British Journal of Pharmacology (2004) 143, 803–818
www.nature.com/bjp

REVIEW

Kinin B_1 receptors: key G-protein-coupled receptors and their role in inflammatory and painful processes

*,[1]João B. Calixto, [1]Rodrigo Medeiros, [1]Elizabeth S. Fernandes, [1]Juliano Ferreira, [1]Daniela A. Cabrini & [1]Maria M. Campos

[1]Department of Pharmacology, Centre of Biological Sciences, Universidade Federal de Santa Catarina, Campus Universitário, Trindade, 88049-900 Florianópolis, SC, Brazil

Kinins are a family of peptides implicated in several pathophysiological events. Most of their effects are likely mediated by the activation of two G-protein-coupled receptors: B_1 and B_2. Whereas B_2 receptors are constitutive entities, B_1 receptors behave as key inducible molecules that may be upregulated under some special circumstances. In this context, several recent reports have investigated the importance of B_1 receptor activation in certain disease models. Furthermore, research on B_1 receptors in the last years has been mainly focused in determining the mechanisms and pathways involved in the process of induction. This was essentially favoured by the advances obtained in molecular biology studies, as well as in the design of selective and stable peptide and nonpeptide kinin B_1 receptor antagonists. Likewise, development of kinin B_1 receptor knockout mice greatly helped to extend the evidence about the relevance of B_1 receptors during pathological states. In the present review, we attempted to remark the main advances achieved in the last 5 years about the participation of kinin B_1 receptors in painful and inflammatory disorders. We have also aimed to point out some groups of chronic diseases, such as diabetes, arthritis, cancer or neuropathic pain, in which the strategic development of nonpeptidic oral-available and selective B_1 receptor antagonists could have a potential relevant therapeutic interest.
British Journal of Pharmacology (2004) **143,** 803–818. doi:10.1038/sj.bjp.0706012

Keywords: Kinins; B1 receptors; inflammation; pain; selective antagonists

Abbreviations: AP-1, activating protein-1; AKT/PKB, protein kinase B; BCG, *Mycobacterium bovis* bacillus Calmette – Guerin; BK, bradykinin; CFA, complete Freund's adjuvant; COX-2, cyclooxygenase-2; CREB, c-AMP response element-binding; DRG, dorsal root ganglia; ERK, extracellular signal-regulated kinase; IL, interleukin; JNK, c-Jun NH_2-terminal kinase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; MEKK, mitogen-activated protein kinase kinase kinase; iNOS, inducible nitric oxide synthase; NF-κB, nuclear factor-κB; OVA, ovalbumin; PAF, platelet-activating factor; PI3-K, phosphatydilinositol-3 kinase; PLC, phospholipase C; PKC, protein kinase C; STZ, streptozotocin; TLR, Toll-like receptors; TNFα, tumour necrosis factor α; VRP, ventral root potential

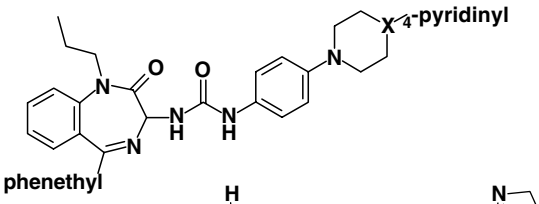
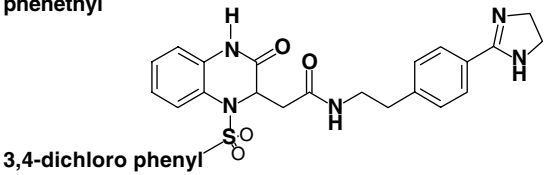
Introduction

Kinins are a group of peptides involved in a series of pathophysiological processes. They are formed in plasma and tissues in response to infection, tissue trauma or inflammatory alterations. Once formed and released, kinins exert most of their biological effects by the activation of two G-protein-coupled receptors, denoted B_1 and B_2. B_2 receptors are distributed in a constitutive manner throughout the central and peripheral tissues and present higher affinity for bradykinin (BK) and Lys-BK peptides. On the other hand, B_1 receptors display high affinity for the metabolites des-Arg^9-BK and Lys-des-Arg^9-BK. It is worth noting that B_1 receptors are not usually expressed under physiological conditions, but may be quickly upregulated under several inflammatory stimuli (see Calixto *et al*., 2000; 2001).

Lewis (1964) demonstrated for the first time that BK was able to evoke all classical signals of inflammation, such as increase in vascular permeability, oedema formation and pain. Soon after, many works pointed out that both B_1 and B_2 receptors are involved in the onset and maintenance of inflammatory and nociceptive alterations (Regoli & Barabé, 1980; Proud & Kaplan, 1988; Ahluwalia & Perretti, 1999; Calixto *et al*., 2000; 2001; Couture *et al*., 2001). For various reasons, research on the involvement of B_2 receptors in nociceptive and inflammatory states has progressed more quickly than that on B_1 receptors. Curiously, the first B_1 receptor antagonist was developed almost 10 years before the first B_2 receptor antagonist, but the characterization of B_1 receptors was delayed, probably due to its complex ability to be induced. Thus, the role of B_2, but not B_1 receptors, in inflammatory and nociceptive processes was widely explored during the 1980s and 1990s. This was favoured by the systematic development of selective peptidic B_2 receptor antagonists by the pharmaceutical companies, as at this time, the industry believed that B_2 receptors were more attractive as pharmacological targets (in comparison to B_1 receptors) for the possible treatment of several pathological states (Regoli

*Author for correspondence; E-mail: calixto@farmaco.ufsc.br or calixto3@terra.com.br
Advance online publication: 1 November 2004

Table 1 Selective kinin B_1 receptor antagonists

Name	*Structure*	*Main characteristics*	*Reference*
[Leu^8]-des-Arg^9-BK	[Leu^8]-des-Arg^9-BK	Peptidic antagonist, first-generation, widely employed for studying the involvement of B_1 receptors in inflammatory and nociceptive models. Mainly active *in vivo* when administered by topical or s.c. routes.	Regoli *et al.* (1977), Regoli & Barabé (1980)
Lys[Leu^8]-des-Arg^9-BK	Lys[Leu^8]-des-Arg^9-BK	Peptidic antagonist, first generation. Presents pharmacological actions similar to those described for [Leu^8]-des-Arg^9-BK. Has higher affinity for rabbit and human B_1 receptors.	Regoli & Barabé (1980), Drapeau *et al.* (1991)
[des-Arg^9]-HOE 140	Des-Arg^9-D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]	Peptidic antagonist, second generation. Presents increased potency and stability in relation to first generation antagonists.	Wirth *et al.* (1991)
[des-Arg^9]-NPC 17731	D-Arg-Arg-Pro-Hyp-Gly-Phe-Ser-DHype-(Transpropyl-Oic)	Peptidic antagonist, second generation. Only a few pharmacological studies have been conducted with this antagonist.	Cabrini & Calixto (1997), Vianna & Calixto (1998)
R-715	AcLys[D-βNal^7,Ile^8]des-Arg^9-BK	Peptidic antagonist, third generation. Has less susceptibility to proteolytic degradation than second generation antagonists. Several recent works have employed this compound to evaluate the involvement of B_1 receptors during pain and inflammation. Active when administered systemically by s.c., i.v. or i.p. routes.	Gobeil *et al.* (1996)
R-954	AcOrn[Oic2, (αMe)Phe^5,D-βNal^7, Ile^8] des-Arg^9-BK	Peptidic antagonist, third generation. Used at the same doses and schedules employed for R-715	Gobeil *et al.* (1996), Neugebauer *et al.* (2002)
B-9858	LysLys[Hyp^3,Igl^5,D-Igl^7,Oic^8] des-Arg^9-BK	Peptidic antagonist, third generation. Extremely potent and long-lasting B_1 receptor antagonist. Some reports indicate that this compound may present B_2 receptor antagonistic properties.	Stewart *et al.* (1996), Rhaleb *et al.* (1992), Larrivee *et al.* (2000)
B-9958	Lys-Lys-Arg-Pro-Hyp-Gly-CpG-Ser-Dtic-CpG	Peptidic antagonist, fourth generation. A highly potent and long-acting B_1 receptor antagonist, without any action on B_2 receptors.	Stewart *et al.* (1996), Gobeil *et al.* (1997)
Benzodiazepine antagonist		Nonpeptidic B_1 receptor antagonist. Presents *in vivo* efficacy against inflammatory pain when administered by the i.p. route.	Wood *et al.* (2003)
Dihydroquinoxalinone antagonist		Nonpeptidic antagonist. It presents higher affinity for human and rabbit B_1 receptors, with low affinity for rat B_1 receptor. *In vivo*, shows antinociceptive actions in a rabbit assay of hyperalgesia.	Su *et al.* (2003)
SSR240612	(2R)-2-[((3R)-3-(1,3-benzodioxol-5-yl)-3-{[(6-methoxy-2-naphtyl)sulfonyl]amino}propanoyl)amino]-3-(4-{[2R,6S)-2,6-dimethylpiperidinyl] methyl} phenyl)-*N*-isopropyl-*N*-methylpropanamide hydrochloride	Nonpeptidic B_1 receptor antagonist. The first B_1 receptor antagonist with proven efficacy when administered by oral route in several models of pain and inflammation.	Gougat *et al.* (2004)

et al., 1998; Stewart *et al*., 1999; Bock & Longmore, 2000). In addition, cloning studies with B_2 receptors were initiated as early as 1991, whereas the B_1 receptor was first cloned only in 1994 (see details below). Other factors have also largely contributed to the delay of the studies on B_1 receptors. Firstly, B_2 receptor knockout mice were generated in 1995 (Borkowski *et al*., 1995), while the generation of B_1 receptor knockout mice was only achieved later in 2000 (Pesquero *et al*., 2000). In addition, the development of nonpeptidic selective B_2 receptor antagonists was achieved almost 10 years before the synthesis of the first nonpeptidic selective B_1 receptor antagonist (see Table 1).

The former relevant *in vivo* studies regarding the role of B_1 receptors in pain and inflammation were initiated only after B_1 receptor cloning studies. At the same time, certain pioneer works provided convincing evidence that B_1 receptors could be functionally upregulated *in vivo* following proinflammatory or trauma stimuli (Perkins *et al*., 1993; Perkins & Kelly, 1993; Davis & Perkins, 1994; Campos & Calixto, 1995; Khasar *et al*., 1995; Perkins *et al*., 1995; Campos *et al*., 1996). The great progress in molecular biology had also made it possible to demonstrate that both B_1 receptor mRNA and protein expression could be markedly enhanced under many stressful conditions. Nowadays, signalling pathways underlying B_1 receptor induction during inflammatory and/or nociceptive alterations are better understood, even though various points in the cascade of B_1 receptor modulation still remain elusive. In addition, a major effort toward the development of selective B_1 receptor antagonists has been undertaken, mainly by the pharmaceutical companies. Most of this effort entails the screening of large compound libraries in order to identify more potent and selective antagonists (see Table 1). In the present review, we will discuss the main advances obtained in the last 5 years on understanding the role of B_1 receptors in pain and inflammation, with special emphasis on some pathophysiological alterations where B_1 receptor induction and/or activation might be of pertinent clinical interest. We will also call attention to some mechanisms involved in the upregulation of B_1 receptors during inflammatory and painful conditions.

The kinin B_1 receptor as the special one

There are, so far, several reasons for describing kinin the B_1 receptor as a special entity in relation to the classical well-known G protein-coupled receptors. Many particular characteristics draw the attention of researchers to B_1 receptors. Without doubt, the most interesting feature regarding this receptor is its complex pattern of expression. As described above, B_1 receptors are normally absent, but may be highly and rapidly upregulated following inflammatory stimuli. This is an unusual characteristic of G protein-coupled receptors. In fact, the pattern of induction of B_1 receptors resembles more closely to tyrosine-kinase-linked receptors. Although some literature data has demonstrated that other G-protein-coupled receptors can be also upregulated – these include the platelet-activating factor (PAF), the neurokinin NK-1, the protease-activated receptors 1 and 4 and occasionally even the kinin B_2 receptor – none of them present the same profile and complexity of induction observed for B_1 receptors (Marceau, 1995; Donaldson *et al*., 1997; Calixto *et al*., 2000; 2001). Interestingly, kinin B_1 receptors seem to be upregulated under the same conditions described for the inducible proinflammatory enzymes cyclooxygenase-2 (COX-2) and the inducible nitric oxide synthase (iNOS). In this way, the stimuli (and also the cellular signalling pathways) shown to be capable of increasing the expression of B_1 receptors are the same as those recognized for stimulating the upregulation of COX-2 and iNOS (Calixto *et al*., 2000; 2001). Furthermore, transcriptional and post-transcriptional tools required for B_1 receptors expression seem to be the same necessary for regulating COX-2 and iNOS enzymes (Zhou *et al*., 1998; 1999; Dixon *et al*., 2000; Haddad *et al*., 2000; Lasa *et al*., 2001; Dixon, 2004). At this moment, it is not easy to explain why a G-protein-coupled receptor behaves as a typical inducible enzyme and this constitutes a very interesting field of research that will probably receive much attention. Despite its inducible profile, it is important to mention that in some tissues, especially at the central nervous system, kinin B_1 receptors can be found constitutively expressed, a feature that is shared with the enzyme COX-2.

Some recent reports have indicated the existence of a crosstalk between B_1 and B_2 receptors. Therefore, evidence has also shown that persistent stimulation of B_2 receptors might result in B_1 receptor upregulation (Campos & Calixto, 1995; Phagoo *et al*., 1999; 2000; Campos *et al*., 2001; Todorov *et al*., 2003; Rashid *et al*., 2004). It is worth noting that stimulation of B_2 receptors leads to transient increases in Ca^{2+} concentration and to the fast desensitization of these receptors. Regarding B_1 receptors, they do not seem to be susceptible to desensitization mechanisms and their stimulation results in sustained elevations of Ca^{2+} concentration (Faussner *et al*., 1999; Marceau *et al*., 2002). Recent studies conducted with fluorescent conjugated proteins have demonstrated that B_1 receptors, in contrast to B_2 receptors, normally do not internalize following agonist stimulation, but they seem to translocate and aggregate (by a caveolae-mediated mechanism) after agonist binding, probably to facilitate the amplification of B_1 receptor-mediated responses. In addition, other relevant points are likely to contribute to the extension of this idea: (1) in some cases, B_1 receptor upregulation appears to be secondary to the downregulation of B_2 receptors; (2) some transduction pathways activated by B_2 receptors are known to be capable of inducing B_1 receptors; and (3) stimulation with both B_1 and B_2 receptor agonists may lead to B_1 receptors induction (Campos *et al*., 1996; 2001; Hayashi *et al*., 1998; Modéer *et al*., 1998; Pan *et al*., 1998; Phagoo *et al*., 1999; 2000). Finally, it has been established that B_1 and B_2 receptor genes are both located at the same chromosome of humans (chromosome 14), rats (chromosome 6) and mice (chromosome 12) (Chai *et al*., 1996; Cayla *et al*., 2002). This feature might also contribute to facilitating a balanced regulation of B_1 and B_2 receptors.

Molecular characterization of B_1 receptor

In the present section, we have remarked on some of the most important findings concerning the molecular biology studies carried out on kinin B_1 receptors.

When, McEachern *et al*. (1991) cloned the B_2 receptor-encoding cDNA from rats, Southern blot analysis indicated that if a distinct gene was responsible for encoding B_1 receptor, it would not be highly homologous to the B_2-enconding gene.

Later, Webb *et al*. (1994) presented important evidence suggesting that mRNA-encoding B_1 receptor was distinct in size and in coding sequence from that encoding B_2 receptor. At the same time, Menke *et al*. (1994) reported the first cloning expression of kinin B_1 receptor from the human embryonic lung fibroblast cDNA library. Afterwards, B_1 receptor has been cloned from rabbit (MacNeil *et al*., 1995), mouse (Pesquero *et al*., 1996), rat (Ni *et al*., 1998a), dog (Hess *et al*., 2001) and monkey (Hess *et al*., 2002). Differences in the amino-acid sequence of B_1 receptors from different species range between 69 and 97%. Greater degrees of homology are observed between mouse and rat (88%) and monkey and human (97%) receptors (Hess *et al*., 2002).

Pharmacological evidence has shown that human, rabbit, mouse and monkey receptors present higher affinity (until 1000 times) for Lys-des-Arg^9-BK in comparison to des-Arg^9-BK, whereas rat and mouse receptors present similar affinities for both B_1 receptor agonists. This difference is also observed for the B_1 receptor antagonists Lys-des-Arg^9-[Leu^8]-BK and des-Arg^9-[Leu^8]-BK (Regoli *et al*., 2001; Hess *et al*., 2002). Recent molecular studies have proposed that these differences in affinity rely on the heterogeneity of the extracellular domain IV of the B_1 receptor. Therefore, it has been proposed that N-terminal lysine preferentially binds to the extracellular domain IV in the human B_1 receptor (Fathy *et al*., 2000; Hess *et al*., 2002). From this, it is worth noting that B_1 receptor agonists and antagonists might present significant differences of affinity and potency depending on the species studied. This is mainly important for the development of antagonists and also for the preclinical studies: selective B_1 receptor antagonists with similar affinities for all species would be preferable.

Molecular biology studies have widely contributed for understanding the inducible characteristic of B_1 receptors. It has demonstrated that 5′-flanking region of B_1 receptor gene (located upstream from the initiation site) holds several putative transcriptional regulatory sequences, including the TATA box and positive and negative control elements, with all characteristics of a core promoter (Bachvarov *et al*., 1996; Yang & Polgar, 1996; Ni *et al*., 1998b; Schanstra *et al*., 1998; Angers *et al*., 2000). Ni *et al*. (1998a, b) and Schanstra *et al*. (1998) have reported an NF-κB-binding domain, although the precise position remains elusive. Ni *et al*. (1998b) have further suggested putative sequences for AP-1 and CREB regulation. These pieces of evidence have largely contributed to the recent advances obtained in the comprehension of the mechanisms and signalling pathways involved in the upregulation of B_1 receptors (see the next section of this review).

Other relevant molecular studies need to be mentioned. For instance, footprint analysis of the promoter region has revealed that it is possibly bound by several sequence-specific DNA binding proteins, like GATA-1, PEA3, AP-1, CAAT, Sp1, Pit-1a, Oct-1 and CREB (Angers *et al*., 2000). With the construction of a minigene (1.8 kb promoter plus exon 1, 1.5 kb intron 1, exon 2 and intron 2), it has been verified that motifs not included in the promoter sequence, such as 5′-UTR and intronic regions, are also required for induction of this gene (Yang *et al*., 2001b). The cotransfection of c-Jun with the minigene causes an increase of the promoter activity in a concentration-dependent manner, probably through interaction with multiple AP-1 sites. On the other hand, the tumour suppressor protein p53 has been demonstrated to suppress B_1 receptor promoter activity in a concentration-dependent manner (Yang *et al*., 2001a). Finally, it has been suggested that the 3′-untranslated region (3′-UTR) containing a poly-adenylation signal in the B_1 receptor gene might be involved in the mRNA stability processes and its ultimate expression, since its absence directly reflects on the half-life of B_1 receptor mRNA (Zhou *et al*., 1999). These data further extends the notion that the kinin B_1 receptor represents an unusual protein-G-coupled receptor which can be modulated by the activation of highly specialized mechanisms.

Main mechanisms involved in the upregulation of B_1 receptors

The induction of B_1 receptors has been associated with the production of inflammatory mediators, stimulation of inflammatory cells, and, finally, activation of several intracellular signalling pathways. Understanding which intracellular signalling pathways mediate B_1 receptor induction is of high interest in the discovery of therapeutic targets. In this section, we have summarized the recent advances made on the mechanisms involved in B_1 receptor upregulation in several inflammatory models (Figure 1).

Primary studies concerning B_1 receptor expression have reported the importance of *in vitro* incubation time in the increase of functional responses to B_1 receptor agonists in many isolated organ preparations. These studies have also shown that the time-dependent B_1 receptor upregulation is sensitive to RNA and protein synthesis inhibitors, suggesting its dependence on *de novo* protein synthesis (see Regoli &

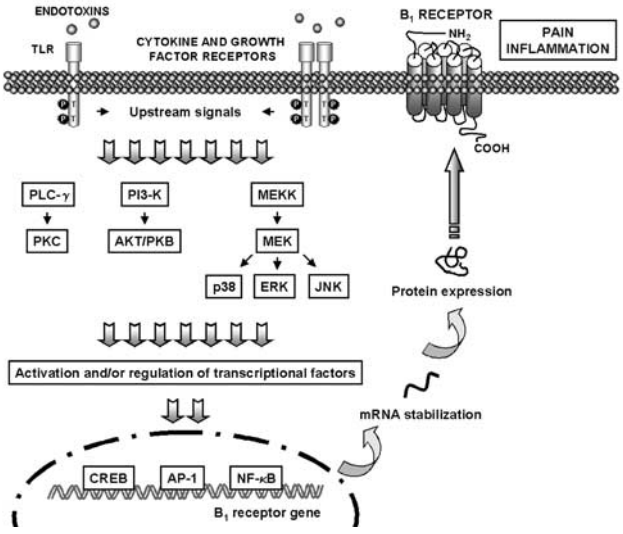


Figure 1 Possible mechanisms underlying B_1 receptor upregulation. Stressful stimuli (such as infection and inflammation) are able to activate several signalling pathways including MAPKs, PKC and PI3-K, which in turn might modulate transcriptional factors (e.g. NF-κB, AP-1 and CREB), and are supposed to be crucial for the regulation of B_1 receptor expression. AP-1, activating protein-1; AKT/PKB, protein kinase B; CREB, c-AMP response element-binding; ERK, extracellular signal-regulated kinase; JNK, c-jun NH_2-terminal kinase; MAPK, mitogen-activated protein kinase; MEKK, mitogen-activated protein kinase kinase kinase; MEK, mitogen-activated protein kinase kinase; NF-κB, nuclear factor-κB; PI3-K, phosphatidylinositol-3 kinase; PLC, phospholipase C; PKC, protein kinase C; TLR, Toll-like receptors.

Table 2 Some possible pathways for pharmacological modulation of B_1 receptor upregulation

Target	*Pharmacological modulation*
mRNA transcription	Actinomycin D
Translocation	Brefeldin A
Translation/ *N*-glycosylation	Cycloheximide
	Tunicamycin
Inhibition of ribonuclease	MAPK inhibitors
Interference with mRNA unstable regions (AUUUA)	Dexamethasone[a]
NF-κB activation	Inhibitors of IκB kinase (PDTC, BAY 117082)
	Proteasome activity inhibitors (TLCK, MG 132)
Cytokine modulation	Inhibitors of cytokines synthesis, antibodies, recombinant receptors
Protein kinase inhibitors	Blockers of TKs, PKC and MAPKs, etc.

For further details, refer to the following reviews: Marceau *et al*. (1998), Calixto *et al*. (2000; 2001), Couture *et al*. (2001).
[a]Dexamethasone is able to block B_1 receptor induction at several points.

Barabé, 1980; Marceau *et al*., 1998). Although the elucidation of the pivotal molecular mechanisms implicated in the regulation of these processes has only just begun, recent literature data has already shown that B_1 receptor upregulation can be modulated at different levels. The main pharmacological strategies applied to the study of the mechanisms involved in the upregulation of B_1 receptors are listed in Table 2.

The determination of distinct signalling pathways involved in the regulation of B_1 receptor expression has been accomplished by several approaches. Gene sequence analysis has revealed that the B_1 receptor promoter presents numerous sequence-specific motifs for different DNA binding proteins, which indicates that B_1 receptor expression could be modulated by several transcriptional factors (Bachvarov *et al*., 1996; Yang & Polgar, 1996; Ni *et al*., 1998b; Schanstra *et al*., 1998; Angers *et al*., 2000). In this context, there is now a considerable amount of experimental evidence indicating that NF-κB, a transcriptional factor that governs the expression of gene-encoding cytokines, chemokines, growth factors, cell adhesion molecules, and some acute phase proteins in health and various pathological states, plays a pivotal role in the regulation of B_1 receptor induction. It has been demonstrated that specific NF-κB blockers are able to prevent B_1 receptor upregulation both *in vitro* (Sardi *et al*., 2000; 2002; Medeiros *et al*., 2001; 2004; Phagoo *et al*., 2001; Sabourin *et al*., 2002) and *in vivo* (Campos *et al*., 1999; Fernandes *et al*., 2003; Passos *et al*., 2004). Indeed, studies conducted by Ni *et al*. (1998b) have revealed the existence of an NF-κB-like binding site on the human B_1 receptor promoter, which seems to be essential for the control of receptor transcription following exposure to certain inflammatory agents such as IL-1β, Tumour necrosis factor α (TNFα) or lipopolysaccharide (LPS). Furthermore, it has been demonstrated that in cultured human lung fibroblasts, B_1 receptor upregulation induced by IL-1β is modulated at the transcriptional level, in a process mediated by the activation of NF-κB (Schanstra *et al*., 1998). Recently, we have verified that tissue damage in the rat portal vein is also able to induce the expression of B_1 receptor, in a process involving IκBα degradation and the subsequent translocation of NF-κB into the nucleus with an increase in NF-κB DNA-binding activity (Medeiros *et al*., 2004).

Protein phosphorylation has also been reported to exert an important role in the regulation of many cellular processes, including gene expression and protein synthesis (Hunter, 1995). Thus, it has been suggested that the activation of some classes of protein kinases is essential for the induction of the B_1 receptor. In fact, Larriveé *et al*. (1998) were the first to show that both spontaneous and IL-1β-induced B_1 receptor upregulation in rabbit aorta are greatly sensitive to inhibitors of tyrosine kinase, extracellular signal-regulated kinase (ERK) or p38 MAPK pathway. In addition, it has been demonstrated that protein kinase C (PKC) activation also critically regulates the B_1 receptor mRNA expression in cultured human lung fibroblasts (Schanstra *et al*., 1998). Other studies have confirmed and extended these previous data. It has been shown that *in vivo* B_1 receptor upregulation induced by inflammatory cytokines IL-1β or TNFα involves the activation of PKC, tyrosine kinase and ERK (Campos *et al*., 1999). Moreover, PKC is also involved in the B_1 receptor upregulation in different isolated tissue preparations (Medeiros *et al*., 2001; Ueno *et al*., 2002). Of interest is the recent data demonstrating that p38 MAPK and c-Jun NH_2-terminal kinase (JNK) are critically involved in the regulation of B_1 receptor mRNA expression induced by vascular tissue trauma (Medeiros *et al*., 2004). Altogether, these data clearly demonstrates the importance of different classes of protein kinases in the modulation of B_1 receptor induction. However, the precise mechanisms through which this regulation occurs is currently a matter of debate. Recently, several studies have suggested that protein kinases may regulate the expression of certain inflammatory genes, acting at both transcriptional and post-transcriptional levels. In addition, the activation of protein kinases has been associated to the increased activation of different transcriptional factors and to the regulation of the half-lives of many inflammatory mediator mRNAs (e.g. COX-2, TNFα and IL-1β) (Dunn *et al*., 2002; Clark *et al*., 2003; Lahti *et al*., 2003; Yang *et al*., 2003). Following this, a recent study has suggested that B_1 receptor expression could also be regulated through the enhancement of mRNA stabilization (Zhou *et al*., 1999). This notion has been extended further by Haddad *et al*. (2000), who demonstrated that B_1 receptor induction in human lung fibroblasts (HEL 299), following incubation with IL-1β and TNFα, is largely dependent on post-transcriptional mechanisms, being this effect greatly sensitive to dexamethasone and p38 MAPK inhibitors. Therefore, it is tempting to suggest that the regulation of B_1 receptor might be controlled both transcriptionally and post-transcriptionally. Therefore, the activation of protein kinases could certainly be involved in the enhancement of both transcriptional factor activity and B_1 receptor mRNA stability.

However, additional studies are still required to better understand and confirm this hypothesis.

The B_1 receptor and its role in development and maintenance of inflammatory diseases

Several pieces of evidence indicate that B_1 receptor induction plays a critical role in inflammatory pathological states. In the next sections, we have highlighted the main recent findings, which implicate B_1 receptors in inflammatory models. Data described herein is summarized in Table 3.

B_1 receptors and infection

The effects of treatment with bacterial endotoxin on B_1 receptor upregulation (both functional and molecular) have been extensively reported (see Marceau *et al*., 1998; Calixto *et al*., 2000; 2001). Likewise, infection seems to constitute a classical signal known for its ability to induce B_1 receptors. A very interesting study conducted by DeBlois & Horlick (2001) suggested that the haemodynamic and inflammatory responses observed after systemic LPS treatment in green monkeys are largely mediated by B_1 activation. The authors reported that the selective B_1 receptor agonist des-Arg^9-BK caused a marked fall in blood pressure, accompanied by an increase in heart rate only in monkeys treated with LPS. In the same way, des-Arg^9-BK-induced skin oedema formation was observed solely in monkeys treated with LPS. The work of Deblois & Horlick (2001) was the first experimental evidence indicating the upregulation of B_1 receptors in non-human primates. In addition, it has been shown that gene deletion of B_1 receptors prevents the endotoxic shock in mice (Pesquero *et al*., 2000). Extending this idea, Ni *et al*. (2003) demonstrated that transgenic overexpression of B_1 receptors renders mice more susceptible to endotoxic shock and greatly potentializes the oedematogenic responses caused by the inflammatory agent carrageenan. Based on these results, it is tempting to speculate that the new generation of nonpeptidic B_1 receptor antagonists (see Table 1) could represent a valuable tool for the control/treatment of situations associated with disseminated infection, such as sepsis.

Studies conducted with rodents confirm and further extend the proposition that endotoxins classically induce B_1 receptors. For instance, B_1 receptors seem to exert an important role in vascular changes and hypotension induced by LPS from *Escherichia coli* in rats (McLean *et al*., 1999). LPS has also been found to markedly increase plasma extravasation induced by the selective B_1 receptor agonist des-Arg^9-BK in the duodenum, ileum and trachea of rats (Wille *et al*., 2001). More recently, Passos *et al*. (2004) have demonstrated the occurrence of kinin B_1 receptor upregulation after the local administration of LPS into the rat paw, in a process involving activation of the transcriptional factor NF-κB, neutrophil migration, and release of inflammatory mediators (e.g. IL-1β, TNFα and PAF). Another recent and relevant publication has shown that incubation of tracheal organ culture for 1 or 4 days with the bacterial products LPS and polyinosininc polycytidylic acid enhances BK- and des-Arg^9-BK-mediated contractile responses by mechanisms largely dependent on protein synthesis, MAPK activation and NF-κB activation (Bachar *et al*., 2004).

More recently, it has been widely demonstrated that LPS and other endotoxins induce several inflammatory alterations by binding to a family of receptors denoted toll-like receptors (TLR). In the case of LPS, it has been reported that their actions are mediated by the activation of TLR4 receptors and the consequent stimulation of NF-κB and several protein kinases, associated with the release of proinflammatory cytokines (e.g. IL-1β or TNFα) (for more details see Beg, 2002; Dobrovolskaia & Vogel, 2002; Akira *et al*., 2003). In this regard, all these signalling pathways have now been demonstrated to be involved in the process of B_1 receptor upregulation.

Another aspect which deserves attention is the recent evidence implicating the B_1 receptor as an adjuvant for *Trypanosoma cruzi* infection. Recent data have shown that intradermal injection of trypomastigotes into the mouse paw results in a long-lasting oedema formation: the first phase of oedematogenic response (3 h) is blocked by the B_2 receptor antagonist Hoe 140, whereas the late phase (24 h) can be prevented by treatment with the selective B_1 receptor antagonist B9858. Late-phase oedema has also been abolished in B_1 receptor knockout mice (Scharfstein *et al*., 2000; Todorov *et al*., 2003). These results have led authors to conclude that B_1 receptors are upregulated following local infection with *T. cruzi* and to point out the importance of B_1 receptors for the progress of Chagas disease. Also of interest are the results from the same group showing that B_1 receptors (as well as B_2 receptors) seem to facilitate the invasion of

Table 3 Inflammatory stimuli associated with B_1 receptors up-regulation

Stimuli	*References*
Infectious stimuli – LPS, polyinosininc polycytidylic acid, trypomastigotes from *T. cruzi*	McLean *et al*. (1999), Deblois & Horkick (2001); Wille *et al*. (2001), Ni *et al*. (2003), Bachar *et al*. (2004), Passos *et al*. (2004), Todorov *et al*. (2003)
Myocardial, intestinal and pancreatic ischemia–reperfusion injury	Mazenot *et al*. (2001), Lagneux *et al*. (2002), Kuebler *et al*. (2003), Souza *et al*. (2004)
Airway stimulation – challenge with OVA to sensitized animals; intrapleural injection of carrageenan; rhinitis	Huang *et al*. (1999), Christiansen *et al*. (2002), Hayashi *et al*. (2002), Eric *et al*. (2003), Landgraf *et al*. (2003)
Type I STZ-induced diabetes	Campos *et al*. (2001), Couture *et al*. (2001), Gabra & Sirois (2002), Abdouh *et al*. (2003), Vianna *et al*. (2003)
Some kinds of cancer – astrocytic tumour; prostatic cancer	Raidoo *et al*. (1999), Barki-Harrington *et al*. (2003), Taub *et al*. (2003)
Cystitis – induced by cyclophosphamide	Bélichard *et al*. (1999)
Injection of some phlogistic agents – zymosan, CFA, carrageenan and kaolin, PAF, the proinflammatory cytokines IL-1β and TNFα	Campos *et al*. (1999), Bélichard *et al*. (2000), Fernandes *et al*. (2003), Fox *et al*. (2003), Seegers *et al*. (2004)

T. cruzi to the host cells. These conclusions are based on evidence indicating that both B_1 and B_2 receptor antagonists are effective in preventing the parasitic infection of Chinese Hamster ovary cells which coexpress B_1 and B_2 receptors (Todorov *et al*., 2003).

Ischaemia–reperfusion lesions

Literature data have suggested that B_1 receptors might exert a pivotal role in postischaemic situations. Thus, it has been shown that B_1 receptors can be induced in the endothelial cells of rabbits (Mazenot *et al*., 2001) or in the hearts of mice (Lagneux *et al*., 2002) submitted to myocardial ischaemia–reperfusion. In the rabbit model, the vascular effects evoked by ischemia–reperfusion can be significantly reduced by a selective B_1 receptor antagonist – [Leu^8]-des-Arg^9-BK (Mazenot *et al*., 2001). In mice, either pharmacological blocking with selective B_1 antagonist [Leu^8]-des-Arg^9-BK or gene deletion of B_1 receptors largely diminishes the infarction's extension, an effect that further reinforces the relevance of B_1 receptors under these conditions. Kuebler *et al*. (2003) have affirmed that B_1 receptors also seem relevant in the ischaemia–reperfusion of rat pancreas. The authors have demonstrated, by means of binding studies, that B_1 receptor expression is augmented by about 22-fold after pancreatic ischaemia–reperfusion injury. They have also shown that the B_1 receptor antagonist CP-0298 (alone or in combination with the B_2 antagonist CP-0597) significantly reduces the number of adherent leukocytes in postcapillary venules (Kuebler *et al*., 2003). Another recent contribution has demonstrated that intestinal ischaemia–reperfusion injury results in a striking increase of B_1 receptor expression in rats (Souza *et al*., 2004). In this model, B_1 receptor upregulation is probably secondary to the activation of B_2 receptors, as it can be significantly blocked by selective B_2 receptor antagonists. It should be noted that all the main inflammatory parameters such as, changes of vascular permeability, neutrophil migration and cytokines production, are completely abolished in B_1 receptor knockout mice.

Ischaemia–reperfusion lesions are associated with a marked migration of inflammatory cells, cytokine generation and activation of kinases and transcriptional factors (e.g. p38 MAPK and NF-κB, respectively) (Jones *et al*., 2003; Lien *et al*., 2003). It is noteworthy that all these signalling pathways have been correlated with the process of B_1 receptor upregulation. Taking into account these considerations, it is tempting to suggest that B_1 receptors modulation could be a very interesting target for the control and/or treatment of the alterations observed after ischaemia–reperfusion injury.

Inflammation of airways

Kinins seem to exert a central role in the control and maintenance of the inflammatory states of the airways. The relevance of B_2 receptors has been widely investigated, but only a few publications have remarked on the role of B_1 receptors in the airways. Thus, some recent reports have pointed out the critical relevance of kinin B_1 receptors in allergen-induced bronchial hyper-responsiveness. Huang *et al*. (1999) have demonstrated that challenge with ovalbumin (OVA) results in a marked increase of B_1 receptor mRNA expression in the lungs of previously sensitized rats. This increase peaks between 2 and 6 h and remains elevated up to 24 h following the challenge with OVA. Another recent report has shown that allergic lung inflammation in ovalbumin-sensitized mice is significantly diminished by the systemic treatment of animals with the selective B_1 receptor antagonist R-954 (Landgraf *et al*., 2003). The same effect of R-954 has been observed in immunocomplex-induced lung inflammation (Landgraf *et al*., 2004). These findings were extended and confirmed by Eric *et al*. (2003), who has demonstrated that treatment with B_1 receptor antagonists R-715 or R-954 significantly reduces lung eosinophilia evoked by OVA in sensitized mice. Importantly, allergen-evoked hyperalgesia in OVA-sensitized rats has been found to be markedly reduced by local treatment with [Leu^8]-des-Arg^9-BK (Lavich *et al*., 2003). In addition, B_1 receptor mRNA levels are consistently increased in carrageenan-induced pleurisy at 3 h, remaining elevated up to 5 h after challenge in rats (Hayashi *et al*., 2002). The same authors have also reported that administration of either of B_1 receptor antagonists [Leu^8]-des-Arg^9-BK or des-Arg^9-D-Arg-[Hyp^3, Thi^5, D-Tic^7, Oic^8]-BK is able to prevent carrageenan-induced exudation. Furthermore, Pesquero *et al*. (2000) have reported that disruption of the B_1R gene in mice results in a marked reduction of the inflammatory responses induced by carrageenan in the pleurisy model in mice. Interestingly, the relevance of B_1 receptors in human airway inflammation has recently been reported by Christiansen *et al*. (2002). These authors have shown that nasal tissue samples from allergic rhinitis present a significantly higher B_1 receptor mRNA expression in comparison to tissue samples obtained from normal subjects.

Some *in vitro* studies conducted with cell lines from airways confirm the data obtained *in vivo* and reinforce the notion that B_1 receptors are critical for the pathological conditions affecting the airways. For instance, B_1 receptors are upregulated by the incubation of IL-1β and TNFα in human embryonic lung fibroblasts HEL 299 (Haddad *et al*., 2000). Likewise, Phagoo *et al*. (2001) have shown that stimulation of human lung fibroblasts with IL-1β or des-Arg^9-bradykinin results in a marked upregulation of B_1 receptors. Moreover, in pulmonary A549 and human bronchial epithelial cells, treatment with the proinflammatory cytokines IL-1β or TNFα causes a remarkable enhancement of B_1R mRNA expression (Newton *et al*., 2002). Very recently, an interesting study conducted by Zhang *et al*. (2004) has demonstrated that the incubation of mouse tracheal segments (for at least 4 days) results in both functional and molecular upregulation of B_1 receptors, these responses being further enhanced by the continuous exposure to the proinflammatory cytokine TNFα. This work also demonstrates that the upregulation of B_1 receptors following TNFα exposure is largely dependent on the activation of MAPKs JNK and ERK 1/2.

Diabetes and B_1 receptor modulation

Type I diabetes constitutes an autoimmune disorder in which insulin production is affected by the destruction of pancreatic β-cells. It has been well documented that inflammatory alterations exert a critical role in the development of diabetes – a massive infiltration of inflammatory cells, the elevated production of proinflammatory cytokines (e.g. IL-1β TNFα) and the generation of free radicals are the main features related to the destruction of β-cells in the pancreas (see Couture *et al*.,

2001; Hohmeier *et al*., 2003). The progress of the diabetes and the constant fluctuation of glucose levels are associated with changes in vascular structure (mainly related to the endothelium) and the production of vasoactive substances. In this context, the kinin system has often been implicated in the pathophysiology of diabetes (Garcia Leme *et al*., 1973; Couture *et al*., 2001). Moreover, the extent to which diabetes may influence the modulation of B_1 receptors has been the matter of several recent publications. Campos *et al*. (2001) have shown that the induction of diabetes with the nitrosamine streptozotocin (STZ) results in a long-lasting (8 weeks) functional upregulation of B_1 receptors in the rat paw, as indicated by a marked increase in the oedematogenic response induced by the selective B_1 receptor agonist des-Arg^9-BK in diabetic rats. This effect is associated with a significant reduction of B_2 receptor-mediated paw oedema. Based on these results, it is possible to assume that during diabetes development, inducible B_1 receptors may be overexpressed, whereas constitutive B_2 receptors are downregulated. The functional induction of B_1 receptors has also been described in the pleural cavity of rats following STZ treatment (Vianna *et al*., 2003). In this model, both mononuclear and neutrophil influx in response to intrapleural injection of des-Arg^9-BK has been found to be markedly enhanced in diabetic rats. In addition, autoradiographic B_1 receptors binding specific sites are significantly augmented in the lungs of STZ (4 days)-treated rats, as compared with control animals (Couture *et al*., 2001; Vianna *et al*., 2003). It has further been demonstrated that intrathecal injection of des-Arg^9-BK results in a biphasic nociceptive response following acute (24 h) administration of STZ (Couture *et al*., 2001). The importance of B_1 receptors in diabetes has also been shown by a series of studies indicating that systemic treatment with the highly selective B_1 receptor antagonists R-715 and R-954 consistently prevents thermal hyperalgesia induced by STZ (Gabra & Sirois, 2002; 2003a, b). Recently, Abdouh *et al*. (2003) have suggested a potential role for B_1 receptors in diabetes-associated retinopathies: a marked increase in autoradiographic B_1 receptor-specific binding sites, associated with the enhancement of des-Arg^9-BK-induced vasodilatation, has been observed in the retinas of STZ (4–21 days)-treated rats. In this regard, it is possible to propose that selective B_1 receptor antagonists would constitute a very attractive strategy for the control of some of the symptoms associated with diabetes, namely inflammatory and neuropathic complications.

Kinin B_1 receptors and cancer

An increasing body of evidence has emerged indicating that kinins and their receptors appear to be involved in cancer (Mahabeer & Bhoola, 2000; Stewart, 2003). The known mitogenic properties of kinins and their ability to activate tyrosine and MAP-kinase cascades could explain, at least in part, the effects of kinins in tumour growth and migration (Mahabeer & Bhoola, 2000; Bhoola *et al*., 2001; Stewart, 2003). In addition, as cancer growth and metastasis are critically dependent on the activation of inflammatory pathways, it is possible to conclude that B_1 receptor upregulation could play an important role in this scenario. Most publications regarding the participation of kinin receptors in cancer indicate a prevalent role for B_2 receptors. Thus, immunohistochemical and autoradiographic studies have pointed out a marked distribution of B_2 receptors in several human and mouse tumour cells (Wang *et al*., 2001; Wu *et al*., 2002). Some studies conducted with MG63 human osteosarcoma cells and mice bearing sarcoma 180 cells have discarded a role for B_1 receptors in cancer (Wang *et al*., 2001; Ishihara *et al*., 2001; 2002). On the other hand, B_1 receptors immunoreactivity has been found to be markedly increased in astrocytes and endothelial cells, as well as in the stromal blood vessels from human astrocytic tumour biopsies (Raidoo *et al*., 1999). In addition, Barki-Harrington *et al*. (2003) have reported that both B_1 and B_2 receptors activation seem to be linked with the mitogenic signalling in androgen-insensitive prostate cancer PC3 cells. This evidence has been further extended by recent results showing that B_1 receptors are present in prostatic intraepithelial neoplasia and malignant lesions, but not in benign prostate tissues (Taub *et al*., 2003). These data permit us to suggest that kinin B_1 receptor antagonists might be potentially useful as adjuvant therapies in some types of cancer. The mechanisms underlying the precise role of B_1 receptors in cancer remain to be investigated and constitute a promising and growing area of research.

Participation of B_1 receptors in other inflammatory states

The modulation of B_1 receptors and their participation in other inflammatory alterations has been demonstrated by several additional contributions. Thus, in the model of cystitis induced by cyclophosphamide in rats, B_1 receptor selective agonists cause a marked contractile response of the urinary bladder, an effect that is not observed in naïve animals (Bélichard *et al*., 1999). Also, a consistent and progressive increase of B_1R mRNA expression in the urinary bladder for up to 48 h following cyclophosphamide treatment has been reported (Bélichard *et al*., 1999). These data suggest that B_1 receptor induction and activation could constitute an important event during the development of cystitis.

In the acute and severe synovitis induced by intra-articular injection of carrageenan and kaolin, a significant increase in B_1R mRNA expression has been observed in the rat knee synovia (Seegers *et al*., 2004). Sainz *et al*. (2004) have reported that in peptidoglycan–polysaccharide-induced arthritis in rats, the selective B_1 receptor antagonist B9858 causes a significant decrease in lymphocyte homing and leukocyte transmigration.

Kinin-induced rat paw oedema is a classical *in vivo* model of inflammation that has been widely used for studying B_1 receptor upregulation. In this model, B_2 receptor agonists induce a marked oedema formation in naïve animals, while the injection of selective B_1 agonists evokes only slight alterations in paw volume. However, systemic or local treatment with several distinct inflammatory stimuli evokes a considerable increase in B_1 receptor-mediated paw oedema. Of note is data showing that acute local treatment with the proinflammatory cytokines IL-1β and TNFα (15–120 min) results in a rapid-onset and time-dependent increase in rat paw oedema caused by the selective B_1 receptor agonists des-Arg^9-BK and des-Arg^{10}-kallidin (Campos *et al*., 1998). The effects of IL-1β and TNFα in the functional upregulation of B_1 receptors in the rat paw have been found to be modulated by the secondary production of inflammatory cytokines, by the activation of several protein kinases, and also by the transcriptional factor NF-κB (Campos *et al*., 1998; 1999). Participation of NF-κB activation in the upregulation of des-Arg^9-BK-induced paw

oedema has also been evidenced 7 days after the surgical removal of adrenal glands (Cabrini *et al*., 2001). Additional evidence has indicated that intraplantar injection of IL-1β induces a striking augmentation of B_1 receptor mRNA expression in the rat paw (Campos *et al*., 2002). In the same publication, it was proposed that B_1 receptor upregulation was directly linked to the activation of chemotatic mediators and neutrophil migration. Likewise, Fernandes *et al*. (2003) have recently shown that PAF, a known chemotatic agent, was capable of inducing both functional and molecular upregulation of B_1 receptors when administered locally (6 h prior) into the rat paw. The mechanisms involved in PAF effects are largely related to the stimulation of PAF receptors, NF-κB activation, and neutrophil migration. Similar findings have been described by Passos *et al*. (2004) following treatment (12 h) with LPS in the rat paw. Recently, Gougat *et al*. (2004) have demonstrated that des-Arg^9-BK produced a marked oedema formation in mice locally pretreated with IL-1β (5 ng/paw, 40 min before). Interestingly, they report that des-Arg^9-BK-induced mouse oedema was dose-dependently inhibited by the systemic treatment (by oral or i.p. routes) with the new nonpeptidic B_1 receptor antagonist SSR240612.

Participation of kinin B_1 receptors in painful processes

Pain may be defined as an unpleasant sensorial and emotional experience associated with actual or perceived tissue damage by a noxious (damaging) stimulus (Watkins & Maier, 2003). As the emotional component of pain is very difficult to measure, especially in experimental animals, the sensorial component of pain (nociception) is more commonly assessed in preclinical and clinical pain studies. BK is among the most potent endogenous algogen substances, and its role in nociceptive processes has been extensively reviewed (Dray & Perkins, 1997; Calixto *et al*., 2000; 2001; Couture *et al*., 2001). BK, acting at B_2 kinin receptors, is able to produce excitation and sensitization of the free endings of C- and Aδ-primary afferent fibres (nociceptors) leading to the production of overt nociception (stimuli-independent pain), hyperalgesia (exaggerated response to a painful stimulus) and allodynia (pain produced by a previously innocuous stimulus) in experimental animals and humans. The activation of B_2 receptors triggers several well-known signalling pathways to produce depolarization and lowering of the threshold of the nociceptors, including phospholipase C and A_2, protein kinase C, formation of COX and lypooxygenase metabolites from arachidonic acid, and activation of vanilloid receptors and tetrodotoxin-resistant sodium currents (Dray & Perkins, 1997; Premkumar & Ahern, 2000; Ferreira *et al*., 2004). On the other hand, the participation of kinin B_1 receptors in pain transmission is a relatively new area of research and is even less explored. B_1 receptors have generally been implicated in the modulation of the persistent and chronic inflammatory hyperalgesia induced by different agents, including cytokines (IL-1β, IL-2 and IL-8, but not TNFα and IL-6), bacterial components (LPS, CFA and *Mycobacterium bovis* bacillus Calmette–Guerin – BCG), irritants (carrageenan and capsaicin), ultra-violet irradiation, and substance P (Perkins & Kelly, 1993; Perkins *et al*., 1993; Davis & Perkins, 1994; Khasar *et al*., 1995; Rupniak *et al*., 1997; De Campos *et al*., 1998; Poole *et al*., 1999; Ganju *et al*., 2001; see for review: Dray & Perkins, 1997; Calixto *et al*., 2000; 2001). However, recent data regarding the constitutive expression of the B_1 receptor in sensory neurones and the participation of B_1 receptors in chronic models of inflammatory and neuropathic pain (especially using knockout mice and selective nonpeptide receptor antagonists) has dramatically improved our comprehension about the critical role of B_1 receptor activation during painful processes. Thus, in the next section, we will focus our attention on these novel ideas and we will try to demonstrate the potential of the B_1 receptor as a new and relevant target for the development of a new class of analgesic drugs.

Expression of B_1 receptors in pain-transmitting neurones

Pain is produced by the stimulation of small-diameter primary afferent fibres that innervate regions of the head and body and arise from cell bodies in trigeminal and dorsal root ganglia (DRG), respectively (Julius & Basbaum, 2001). In a first attempt to identify B_1 receptors in sensory neurons, Davis *et al*. (1996) were unable to detect specific any binding to [^{3}H]-des-Arg^{10}-kallidin rat DRG cultured for 7–8 days. Afterwards, expression of B_1 receptor mRNA was detected in freshly isolated mouse and rat DRG by means of RT–PCR (Seabrook *et al*., 1997; Levy & Zochodne, 2000; Yamaguchi-Sase *et al*., 2003). The constitutive expression of B_1 receptors in rat and mouse sensory neurones was further confirmed by the use of immunohistochemical staining (Ma *et al*., 2000; Wotherspoon & Winter, 2000). Furthermore, peripheral B_1-containing neurons were found in peripheral nerve terminals, such as those that innervate rat urinary bladder (Wotherspoon & Winter, 2000). B_1 receptor staining was also localized in rat, mouse and monkey trigeminal and DRG ganglia (Ma *et al*., 2000; Wotherspoon & Winter, 2000; Shughrue *et al*., 2003; Rashid *et al*., 2004). B_1 receptors were predominantly expressed by small diameter DRG neurones, colocalized with calcitonin gene-related peptide or isolectin B4 (Ma, 2001).

It has been well demonstrated that both B_1 receptor mRNA and protein are constitutively present in the spinal cord of rats, mice, monkeys and humans (Couture & Lindsey, 2000; Wotherspoon & Winter, 2000; Ma & Heavens, 2001; Shughrue *et al*., 2003). It is worth noting that B_1 receptor immunoreactivity has been identified in the superficial layers of the dorsal horn, confined mainly to the spinal terminals of primary afferent fibres (Couture & Lindsey, 2000; Wotherspoon & Winter, 2000; Ma & Heavens, 2001). Subsets of dorsal horn neurons project axons and transmit pain messages to higher brain structures related by the somatic, affective and autonomic responses to pain (Hunt & Mantyh, 2001). Of interest is the fact that basal B_1 receptor expression has been described in several structures related to pain transmission and modulation, including the somatosensory cortex and thalamus (Ongali *et al*., 2003; Shughrue *et al*., 2003). However, the function of B_1 receptors in these regions remains obscure.

Role of kinin B_1 receptor in acute and chronic painful processes

Much about the earlier studies on the role of B_1 receptors in physiological and pathological processes has been elucidated on the basis of functional studies or the use of selective receptor antagonists. However, because of the problems of

selectivity and agonist activity, and the rapid degradation of some of the antagonists, advances in understanding the role played by B_1 receptors in most physiological and pathological processes has been hampered. The generation of the B_1 knockout mouse has made it possible to expand our current knowledge regarding the contribution of this receptor in nociceptive processes (Pesquero *et al*., 2000). B_1 receptor-deficient mice present hypoalgesia against the acute overt nociception induced by capsaicin or formalin and by high intensity heat stimuli (Pesquero *et al*., 2000). This evidence confirms and extends previous data that demonstrates the role of B_1 receptor in acute pain. For instance, intraplantar or systemic treatment with the selective B_1 receptor antagonist des-Arg^9-Leu^8-BK was found to be capable of reducing capsaicin, glutamate, and first-phase of formalin-induced pain (Shibata *et al*., 1989; Corrêa & Calixto, 1993; Sufka & Roach, 1996; Beirith *et al*., 2003; J.B. Calixto, unpublished results). Since the nociceptive behaviours of the former tests are very short (lasting up to 10 min), it seems improbable that B_1 receptor expression depends on *de novo* protein synthesis (see details above) and the involvement of constitutively expressed receptors is indicated. However, the peripheral injection of B_1 receptor agonists rarely induces nociception in naïve animals (Perkins & Kelly, 1994; Khasar *et al*., 1995; Ganju *et al*., 2001; Fox *et al*., 2003; Ferreira *et al*., 2004). Moreover, so far, there is no functional evidence showing that B_1 receptor agonists directly activate peripheral terminals or the cell bodies of sensory neurones (Dray *et al*., 1992; Davis *et al*., 1996; Seabrook *et al*., 1997; Brand *et al*., 2001). Thus, it has been suggested that B_1 receptors on the peripheral sensory nerve terminals need some stimulation to prime their nociceptive action (Ma, 2001). In fact, des-Arg^9-BK is able to produce overt nociception when intraplantarly coadministered with formalin (Campos *et al*., 1995; De Campos *et al*., 1998). The mechanisms involved in this short-lasting functional induction of B_1 receptor remain elusive and require further study.

Studies carried out with B_1 receptor knockout mice have also confirmed the important role played by B_1 receptors in the development and maintenance of chronic pain. Chronic pain differs substantially from acute pain, not only in terms of the persistence, but also in relation to the maladaptive neuroplasticity described at various levels of the nervous system (Woolf & Mannion, 1999). Peripheral injection of CFA has been used as an experimental animal model of arthritis, causing persistent hyperalgesia and allodynia to mechanical and thermal stimuli that is developed as early as 2 h after its administration and persists for weeks. As observed in humans, this nociceptive behaviour takes place on both ipsilateral and contralateral sides of the injection, an effect which is mediated by local nociceptor sensitization and systemic neuronal (such as central sensitization) and immune (such as increase in cytokine serum level) mechanisms (Shenker *et al*., 2001). Gene deletion of the B_1 receptor reduces ipsilateral, and in particular contralateral, thermal hyperalgesia and mechanical allodynia induced by CFA (Ferreira *et al*., 2001). These findings confirm previous studies which have indicated that the B_1 receptor antagonists des-Arg^9-Leu^8-BK, des-Arg^{10}-Hoe 140 and B9858 are capable of inhibiting CFA-induced nociception in rats, mice and rabbits, respectively (Perkins *et al*., 1993; Panesar *et al*., 1998; Mason *et al*., 2002). Moreover, intraplantar injection of des-Arg^9-BK induces contralateral mechanical hyperalgesia and allodynia in rats pretreated with intraplantar CFA (Khasar *et al*., 1995; Fox *et al*., 2003). Interestingly, in the experiment of Fox *et al*. (2003), B_1 receptor-immunoreactivity was significantly increased 24 h after CFA administration in both ipsilateral and contralateral small DRG neurons. It is worth noting that the nonpeptidic B_1 receptor antagonist derived from dihydroquinoxalinone produces potent antinociceptive action in the model of CFA-induced hyperalgesia in rabbits (Su *et al*., 2003).

Painful neuropathies may result from nerve injury, chronic treatment with certain drugs and metabolic disorders (Woolf & Mannion, 1999). As the mechanisms underlying these syndromes are not fully understood, available therapy does not provide satisfactory pain relief and patients suffer from chronic intractable pain. Several studies have demonstrated the important role played by kinins and their receptors in neuropathic pain induction. Increased levels of B_1 receptor mRNA or protein have been found in dorsal root ganglion after sciatic nerve constriction injury in rats and mice (Petersen *et al*., 1998; Eckert *et al*., 1999; Levy & Zochodne, 2000; Yamaguchi-Sase *et al*., 2003; Rashid *et al*., 2004). Very recently, it has been reported that B_1 receptors are newly expressed after nerve injury, mainly in myelinated DRG neurons, whereas B_2 receptor expression drastically decreases in DRG (Rashid *et al*., 2004). Importantly, systemic administration of B_1 receptor antagonist des-Arg^9-Leu^8-BK is able to reduce the thermal hyperalgesia and mechanical allodynia produced by sciatic nerve constriction in rats (Levy & Zochodne, 2000; Yamaguchi-Sase *et al*., 2003). Also, the gene deletion of B_1 receptors practically abolishes the nociceptive hypersensitivity produced by sciatic nerve injury in mice (J.B. Calixto, unpublished results). This effect appears as early as 1 day after lesion and remains significant up to 28 days, suggesting that the B_1 receptor is involved in both development and maintenance of neuropathic pain symptoms. Corroborating these data, intraplantar administration of Lys-des-Arg^9-BK 7 days after sciatic nerve injury in mice is able to induce both nociception and activation of ERK in DRG neurones (Rashid *et al*., 2004). Interestingly, oral treatment with the newly developed nonpeptide B_1 receptor antagonist SSR240612 is capable of reducing the thermal hyperalgesia produced by sciatic nerve injury in rats (Gougat *et al*., 2004).

Painful neuropathy may also be developed in relation to diabetes (Woolf & Mannion, 1999). As discussed earlier, the B_1 receptor seems to be implicated in type I diabetes complications (for review see: Couture *et al*., 2001; Gabra & Sirois, 2003a, b). In fact, thermal hyperalgesia produced by STZ in mice is blocked by the systemic treatment of the selective B_1 receptor antagonists R715 or R954 (Gabra & Sirois, 2003a, b). In addition, acute administration of des-Arg^9-BK significantly potentiates diabetes-induced hyperalgesia. Considering these data, it is possible to suggest that the use of selective B_1 receptor antagonists could represent a novel approach for the treatment of chronic pain of inflammatory and neuropathic origin.

Other recent evidence for the involvement of B_1 receptors in painful processes

Other relevant studies have addressed the involvement of B_1 receptors in various models of acute and chronic pain. For instance, intraplantar injection of zymozam produces local increase in the B_1 receptor mRNA expression and mechanical

hyperalgesia that has been reversed by the B_1 receptor antagonists des-Arg^9-Leu^8-BK and R715 (Bélichard *et al*., 2000). Moreover, intraplantar injection of the B_1 receptor agonist des-Arg^9-BK is able to produce hyperalgesia 1 h following administration of IL1-β at the same site, a response that seems to be dependent on p38 MAPK activation (Ganju *et al*., 2001). A recent report has demonstrated that peripheral B_1 receptor is also involved in the orofacial nociception caused by formalin in rats (Chichorro *et al*., 2004). B_1 receptors seem likely to be involved in visceral pain production, since the antagonism of the B_1 receptor reduces the late viscero-visceral hyper-reflexia induced by turpentine inflammation. Another recent study has shown that the benzodiazepine-derived nonpeptide B_1 receptor antagonists are able to reduce carrageenan-induced hyperalgesia in rats, apart from their poor bioavailability after systemic treatment (Wood *et al*., 2003). Moreover, oral treatment with the nonpeptide B_1 receptor antagonist SSR240612 is capable of reducing the nociception produced by formalin and ultra-violet application (Gougat *et al*., 2004). Based on these data, it is possible to infer that B_1 receptors (expressed constitutively or upregulated) might be related to acute and chronic pain of somatic and visceral origin.

The spinal cord is an important site for B_1 receptor nociceptive action

As previously demonstrated, B_1 receptors have been identified in the spinal cord (Couture & Lindsey, 2000; Wotherspoon & Winter, 2000; Ma & Heavens, 2001; Shughrue *et al*., 2003). Using an *in vitro* spinal cord preparation, Pesquero *et al*. (2000) have shown that B_1 receptor stimulation increases the C fibre component, but not the Aβ fibre-component, of the ventral root potential (VRP) produced by electrical excitation of naïve mouse dorsal root. This indicates that the B_1 receptor functions specifically in nociceptive synaptic pathways and that it may be involved with some forms of central sensitization. However, B_1 receptor activation is not able to produce direct ongoing activation of VRP in rats (Dunn & Rang, 1990). Thus, it is possible to suggest that B_1 receptor activity is not sufficient for the depolarization of unstimulated spinal cord, but that it produces excitation only following C-fibre activation. In fact, intrathecal administration of B_1 receptor agonists induces hyperalgesia after peripheral thermal or mechanical stimulation in mice and rats (Ferreira *et al*., 2002; Fox *et al*., 2003). In addition, the B_1 receptor seems to be involved in the late component of the hyperalgesia induced by bradykinin, a potent activator of C fibres (Ferreira *et al*., 2002; Sot *et al*., 2002). Moreover, repetitive electrical stimulation of the dorsal root produces an increase in the VRP, a use-dependent facilitation plasticity of the spinal cord neurones named wind-up. Interestingly, in an experiment on B_1 receptor knockout mice conducted by Pesquero *et al*. (2000), wind-up was significantly reduced (by about 50%) in comparison with the wild-type littermates. These data indicate that the nociceptive impairment observed in knockout B_1 receptor mice might be attributed, at least in part, to a deficit in the pathological plasticity of the spinal neurones. Indeed, it has been shown that spinal B_1 receptor activation greatly contributes to the inflammatory phase of formalin-induced pain and to the chronic inflammatory pain caused by CFA or sciatic nerve injury in mice and rats (McNair *et al*., 2001; Ferreira *et al*., 2002; Fox *et al*., 2003). Moreover, intrathecal administration of B_1 receptor agonist produces thermal hyperalgesia in hyperglycaemic rats (Couture *et al*., 2001). The data described above strongly supports the notion that the spinal cord represents a relevant site of action for kinins acting at B_1 receptors to produce nociception in both acute and chronic painful processes.

Possible role of B_1 receptors expressed in nonsensory neurons in pain production

Apart from their expression in DRG neurones, B_1 receptors might be expressed and induced in other cells that are involved in pain production, especially in chronic situations. For example, B_1 receptor immunoreactivity has been found in non-neuronal DRG satellite cells after nerve injury in mice (Rashid *et al*., 2004). Satellite cells (such as fibroblast-like cells and Schwann cells) are closely associated with neurons and may regulate the function of nociceptors (Heblich *et al*., 2001). However, the role of B_1 receptors in these cells continues to be elusive.

Some painful processes are mediated by sympathetic activity (Woolf & Mannion, 1999). Interestingly, functional B_1 receptors are expressed in sympathetic ganglia, since their activation is able to depolarize superior cervical ganglia neurones *in vitro* (Seabrook *et al*., 1995; 1997). Postganglionic sympathetic terminals have been demonstrated to be involved in B_1 receptor agonist-induced hyperalgesia (Khasar *et al*., 1995). Thus, sympathetic fibres seem to be important to the nociceptive action of B_1 receptor agonists. Glial cells are also involved in pain production, as their activation results in the release of several pronociceptive mediators (including prostaglandins, glutamate and cytokines) (Watkins & Maier, 2003). Furthermore, B_1 receptor upregulation has been reported in rat primary cultured microglia after BK stimulation (Noda *et al*., 2003). Moreover, the B_1 receptor agonist des-Arg^9-BK elicits outward membrane current and increases in intracellular calcium in cultured rat astrocytes (Gimpl *et al*., 1992). Apart from its participation in nociception, the role of glial cell activation by B_1 receptors during painful processes is so far unknown.

Concluding remarks

In the present review, we have described the main advances in the involvement of kinin B_1 receptors in painful and inflammatory processes. The most recent studies are supported by evidence obtained with selective B_1 receptor agonists or antagonists in various distinct models of pain and inflammation. Nevertheless, greater progress achieved in relation to B_1 receptors implies a better understanding of the mechanisms related to their induction. As discussed previously, although pharmacological data suggested early on that B_1 receptors were inducible molecules, only recently has this hypothesis been confirmed by molecular biology studies. In fact, combined functional and molecular studies have permitted a step forward in identifying the signalling pathways implicated in the upregulation of B_1 receptors. These studies have demonstrated that B_1 receptor induction is the result of a strictly regulated cascade of events which involves an interaction among transcriptional factors, protein kinases, cytokines

and inflammatory/neuronal cells. Understanding these pathways brings new possibilities for the control of B_1 receptor expression during pathological states. In addition, the efforts to generate mice lacking B_1 receptor gene, together with the recent development of selective peptidic and nonpeptidic B_1 receptor antagonists, has opened up an important option for the development of clinically relevant drugs for the treatment of inflammatory and painful conditions.

With basis on the literature data presented in this review, it is clear that B_1 receptors occupy a relevant place in the inflammatory scenario. In addition, without doubt, B_1 receptors seem to be deeply involved in both acute and chronic nociceptive processes. Given the large number of diseases in which B_1 receptors are expected to be involved, there is much hope that orally active and selective B_1 receptor antagonists could be clinically tested and proved useful in the management of important pathologies, especially in relation to inflammation and pain. In spite of that, the real effectiveness of selective kinin B_1 receptors antagonists for treating inflammatory/nocipetive alterations remains to be investigated by future studies.

R.M. and J.F. are Ph.D. students holding grants from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico). E.S.F. is a Ph.D. student receiving a grant from CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) D.A.C. and M.M.C. are holders of post-doctoral fellowships from CNPq and CAPES, respectively. Works from our laboratory were supported by grants of CNPq, CAPES and PRONEX.

References

ABDOUH, M., KHANJARI, A., ABDELAZZIZ, N., ONGALI, B., COUTURE, R. & HASSÉSSIAN, H.M. (2003). Early upregulation of kinin B_1 receptors in retinal microvessels of the streptozotocin-diabetic rats. *Br. J. Pharmacol.,* **140,** 33–40.

AHLUWALIA, A. & PERRETTI, M. (1999). B_1 receptors as a new inflammatory target. Could this B the 1? *Trends Pharmacol. Sci.,* **20,** 100–104.

AKIRA, S., YAMAMOTO, M. & TAKEDA, K. (2003). Role of adapters in Toll-like receptor signalling. *Biochem. Soc. Trans.,* **31,** 637–642.

ANGERS, M., DROUIN, R., BACHVAROVA, M., PARADIS, I., MARCEAU, F. & BACHVAROV, D.R. (2000). *In vivo* protein-DNA interactions at the kinin B_1 receptor gene promoter: no modification on interleukin-1 beta or lypopolysaccharide induction. *J. Cell. Biochem.,* **78,** 278–296.

BACHAR, O., ADNER, M., UDDMAN, R. & CARDELL, L.O. (2004). Toll-like receptor stimulation induces airway hyper-responsiveness to bradykinin, an effect mediated by JNK and NF-kappaB signaling pathways. *Eur. J. Immunol.,* **34,** 1196–1207.

BACHVAROV, D.R., HESS, J.F., MENKE, J.G., LARRIVEE, J.F. & MARCEAU, F. (1996). Structure and genomic organization of the human B_1 receptor gene for kinins (BDKRB1). *Genomics,* **33,** 374–381.

BARKI-HARRINGTON, L., BOOKOUT, A.L., WANG, G., LAMB, M.E., LEEB-LUNDBERG, L.M. & DAAKA, Y. (2003). Requirement for direct cross-talk between B_1 and B_2 kinin receptors for the proliferation of androgen-insensitive prostate cancer PC3 cells. *Biochem. J.,* **371,** 581–587.

BEG, A.A. (2002). Endogenous ligands of Toll-like receptors: implications for regulating inflammatory and immune responses. *Trends Immunol.,* **23,** 509–512.

BEIRITH, A., SANTOS, A.R. & CALIXTO, J.B. (2003). The role of neuropeptides and capsaicin-sensitive fibres in glutamate-induced nociception and paw oedema in mice. *Brain Res.,* **969,** 110–116.

BÉLICHARD, P., LANDRY, M., FAYE, P., BACHVAROV, D.R., BOUTHILLIER, J., PRUNEAU, D. & MARCEAU, F. (2000). Inflammatory hyperalgesia induced by zymosan in the plantar tissue of the rat: effect of kinin receptor antagonists. *Immunopharmacology,* **46,** 139–147.

BÉLICHARD, P., LUCCARINI, JM., DEFRENE, E., FAYE, P., FRANCK, R.M., DUCOS, H., PAQUET, J.L. & PRUNEAU, D. (1999). Pharmacological and molecular evidence for kinin expression in urinary bladder of cyclophosphamide-treated rats. *Br. J. Pharmacol.,* **128,** 213–219.

BHOOLA, K.D., RAMSAROOP, R., PLENDL, J., CASSIM, B., DLAMINI, Z. & NAICKER, S. (2001). Kallikrein and kinin receptor expression in inflammation and cancer. *Biol. Chem.,* **382,** 77–89.

BOCK, M.G. & LONGMORE, J. (2000). Bradykinin antagonists: new opportunities. *Curr. Opin. Chem. Biol.,* **4,** 401–406.

BORKOWSKI, J.A., RANSOM, R.W., SEABROOK, G.R., TRUMBAUER, M., CHEN, H., HILL, R.G., STRADER, C.D. & HESS, J.F. (1995). Targeted disruption of a B_2 bradykinin receptor gene in mice eliminates bradykinin action in smooth muscle and neurons. *J. Biol. Chem.,* **270,** 13706–13710.

BRAND, M., KLUSCH, A., KURZAI, O., VALDEOLMILLOS, M., SCHMIDT, R.F. & PETERSEN, M. (2001). No evidence for bradykinin B_1 receptors in rat dorsal root ganglion neurons. *NeuroReport,* **12,** 3165–3168.

CABRINI, D.A. & CALIXTO, J.B. (1997). Characterization of des-Arg^9-bradykinin-induced contraction in guinea-pig gallbladder *in vitro*. *Eur. J. Pharmacol.,* **331,** 31–38.

CABRINI, D.A., CAMPOS, M.M., TRATSK, K.S., MERINO, V.F., SILVA JR, J.A., SOUZA, G.E., AVELLAR, M.C., PESQUERO, J.B. & CALIXTO, J.B. (2001). Molecular and pharmacological evidence for modulation of kinin B_1 receptor expression by endogenous glucocorticoids hormones in rats. *Br. J. Pharmacol.,* **132,** 567–577.

CALIXTO, J.B., CABRINI, D.A., FERREIRA, J. & CAMPOS, M.M. (2000). Kinins in pain and inflammation. *Pain,* **87,** 1–5.

CALIXTO, J.B., CABRINI, D.A., FERREIRA, J. & CAMPOS, M.M. (2001). Inflammatory pain: kinins and antagonists. *Curr. Opin. Anaesthesiol.,* **14,** 519–526.

CAMPOS, M.M., CABRINI, D.A., CARDOZO, A.H., RAE, G.A., TORO, J.H. & CALIXTO, J.B. (2001). Changes in paw oedema triggered *via* bradykinin B(1) and B(2) receptors in streptozotocin-diabetic rats. *Eur. J. Pharmacol.,* **416,** 169–177.

CAMPOS, M.M. & CALIXTO, J.B. (1995). Involvement of B_1 and B_2 receptors in bradykinin-induced rat paw oedema. *Br. J. Pharmacol.,* **114,** 1005–1013.

CAMPOS, M.M., MATA, L.V. & CALIXTO, J.B. (1995). Expression of B_1 kinin receptors mediating paw edema and formalin-induced nociception. *Modulation by glucocorticoids. Can. J. Physiol. Pharmacol.,* **73,** 812–819.

CAMPOS, M.M., SOUZA, G.E. & CALIXTO, J.B. (1996). Upregulation of B_1 receptor mediating des-Arg^9-BK-induced rat paw oedema by systemic treatment with bacterial endotoxin. *Br. J. Pharmacol.,* **117,** 793–798.

CAMPOS, M.M., SOUZA, G.E. & CALIXTO, J.B. (1998). Modulation of kinin B_1 but not B_2 receptors-mediated rat paw edema by IL-1beta and TNFalpha. *Peptides,* **19,** 1269–1276.

CAMPOS, M.M., SOUZA, G.E. & CALIXTO, J.B. (1999). *In vivo* B_1 kinin-receptor upregulation. Evidence for involvement of protein kinases and nuclear factor kappaB pathways. *Br. J. Pharmacol.,* **127,** 1851–1859.

CAMPOS, M.M., SOUZA, G.E., RICCI, N.D., PESQUERO, J.L., TEIXEIRA, M.M. & CALIXTO, J.B. (2002). The role of migrating leukocytes in IL-1 beta-induced up-regulation of kinin B_1 receptors in rats. *Br. J. Pharmacol.,* **135,** 1107–1114.

CAYLA, C., MERINO, V.F., CABRINI, D.A., SILVA JR, J.A., PESQUERO, J.B. & BADER, M. (2002). Structure of the mammalian kinin receptor gene locus. *Int. Immunopharmacol.,* **2,** 1721–1727.

CHAI, K.X., NI, A., WANG, D., WARD, D.C., CHAO, J. & CHAO, L. (1996). Genomic DNA sequence, expression, and chromosomal localization of the human B_1 bradykinin receptor gene $BDKRB_1$. *Genomics,* **31,** 51–57.

CHICHORRO, J.G., LORENZETTI, B.B. & ZAMPRONIO, A.R. (2004). Involvement of bradykinin, cytokines, sympathetic amines and prostaglandins in formalin-induced orofacial nociception in rats. *Br. J. Pharmacol.,* **141,** 1175–1184.

CHRISTIANSEN, S.C., EDDLESTON, J., WOESSNER, K.M., CHAMBERS, S.S., YE, R., PAN, Z.K. & ZURAW, B.L. (2002). Up-regulation of functional kinin B_1 receptors in allergic airway inflammation. *J. Immunol.,* **169,** 2054–2060.

CLARK, A.R., DEAN, J.L. & SAKLATVALA, J. (2003). Post-transcriptional regulation of gene expression by mitogen-activated protein kinase p38. *FEBS Lett.,* **546,** 37–44.

CORRÊA, C.R. & CALIXTO, J.B. (1993). Evidence for participation of B_1 and B_2 kinin receptors in formalin-induced nociceptive response in the mouse. *Br. J. Pharmacol.,* **110,** 193–198.

COUTURE, R. & LINDSEY, C.J. (2000). Brain kallikrein–kinin system: from receptors to neuronal pathways and physiological functions. In: *Handbook of Chemical Anatomy: Peptide Receptors,* ed. QUIRION, R., BJ, A. & H, T. pp. 241–298. The Netherlands: Elsevier..

COUTURE, R., HARRISSON, M., VIANNA, R.M. & CLOUTIER, F. (2001). Kinin receptors in pain and inflammation. *Eur. J. Pharmacol.,* **429,** 161–176.

DAVIS, A.J. & PERKINS, M.N. (1994). Induction of B_1 receptors *in vivo* in a model of persistent inflammatory mechanical hyperalgesia in the rat. *Neuropharmacology,* **33,** 127–133.

DAVIS, C.J., NAEEM, S., PHAGOO, S.B., CAMPBELL, E.A., URBAN, L. & BURGESS, G.M. (1996). B_1 bradykinin receptors and sensory neurones. *Br. J. Pharmacol.,* **118,** 1469–1476.

DE CAMPOS, R.O., HENRIQUES, M.G. & CALIXTO, J.B. (1998). Systemic treatment with *Mycobacterium bovis* bacillus Calmette–Guerin (BCG) potentiates kinin B_1 receptor agonist-induced nociception and oedema formation in the formalin test in mice. *Neuropeptides,* **32,** 393–403.

DEBLOIS, D. & HORLICK, R.A. (2001). Endotoxin sensitization to kinin B_1 receptor agonist in a non-human primate model: haemodynamic and pro-inflammatory effects. *Br. J. Pharmacol.,* **132,** 327–335.

DIXON, D.A. (2004). Dysregulated post-transcriptional control of COX-2 gene expression in cancer. *Curr. Pharm. Des.,* **10,** 635–646.

DIXON, D.A., KAPLAN, C.D., MCINTYRE, T.M., ZIMMERMAN, G.A. & PRESCOTT, S.M. (2000). Post-transcriptional control of cyclooxygenase-2 gene expression. The role of the 3′-untranslated region. *J. Biol. Chem.,* **275,** 11750–11757.

DOBROVOLSKAIA, M.A. & VOGEL, S.N. (2002). Toll receptors, CD14, and macrophage activation and deactivation by LPS. *Microbes Infect.,* **4,** 903–914.

DONALDSON, L.F., HANLEY, M.R. & VILLABRANCA, A.C. (1997). Inducible receptors. *Trends Pharmacol. Sci.,* **18,** 171–181.

DRAPEAU, G., DEBLOIS, D. & MARCEAU, F. (1991). Hypotensive effects of Lys-des-Arg^9-Bradykinin and metabolically protected agonists of b1 receptors for kinins. *J. Pharmacol. Exp. Ther.,* **259,** 997–1003.

DRAY, A., PATEL, I.A., PERKINS, M.N. & RUEFF, A. (1992). Bradykinin-induced activation of nociceptors: receptor and mechanistic studies on the neonatal rat spinal cord-tail preparation *in vitro*. *Br. J. Pharmacol.,* **107,** 1129–1134.

DRAY, A. & PERKINS, M. (1997). Kinins and pain. In: *The Handbook of Immunopharmacology: the Kinin System,* ed. Farmer, S.G. pp 157–172. London: Academic Press.

DUNN, P.M. & RANG, H.P. (1990). Bradykinin-induced depolarization of primary afferent terminals in the neonatal rat spinal cord *in vitro*. *Br. J. Pharmacol.,* **100,** 656–660.

DUNN, C., WILTSHIRE, C., MACLAREN, A. & GILLESPIE, D.A. (2002). Molecular mechanism and biological functions of c-Jun N-terminal kinase signalling *via* the c-Jun transcription factor. *Cell. Signal.,* **14,** 585–593.

ECKERT, A., VON BANCHET, G.S., SOPPER, S. & PETERSEN, M. (1999). Spatio-temporal pattern of induction of bradykinin receptors and inflammation in rat dorsal root ganglia after unilateral nerve ligation. *Pain,* **83,** 487–497.

ERIC, J., BKAILY, G., BKAILY, G.B., VOLKOV, L., GABRA, B.H. & SIROIS, P. (2003). Des-Arg^9-bradykinin increases intracellular Ca^{2+} in bronchoalveolar eosinophils from ovalbumin-sensitized and -challenged mice. *Eur. J. Pharmacol.,* **475,** 129–137.

FATHY, D.B., KYLE, D.J. & LEEB-LUNDBERG, L.M. (2000). High-affinity binding of peptide agonists to the human B_1 bradykinin receptor depends on interaction between the peptide N-terminal L-lysine and the fourth extracellular domain of the receptor. *Mol. Pharmacol.,* **57,** 171–179.

FAUSSNER, A., BATHON, J.M. & PROUD, D. (1999). Comparison of the responses of B_1 and B_2 kinin receptors to agonist stimulation. *Immunopharmacology,* **45,** 13–20.

FERNANDES, E.S., PASSOS, G.F., CAMPOS, M.M., ARAÚJO, J.G., PESQUERO, J.L., AVELLLAR, M.C., TEIXEIRA, M.M. & CALIXTO, J.B. (2003). Mechanisms underlying the modulatory action of platelet activating factor (PAF) on the upregulation of kinin B_1 receptors in the rat paw. *Br. J. Pharmacol.,* **139,** 973–981.

FERREIRA, J., CAMPOS, M.M., ARAÚJO, R., BADER, M., PESQUERO, J.B. & CALIXTO, J.B. (2002). The use of kinin B_1 and B_2 receptor knockout mice and selective antagonists to characterize the nociceptive responses caused by kinins at the spinal cord. *Neuropharmacology,* **43,** 1188–1197.

FERREIRA, J., CAMPOS, M.M., PESQUERO, J.B., ARAÚJO, R.C., BADER, M. & CALIXTO, J.B. (2001). Evidence for the participation of kinins in Freund's adjuvant-induced inflammatory and nociceptive responses in kinin B_1 and B_2 receptor knockout mice. *Neuropharmacology,* **41,** 1006–1012.

FERREIRA, J., SILVA, G.L. & CALIXTO, J.B. (2004). Involvement of vanilloid receptors in the overt nociception induced by B_2 kinin receptor activation in mice. *Br. J. Pharmacol.,* **141,** 787–794.

FOX, A., WOTHERSPOON, G., MCNAIR, K., HUDSON, L., PATEL, S., GENTRY, C. & WINTER, J. (2003). Regulation and function of spinal and peripheral neuronal B_1 bradykinin receptors in inflammatory mechanical hyperalgesia. *Pain,* **104,** 683–691.

GABRA, B.H. & SIROIS, P. (2002). Role of bradykinin B_1 receptors in diabetes-induced hyperalgesia in streptozotocin-treated mice. *Eur. J. Pharmacol.,* **457,** 115–124.

GABRA, B.H. & SIROIS, P. (2003a). Kinin B_1 receptor antagonists inhibit diabetes-induced hyperalgesia in mice. *Neuropeptides,* **37,** 36–44.

GABRA, B.H. & SIROIS, P. (2003b). Beneficial effect of chronic treatment with the selective bradykinin B_1 receptor antagonists, R-715 and R-954, in attenuating streptozotocin-diabetic thermal hyperalgesia in mice. *Peptides,* **24,** 1131–1139.

GANJU, P., DAVIS, A., PATEL, S., NUNEZ, X. & FOX, A. (2001). p38 stress-activated protein kinase inhibitor reverses bradykinin B(1) receptor-mediated component of inflammatory hyperalgesia. *Eur. J. Pharmacol.,* **421,** 191–199.

GARCIA LEME, J., HAMAMURA, L., MIGLIORINI, R.H. & LEITE, M.P. (1973). Experimental diabetes and inflammatory reactions in the rat. *Agents Actions,* **3,** 380–381.

GIMPL, G., WALZ, W., OHLEMEYER, C. & KETTENMANN, H. (1992). Bradykinin receptors in cultures astrocytes from neonatal brain are linked to physiological responses. *Neurosci. Lett.,* **144,** 139–142.

GOBEIL, F., NEUGEBAUER, W., FILTEAU, C., JUKIC, D., ALLOGHO, S.N., PHENG, L.H., NGUYEN-LE, X.K., BLOUIN, D. & REGOLI, D. (1996). Structure–activity studies of B_1 receptor-related peptides. *Antagonists Hypertension,* **28,** 833–839.

GOBEIL, F., NEUGBAUER, W., NGUEYEN-LE, X.K., NSA ALLOGHO, S., PHENG, L.H., BLOUIN, D., WHALLEY, E.T. & REGOLI, D. (1997). Pharmacological profiles of the human and rabbit B_1 receptors. *Can. J. Physiol. Pharmacol.,* **75,** 591–595.

GOUGAT, J., FERRARI, B., SARRAN, L., PLANCHENAULT, C., PONCELET, M., MARUANI, J., ALONSO, R., CUDENNEC, A., CROCI, T., GUAGNINI, F., URBAN-SZABO, K., MARTINOLLE, J.P., SOUBRIE, P., FINANCE, O. & LE FUR, G. (2004). 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], a new nonpeptide antagonist of the bradykinin B_1 receptor: Biochemical and pharmacological characterization. *J. Pharmacol. Exp. Ther.,* **309,** 661–669.

HADDAD, E.B., FOX, A.J., ROUSELL, J., BURGESS, G., MCINTYRE, P., BARNES, P.J. & CHUNG, K.F. (2000). Post-transcriptional regulation of bradykinin B_1 and B_2 receptor gene expression in human lung fibroblasts by tumor necrosis factor-alpha: modulation by dexamethasone. *Mol. Pharmacol.,* **57,** 1123–1131.

HAYASHI, I., AMANO, H., ISHIHARA, K., KUMAGAI, Y., YOSHIMURA, H. & MAJIMA, M. (2002). The role of kinin B_1 in the plasma extravasation of carragenin-induced pleurisy. *Life Sci.,* **70,** 937–949.

HAYASHI, R., YAMASHITA, N., MATSUI, S., MARUYAMA, M., SUGIYAMA, E. & SUGIYAMA, S. (1998). Bradykinin stimulates interleukin-8 production by human lung fibroblasts. *Immunology,* **95,** 507–511.

HEBLICH, F., ENGLAND, S. & DOCHERTY, R.J. (2001). Indirect actions of bradykinin on neonatal rat dorsal root ganglion neurones: a role for non-neuronal cells as nociceptors. *J. Physiol.,* **536,** 111–121.

HESS, J.F., HEY, P.J., CHEN, T.B., O'BRIEN, J., OMALLEY, S.S., PETTIBONE, D.J. & CHANG, R.S. (2001). Molecular cloning and pharmacological characterization of the canine B_1 and B_2 bradykinin receptors. *Biol. Chem.,* **382,** 123–129.

HESS, J.F., HEY, P.J., CHEN, T.B., PETTIBONE, D.J. & CHANG, R.S. (2002). Molecular and pharmacological diversity of the kinin B_1 receptor. *Int. Immunopharmacol.,* **2,** 1747–1754.

HOHMEIER, H.E., TRAN, V.V., CHEN, G., GASA, R. & NEWGARD, C.B. (2003). Inflammatory mechanisms in diabetes; lessons form the beta-cell. *Int. J. Obes. Relat. Metab. Disord.,* **27,** S12–S16.

HUANG, T.J., HADDAD, E.B., FOX, A.J., SALMON, M., JONES, C., BURGESS, G. & CHUNG, K.F. (1999). Contribution of bradykinin B_1 and B_2 receptors in allergen-induced bronchial hyperresponsiveness. *Am. J. Respir. Crit. Care Med.,* **160,** 1717–1723.

HUNT, S.P. & MANTYH, P.W. (2001). The molecular dynamics of pain control. *Nat. Rev. Neurosci.,* **2,** 83–91.

HUNTER, T. (1995). Protein kinases and phosphatases: the Yin and Yang of protein phosphorylation and signaling. *Cell,* **80,** 225–236.

ISHIHARA, K., HAYASH, I., YAMASHINA, S. & MAJIMA, M. (2001). A potential role of bradykinin in angiogenesis and growth of S-180 mouse tumors. *Jpn. J. Pharmacol.,* **87,** 318–326.

ISHIHARA, K., KAMATA, M., HAYASHI, I., YAMASHINA, S. & MAJIMA, M. (2002). Roles of bradykinin in vascular permeability and angiogenesis in solid tumor. *Int. Immunopharmacol.,* **2,** 499–509.

JONES, W.K., BROWN, M., REN, X., HE, S. & MCGUINNESS, M. (2003). NF-kappaB as an integrator of diverse signaling pathways: the heart of myocardial signaling? *Cardiovasc. Toxicol.,* **3,** 229–254.

JULIUS, D. & BASBAUM, A.I. (2001). Molecular mechanisms of nociception. *Nature,* **413,** 203–210.

KHASAR, S.G., MIAO, F.J. & LEVINE, J.D. (1995). Inflammation modulates the contribution of receptor-subtypes to bradykinin-induced hyperalgesia in the rat. *Neuroscience,* **69,** 685–690.

KUEBLER, J.F., SCHREMMER-DANNINGER, E., BHOOLA, K.D., ROSCHER, A.A., MESSMER, K. & HOFFMANN, T.F. (2003). Kinin-B_1 receptors in ischaemia-induced pancreatitis: functional importance and cellular localisation. *Biol. Chem.,* **384,** 1311–1319.

LAGNEUX, C., BADER, M., PESQUERO, J.B., DEMENGE, P. & RIBUOT, C. (2002). Detrimental implication of B_1 receptors in myocardial ischemia: evidence from pharmacological blockade and gene knockout mice. *Int. Immunopharmacol.,* **2,** 815–822.

LAHTI, A., JALONEN, U., KANKAANRANTA, H. & MOILANEN, E. (2003). c-Jun NH_2-terminal kinase inhibitor anthra(1,9-cd)pyrazol-6(2H)-one reduces inducible nitric-oxide synthase expression by destabilizing mRNA in activated macrophages. *Mol. Pharmacol.,* **64,** 308–315.

LANDGRAF, R.G., JANCAR, S., STEIL, A.A. & SIROIS, P. (2004). Modulation of allergic and immune complex-induced lung inflammation by bradykinin receptor antagonists. *Inflamm. Res.,* **53,** 78–83.

LANDGRAF, R.G., SIROIS, P. & JANCAR, S. (2003). Differential modulation of murine lung inflammation by bradykinin B_1 and B_2 selective receptor antagonists. *Eur. J. Pharmacol.,* **460,** 75–83.

LARRIVÉE, J.-F., BACHVAROV, D.R., HOULE, F., LANDRY, J., HUOT, J. & MARCEAU, F. (1998). Role of the mitogen-activated protein kinases in the expression of the kinin B_1 receptors induced by tissue injury. *J. Immunol.,* **160,** 1419–1426.

LARRIVEÉ, J.F., GERA, L., HOULE, S., BOUTHILLIER, J., BACHVAROV, D.R., STEWART, J.M. & MARCEAU, F. (2000). Non-competitive pharmacological antagonism at the rabbit B_1 receptor. *Br. J. Pharmacol.,* **131,** 885–892.

LASA, M., BROOK, M., SAKLATVALA, J. & CLARK, A.R. (2001). Dexamethasone destabilizes cyclooxygenase 2 mRNA by inhibiting mitogen-activated protein kinase p38. *Mol. Cell. Biol.,* **21,** 771–780.

LAVICH, T.R., CORDEIRO, R.S., CALIXTO, J.B., SILVA, P.M. & MARTINS, M.A. (2003). Combined action of vasoactive amines and bradykinin mediates allergen-evoked thermal hyperalgesia in rats. *Eur. J. Pharmacol.,* **462,** 185–192.

LEVY, D. & ZOCHODNE, D.W. (2000). Increased mRNA expression of the B_1 and B_2 bradykinin receptors and antinociceptive effects of their antagonists in an animal model of neuropathic pain. *Pain,* **86,** 265–271.

LEWIS, G.P. (1964). Plasma kinins and inflammation. *Metabolism,* **13,** 1256–1263.

LIEN, Y.H., LAI, L.W. & SILVA, A.L. (2003). Pathogenesis of renal ischemia/reperfusion injury: lessons from knockout mice. *Life Sci.,* **74,** 543–552.

MA, Q.-P. (2001). The expression of bradykinin B_1 receptors on primary sensory neurones that give rise to small calibre sciatic nerve fibres in rats. *Neuroscience,* **107,** 665–673.

MA, Q.-P. & HEAVENS, R. (2001). Basal expression of bradykinin B(1) receptor in the spinal cord in humans and rats. *NeuroReport,* **12,** 2311–2314.

MA, Q.-P., HILL, R. & SIRINATHSINGHJI, D. (2000). Basal expression of bradykinin B_1 receptor in peripheral sensory ganglia in the rat. *NeuroReport,* **11,** 4003–4005.

MACNEIL, T., BIERILO, K.K., MENKE, J.G. & HESS, J.F. (1995). Cloning and pharmacological characterization of a rabbit bradykinin B_1 receptor. *Biochim. Biophys. Acta.,* **1264,** 223–228.

MAHABEER, R. & BHOOLA, K.D. (2000). Kallikrein and kinin receptor genes. *Pharmacol. Ther.,* **88,** 77–89.

MARCEAU, F. (1995). Kinin B_1 receptors: a review. *Immunopharmacology,* **30,** 1–26.

MARCEAU, F., HESS, J.F. & BACHVAROV, D.R. (1998). The B_1 receptors for kinins. *Pharmacol. Rev.,* **50,** 357–386.

MARCEAU, F., SABOURIN, T., HOULE, S., FORTIN, J.P., PETITCLERC, E., MOLINARO, G. & ADAM, A. (2002). Kinin receptors: functional aspects. *Int. Immunopharmacol.,* **2,** 1729–1739.

MASON, G.S., CUMBERBATCH, M.J., HILL, R.G. & RUPNIAK, N.M.J. (2002). The bradykinin B_1 receptor antagonist B9858 inhibits a nociceptive spinal reflex in rabbits. *Can. J. Physiol. Pharmacol.,* **80,** 264–268.

MAZENOT, C., LOUFRANI, L., HENRION, D., RIBUOT, C., MULLER-ESTERL, W. & GODIN-RIBUOT, D. (2001). Endothelial kinin B_1-receptors are induced by myocardial ischaemia–reperfusion in the rabbit. *J. Physiol.,* **530,** 69–78.

MCEACHERN, A.E., SHELTON, E.R., BHAJTA, S., OBERNOLT, R., BACH, C., ZUPPAN, P., FUJISAKI, J., ALDRISH, R.W. & JARNAGIN, K. (1991). Expression cloning of rat B_2 bradykinin receptor. *Proc. Natl. Acad. Sci. U.S.A.,* **88,** 7724–7728.

MCLEAN, P.G., PERRETTI, M. & AHLUWALIA, A. (1999). Inducible expression of the kinin B_1 receptor in the endotoxemic heart: mechanisms of des-Arg^9-bradykinin-induced coronary vasodilation. *Br. J. Pharmacol.,* **128,** 275–282.

MCNAIR, K., KESINGLAND, A., URBAN, L. & FOX, A. (2001). The role of central and peripheral bradykinin B_1 receptors in models of neuropathic and inflammatory pain in the rat. *Br. J.Pharmacol.,* **134,** p158.

MEDEIROS, R., CABRINI, D.A. & CALIXTO, J.B. (2001). The *in vivo* and *ex vivo* roles of cyclooxygenase-2, nuclear factor-κB and protein kinases pathways in the up-regulation of B_1 receptor mediated contraction of the rabbit aorta. *Regul. Pept.,* **97,** 121–130.

MEDEIROS, R., CABRINI, D.A., FERREIRA, J., FERNANDES, E.S., MORI, M.A., PESQUERO, J.B., BADER, M., AVELLAR, M.C., CAMPOS, M.M. & CALIXTO, J.B. (2004). Bradykinin B_1 receptor expression induced by tissue damage in the rat portal vein. A critical role for mitogen-activated protein kinase and nuclear factor-κB signaling pathways. *Circ. Res.,* **94,** 1375–1382.

MENKE, J.G., BORKOWSKI, J.A., BIERILO, K.K., MACNEIL, T., DERRICK, A.W., SCHNECK, K.A., RANSOM, R.W., STRADER, C.D., LINEMEYER, D.L. & HESS, J.F. (1994). Expression cloning of a human B_1 bradykinin receptor. *J. Biol. Chem.,* **269,** 21583–21586.

MODÉER, T., ANDUREN, I. & YUCEL-LINDBERG, T. (1998). Bradykinin synergistically stimulates interleukin 6 production in human gingival fibroblasts challenged with interleukin 1 or tumour necrosis factor alpha. *Cytokine,* **10,** 26–31.

NEUGEBAUER, W., BLAIS, P.A., HALLE, S., FILTEAU, C., REGOLI, D. & GOBEIL JR, F. (2002). Kinin B_1 receptor antagonists with multi-enzymatic resistance properties. *Can. J. Physiol. Pharmacol.,* **80,** 287–292.

NEWTON, R., EDDLESTON, J., HADDAD, E.-B., HAWISA, S., MAK, J., LIM, S., FOX, A.J., DONNELY, L.E. & CHUNG, K.F. (2002). Regulation of kinin receptors in airway epithelial cells by inflammatory cytokines and dexamethasone. *Eur. J. Pharmacol.,* **441,** 193–202.

NI, A., CHAI, K.X., CHAO, L. & CHAO, J. (1998a). Molecular cloning and expression of rat bradykinin B_1 receptor. *Biochim. Biophys. Acta,* **1442,** 177–185.

NI, A., CHAO, L. & CHAO, J. (1998b). Transcription factor nuclear factor κB regulates the inducible expression of the human B_1 receptor gene in inflammation. *J. Biol. Chem.,* **273,** 2784–2791.

NI, A., YIN, H., AGATA, J., YANG, Z., CHAO, L. & CHAO, J. (2003). Overexpression of kinin B_1 receptors induces hypertensive response to des-Arg^9-bradykinin and susceptibility to inflammation. *J. Biol. Chem.,* **278,** 219–225.

NODA, M., KARIURA, Y., AMANO, T., MANAGO, Y., NISHIKAWA, K., AOKI, S. & WADA, K. (2003). Expression and function of bradykinin receptors in microglia. *Life Sci.,* **72,** 1573–1581.

ONGALI, B., CAMPOS, M.M., BREGOLA, G., RODI, D., REGOLI, D., THIBAULT, G., SIMONATO, M. & COUTURE, R. (2003). Autoradiographic analysis of rat brain kinin B_1 and B_2 receptors: normal distribution and alterations induced by epilepsy. *J. Comp. Neurol.,* **461,** 506–519.

PAN, Z.K., YE, R.D., CHRISTIANSEN, S.C., JAGELS, M.A., BOKOCH, G.M. & ZURAW, B.L. (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.

PANESAR, M.S., GENTRY, C.T., URBAN, L., WALKER, K. & FOX, A. (1998). A model of persistent, inflammation-induced mechanical hyperalgesia in the mouse. *Br. J. Pharmacol.,* **125** (Suppl.), 122.

PASSOS, G.F., FERNANDES, E.S., CAMPOS, M.M., ARAÚJO, J.G., PESQUERO, J.L., SOUZA, G.E., AVELLAR, M.C., TEIXEIRA, M.M. & CALIXTO, J.B. (2004). Kinin B_1 receptor up-regulation after lipopolysaccharide administration: role of proinflammatory cytokines and neutrophil influx. *J. Immunol.,* **172,** 1839–1847.

PERKINS, M.N. & KELLY, D. (1993). Induction of bradykinin-B_1 receptors '*in vivo*' in a model of ultra-violet irradiation-induced thermal hyperalgesia in the rat. *Br. J. Pharmacol.,* **110,** 1441–1444.

PERKINS, M.N. & KELLY, D. (1994). Interleukin-1β induced-desArg^9-bradykinin-mediated thermal hyperalgesia in the rat. *Neuropharmacology,* **33,** 657–660.

PERKINS, M.N., CAMPBELL, E. & DRAY, A. (1993). Antinociceptive activity of the bradykinin B_1 and B_2 receptor antagonists, des-Arg^9,[Leu^8]-BK and Hoe 140, in two models of persistent hyperalgesia in the rat. *Pain,* **53,** 191–197.

PERKINS, M.N., KELLY, D. & DAVIS, A.J. (1995). Bradykinin B_1 and B_2 receptor mechanisms and cytokine-induced hyperalgesia in the rat. *Can. J. Physiol. Pharmacol.,* **73,** 832–836.

PESQUERO, J.B., ARAUJO, R.C., HEPPENSTALL, P.A., STUCKY, C.L., SILVA, JR., J.A, WALTHER, T., OLIVEIRA, S.M., PESQUERO, J.L., PAIVA, A.C., CALIXTO, J.B., LEWIN, G.R. & BADER, M. (2000). Hypoalgesia and altered inflammatory responses in mice lacking kinin B_1 receptors. *Proc. Natl. Acad. Sci. U.S.A.,* **97,** 8140–8145.

PESQUERO, J.B., PESQUERO, J.L., OLIVEIRA, S.M., ROSCHER, A.A., METZGER, R., GANTEN, D. & BADER, M. (1996). Molecular cloning and functional characterization of a mouse bradykinin B_1 receptor gene. *Biochem. Biophys. Res. Commun.,* **224,** 281.

PETERSEN, M., ECKERT, A.S., VON BANCHET, G.S., HEPPELMANN, B., KLUSCH, A. & KNIFFKI, K.D. (1998). Plasticity in the expression of bradykinin binding sites in sensory neurons after mechanical nerve injury. *Neuroscience,* **83,** 949–959.

PHAGOO, S.B., POOLE, S. & LEEB-LUNDBERG, L.M. (1999). Autoregulation of bradykinin receptors: agonists in the presence of interleukin-1beta shift the repertoire of receptor subtypes from B_2 to B_1 in human lung fibroblasts. *Mol. Pharmacol.,* **56,** 325–333.

PHAGOO, S.B., REDDI, K., ANDERSON, K.D., LEEB-LUNDBERG, L.M. & WARBURTON, D. (2001). Bradykinin B_1 receptor up-regulation by interleukin-1β agonist occurs through independent and synergistic intracellular signaling mechanisms in human lung fibroblasts. *J. Pharmacol. Exp. Ther.,* **298,** 77–85.

PHAGOO, S.B., YAQOOB, M., HERRERA-MARTINEZ, E., MCINTYRE, P., JONES, C. & BURGESS, G.M. (2000). Regulation of bradykinin receptor gene expression in human lung fibroblasts. *Eur. J. Pharmacol.,* **397,** 237–246.

POOLE, S., LORENZETTI, B.B., CUNHA, J.M., CUNHA, F.Q. & FERREIRA, S.H. (1999). Bradykinin B_1 and B_2 receptors, tumour necrosis factor alpha and inflammatory hyperalgesia. *Br. J. Pharmacol.,* **126,** 649–656.

PREMKUMAR, L.S. & AHERN, G.P. (2000). Induction of vanilloid receptor channel activity by protein kinase C. *Nature,* **408,** 985–990.

PROUD, D. & KAPLAN, A.P. (1988). Kinin formation: mechanisms and role in inflammatory disorders. *Annu. Rev. Immunol.,* **6,** 49–83.

RAIDOO, D.M., SWANT, S., MAHABEER, R. & BHOOLA, K.D. (1999). Kinin receptors are expressed in human astrocytic tumour cells. *Immunopharmacology,* **43,** 255–263.

RASHID, M.H., INOUE, M., MATSUMOTO, M. & UEDA, H. (2004). Switching of bradykinin-mediated nociception following partial sciatic nerve injury in mice. *J. Pharmacol. Exp. Ther.,* **308,** 1158–1164.

REGOLI, D. & BARABE, J. (1980). Pharmacology of bradykinin and related kinins. *Pharmacol. Rev.,* **32,** 1–46.

REGOLI, D., BARABÉ, J. & PARK, W.K. (1977). Receptors for bradykinin in rabbit aortae. *Can. J. Physiol. Pharmacol.,* **55,** 855–867.

REGOLI, D., NSA ALLOGHO, S., RIZZI, A. & GOBEIL, F.J. (1998). Bradykinin receptors and their antagonists. *Eur. J. Pharmacol.,* **348,** 1–10.

REGOLI, D., RIZZI, A., PERRON, S.L. & GOBEIL, F. (2001). Classification of kinin receptors. *Biol. Chem.,* **382,** 31–35.

RHALEB, N.E., ROUISSI, N., JUKIC, D., REGOLI, D., HENKE, S., BREIPOHL, G. & KNOLLE, J. (1992). Pharmacological characterization of a new potent B_2 receptor antagonist (HOE 140): D-Arg[Hyp^3, Thi^5, D Tic^7, Oic^8]BK. *Eur. J. Pharmacol.,* **163,** 263–272.

RUPNIAK, N.M.J., BOYCE, S., WEBB, J.K., WILLIAMS, A.R., CARLSON, E.J., HILL, R.G., BORKOWSKI, J.A. & HESS, J.F. (1997). Effects of the bradykinin B_1 receptor antagonist des-Arg^9-[Leu^8]-bradykinin and genetic disruption of the B_2 receptor on nociception in rats and mice. *Pain,* **71,** 89–97.

SABOURIN, T., MORISSETTE, G., BOUTHILLIER, J., LEVESQUE, L. & MARCEAU, F. (2002). Expression of kinin B_1 receptor in fresh or cultured rabbit aortic smooth muscle: role of NF-κB. *Am. J. Physiol. Heart. Circ. Physiol.,* **283,** H227–H237.

SAINZ, I.M., UKNIS, A.B., ISORDIA-SALAS, I., DELA CADENA, R.A., PIXLEY, R.A. & COLMAN, R.W. (2004). Interactions between bradykinin (BK) and cell adhesion molecule (CAM) expression in peptidoglycan–polysaccharide (PG-PS)-induced arthritis. *FASEB J., Published online March,* **4,** 2004.

SARDI, S.P., ERRASTI, A.E., REY-ARES, V., ROGINES-VELO, M.P. & ROTHLIN, R.P. (2000). Bradykinin B_1 receptor in isolated human umbilical vein: an experimental model of the *in vitro* up-regulation. *Acta Pharmacol. Sinica,* **21,** 105–110.

SARDI, S.P., REY-ARES, V., PUJOL-LEREIS, V.A., SERRANO, S.A. & ROTHLIN, R.P. (2002). Further pharmacological evidence of nuclear factor-κB pathway involvement in bradykinin B_1 receptor-sensitized responses in human umbilical vein. *J. Pharmacol. Exp. Ther.,* **301,** 975–980.

SCHANSTRA, J.P., BATAILLÉ, E., CASTAÑO, M.E.M., BARASCUD, Y., HIRTZ, C., PESQUERO, J.B., PECHER, C., GAUTHIER, F., GIROLAMI, J.-P. & BASCANDS, J.-L. (1998). The B_1 -agonist [des-Arg^{10}]-kallidin activates transcription factor NF-κB and induces homologous up-regulation of the bradykinin B_1-receptor in cultured human lung fibroblasts. *J. Clin. Invest.,* **101,** 2080–2091.

SCHARFSTEIN, J., SCHMITZ, V., MORANDI, V., CAPELLA, M.M., LIMA, A.P., MORROT, A., JULIANO, L. & MULLER-ESTERL, W. (2000). Host cell invasion by *Trypanosoma cruzi* is potentiated by activation of bradykinin B_2 receptors. *J. Exp. Med.,* **192,** 1289–1300.

SEABROOK, G.R., BOWERY, B.J., HEAVENS, R., BROWN, N., FORD, H., SIRINATHSINGHJI, D.J.S., BORKOWSKI, J.A., HESS, J.F., STRADER, C.D. & HILL, R.G. (1997). Expression of B_1 and B_2 bradykinin receptor mRNA and their function roles in sympathetic ganglia and sensory root ganglia neurones from wild-type and B_2 receptor knockout mice. *Neuropharmacology,* **36,** 1009–1017.

SEABROOK, G.R., BOWERY, B.J. & HILL, R.G. (1995). Bradykinin receptors in mouse and rat isolated superior cervical ganglia. *Br. J. Pharmacol.,* **115,** 368–372.

SEEGERS, H.C., AVERY, P.S., MCWILLIAMS, D.F., HAYWOOD, L. & WALSH, D.A. (2004). Combined effect of bradykinin B_2 and neurokinin-1 receptor activation on endothelial cell proliferation in acute synovitis. *FASEB J.,* **18,** 762–764.

SHENKER, N., HAIGH, R., ROBERTS, E., MAPP, P., HARRIS, A. & BLAKE, D. (2001). Pain, neurogenic inflammation and symmetry in medical practice. *Pain Rev.,* **8,** 27–34.

SHIBATA, M., OHKUBO, T., TAKAHASHI, H. & INOKI, R. (1989). Modified formalin test: characteristic biphasic pain response. *Pain,* **38,** 347–352.

SHUGHRUE, P.J., KY, B. & AUSTIN, C.P. (2003). Localization of B_1 bradykinin receptor mRNA in the primate brain and spinal cord: an *in situ* hybridization study. *J. Comp. Neurol.,* **465,** 372–384.

SOT, U., MISTEREK, K., GUMULKA, S.W. & DOROCIAK, A. (2002). Intrathecal bradykinin administration: opposite effects on nociceptive transmission. *Pharmacology,* **66,** 76–80.

SOUZA, D.G., LOMEZ, E.S.L., PINHO, V., PESQUERO, J.B., BADER, M., PESQUERO, J.L. & TEIXEIRA, M.M. (2004). Role of bradykinin B_2 and B_1 receptors in the local, remote, and systemic inflammatory responses that follow intestinal ischemia and reperfusion injury. *J. Immunol.,* **172,** 2542–2548.

STEWART, J.M. (2003). Bradykinin antagonists as anti-cancer agents. *Curr. Pharm. Design,* **9,** 2036–2042.

STEWART, J.M., GERA, L., HANSON, W., ZUZACK, J.S., BUCKARD, M., MCCULLOUGH, R. & WHALLEY, E.T. (1996). A new generation of bradykinin antagonists. *Immunopharmacology,* **33,** 51–60.

STEWART, J.M., GERA, L., YORK, E.J., CHAN, D.C. & BUNN, P. (1999). Bradykinin antagonists: present progress and future prospects. *Immunopharmacology,* **43,** 155–161.

SU, D.S., MARKOWITZ, M.K., DIPARDO, R.M., MURPHY, K.L., HARRELL, C.M., O'MALLEY, S.S., RANSOM, R.W., CHANG, R.S., HA, S., HESS, F.J., PETTIBONE, D.J., MASON, G.S., BOYCE, S., FREIDINGER, R.M. & BOCK, M.G. (2003). Discovery of a potent, non-peptide bradykinin B_1 receptor antagonist. *J. Am. Chem. Soc.,* **125,** 7516–7517.

SUFKA, K.J. & ROACH, J.T. (1996). Stimulus properties and antinociceptive effects of selective bradykinin B_1 and B_2 receptor antagonists in rats. *Pain,* **66,** 99–103.

TAUB, J.S., GUO, R., LEEB-LUNDBERG, L.M., MADDEN, J.F. & DAAKA, Y. (2003). Bradykinin receptor subtype 1 expression and function in prostate cancer. *Cancer Res.,* **63,** 2037–2041.

TODOROV, A.G., ANDRADE, D., PESQUERO, J.B., ARAÚJO, R.DE C., BADER, M., STEWART, J., GERA, L., MULLER-ESTERL, W., MORANDI, V., GOLDENBERG, R.C., NETO, H.C. & SCHARFSTEIN, J. (2003). *Trypanosoma cruzi* induces edematogenic responses in mice and invades cardiomyocytes and endothelial cells *in vitro* by activating distinct kinin receptor (B_1/B_2) subtypes. *FASEB J.,* **17,** 73–75.

UENO, A., DEKURA, E., KOSUGI, Y., YOSHIMURA, M., NARABA, H., KOJIMA, F. & OH-ISHI, S. (2002). Effects of dexamethasone and protein kinase C inhibitors on the induction of bradykinin B_1 mRNA and the bradykinin B_1 receptor-mediated contractile response in isolated rat ileum. *Biochem. Pharmacol.,* **63,** 2043–2053.

VIANNA, R.M. & CALIXTO, J.B. (1998). Characterization of the receptor and the mechanisms underlying the inflammatory response induced by des-Arg^9-BK in mouse pleurisy. *Br. J. Pharmacol.,* **123,** 281–291.

VIANNA, R.M., ONGALI, B., REGOLI, D., CALIXTO, J.B. & COUTURE, R. (2003). Up-regulation of kinin B_1 receptor in the lung of streptozotocin-diabetic rat: autoradiographic and functional evidence. *Br. J. Pharmacol.,* **138,** 13–22.

WANG, J.W., SU, W., LAW, Y.P., LU, C.H., CHEN, Y.C., WANG, J.L., CHANG, H.J., CHEN, W.C. & JAN, C.R. (2001). Mechanism of bradykinin-induced Ca(2+) mobilization in MG63 human osteosarcoma cells. *Horm. Res.,* **55,** 265–270.

WATKINS, L.R. & MAIER, S.F. (2003). Glia: a novel drug discovery target for clinical pain. *Nat. Rev. Drug Discov.,* **2,** 973–985.

WEBB, M., MCINTYRE, P. & PHILLIPS, E. (1994). B_1 and B_2 bradykinin receptors encoded by distinct mRNAs. *J. Neurochem.,* **62,** 1247–1253.

WILLE, P.R., VITOR, R., GABILAN, N.H. & NICOLAU, M. (2001). Plasma extravasation mediated by lipopolysaccharide-induction of kinin B_1 receptors in rat tissues. *Mediat. Inflamm.,* **10,** 163–167.

WIRTH, K., BREIPOHL, G., STECHL, J., KNOLLE, J., HENKE, S. & SCHOLKENS, B. (1991). DesArg^9-D-Arg[Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin (desArg^{10}-[Hoe140]) is a potent bradykinin B_1 receptor antagonist. *Eur. J. Pharmacol.,* **205,** 217–218.

WOOD, M.R., KIM, J.J., HAN, W., DORSEY, B.D., HOMNICK, C.F., DIPARDO, R.M., KUDUK, S.D., MACNEIL, T., MURPHY, K.L., LIS, E.V., RANSOM, R.W., STUMP, G.L., LYNCH, J.J., O'MALLEY, S.S., MILLER, P.J., CHEN, T.B., HARRELL, C.M., CHANG, R.S., SANDHU, P., ELLIS, J.D., BONDISKEY, P.J., PETTIBONE, D.J., FREIDINGER, R.M. & BOCK, M.G. (2003). Benzodiazepines as potent and selective bradykinin B_1 antagonists. *J. Med. Chem.,* **46,** 1803–1806.

WOOLF, C.J. & MANNION, R.J. (1999). Neuropathic pain: aetiology, symptoms, mechanisms, and management. *Lancet,* **353,** 1959–1964.

WOTHERSPOON, G. & WINTER, J. 2000. Bradykinin B_1 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., MULLER-ESTERL, W. & MAEDA, H. (2002). Identification of bradykinin receptors in clinical cancer specimens and murine tumor tissues. *Int. J. Cancer,* **98,** 29–35.

YAMAGUCHI-SASE, S., HAYASHI, I., OKAMOTO, H., NARA, Y., MATSUZAKI, S., HOKA, S. & MAJIMA, M. (2003). Amelioration of hyperalgesia by kinin receptor antagonists or kininogen deficiency in chronic constriction nerve injury in rats. *Inflamm. Res.,* **52,** 164–169.

YANG, X. & POLGAR, P. (1996). Genomic structure of the human bradykinin B_1 receptor gene and preliminary characterization of its regulatory regions. *Biochem. Biophys. Res. Commun.,* **222,** 18–25.

YANG, S.H., SHARROCKS, A.D. & WHITMARSH, A.J. (2003). Transcriptional regulation by the MAP-kinase signaling cascades. *Gene,* **320,** 3–21.

YANG, X., TAYLOR, L. & POLGAR, P. (2001a). p53 down-regulates human bradykinin B_1 receptor gene expression. *J. Cell. Biochem.,* **82,** 38–45.

YANG, X., TAYLOR, L., YU, J., FENTON, M.J. & POLGAR, P. (2001b). Mediator caused induction of a human bradykinin B_1 receptor minigene: participation of c-jun in the process. *J. Cell. Biochem.,* **82,** 163–170.

ZHANG, Y., ADNER, M. & CARDELL, L.O. (2004). Up-regulation of bradykinin receptors in a murine *in-vitro* model of chronic airway inflammation. *Eur. J. Pharmacol.,* **489,** 117–126.

ZHOU, X., POLGAR, P. & TAYLOR, L. (1998). Roles for interleukin-1beta, phorbol ester and a post-transcriptional regulator in the control of bradykinin B_1 receptor gene expression. *Biochem. J.,* **330,** 361–366.

ZHOU, X., PRADO, G.N., CHAI, M., YANG, X., TAYLOR, L. & POLGAR, P. (1999). Posttranscriptional destabilization of the bradykinin B_1 receptor messenger RNA: cloning and functional characterization of the 3′-untranslated region. *Mol. Cell. Biol. Res. Commun.,* **1,** 29–35.

(Received May 21, 2004
Revised August 3, 2004
Accepted September 10, 2004)