzyme in CHO cells. *Circ Res* **81**:848–856.
- Minsker DH, Bagdon WJ, MacDonald JS, Robertson RT, and Bokelman DL (1990) Maternotoxicity and fetotoxicity of an angiotensin-converting enzyme inhibitor, enalapril, in rabbits. *Fundam Appl Toxicol* **14**:461–470.
- Miyamoto A, Laufs U, Pardo C, and Liao JK (1997) Modulation of bradykinin receptor ligand binding affinity and its coupled G-proteins by nitric oxide. *J Biol Chem* **272**:19601–19608.
- Molinaro G, Cugno M, Perez M, Lepage Y, Gervasi N, Agostoni A, and Adam A (2002) Angiotensin-converting enzyme inhibitor-associated angioedema is characterized by a slower degradation of des-arginine<sup>9</sup>-bradykinin. *J Pharmacol Exp Ther* **303**:232–237.
- Mukae S, Aoki S, Itoh S, Iwata T, Ueda H, and Katagiri T (2000) Bradykinin B<sub>2</sub> receptor gene polymorphism is associated with angiotensin-converting enzyme inhibitor-related cough. *Hypertension* **36**:127–131.
- Mukae S, Itoh S, Aoki S, Iwata T, Nishio K, Sato R, and Katagiri T (2002) Association of polymorphisms of the renin-angiotensin system and bradykinin B<sub>2</sub> receptor with ACE-inhibitor-related cough. *J Hum Hypertens* **16**:857–863.
- Mulatero P, Williams TA, Milan A, Paglieri C, Rabbia F, Fallo F, and Veglio F (2002) Blood pressure in patients with primary aldosteronism is influenced by bradykinin (B<sub>2</sub>) receptor and alpha-adducin gene polymorphisms. *J Clin Endocrinol Metab* **87**:3337–3343.
- Munoz CM, Cotechia S, and Leeb-Lundberg LMF (1993) B<sub>2</sub> kinin receptor-mediated internalization of bradykinin in DDT<sub>1</sub> MF-2 smooth muscle cells is paralleled by a sequestration of the occupied receptors. *Arch Biochem Biophys* **301**:336–344.
- Munoz CM and Leeb-Lundberg LM (1992) Receptor-mediated internalization of bradykinin: DDT<sub>1</sub> MF-2 smooth muscle cells process internalized bradykinin via multiple degradative mechanisms. *J Biol Chem* **267**:303–309.
- Naclerio RM, Proud D, Lichtenstein LM, Kagey-Sobotka A, Hendley JO, Sorrentino J, and Gwaltney JM (1988) Kinins are generated during experimental rhinovirus colds. *J Infect Dis* **157**:133–142.
- Nadar R, Derrick A, Naidoo S, Naidoo Y, Hess F, and Bhoola K (1996) Immunoreactive B<sub>1</sub> receptors in human transbronchial tissue. *Immunopharmacology* **33**:317–320.
- Naicker S, Naidoo S, Ramsarop R, Moodley D, and Bhoola K (1999) Tissue kallikrein and kinins in renal disease. *Immunopharmacology* **44**:183–192.
- Naidoo S, Ramsarop R, Naidoo Y, and Bhoola KD (1996) The status of B<sub>2</sub> receptors in acute renal transplant rejection. *Immunopharmacology* **33**:157–160.
- Nakhostine N, Ribout C, Lamontagne D, Nadeau R, and Couture R (1993) Mediation by B<sub>1</sub> and B<sub>2</sub> receptors of vasodepressor responses to intravenously administered kinins in anaesthetized dogs. *Br J Pharmacol* **110**:71–76.
- Nardone J and Hogan PG (1994) Delineation of a region in the B<sub>2</sub> bradykinin receptor that is essential for high-affinity agonist binding. *Proc Natl Acad Sci USA* **91**:4417–4421.
- Narotam JK, Rodell TC, Nadvi SS, Bhoola KD, Troha JM, Parbhoo Singh R, and van Dellen JP (1998) Traumatic brain contusions: a clinical role for the kinin antagonist CP-0127. *Acta Neurochir (Wien)* **140**:793–802.
- Ni A, Chai KX, Chao L, and Chao J (1998a) Molecular cloning and expression of rat bradykinin B<sub>1</sub> receptor. *Biochim Biophys Acta* **1442**:177–185.
- Ni A, Chao L, and Chao J (1998b) Transcription factor nuclear factor  $\kappa$ B regulates



- the inducible expression of the human B<sub>1</sub> receptor gene in inflammation. *J Biol Chem* **273**:2784–2791.
- Ni A, Yin H, Agata J, Yang Z, Chao L, and Chao J (2003) Overexpression of kinin B<sub>1</sub> receptors induces hypertensive response to des-Arg<sup>9</sup>-bradykinin and susceptibility to inflammation. *J Biol Chem* **278**:219–225.
- Nicolau M, Feltrin MR, and Regoli D (1996) Induction of bradykinin B<sub>1</sub> hypotensive receptors in rats by lipopolysaccharide. *Can J Physiol Pharmacol* **74**:337–340.
- Novotny EA, Bednar DL, Connolly MA, Connor JR, and Stormann TM (1994) Mutation of aspartate residues in the third extracellular loop of the rat B2 bradykinin receptor decreases affinity for bradykinin. *Biochem Biophys Res Commun* **201**:523–530.
- Nussberger J, Cugno M, Cicardi M, and Agostoni A (1999) Local bradykinin generation in hereditary angioedema. *J Allergy Clin Immunol* **104**:1321–1322.
- Oda CE and Goodfriend TL (1979) Bradykinin receptors, in *Handbook of Experimental Pharmacology* (Erdős EG ed), vol XXV, pp 287–300, Springer-Verlag, Berlin, Germany.
- Olejek A, Mazurek U, Michalski B, Kusmierz D, Orchel J, Witek A, and Wilczok T (2000) Profiles of expression of genes coding kininogen and kinin receptors as a marker of tissue pathology in cervical cancer coexisting with HPV infection. *Wiad Lek* **53**:247–254.
- Olejek A, Mazurek U, Orchel J, Rzepoluch J, and Wilczok T (2001) The assessment of expression of kininogen and bradykinin receptors genes in different vulvar pathologies by QPCR (TaqMan). *Ginekolog Pol* **72**:717–722.
- Ongali B, Campos MM, Bregola G, Rodi D, Regoli D, Thibault G, Simonato M, and Couture R (2003) Autoradiographic analysis of rat brain kinin B<sub>1</sub> and B<sub>2</sub> receptors: normal distribution and alterations induced by epilepsy. *J Comp Neurol* **461**:506–519.
- Otterbein L, Lowe VC, Kyle DJ, and Noronha-Blob L (1993) Additive effects of a bradykinin antagonist, NPC 17761 and a leumedin, NPC 15669, on survival in animal models of sepsis. *Agents Actions* **39**:C125–C127.
- Owen NE and Villereal ML (1983) Lys-bradykinin stimulates Na<sup>+</sup> influx and DNA synthesis in cultured human fibroblasts. *Cell* **32**:979–985.
- Palczewski K, Kumakura T, Hori T, Behnke CA, Motoshima H, Fox BA, Le Trong I, Teller DC, Okada T, Stenkamp RE, et al. (2000) Crystal structure of rhodopsin: a G protein-coupled receptor. *Science (Wash DC)* **289**:739–745.
- Palmer RM, Ferrige AG, and Moncada S (1987) Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature (Lond)* **327**:524–526.
- Pan ZK, Ye RD, Christiansen SC, Jagels MA, Bokoch GM, and Zuraw BL (1998) Role of the Rho GTPase in bradykinin-stimulated nuclear factor-κB activation and IL-1β gene expression in cultured human epithelial cells. *J Immunol* **160**:3038–3045.
- Pan ZK, Zuraw BL, Lung CC, Prossnitz ER, Browning DD, and Ye RD (1996) Bradykinin stimulates NF-κB activation and interleukin 1β gene expression in cultured human fibroblasts. *J Clin Invest* **98**:2042–2049.
- Parenti A, Morbidelli L, Ledda F, Granger HJ, and Ziche M (2001) The bradykinin/B1 receptor promotes angiogenesis by up-regulation of endogenous FGF-2 in endothelium via the nitric oxide synthase pathway. *FASEB J* **15**:1487–1489.
- Parries G, Hoebel R, and Racher E (1987) Opposing effects of a ras oncogene of growth factor-stimulated phosphoinositide hydrolysis: desensitization to platelet-derived growth factor and enhanced sensitivity to bradykinin. *Proc Natl Acad Sci USA* **84**:2648–2652.
- Passos GF, Fernandez ES, Campos MM, Araujo JGVC, Pesquero JL, Souza GEP, Avellar MCW, Teixeira MM, and Calixto JB (2004) Kinin B<sub>1</sub> receptor up-regulation after lipopolysaccharide administration: role of proinflammatory cytokines and neutrophil influx. *J Immunol* **172**:1839–1847.
- Patel KV and Schrey MP (1992) Inhibition of DNA synthesis and growth in human breast stromal cells by bradykinin: evidence for independent roles of B1 and B2 receptors in the respective control of cell growth and phospholipid hydrolysis. *Cancer Res* **52**:334–340.
- Pe'er I and Beckmann JS (2004) On the applicability of a haplotype map to unassayed populations. *Hum Genet* **114**:214–217.
- Perkins MN, Campbell E, and Dray A (1993) Antinociceptive activity of the bradykinin B1 and B2 receptor antagonists, des-Arg<sup>9</sup>[Leu<sup>8</sup>]-BK and Hoe 140, in two models of persistent hyperalgesia in the rat. *Pain* **53**:191–197.
- Perkins MN and Kelly D (1993) Induction of bradykinin B<sub>1</sub> receptors in vivo in a model of ultra-violet irradiation-induced thermal hyperalgesia in the rat. *Br J Pharmacol* **110**:1441–1444.
- Perlman S, Schambye HT, Rivero RA, Greenlee WJ, Hjorth SA, and Schwartz TW (1995) Functional and molecular interaction with the AT1 receptor. *J Biol Chem* **270**:1493–1496.
- Pesquero JB, Araujo RC, Heppenstall PA, Stucky CL, Silva JA Jr, Walther T, Oliveira SM, Pesquero JL, Paiva AC, Calixto JB, et al. (2000) Hypoalgesia and altered inflammatory responses in mice lacking kinin B1 receptors. *Proc Natl Acad Sci USA* **97**:8140–8145.
- Pesquero JB, Lindsey CJ, Paiva AC, Ganten D, and Bader M (1996a) Transcriptional regulatory elements in the rat bradykinin B2 receptor gene. *Immunopharmacology* **33**:36–41.
- Pesquero JB, Lindsey CJ, Zeh K, Paiva AC, Ganten D, and Bader M (1994) Molecular structure and expression of rat bradykinin B2 receptor gene. Evidence for alternative splicing. *J Biol Chem* **269**:26920–26925.
- Pesquero JB, Pesquero JL, Oliveira SM, Roscher AA, Metzger R, Ganten D, and Bader M (1996b) Molecular cloning and functional characterization of a mouse bradykinin B1 receptor gene. *Biochem Biophys Res Commun* **220**:219–225.
- Phagoo SB, Poole S, and Leeb-Lundberg LMF (1999) Autoregulation of bradykinin receptors: agonists in the presence of interleukin-1β shift the repertoire of receptor subtypes from B<sub>2</sub> to B<sub>1</sub> in human lung fibroblasts. *Mol Pharmacol* **56**:325–333.
- Pizard A, Blaukat A, Michineau S, Dikic I, Müller-Esterl W, Alhenc-Gelas F, and Rajerison RM (2001) Palmitoylation of the human bradykinin B2 receptor influences ligand efficacy. *Biochemistry* **40**:15743–15751.
- Pizard A, Blaukat A, Müller-Esterl W, Alhenc-Gelas F, and Rajerison RM (1999) Bradykinin-induced internalization of the human B2 receptor requires phosphorylation of three serine and two threonine residues at its carboxyl tail. *J Biol Chem* **274**:12738–12747.
- Pizard A, Marchetti J, Allegrini J, Alhenc-Gelas F, and Rajerison RM (1998) Negative cooperativity in the human bradykinin B<sub>2</sub> receptor. *J Biol Chem* **273**:1309–1315.
- Polosa R, Cacciola RR, and Holgate ST (1992) The response of the airways in asthmatic patients to kinin inhalations define a type-B2 receptor profile. *Ann Ital Med Int* **7**:220–225.
- Polosa R and Holgate ST (1990) Comparative airway response to inhaled bradykinin, kallidin and [des-Arg<sup>9</sup>]bradykinin in normal and asthmatic subjects. *Am Rev Respir Dis* **142**:1367–1371.
- Poole S, Lorenzetti BB, Cunha JM, Cunha FQ, and Ferreira SH (1999) Bradykinin B<sub>1</sub> and B<sub>2</sub> receptors, tumour necrosis factor α and inflammatory hyperalgesia. *Br J Pharmacol* **126**:649–656.
- Powell SJ, Slynn G, Thomas C, Hopkins B, Briggs I, and Graham A (1993) Human bradykinin B2 receptor: nucleotide sequence analysis and assignment to chromosome 14. *Genomics* **15**:435–438.
- Prado GN, Mierke DF, Pellegrini M, Taylor L, and Polgar P (1998) Motif mutation of bradykinin B2 receptor second intracellular loop and proximal C terminus is critical for signal transduction, internalization and resensitization. *J Biol Chem* **273**:33548–33555.
- Prado GN, Taylor L, and Polgar P (1997) Effects of intracellular tyrosine residue mutation and carboxyl terminus truncation on signal transduction and internalization of the rat bradykinin B2 receptor. *J Biol Chem* **272**:14638–14642.
- Prado GN, Taylor L, Zhou X, Ricupero D, Mierke DF, and Polgar P (2002) Mechanisms regulating the expression, self-maintenance and signaling-function of the bradykinin B2 and B1 receptors. *J Cell Physiol* **193**:275–286.
- Prasad A, Husain S, Schenke W, Mincemoyer R, Epstein N, and Quyyumi AA (2000) Contribution of bradykinin receptor dysfunction to abnormal coronary vasomotion in humans. *J Am Coll Cardiol* **36**:1467–1473.
- Prat A, Weinrib L, Becher B, Poirier J, Duquette P, Couture R, and Antel JP (1999) Bradykinin B1 receptor expression and function on T lymphocytes in active multiple sclerosis. *Neurology* **53**:2087–2092.
- Pretorius M, Rosenbaum D, Vaughan DE, and Brown NJ (2003) Angiotensin-converting enzyme inhibition increases human vascular-type plasminogen activator release through endogenous bradykinin. *Circulation* **107**:579–585.
- Proud D, Bathon JM, Toggias AG, and Naclerio RM (1995) Inhibition of the response to nasal provocation with bradykinin by HOE-140: efficacy and duration of action. *Can J Physiol Pharmacol* **73**:820–826.
- Proud D, Reynolds CJ, Lacapra S, Kagey-Sobotka A, Lichtenstein LM, and Naclerio RM (1988) Nasal provocation with bradykinin induces symptoms of rhinitis and a sore throat. *Am Rev Respir Dis* **137**:613–616.
- Proud D, Toggias A, Naclerio RM, Crush SA, Norman PS, and Lichtenstein LM (1983) Kinins are generated *in vivo* following nasal airway challenge of allergic individuals with allergen. *J Clin Invest* **72**:1678–1685.
- Pruneau D, Luccarini JM, Defrène E, Paquet JL, and Béliard P (1996) Characterization of bradykinin receptors from juvenile pig coronary artery. *Eur J Pharmacol* **297**:53–60.
- Pruneau D, Paquet JL, Luccarini JM, Defrène E, Fouchet C, Franck R-M, Loeffler B, Robert C, Béliard P, Duclos H, et al. (1999) Pharmacological profile of LF 16-0687, a new potent non-peptide bradykinin B<sub>2</sub> receptor antagonist. *Immunopharmacology* **43**:187–194.
- Pyne S and Pyne NJ (1995) Bradykinin-stimulated phosphatidylcholine hydrolysis in airway smooth muscle: the role of Ca<sup>2+</sup> and protein kinase C. *Biochem J* **311**:637–642.
- Qadri F, Häuser W, Jöhren O, and Dominiak P (2002) Kinin B<sub>1</sub> and B<sub>2</sub> receptor mRNA expression in the hypothalamus of spontaneously hypertensive rats. *Can J Physiol Pharmacol* **80**:258–263.
- Quitterer U, Abdalla S, Jarnagin K, and Müller-Esterl W (1996) Na<sup>+</sup> ions binding to the bradykinin B2 receptor suppress agonist-independent receptor activation. *Biochemistry* **35**:13368–13377.
- Racchi M, Ianna P, Binetti G, Trabucchi M, and Govoni S (1998) Bradykinin-induced amyloid precursor protein secretion: a protein kinase C-independent mechanism that is not altered in fibroblasts from patients with sporadic Alzheimer's disease. *Biochem J* **330**:1271–1275.
- Raidoo DM and Bhoola KD (1997) Kinin receptors on human neurons. *J Neuroimmunol* **77**:39–44.
- Raidoo DM, Ramchurren N, Naidoo Y, Naidoo S, Müller-Esterl W, and Bhoola KD (1996) Visualisation of bradykinin B2 receptors on human brain neurons. *Immunopharmacology* **33**:104–107.
- Raidoo DM, Ramsaroop R, Naidoo S, Müller-Esterl W, and Bhoola KD (1997) Kinin receptors in human vascular tissue: their role in atheromatous disease. *Immunopharmacology* **36**:153–160.
- Raidoo DM, Sawant S, Mahabeer R, and Bhoola KD (1999) Kinin receptors are expressed in human astrocytic tumour cells. *Immunopharmacology* **43**:255–263.
- Rashid MH, Inoue M, Matsumoto M, and Ueda H (2004) Switching of bradykinin-mediated nociception following partial sciatic nerve injury in mice. *J Pharmacol Exp Ther* **308**:1158–1164.
- Rees GL and Eccles R (1994) Sore throat following nasal and oropharyngeal bradykinin challenge. *Acta Oto-Laryngol* **114**:311–314.
- Regoli D and Barabé J (1980) Pharmacology of bradykinin and related kinins. *Pharmacol Rev* **32**:1–46.
- Regoli D, Barabé J, and Park WK (1977) Receptors for bradykinin in rabbit aortae. *Can J Physiol Pharmacol* **55**:855–867.
- Regoli D, Marceau F, and Barabé J (1978) De novo formation of vascular receptors for bradykinin. *Can J Physiol Pharmacol* **56**:674–677.
- Regoli D, Park WK, and Rioux F (1974) Pharmacology of angiotensin. *Pharmacol Rev* **26**:69–123.
- Regoli DC, Marceau F, and Lavigne J (1981) Induction of B<sub>1</sub>-receptors for kinins in the rabbit by a bacterial lipopolysaccharide. *Eur J Pharmacol* **71**:105–115.

- Reich DE, Cargill M, Bolk S, Ireland J, Sabeti PC, Richter DJ, Lavery T, Kouyoumjian R, Farhadian SF, Ward R, et al. (2001) Linkage disequilibrium in the human genome. *Nature (Lond)* **411**:199–204.
- Relton JK, Beckey VE, Hanson WL, and Whalley ET (1997) CP-0597, a selective bradykinin B<sub>2</sub> receptor antagonist, inhibits brain injury in a rat model of reversible middle cerebral artery occlusion. *Stroke* **28**:1430–1436.
- Reynolds CJ, Togias A, and Proud D (1999) Airway neural responses to kinin. Tachyphylaxis and role of receptor subtypes. *Am J Respir Crit Care Med* **159**:431–438.
- Rhaleb NE, Gobeil F, and Regoli D (1992) Non-selectivity of new bradykinin antagonists for B<sub>1</sub> receptors. *Life Sci* **51**:PL125–PL129.
- Ricupero DA, Romero JR, Rishikof DC, and Goldstein RH (2000) Des-Arg<sup>10</sup>-kallidin engagement of the B<sub>1</sub> receptor stimulates type I collagen synthesis via stabilization of connective tissue growth factor mRNA. *J Biol Chem* **275**:12475–12480.
- Risch NJ (2000) Searching for genetic determinants in the new millennium. *Nature (Lond)* **405**:847–856.
- Rizzi A, Calo G, Amadesi S, and Regoli D (1997) Kinin B<sub>1</sub> and B<sub>2</sub> receptors in pig vessels: characterization of two monoreceptor systems. *Naunyn-Schmiedeberg's Arch Pharmacol* **356**:662–670.
- Roberts RA and Gullick WJ (1989) Bradykinin receptor number and sensitivity to ligand stimulation of mitogenesis is increased by expression of a mutant ras oncogene. *J Cell Sci* **94**:527–535.
- Rocha e Silva M (1970) *Kinin Hormones*. Charles C Thomas Publisher, Ltd., Springfield, IL.
- Rocha e Silva M, Beraldo WT, and Rosenfeld G (1949) Bradykinin, a hypotensive and smooth muscle stimulating factor released from plasma by snake venoms and by trypsin. *Am J Physiol* **156**:261–273.
- Roscher AA, Klier C, Dengler R, Faussner A, and Müller-Esterl W (1990) Regulation of bradykinin action at the receptor level. *J Cardiovasc Pharmacol* **15** (Suppl 6):S39–S43.
- Roscher AA, Manganiello VC, Jelsema CL, and Moss J (1984) Autoregulation of bradykinin receptors and bradykinin-induced prostacyclin formation in human fibroblasts. *J Clin Invest* **74**:552–558.
- Ross D and Joyner WL (1997) Resting distribution and stimulated translocation of protein kinase C isoforms alpha, epsilon and zeta in response to bradykinin and TNF in human endothelial cells. *Endothelium* **5**:321–332.
- Ruggiero M, Srivastava SK, Fleming TP, Ron D, and Eva A (1989) NIH3T3 fibroblasts transformed by the dbl oncogene show altered expression of bradykinin receptors: effect on inositol lipid turnover. *Oncogene* **4**:767–771.
- Sabourin T, Bastien L, Bachvarov DR, and Marceau F (2002a) Agonist-induced translocation of the kinin B<sub>1</sub> receptor to caveolae-related rafts. *Mol Pharmacol* **61**:546–553.
- Sabourin T, Guay K, Houle S, Bouthillier J, Bachvarov DR, Adam A, and Marceau F (2001) Absence of ligand-induced regulation of kinin receptor expression in the rabbit. *Br J Pharmacol* **133**:1154–1162.
- Sabourin T, Morissette G, Bouthillier J, Levesque L, and Marceau F (2002b) Expression of kinin B<sub>1</sub> receptor in fresh or cultured rabbit aortic smooth muscle: role of NF-κB. *Am J Physiol* **283**:H227–H237.
- Saifudeen Z, Dipp S, and El-Dahr SS (2002) A role for p53 in terminal epithelial cell differentiation. *J Clin Invest* **109**:1021–1030.
- Saifudeen Z, Du H, Dipp S, and El-Dahr SS (2000) The bradykinin type 2 receptor is a target for p53-mediated transcriptional activation. *J Biol Chem* **275**:15557–15562.
- Salvino JM, Seoane PR, Douty BD, Awad MMA, Dolle RE, Houck WT, Faunce DM, and Sawutz DG (1993) Design of potent non-peptide competitive antagonists of the human bradykinin B<sub>2</sub> receptor. *J Med Chem* **36**:2583–2584.
- Samadifam R, Teixeira C, Bkaily G, Sirois P, de Brum-Fernandez A, and D'Orléans-Juste P (2000) Contribution of B<sub>2</sub> receptors for bradykinin in arthus reaction-induced plasma extravasation in wild-type or B<sub>2</sub> transgenic knockout mice. *Br J Pharmacol* **129**:1732–1738.
- Sardi SP, Daray FM, Errasti AE, Pelorosso FG, Pujol-Lereis VA, Rey-Ares V, Rogines-Velo MP, and Rothlin RP (1999) Further pharmacological characterization of bradykinin B<sub>1</sub> receptor up-regulation in human umbilical vein. *J Pharmacol Exp Ther* **290**:1019–1025.
- Sardi SP, Rey-Ares V, Pujol-Lereis VA, Serrano SA, and Rothlin RP (2002) Further pharmacological evidence of nuclear factor-κB pathway involvement in bradykinin B<sub>1</sub> receptor-sensitized responses in human umbilical vein. *J Pharmacol Exp Ther* **301**:975–980.
- Sarker MH, Hu DE, and Fraser PA (2000) Acute effects of bradykinin on cerebral microvascular permeability in the anaesthetized rat. *J Physiol* **528**:177–187.
- Sawutz DG, Salvino JM, Dolle RE, Casiano F, Ward SJ, Houck WT, Faunce DM, Douty BD, Baizman E, Awad MMA, et al. (1994) The nonpeptide WIN 64338 is a bradykinin B<sub>2</sub> receptor antagonist. *Proc Natl Acad Sci* **91**:4693–4697.
- Schaefer RM, Fink E, Schaefer L, Barkhausen R, Kulzer P, and Heidland A (1993) Role of bradykinin in anaphylactoid reactions during hemodialysis with AN69 dialyzers. *Am J Nephrol* **13**:473–477.
- Schanstra JP, Bataille E, Marin Castano ME, Barascud Y, Hirtz C, Pesquero JB, Pecher C, Gauthier F, Girolami JP, and Bascands JL (1998) The B<sub>1</sub>-agonist [des-Arg<sup>10</sup>]-kallidin activates transcription factor NF-κB and induces homologous upregulation of the bradykinin B<sub>1</sub>-receptor in cultured human lung fibroblasts. *J Clin Invest* **101**:2080–2091.
- Schanstra JP, Duchene J, Pradaude F, Bruneval P, Tack I, Chevalier J, Girolami JP, and Bascands JL (2003) Decreased renal NO excretion and reduced glomerular tuft area in mice lacking the bradykinin B<sub>2</sub> receptor. *Am J Physiol* **284**:H1904–H1908.
- Schanstra JP, Marin-Castano ME, Pradaude F, Tack I, Ader JL, Girolami JP, Bascands JL, and Jeunier B (2000) Bradykinin B<sub>1</sub> receptor-mediated changes in renal hemodynamics during endotoxin-induced inflammation. *J Am Soc Nephrol* **11**:1208–1215.
- Schanstra JP, Neau E, Drogoz P, Arevalo Gomez MA, Lopez Novoa JM, Calise D, Pecher C, Bader M, Girolami JP, and Bascands JL (2002) In vivo bradykinin B<sub>2</sub> receptor activation reduces renal fibrosis. *J Clin Invest* **110**:371–379.
- Schild HO (1973) Receptor classification with special reference to beta-adrenergic receptors, in *Drug Receptors* (Rang HP ed) pp 29–36, University Park Press, Baltimore, MD.
- Schmidlin F, Loeffler S, Bertrand C, Landry Y, and Gies J-P (2000) PLA<sub>2</sub> phosphorylation and cyclooxygenase-2 induction, through p38 MAP kinase pathway, is involved in the IL-1β-induced bradykinin B<sub>2</sub> receptor gene transcription. *Naunyn-Schmiedeberg's Arch Pharmacol* **361**:247–254.
- Schmidlin F, Scherrer D, Daeffler L, Bertrand C, Landry Y, and Gies JP (1998) Interleukin-1β induces bradykinin B<sub>2</sub> receptor gene expression through a prostanoic cyclic AMP-dependent pathway in human bronchial smooth muscle cells. *Mol Pharmacol* **53**:1009–1015.
- Schneck KA, Hess JF, Stonesifer GY, and Ransom RW (1994) Bradykinin B<sub>1</sub> receptors in rabbit aorta smooth muscle cells in culture. *Eur J Pharmacol* **266**:277–282.
- Scholzen TE, Stander S, Riemann H, Brzoska T, and Luger TA (2003) Modulation of cutaneous inflammation by angiotensin-converting enzyme. *J Immunol* **170**:3866–3873.
- Schremmer-Danninger E, Hermann A, Fink E, Fritz H, and Roscher AA (1999) Identification and occurrence of mRNAs for components of the kallikrein-kinin system in human skin and in skin diseases. *Immunopharmacology* **43**:287–291.
- Schroeder C, Beug H, and Müller-Esterl W (1997) Cloning and functional characterization of the ornithokinin receptor. Recognition of the major kinin receptor antagonist, HOE140, as a full agonist. *J Biol Chem* **272**:12475–12481.
- Simple PF (1995) Putative mechanisms of cough after treatment with angiotensin converting enzyme inhibitors. *J Hypertens* **13** (Suppl 3):S17–S21.
- Sharma JN (1993) Therapeutic prospects of bradykinin receptor antagonists. *Gen Pharmacol* **24**:267–274.
- Sharma JN and Al-Dhalmawi GS (2003) Bradykinin receptor antagonists: therapeutic implications. *Drugs* **6**:581–586.
- Shin J, Cho H, Hwang SW, Jung J, Shin CY, Lee SY, Kim SH, Lee MG, Choi YH, Kim J, et al. (2002) Bradykinin-12-lipoxygenase-VR1 signaling pathway for inflammatory hyperalgesia. *Proc Natl Acad Sci USA* **99**:10150–10155.
- Shughrue PJ, Ky B, and Austin CP (2003) Localization of B<sub>1</sub> bradykinin receptor mRNA in the primate brain and spinal cord: an in situ hybridization study. *J Comp Neurol* **465**:372–384.
- Silva JA Jr, Araujo RC, Baltatu O, Oliveira SM, Tschöpe C, Fink E, Hoffmann S, Plehm R, Chai KX, Chao L, et al. (2000) Reduced cardiac hypertrophy and altered blood pressure control in transgenic rats with the human tissue kallikrein gene. *FASEB J* **14**:1858–1860.
- Silvestre JS, Bergaya S, Tamarat R, Duriez M, Boulanger C, and Levy BI (2001) Proangiogenic effect of angiotensin-converting enzyme inhibition is mediated by the bradykinin B<sub>2</sub> receptor pathway. *Circ Res* **89**:678–683.
- Smith JAM, Webb C, Holford J, and Burgess GM (1995) Signal transduction pathways for B<sub>1</sub> and B<sub>2</sub> bradykinin receptors in bovine pulmonary artery endothelial cells. *Mol Pharmacol* **47**:525–534.
- Sobey CG (2003) Bradykinin B<sub>2</sub> receptor antagonism: a new direction for acute stroke therapy? *Br J Pharmacol* **139**:1369–1371.
- Soskic V, Nyakatura E, Roos M, Müller-Esterl W, and Godovac-Zimmermann J (1999) Correlations in palmitoylation and multiple phosphorylation of rat bradykinin B<sub>2</sub> receptor isolated from chinese hamster ovary cells. *J Biol Chem* **274**:8539–8545.
- Souza DG, Lomez ESL, Pinho V, Pesquero JB, Bader M, Pesquero JL, and Teixeira MM (2004) Role of bradykinin B<sub>2</sub> and B<sub>1</sub> receptors in the local, remote and systemic inflammatory responses that follow intestinal ischemia and reperfusion injury. *J Immunol* **172**:2542–2548.
- Spillmann F, Altmann C, Scheeler M, Barbosa M, Westermann D, Schultheiss HP, Walther T, and Tschöpe C (2002) Regulation of cardiac bradykinin B<sub>1</sub>- and B<sub>2</sub>-receptor mRNA in experimental ischemic, diabetic and pressure-overload-induced cardiomyopathy. *Int Immunopharmacol* **2**:1823–1832.
- Squire IB, O'Kane KP, Anderson N, and Reid JL (2000) Bradykinin B<sub>2</sub> receptor antagonism attenuates blood pressure response to acute angiotensin-converting enzyme inhibition in normal men. *Hypertension* **36**:132–136.
- Steranka LR, Manning DC, Dehaas CJ, Ferkany JW, Borosky SA, Connor JR, Vavrek RJ, Stewart JM, and Snyder SH (1988) Bradykinin as a pain mediator: receptors are localized to sensory neurons and antagonists have analgesic actions. *Proc Natl Acad Sci USA* **85**:3245–3249.
- Stewart JM (1995) Bradykinin antagonists: development and applications. *Biopolymers* **37**:143–155.
- Stewart JM (2003) Bradykinin antagonists as anti-cancer agents. *Curr Pharm Des* **9**:2036–2042.
- Stewart JM, Chan DC, Simkeviciene V, Bunn PA Jr, Helfrich B, York EJ, Taraseviciene-Stewart L, Bironaite D, and Gera L (2002a) Bradykinin antagonists as new drugs for prostate cancer. *Int Immunopharmacol* **2**:1781–1786.
- Stewart JM, Gera L, Chan DC, Bunn PA Jr, York EJ, Simkeviciene V, and Helfrich B (2002b) Bradykinin-related compounds as new drugs for cancer and inflammation. *Can J Physiol Pharmacol* **80**:275–280.
- Stewart JM, Gera L, Chan DC, Whalley ET, Hanson WL, and Zuzack JS (1997) Potent, long-acting bradykinin antagonists for a wide range of applications. *Can J Physiol Pharmacol* **75**:719–724.
- Stewart JM, Gera L, Hanson W, Zuzack JS, Burkard M, McCullough R, and Whalley ET (1996) A new generation of bradykinin antagonists. *Immunopharmacology* **31**:51–60.
- Stewart JM, Gera L, York EJ, Chan DC, and Bunn PA Jr (1999) Bradykinin antagonists: present progress and future prospects. *Immunopharmacology* **43**:155–161.
- Su DS, Markowitz MK, DiPardo RM, Murphy KL, Harrell CM, O'Malley SS, Ransom RW, Chang RS, Ha S, Hess FJ, et al. (2003) Discovery of a potent, non-peptide bradykinin B<sub>1</sub> receptor antagonist. *J Am Chem Soc* **125**:7516–7517.
- Sulpizio A, Pullen MA, Edwards RM, and Brooks DP (2004) The effect of acute



- angiotensin converting enzyme and neutral endopeptidase 24.11 inhibition on plasma extravasation in the rat. *J Pharmacol Exp Ther* **309**:1141–1147.
- Takano M, Kondo J, Yayama K, Otani M, Sano K, and Okamoto H (1997) Molecular cloning of cDNAs for mouse low-molecular-weight and high-molecular-weight prekininogens. *Biochim Biophys Acta* **1352**:222–230.
- Tancredi M, Galoppini C, Meini S, Quartara L, Maggi CA, and Rovero P (1997) Synthesis and biological activity of new bradykinin pseudopeptide B1 receptor agonists containing alkylic spacers. *Bioorg Med Chem Lett* **7**:2661–2664.
- Taub JS, Guo R, Leeb-Lundberg LMF, Madden JF, and Daaka Y (2003) Bradykinin receptor subtype 1 expression and function in prostate cancer. *Cancer Res* **63**:2037–2041.
- Teater S and Cuthbert AW (1997) Induction of bradykinin B<sub>1</sub> receptors in rat colonic epithelium. *Br J Pharmacol* **121**:1005–1011.
- Tilly BC, van Paridon PA, Verlaan I, Wirtz KW, de Laat SW, and Moolenaar WH (1987) Inositol phosphate metabolism in bradykinin-stimulated human A431 carcinoma cells. Relationship to calcium signalling. *Biochem J* **244**:129–135.
- Tipper S, Quitterer U, Kolm V, Faussner A, Roscher A, Mosthaf L, Müller-Esterl W, and Haring H (1994) Bradykinin induces translocation of the protein kinase C isoforms alpha, epsilon and zeta. *Eur J Biochem* **225**:297–304.
- Toussi CR and Ferreira SH (1997) Bradykinin-induced knee joint incapacitation involves bradykinin B<sub>2</sub> receptor mediated hyperalgesia and bradykinin B<sub>1</sub> receptor-mediated nociception. *Eur J Pharmacol* **326**:61–65.
- Trabold F, Pons S, Hagege AA, Bloch-Faure M, Alhenc-Gelas F, Giudicelli JF, Richer-Giudicelli C, and Meneton P (2002) Cardiovascular phenotypes of kinin B2 receptor- and tissue kallikrein-deficient mice. *Hypertension* **40**:90–95.
- Trivisani M, Amadesi S, Schmidlin F, Poblete MT, Bardella E, Maggioro B, Harrison S, Figueroa CD, Tognetto M, Navarra G, et al. (2003) Bradykinin B2 receptors mediate contraction in the normal and inflamed human gallbladder in vitro. *Gastroenterology* **125**:126–135.
- Trivisani M, Schmidlin F, Tognetto M, Nijkamp FP, Gies JP, Frossard N, Amadesi S, Folkerts G, and Geppetti P (1999) Evidence for in vitro expression of B<sub>1</sub> receptor in the mouse trachea and urinary bladder. *Br J Pharmacol* **126**:1293–1300.
- Triffeliff A, Amrani Y, Landry Y, and Gies JP (1993) Comparative action of new highly potent bradykinin receptor antagonists in the guinea-pig trachea. *Eur J Pharmacol* **239**:227–229.
- Triffeliff A, Lach E, Dumont P, and Gies JP (1994) Bradykinin binding sites in healthy and carcinomatous human lung. *Br J Pharmacol* **111**:1228–1232.
- Tropea MM, Gummelt D, Herzog MCS, and Leeb-Lundberg LMF (1993) B1 and B2 kinin receptors in rabbit superior mesenteric artery smooth muscle cells: receptor-specific stimulation of inositol phosphate formation and arachidonic acid release by des-Arg<sup>9</sup>-bradykinin and bradykinin. *J Pharmacol Exp Ther* **264**:930–937.
- Tschöpe C, Heringer-Walther S, Koch M, Spillmann F, Wendorf M, Leitner E, Schultheiss H-P, and Walther T (2000) Upregulation of bradykinin B1-receptor expression after myocardial infarction. *Br J Pharmacol* **129**:1537–1538.
- Tschöpe C, Spillmann F, Altmann C, Koch M, Westermann D, Dhayat N, Dhayat S, Bascands JL, Gera L, Hoffmann S, et al. (2004) The bradykinin B<sub>1</sub> receptor contributes to the cardioprotective effects of AT<sub>1</sub> blockade after experimental myocardial infarction. *Cardiovasc Res* **61**:559–569.
- Tsutsui M, Onoue H, Iida Y, Smith L, O'Brien T, and Katusic ZS (2000) B(1) and B(2) bradykinin receptors on adventitial fibroblasts of cerebral arteries are coupled to recombinant eNOS. *Am J Physiol Heart Circ Physiol* **278**:H367–H372.
- Turner P, Dear J, Scadding G, and Foreman JC (2001) Role of kinins in seasonal allergic rhinitis: icatibant, a bradykinin B<sub>2</sub> receptor antagonist, abolishes the hyperresponsiveness and nasal eosinophilia induced by antigen. *J Allergy Clin Immunol* **107**:105–113.
- Turner PJ, Dear JW, and Foreman JC (2000) Involvement of kinins in hyperresponsiveness induced by platelet activating factor in the human nasal airway. *Br J Pharmacol* **129**:525–532.
- Uknis AB, DeLa Cadena RA, Janardham R, Sartor RB, Whalley ET, and Colman RW (2001) Bradykinin receptor antagonists type 2 attenuate the inflammatory changes in peptidoglycan-induced acute arthritis in the Lewis rat. *Inflamm Res* **50**:149–155.
- Valdes G, Germain AM, Cortthorn J, Chacon C, Figueroa CD, and Müller-Esterl W (2001) Tissue kallikrein and bradykinin B2 receptor in human uterus in luteal phase and in early and late gestation. *Endocrine* **16**:207–215.
- Vavrek RJ and Stewart JM (1985) Competitive antagonists of bradykinin. *Peptides* **6**:161–164.
- Velarde V, Ullian ME, Morinelli TA, Mayfield RK, and Jaffa AA (1999) Mechanisms of MAPK activation by bradykinin in vascular smooth muscle cells. *Am J Physiol* **277**:C253–C261.
- Venema RC (2002) Post-translational mechanisms of endothelial nitric oxide synthase regulation by bradykinin. *Int Immunopharmacol* **2**:1755–1762.
- Venema VJ, Ju H, Sun J, Eaton DC, Marrero MB, and Venema RC (1998) Bradykinin stimulates the tyrosine phosphorylation and bradykinin B2 receptor association of phospholipase C gamma 1 in vascular endothelial cells. *Biochem Biophys Res Commun* **246**:70–75.
- Verresen L, Fink E, Lemke HD, and Vanrenterghem Y (1994) Bradykinin is a mediator of anaphylactoid reactions during hemodialysis with AN69 membranes. *Kidney Int* **45**:1497–1503.
- Vianna RMJ, Ongali B, Regoli D, Calixto JB, and Couture R (2003) Up-regulation of kinin B<sub>1</sub> receptor in the lung of streptozotocin-diabetic rat: autoradiographic and functional studies. *Br J Pharmacol* **138**:13–22.
- Voyno-Yasenetskaya TA, Tkachuk VA, Cheknyova EG, Panchenko MP, Grigorian GY, Vavrek RJ, Stewart JM, and Ryan US (1989) Guanine nucleotide-dependent, pertussis toxin-insensitive regulation of phosphoinositide turnover by bradykinin in bovine pulmonary artery endothelial cells. *FASEB J* **3**:44–51.
- Wahl M, Whalley ET, Unterberg A, Schilling L, Parsons AA, Baethmann A, and Young AR (1996) Vasomotor and permeability effects of bradykinin in the cerebral microcirculation. *Immunopharmacology* **33**:257–263.
- Wang B, Dang A, and Liu G (2001) Genetic variation in the promoter region of the beta2 bradykinin receptor gene is associated with essential hypertension in a Chinese Han population. *Hypertens Res* **24**:299–302.
- Wang DZ, Chao L, and Chao J (1997) Hypertension in transgenic mice overexpressing human bradykinin B<sub>2</sub> receptor. *Hypertension* **29**:488–493.
- Warner KK, Visconti JA, and Tschampel MM (2000) Angiotensin II receptor blockers in patients with ACE inhibitor-induced angioedema. *Ann Pharmacother* **34**:526–528.
- Weldon SM, Winkquist RJ, and Madwed JB (1995) Differential effects of L-NAME on blood pressure and heart rate responses to acetylcholine and bradykinin in cynomolgus primates. *J Pharmacol Exp Ther* **272**:126–133.
- Werle E and Grunz M (1939) Zur kenntnis der darmkontrahierenden uteruserregenden und blutdrucksenkenden substanz DK. *Biochem Z* **301**:429–436.
- Whalley ET, Clegg S, Stewart JM, and Vavrek RJ (1987) The effect of kinin agonists and antagonists on the pain response of the human blister base. *Naunyn-Schmiedeberg's Arch Pharmacol* **336**:652–655.
- Wilk-Blaszczak MA, Gutowski S, Sternweis PC, and Belardetti F (1994) Bradykinin modulates potassium and calcium currents in neuroblastoma hybrid cells via different pertussis toxin-insensitive pathways. *Neuron* **12**:109–116.
- Williams AG, Dhamrait SS, Wootton PT, Day SH, Hawe E, Payne JR, Myerson SG, World M, Budgett R, Humphries SE, et al. (2004) Bradykinin receptor gene variant and human physical performance. *J Appl Physiol* **96**:938–942.
- Witherow FN, Helmy A, Webb DJ, Fox KA, and Newby DE (2001) Bradykinin contributes to the vasodilator effects of chronic angiotensin-converting enzyme inhibition in patients with heart failure. *Circulation* **104**:2177–2181.
- Wojciak-Stothard B and Ridley AJ (2002) Rho GTPases and the regulation of endothelial permeability. *Vascul Pharmacol* **39**:187–199.
- Wolsing DH and Rosenbaum JS (1991) Bradykinin-stimulated inositol phosphate production in NG108-15 cells is mediated by a small population of binding sites which rapidly desensitize. *J Pharmacol Exp Ther* **257**:621–633.
- Wood MR, Kim JJ, Han W, Dorsey BD, Homnick CF, DiPardo RM, Kuduk SD, MacNeil T, Murphy KL, Lis EV, et al. (2003) Benzodiazepines as potent and selective bradykinin B<sub>1</sub> antagonists. *J Med Chem* **46**:1803–1806.
- Worden FP and Kalemkerian GP (2000) Therapeutic advances in small cell lung cancer. *Exp Opin Investig Drugs* **9**:565–579.
- Wotherspoon G and Winter J (2000) Bradykinin B<sub>1</sub> receptor is constitutively expressed in the rat sensory nervous system. *Neurosci Lett* **294**:175–178.
- Wu J, Akaike T, Hayashida K, Miyamoto Y, Nakagawa T, Miyakawa K, Müller-Esterl W, and Maeda H (2002) Identification of bradykinin receptors in clinical cancer specimens and murine tumor tissues. *Int J Cancer* **98**:29–35.
- Xie P, Browning DD, Hay N, Mackman N, and Ye RD (2000) Activation of NF- $\kappa$ B by bradykinin through a G $\alpha_q$ - and G $\beta\gamma$ -dependent pathway that involves phosphoinositide 3-kinase and Akt. *J Biol Chem* **275**:24907–24914.
- Xing M, Tao L, and Insel PA (1997) Role of extracellular signal-regulated kinase and PKC alpha in cytosolic PLA2 activation by bradykinin in MDCK-D1 cells. *Am J Physiol* **272**:C1380–C1387.
- Xiong W, Chao J, and Chao L (1995) Muscle delivery of human kallikrein gene reduces blood pressure in hypertensive rats. *Hypertension* **25**:715–719.
- Yamamoto Y and Gaynor RB (2001) Therapeutic potential of inhibition of the NF- $\kappa$ B pathway in the treatment of inflammation and cancer. *J Clin Investig* **107**:135–142.
- Yanaga F, Hirata M, and Koga T (1991) Evidence for coupling of bradykinin receptors to a guanine-nucleotide binding protein to stimulate arachidonic liberation in the osteoblast-like cell line, MC3T3-E1. *Biochim Biophys Acta* **1094**:139–146.
- Yang X and Polgar P (1996) Genomic structure of the human bradykinin B1 receptor gene and preliminary characterization of its regulatory regions. *Biochem Biophys Res Commun* **222**:718–725.
- Yang X, Taylor L, and Polgar P (1998) Mechanisms in the transcriptional regulation of bradykinin B1 receptor gene expression. Identification of a minimum cell-type specific enhancer. *J Biol Chem* **273**:10763–10770.
- Yang X, Taylor L, and Polgar P (2001a) p53 down-regulates human bradykinin B1 receptor gene expression. *J Cell Biochem* **82**:38–45.
- Yang XP, Liu YH, Mehta D, Cavanis MA, Shesley E, Xu J, Liu F, and Carretero OA (2001b) Diminished cardioprotective response to inhibition of angiotensin-converting enzyme and angiotensin II type 1 receptor in B<sub>2</sub> kinin receptor gene knockout mice. *Circ Res* **88**:1072–1079.
- Yang XP, Liu YH, Scicli GM, Webb CR, and Carretero OA (1997) Role of kinins in the cardioprotective effect of preconditioning: study of myocardial ischemia/reperfusion injury in B<sub>2</sub> kinin receptor knockout mice and kininogen-deficient rats. *Hypertension* **30**:735–740.
- Yano K, Higashida H, Hattori H, and Nozawa Y (1985) Bradykinin-induced transient accumulation of inositol trisphosphate in neuron-like cell line NG108-15 cells. *FEBS Lett* **181**:403–406.
- Yaqoob M, Snell CR, and Burgess GM (1995) Carbohydrate analysis of the B2 bradykinin receptor from rat uterus. *J Neurochem* **65**:1290–1296.
- Youngman MA, Carson JR, Lee JS, Dax SL, Zhang S-P, Colburn RW, Stone DJ, Codd EE, and Jetter MC (2003) Synthesis and structure-activity relationships of aroylpyrrole alkylamide bradykinin (B<sub>2</sub>) antagonists. *Bioorg Med Chem Lett* **13**:1341–1344.
- Zausinger S (2003) Bradykinin receptor antagonists in cerebral ischemia and trauma. *IDrugs* **6**:970–975.
- Zausinger S, Lumenta DB, Pruneau D, Schmid-Elsaesser R, Plesnila N, and Baethmann A (2002) Effects of LF 16-0687 Ms, a bradykinin B<sub>2</sub> receptor antagonist, on brain edema formation and tissue damage in a rat model of temporary focal cerebral ischemia. *Brain Res* **950**:268–278.
- Zausinger S, Lumenta DB, Pruneau D, Schmid-Elsaesser R, Plesnila N, and Baethmann A (2003) Therapeutical efficacy of a novel non-peptide bradykinin B<sub>2</sub> receptor antagonist on brain edema formation and ischemic tissue damage in focal cerebral ischemia. *Acta Neurochir* **86**:205–207.
- Zee RY, Rao VS, Paster RZ, Sweet CS, and Lindpaintner K (1998) Three candidate genes and angiotensin-converting enzyme inhibitor-related cough: a pharmacogenetic analysis. *Hypertension* **31**:925–928.



- Zhao WQ, Ravindranath L, Mohamed AS, Zohar O, Chen GH, Lyketsos CG, Etcheberrigaray R, and Alkon DL (2002) MAP kinase signaling cascade dysfunction specific to Alzheimer's disease in fibroblasts. *Neurobiol Dis* **11**:166–183.
- Zhou X, Polgar P, and Taylor L (1998) Roles for interleukin-1beta, phorbol ester and a post-transcriptional regulator in the control of bradykinin B1 receptor gene expression. *Biochem J* **330**:361–366.
- Zhou X, Prado GN, Chai M, Yang X, Taylor L, and Polgar P (1999) Posttranscriptional destabilization of the bradykinin B1 receptor messenger RNA: cloning and functional characterization of the 3'-untranslated region. *Mol Cell Biol Res Commun* **1**:29–35.
- Zhou X, Prado GN, Taylor L, Yang X, and Polgar P (2000) Regulation of inducible bradykinin B1 receptor gene expression through absence of internalization and resensitization. *J Cell Biochem* **78**:351–362.
- Zhu P, Zaugg CE, Hornstein PS, Allegrini PR, and Buser PT (1999) Bradykinin-dependent cardioprotective effects of losartan against ischemia and reperfusion in rat hearts. *J Cardiovasc Pharmacol* **33**:785–790.
- Zhu YM, Bradbury DA, Pang L, and Knox AJ (2003) Transcriptional regulation of interleukin (IL)-8 by bradykinin in human airway smooth muscle cells involves prostanoid-dependent activation of AP-1 and nuclear factor (NF)-IL-6 and prostanoid-independent activation of NF- $\kappa$ B. *J Biol Chem* **278**:29366–29375.
- Zuccollo A, Navarro M, Frontera M, Cueva F, Carattino M, and Catanzaro OL (1999) The involvement of kallikrein-kinin system in diabetes type I (insulinitis). *Immunopharmacology* **45**:69–74.
- Zwick E, Daub H, Aoki N, Yamaguchi-Aoki Y, Tinhofer I, Maly K, and Ullrich A (1997) Critical role of calcium-dependent epidermal growth factor receptor transactivation in PC12 cell membrane depolarization and bradykinin signaling. *J Biol Chem* **272**:24767–24770.
- Zychma MJ, Gumprecht J, Trautsolt W, Szydłowska I, and Grzeszczak W (2003) Polymorphic genes for kinin receptors, nephropathy and blood pressure in type 2 diabetic patients. *Am J Nephrol* **23**:112–116.
- Zychma MJ, Gumprecht J, Zukowska-Szzechowska E, and Grzeszczak W (1999) Polymorphisms in the genes encoding for human kinin receptors and the risk of end-stage renal failure: results of transmission/disequilibrium test. The End-Stage Renal Disease Study Group. *J Am Soc Nephrol* **10**:2120–2124.