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REVIEW ARTICLE

THE THERAPEUTIC POTENTIAL OF BRADYKININ B_1 RECEPTOR ANTAGONISTS IN CHRONIC PAIN

S. Farkas and J. Éles

Research Division, Gedeon Richter, Plc, Budapest, Hungary

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SUMMARY

After the rise and fall of cyclooxygenase-2 (COX-2)-selective inhibitors as safe replacements for nonsteroidal antiinflammatory drugs (NSAIDs), particularly for the treatment of chronic painful conditions in the elderly, there is still a great need for safer drugs that can alleviate both inflammation and pain. Des-arginine metabolites of bradykinin-related peptides are involved in the propagation and maintenance of inflammation and pain via activation of bradykinin B_1 receptors, the bradykinin receptor subtype that is hardly detectable in healthy tissues but induced during inflammation. Due to the really inducible nature of the receptor at peripheral sites, orally acting B_1 receptor antagonists hold the promise of being a safe substitute for NSAIDs, especially for chronic treatment. In addition, nonclinical research suggests the potential utility of central nervous system-penetrating B_1 receptor antagonists in a broader range of chronic pain states, including neuropathic pain, and involving a central sensitization process. However, exploitation of the therapeutic utility has been hindered by the extreme difficulties related to optimization for an inducible peptide–receptor with species-specific differences in physiology and pharmacology. The scientific literature implicating therapeutic utility for B_1 receptor antagonists and the ongoing efforts for exploiting this target are reviewed in this article.

Correspondence: S. Farkas, Research Division, Gedeon Richter, Plc, H-1475, P.O. Box 27, Budapest, Hungary. E-mail: s.farkas@richter.hu.

INTRODUCTION

Bradykinin and related peptides, kinins, have long been acknowledged as algogenic mediators released at sites of inflammation and/or tissue injury. The inflammatory response functions as a two-edged sword in several pathophysiological processes. Namely, it serves the beneficial purpose of defense and repair, either by eliciting pain and consequent avoidance of further injury, or by promoting cellular responses serving immunological defense against intruders, like infections or tumor cells, as well as by promoting repair and healing of the damaged tissues. However, this homeostatic response can go wrong in many pathological situations, such that pain, inflammation and/or an overly active immune response becomes a chronic condition, promoting maintenance of a disease state instead of healing. Therefore, it is a great challenge for pharmacotherapy to effectively mitigate chronic pain and inflammation without impairing the defense and repair functions. Inflammation is a very complex programmed process, in which multiple humoral mediators play in concert to initiate, propagate, maintain and resolve this state and its various phases. The kallikrein–kinin system is one of the multiple systems that play a role in the initiation, propagation and maintenance of inflammation and pain, and as such, may provide a promising target for pharmacological intervention (1).

THE KALLIKREIN–KININ SYSTEM

The kallikrein–kinin system is part of a humoral defense system and participates in the inflammatory response in various organs. It represents a proteolytic complex that, when activated, triggers the release of kinins. In mammals, endogenous kinins include the nonapeptide bradykinin (BK; Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg), the decapeptide lysyl-bradykinin (LysBK; Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg; also termed kallidin) and their respective carboxy-terminal (right end) des-arginine metabolites: des-arginine-bradykinin (DABK) and lysyl-des-arginine-bradykinin (LysDABK). There are two types of kallikreins: plasma and tissue kallikreins. Both are serine protease enzymes that cleave precursor proteins, "kininogens", to release kinins. The plasma kallikrein system is involved in triggering the intrinsic coagulation cascade and consists of three serine proenzymes (factors FXII, FXI and prekallikrein) and high-molecular-weight kininogen (HMWK). Contact between plasma and a negatively charged surface, which occurs at sites of blood vessel injury, induces the activation of factor XII to factor XIIa, subsequently stimulating the conversion of prekallikrein to kallikrein, followed by the

cleavage of HMWK to release BK, in addition to triggering the coagulation cascade. Another mechanism for initiation of the activation of the kallikrein–kinin system depends on binding of components of the contact activation cascade on the surface of cells, such as leukocytes, platelets, endothelial cells and myocytes (1, 2).

The tissue kallikrein system is widely distributed throughout the body. Tissue kallikrein preferentially cleaves low-molecular-weight kininogen to generate LysBK. The mechanisms that regulate the tissue kallikrein activity are poorly understood (1). Regulation mechanisms include secretion and codistribution of a kallikrein inhibitor protein, kallistatin (3, 4). However, abundant evidence indicates that the kallikrein–kinin system is activated in response to tissue injury, infection and inflammation, leading to local release of kinins.

BRADYKININ RECEPTORS

The released kinins exert various effects via two G protein-coupled receptors –the bradykinin B_1 and B_2 receptors (5, 6)–, the existence of which has been confirmed by expression cloning experiments in several species, including mice (7, 8), rats (9, 10), rabbits (11, 12), dogs (13) and humans (14-16). Although the existence of a third bradykinin receptor (B_3) had been postulated (17), it could not be validated by cloning and was finally refuted (18). The released primary kinin peptides, BK and LysBK, are metabolized by cleavage at several sites (19). However, two metabolic pathways are dominant. Primary kinins are either metabolized to inactive peptides by angiotensin-converting enzyme (ACE, kininase II) or converted by carboxypeptidase enzymes (kininase I) via removal of the carboxy-terminal arginine residue to the des-arginine metabolites (20), DABK and LysDABK, which are also biologically active kinins with altered affinities for the bradykinin receptor subtypes. Affinities of the primary kinins and their des-arginine metabolites for the two subtypes of bradykinin receptors cloned from different species, as well as their functional agonist potencies at the human receptors, are summarized in Table I.

In principle, the primary kinins are potent and selective agonists for B_2 receptors, but have low affinity for the B_1 receptor. On the contrary, the des-arginine products are potent agonists of the B_1 receptor, but have mostly low affinity for B_2 receptors. In particular, the affinity of BK is so low for B_1 receptors that these receptors are in fact not bradykinin but "only" kinin receptors. It is worth noting here that estimated BK + LysBK and DABK + LysDABK tissue concentrations in inflamed rat (27) or human (28) tissues, or in rabbit plasma, after treatment with bacterial lipopolysaccharides (29) range at approximately 0.05-2 nmol/kg (or nmol/L), and hence, hardly exceed the low nanomolar range. Formerly, plasma and tissue concentrations of "BK-related material" were assessed by radioimmunoassay or bioassay in biological fluids from healthy or diseased patients/volunteers and were found to range from subnanomolar to 55 nM (58 ng/mL) (6), the latter being observed in synovial fluid from rheumatoid arthritis (RA) patients during exacerbation (30). Interestingly, although DABK has equal or higher affinity than LysDABK for rodent (rat and mouse) and dog B_1 receptors, it has two orders of magnitude lower affinity in rabbits and negligible relative affinity in humans. Despite this, DABK appeared as a potent functional B_1 receptor agonist at cloned recombinant human B_1 receptors. However, in functional studies of native B_1 receptors in human umbilical vein endothelial cells, DABK had only moderate potency, two orders of magnitude less than LysDABK (see Table I). On the other hand, LysBK appeared to have rather high affinity but low functional potency at human B_1 receptors. In brief, both BK and LysBK are potent and selective B_2 receptor agonists, whereas LysDABK is a potent and selective B_1 receptor agonist in all species. DABK is also a potent and selective B_1 receptor agonist in mice, rats and dogs, but not in rabbits, monkeys and humans. The B_1 receptor-stimulating des-arginine products are also inactivated, mainly by ACE. However, while the natural B_2 receptor agonists have short half-lives (e.g., 10-50 s for BK), the natural B_1 receptor agonists have 4- to 12-fold longer half-lives (31, 32). As a consequence, in circumstances associated with chronic activation of the kallikrein–kinin system the B_1 receptor agonist peptides are more prone to accumulation.

Table I. *Affinity and functional potency values of natural kinin peptides in various species.*

	Species	Affinities (K_i, nM)			
		BK	LysBK	DABK	LysDABK
B_1	Mouse (8, 21)	> 1,000	510	0.3-0.7	1.3-1.7
	Rat (10)	120	–	2.0	2.4
	Rabbit (12)	> 5,000	19	32	0.23
	Dog (13)	520	440	4.7	1.6
	Rhesus (22)	2,200	110	350	0.17
	Human (23)	> 10,000	1.35	1370	0.12
B_2	Mouse (7)	0.48	0.52	8.1	6,400
	Rat (24)	0.07	0.09	> 100,000	–
	Rabbit (11)	≈ 3	≈ 1.5	> 1,000	> 1,000
	Dog (13)	3.9	2.1	> 10,000	> 10,000
	Human (7, 24)	0.12-0.54	0.30-0.63	8,100 69,000	> 30,000
		Functional potencies at recombinant receptors (EC_{50}, nM)			
		BK	LysBK	DABK	LysDABK
B_1	Human (25)	1,000	243	8.6	7.9
B_2	Human (25)	2	10	> 3,000	> 10,000
		Functional potencies on human umbilical vein (EC_{50}, nM)			
		BK	LysBK	DABK	LysDABK
B_1	Human (26)	> 5,000	> 5,000	204.2	2.5
B_2	Human (26)	2.5	1.5	> 5,000	> 5,000

BK, bradykinin; LysBK, lysyl-bradykinin; DABK, des-arginine-bradykinin; LysDABK, lysyl-des-arginine-bradykinin.

The regulation of the expression and activity of B_1 and B_2 receptors is remarkably different. Bradykinin B_2 receptors are constitutively expressed in a wide variety of tissues and cell types. Moreover, B_2 receptors, when exposed to bradykinin, undergo rapid desensitization and internalization (19). Therefore, they are more predestined for mediating acute-phase responses immediately following injury and initiation of inflammation. In contrast, B_1 receptors are expressed at a very low level, if at all, in healthy tissues, but undergo massive upregulation (or if they lack constitutive expression, "induction") upon tissue injury or inflammation. Nevertheless, B_1 receptors are not prone to desensitization or agonist-induced recep-

tor internalization. These features, i.e., activation by secondary kinins, which have a longer half-life, and the inducible character, predestine B_1 receptors more for mediating subacute and chronic phases of inflammation and associated pain. However, it is worth noting that there are some exceptions to this general rule. The constitutive presence of B_1 receptors in blood vessels of dogs (33-35) and in the sensory neurons, spinal cord and other central nervous system (CNS) sites of rats, primates and humans has also been demonstrated (36-41). Moreover, upregulation of B_2 receptors has also been described under some conditions, for example in neuropathies (41, 42), simultaneously with B_1 receptor induction. However, desensitization and internalization remain limiting factors of long-term effects via B_2 receptors, particularly in inflammation.

Several proinflammatory/injury mediators have a documented role in induction of B_1 receptors, including growth factors, such as epidermal growth factor (EGF) (43) and glial cell line-derived neurotrophic factor (GDNF) (44), interferon-γ (45) and cytokines, including TNF-α, IL-1β, IL-6 and IL-8 (46-48). Moreover, B_1 receptors are autoinduced by B_1 and B_2 kinin agonists. Furthermore, IL-1β and LysDABK can act synergistically in a supra-additive fashion to boost B_1 receptor induction (49), and priming with bradykinin also leads to B_1 receptor induction in vivo (50), as well as in vitro (46, 51), and to evolving function of B_1 receptors. The induction elicited by B_2, but not B_1, receptor agonists appeared to be IL-1β-dependent (46). The induction mechanisms involve activation of nuclear factor NF-κB-mediated promoter regulation (52, 53); however, several other mechanisms have also been postulated (54, 55). Topical or systemic exposure to bacterial endotoxins, such as lipopolysaccharide (LPS), is also a strong inducer, mostly via stimulation of cytokine release (56). In turn, kinins stimulate the synthesis and release of nitric oxide (NO), cytokines (57), arachidonic acid, prostaglandins (58), prostacyclin (PGI_2) (59, 60), leukotrienes (59), as well as other chemotactic factors (61).

The presence of both B_1 and B_2 kinin receptors has been documented in diverse tissues and cell types, including endothelial cells (62), vascular (59, 63), gastrointestinal (64, 65) and smooth muscle cells, fibroblasts (46, 66), osteoblasts (67), mucosal epithelium (68, 69), glandular tissues/cells (68, 70) and various blood and inflammatory cells, i.e., neutrophils (71, 72) and eosinophils (73), granulocytes, lymphocytes (45), dendritic cells (74), macrophages (68, 69) and mast cells (75), both in animals and humans. Moreover, expression of both kinin receptors was also shown in the CNS. Constitutive B_1 receptor expression was found in neurons of the thalamus, hypothalamus and spinal cord of rats, monkeys and humans (36, 39, 40, 76, 77). Bradykinin B_1 receptor expression was also found in sensory ganglia (37, 78), and in the spinal cord, B_1 receptor immunoreactivity is confined to spinal dorsal horn neurons and central terminals of afferent fibers (39). Most attempts were unsuccessful at demonstrating a direct functional effect of B_1 receptors on peripheral sensory nerves (79, 80) or nociceptors, even after inducing stimuli. However, recently, potentiation of heat-evoked current by DABK could be demonstrated in GDNF-treated sensory neurons (44). This contrasts with the well-documented constitutive presence and sensitizing function of B_2 receptors on primary afferents (81). Bradykinin B_1 and B_2 receptors are also present in glial cells (82).

The cellular effects of kinin receptor activation include either contraction or relaxation of smooth muscle cells, production and release of NO and inflammatory mediators, direct or indirect activation and sensitization of sensory nerves (83), and stimulation of cell proliferation or collagen synthesis (84, 85). These effects occur via numerous signaling pathways. At first glance, the major signals generated by the B_1 and B_2 receptors appear to be identical, with the reservation that normally only B_2 receptor-mediated effects can be observed, but a switch occurs to major involvement of B_1 receptors under inflammatory conditions. However, differential signaling pathways for B_1 and B_2 receptors have also been postulated (62). Signaling via activation of phospholipase C (PLC), leading to the formation of diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3), which increases cytoplasmic Ca^{2+} concentrations, is well defined in numerous cell types (83). DAG activates protein kinase C (PKC), which modulates the function of other proteins, such as the thermo- and capsaicin-sensitive transient receptor potential channel TRPV1 of nociceptors. The increased cytoplasmic Ca^{2+} concentrations participate in the release of various mediators (cytokines, chemokines, NO) or elicit contraction of smooth muscle cells. Release of NO from the endothelium mediates vasodilation of blood vessels. Metabolism of DAG yields products of the arachidonic acid cascade. Kinin agonists also activate phospholipase PLA2, which releases arachidonic acid, leading to formation of vasodilator prostaglandins such as PGI_2 (83). NO and PGI_2 are thought to be major endothelium-derived vasodilators, but endothelial-derived hyperpolarizing factors also play a role (86). Furthermore, several signaling pathways of kinin receptors involving the extracellular signal-regulated kinase and mitogen-activated protein kinase (ERK/MAPK) and β-arrestin pathways are reviewed and discussed in more detail elsewhere (62, 83, 87).

EXPERIMENTAL TOOLS FOR ELUCIDATING THE ROLES OF B_1 RECEPTORS

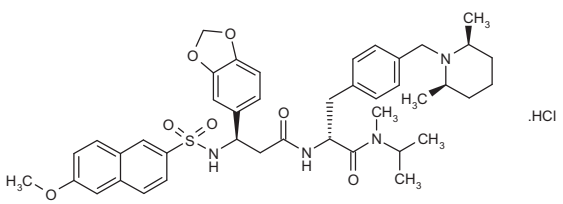
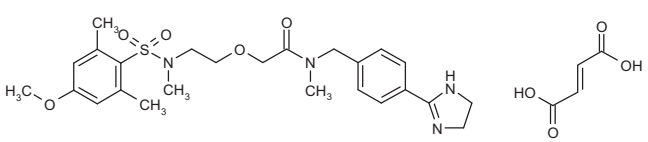
Exploration of the role of B_1 receptors in pathophysiology and exploitation of their potential therapeutic utility has been hindered by the difficulty of making good bioavailable pharmacological agents (peptidomimetics) for this peptide receptor and also by the significant species-specific differences in the sequence, structure and pharmacological sensitivity of B_1 receptors. Amino acid sequence identities of B_1 receptors of different species compared to the human sequence are as follows: mouse 68% (21), rat 71% (10), dog 76% (13) and rabbit 78% (12), whereas rhesus and African green monkeys have a very high sequence identity of 97% (22). For comparison, the sequence identity between mouse and rat B_1 receptors is 89% (10), and that between human B_1 and B_2 receptors is only 36% (16). Pharmacological studies with peptide ligands suggested that there are two groups of B_1 receptor subtypes or classes defined by species. One group –"rodent-type"– comprises rat, mouse, hamster and dog, and the other –"human-type"– comprises rabbit, pig, monkey and human (88, 89). Therefore, several classes of new B_1 receptor antagonists designed to act on human B_1 receptors are inactive or weakly active on rodent receptors and thus cannot be used in rats and mice, the most feasible species for nonclinical studies. To circumvent this obstacle, researchers have either used antagonists as pharmacological tools, which are also potent against rodent B_1 receptors, or applied non-rodent animal models using either rabbits, or exceptionally monkeys, for in vivo pharmacological experiments, or developed transgenic animal approaches.

Pharmacological tools

Apart from the use of B_1 receptor knockout mice, the vast majority of studies used peptide or nonpeptide B_1 receptor antagonists to reveal the participation of B_1 receptors in pathophysiological processes. For proper interpretation of the results, it is essential to consider the properties, i.e., virtues and limitations, of these pharmacological tools. The agonists used in exploration of the field were mostly restricted to the natural peptide agonists DABK and LysDABK, although recently, further optimized synthetic peptide agonists were also proposed (90). Table II summarizes the main characteristics of most B_1 receptor antagonist compounds used for in vivo studies.

The first potent B_1 receptor antagonists, DALBK and LysDALBK, were obtained by replacing phenylalanine at position 8 with leucine in the natural B_1 receptor agonists. Although they are pure antagonists at human and rabbit B_1 receptors, in rats and mice they proved to be partial agonists with considerable intrinsic activity (see references in Table II). Some early studies on analgesic effects in rats revealed dose-dependent effects for DALBK, with bell-shaped dose–response curves peaking around 10-30 nmol/kg s.c. (102, 103) or 100 nmol/kg i.p. (104). This finding may be due to the fact that at low doses it behaves as an antagonist but at higher doses the agonist effect becomes significant (19). On the other hand, much higher doses of DALBK have often been used in various studies with apparent analgesic effects. The interpretation of these effects may be questioned, since low doses of the agonist DABK can also cause analgesia in certain circumstances (105). Therefore, conclusions drawn with pure antagonists may be more reliable. A second gener-

Table II. *Selective B_1 receptor antagonists used for elucidation of function in vivo.*

Name/code	Structure	Characteristics
Peptide, first-generation		
des-Arg9-[Leu8]-BK (DALBK; B6769)	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu	Most extensively studied in vivo in rodents. Effective by topical, i.v., i.p., s.c. administration. K_i: 3 nM (rat) (10), 4 nM (mouse), 90 nM (rabbit), 130 nM (human) (8). Partial agonist at mouse, rat and dog but pure antagonist at rabbit and human B_1 receptors (13, 25, 33, 64, 91, 92).
Lys-des-Arg9-[Leu8]-BK (LysDALBK)	Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu	Rabbit/human-preferring antagonist. K_i: 7.5 nM (mouse), 0.4 nM (rabbit), 0.4 nM (human) (8). Partial agonist at "rodent-type" but not at "human-type" B_1 receptors.
Peptide, second-generation		
des-Arg-Hoe 140	[D-Arg]-Arg-Pro-Hyp-Gly-Thi-Ser-[D-Tic]-Oic	More stable than first-generation ligands (93). K_i: 24 nM (mouse), 20 nM (rabbit), 20 nM (human). Weak partial agonist at rat B_1 receptors (89). Occasionally used in vivo (94).
Peptide, third-generation		
R-715	[Ac-Lys]-Arg-Pro-Pro-Gly-Phe-Ser-[β-D-Nal]-Ile	More stable and potent than first-generation ligands. K_b: 3 nM (rabbit and human), 100 nM (mouse). Pure antagonist! (95-97). Second most widely used peptide B_1 receptor antagonist in vivo after DALBK. Effective by topical, i.v., i.p. and s.c. administration. Considered to be not brain penetrant (98).
R-954	[Ac-Orn]-Arg-Oic-Pro-Gly-[αMe-Phe]-Ser-[β-D-Nal]-Ile	Features similar to R-715. Less widely used. More stable against kininase II from platelets (97).
Nonpeptide		
SSR-240612	 .HCl	Potent and selective B_1 receptor antagonist in all species. K_i: 0.5 nM (human); K_b: 0.4 nM (rat), 1.3 nM (rabbit). Weak B_2 receptor antagonist, K_i: 358 nM. Orally active in a variety of in vivo models (99). May be brain penetrant to some extent (?) (100). Most widely used nonpeptide B_1 receptor antagonist.
LF-22-0542		Potent and selective B_1 receptor antagonist in all species. K_b: 0.6 nM (human), 2 nM (mouse), 3 nM (rat). Not orally active but effective by s.c. and i.p. route (101). Considered to be brain penetrant (98).

ation of B_1 receptor antagonists with somewhat improved metabolic stability is represented by the des-arginine product of the peptidic B_2 receptor antagonist Hoe-140. However, the third-generation peptidic B_1 receptor antagonists R-715 and R-954, which are devoid of partial agonist effects and are metabolically more stable, are better research tools for elucidating functions of B_1 receptors. SSR-240612 was the first orally active nonpeptide B_1 receptor antagonist ligand, which has recently gained popularity as a research tool, as it is almost equally potent on rat and human B_1 receptors (99). Its brain penetration has not been publicly documented, but some pharmacodynamic studies suggest access to central B_1 receptors (100). LF-22-0542 is another nonpeptide B_1 receptor antagonist with high potency on rodent B_1 receptors (101). Although not documented, it is discussed as a brain-penetrating antagonist (98). Nevertheless, it is effective only by the parenteral route of administration. Although L-F22-0542 caused minimal sedation and motor impairment at a high dose (100 mg/kg s.c.), there appears to be sufficient separation from analgesic effects (≥ 10 mg/kg) (101). These nonpeptide antagonists are also devoid of partial agonist activity and their recent use has greatly contributed to exploration of B_1 receptor functions.

Non-rodent in vivo experiments

Many nonpeptide B_1 receptor antagonists with potent activity at the human receptor also have high potency at rabbit but low potency at rat and mouse receptors. For in vivo pharmacodynamic characterization of such compounds, some research groups invented novel preclinical models in rabbits. For example, a rabbit spinal reflex measurement after induction with complete Freund's adjuvant (CFA) was established by Merck scientists (106) and a CFA-induced mechanical hyperalgesia test was applied recently by Amgen scientists. We also published the use of a new rabbit model measuring weight-bearing incapacitance of forelimbs after CFA-induced arthritis (107). Due to the high sequence homology and pharmacological similarity between monkey and human B_1 receptors, a few studies were performed in monkeys. Merck researchers used African green monkeys (108) or rhesus monkeys (109) for in vivo B_1 receptor antagonist potency studies on the hypotensive effects of the B_1 receptor agonist LysDABK after priming with LPS. Anti-hyperalgesic effects were measured in rhesus monkeys in a carrageenan-induced thermal hyperalgesia test by an Elan research group (89).

Transgenic animals

Bradykinin B_1 receptor knockout mice, which were reported to develop in good health but exhibited hypoalgesia and blunted inflammatory responses (110), have been extensively used for exploration of B_1 receptor function by academic groups. For studying the effects of specific human receptor antagonists, "humanized" transgenic mice and rats have been developed. Merck scientists generated a transgenic rat that overexpresses human B_1 receptors in neural tissues (111) for the study of human B_1 receptor occupancy in the rat brain. Unfortunately, these rats were unsuitable for pain studies and attempts to develop a brain-penetrating radioligand have failed. However, this rat model appeared to be suitable for ex vivo occupancy studies (112). For analgesia studies, both Novartis and Merck researchers have developed and used a mouse line in which the mouse B_1 receptor is replaced with the human B_1 receptor and its expression is regulated by the same promoter that is inducible by inflammatory stimuli (113, 114).

PATHOPHYSIOLOGICAL ROLES OF KININ RECEPTORS WITH PARTICULAR RESPECT TO B_1 RECEPTORS

Exogeneous injection of BK into animal or human tissues elicits all the classical cardinal signs of inflammation: redness, local heat, swelling and pain (115-117). In line with these symptoms, kinins are involved in cardiovascular regulation and mediation of inflammation and pain transmission.

Cardiovascular functions

Systemic injection or infusion of BK or LysBK elicits vasodilating and hypotensive effects in various animal species (33, 108, 118, 119) and man (120, 121), despite the fact that in vitro numerous major blood vessels respond to administration of kinins with constriction (6, 122). The vasorelaxant actions of kinins are mediated via endothelium-dependent and -independent mechanisms (34). However, the dominant net systemic effect is hypotension. The major endothelium-dependent vasorelaxation involves release of NO and secondary stimulation of cGMP production, although signaling via PGI_2 and endothelium-derived hyperpolarizing factors, e.g., epoxyeicosatrienoic acids, has also been postulated (34, 123). In contrast, DABK or LysDABK do not exhibit hypotensive actions without previous priming with inflammatory stimuli in most species, except dogs, due to the lack of constitutive expression of B_1 receptors, but display comparable hypotensive actions to BK after systemic inflammatory induction by LPS treatment (33, 108, 124, 125). Accordingly, B_2 receptor knockout mice and B_2 receptor antagonist-treated rats and mice have elevated basal blood pressure (126). Furthermore, they develop cardiomyopathy (127), are prone to further hypertension exacerbated by a high-salt diet (126, 128) and have disrupted preconditioning protection under conditions of repeated ischemia–reperfusion (129). These features clearly indicate the involvement of B_2 receptors in normal cardiovascular regulation and that long-term administration of B_2 receptor antagonists might have detrimental consequences with regard to cardiovascular risks. This is the main reason why B_2 receptor antagonists, although they could be antiinflammatory analgesics, have not gained importance in therapy as systemic treatment, except for the peptide antagonist icatibant (HOE-140) for the treatment of the acute condition hereditary- or ACE inhibitor-induced angioedema (130). Another B_2 receptor antagonist (anatibant, LF-16-0687) has also been developed for the treatment of an acute condition, traumatic brain injury (131). Furthermore, recently, the B_2 receptor antagonist MEN-16132 (fasitibant chloride hydrochloride) (132) has entered clinical development as a topical (intra-articular) treatment for osteoarthritis (OA) (ClinicalTrials.gov Identifier NCT01091116).

On the contrary, B_1 receptor knockout mice are healthy and normotensive (110). This is also in contrast with the phenotype of COX-2 knockout mice. It is worth noting that the COX-2 enzyme, like B_1 receptors, had been proposed to be constitutively expressed and thus not to participate in physiological but only in pathological processes. Nevertheless, COX-2 knockout mice exhibit elevated blood pressure, accelerated thrombogenesis (133), renal abnormalities, cardiac fibrosis and increased mortality (134), which is in line with the clinical observation of an increased incidence of myocardial infarction and stroke in patients treated with COX-2 inhibitors.

Kinins are believed to exert hypotensive and cardioprotective effects normally exclusively via the B_2 receptor. However, when B_2 receptors are pharmacologically or genetically ablated, the B_1 receptor under-

goes a compensatory overexpression and may take over their role (135, 136), suggesting that simultaneous blockade of both B_2 and B_1 receptors might cause more adverse cardiovascular effects than blocking B_2 receptors alone.

The major interest of researchers and drug companies turned in the last decade to the B_1 receptor and its antagonism as a promising drug target due to its special inducible nature (54, 83, 137, 138). However, in the context of therapeutic utility, it is not enough that B_1 receptors are not involved in physiological processes if B_1 receptor antagonists have detrimental consequences in pathological situations when B_1 receptors are induced. Therefore, numerous studies have been aimed at assessing the function of B_1 receptors in pathological situations by administering B_1 receptor antagonists or knockout of B_1 receptors. The results are equivocal, with more hints for "salutary" than for "detrimental" effects, as summarized in Table III.

The controversies may be related to the fact that in most cases, when the inflammation itself is pathogenic and destructive, B_1 receptor-mediated reduction of inflammation is salutary, but in some severe conditions B_1 receptors may contribute to tissue reparation, which might be impaired by B_1 receptor ablation. It is worth noting that all the detrimental effects were seen in very severe conditions. The most troublesome finding in the context of side effect liability of B_1 receptor antagonists was enhanced atherosclerosis (154). However, this observation was also made in a seriously challenged condition, i.e., high-fat diet and knockout of apolipoprotein E (Apo-E). Bradykinin B_1 receptors are upregulated in atherosclerotic plaques of human blood vessels and it has been proposed that inflammation, B_1 receptor stimulation and leukocyte migration may play a pathogenic role in early stages of atherosclerosis, suggesting that B_1 receptor antagonists may be useful for the prevention of atherosclerosis (157, 158). To sum up, B_1 receptor antagonism may be a two-edged sword with beneficial effects in early stages of atherosclerosis or when an inflammatory condition, such as rheumatoid arthritis (RA) (159), is the main pathogenic factor, but may be detrimental in severely progressed stages, or when serious dyslipidemia is the main pathogenic factor.

Central B_1 receptors also appear to be involved in pathological cardiovascular regulation. Bradykinin B_1 receptors are upregulated and kinin concentrations are increased in the hypothalamus of spontaneously hypertensive rats (SHR; a model of essential hypertension) (160, 161). Intracerebroventricular (i.c.v.) injection of LysDABK increases the blood pressure of both normal rats (Wistar–Kyoto, WKY) and SHR, which is in line with the constitutive expression of centrally located B_1 receptors (162). However, i.c.v. administration of B_1 receptor antagonists (DALBK or R-715) or antisense oligonucleotides for B_1 receptors, or systemic administration of brain-penetrating B_1 receptor antagonists (SSR-240612 or LF-22-0542), but not non-penetrating antagonists (R-715), markedly reduced blood pressure in hypertensive (either SHR or diabetes-induced), but not in normotensive control (WKY) rats (98, 100, 162, 163). These data sug-

Table III. *Salutary and detrimental effects of pharmacological or genetic ablation of B_1 receptors in models of pathological conditions.*

Ablation	Species	Condition/finding
Salutary effects		
KO	Mouse	Reduced infarct size in I/R injury of isolated heart (in vitro) (139)
KO	Mouse	Reduced myocardial infarct size following I/R injury (in vivo) (140)
KO & B1a	Mouse	Protection (reduced infarct) in a transient focal brain ischemia model (141)
KO & B1a	Mouse	Protection (including preserved renal function) against renal I/R injury (142, 143)
B1a	Mouse	Protection (including glomerular and tubular lesions, renal fibrosis, renal function) in nephrotoxic serum-induced glomerulonephritis model (144)
KO & B1a	Mouse	Protection against ureteral obstruction-induced nephropathy and fibrosis (145, 146)
B1a	Rat	Protection against I/R bowel injury and associated hypotension (147)
KO	Mouse	Attenuation of streptozotocin-induced diabetic cardiomyopathy (including improved systolic and diastolic function) (148)
KO	Mouse	Reduced (but B_2 KO-enhanced) the hypotensive effects in lipopolysaccharide-induced model of endotoxemic shock (149)
B1a	Rat	Reduced plasma extravasation (microvascular permeability in numerous tissues including retina) in streptozotocin-induced diabetic vasculopathy (150-152)
Detrimental effects		
KO & B1a	Mouse	Revascularization and recovery of blood flow blunted after excision of femoral artery (153)
KO	Mouse	Enhanced formation of atherosclerotic plaques in animals provoked by cholesterol rich-diet and deletion of apolipoprotein E gene (154) (N.B. These animals show considerable damage without deleting the B_1 receptor)
B1a	Rat	Reduced survival rate following pancreatitis induced by I/R (155)
KO	Mouse	Enhanced (but B_2 KO-reduced) the hypotensive effect and mortality in lipopolysaccharide-induced model of endotoxemic shock (156)

KO, mice born with deleted bradykinin B_1 receptor gene ($^{-/-}$) compared to wild-type ($^{+/+}$); B1a, treatment with B_1 receptor antagonist compared to vehicle.

gest a beneficial (antihypertensive) rather than an adverse role for B_1 receptor antagonists with central disposition in pathological states of hypertension.

Overall, the above data, including studies with normal knockout animals, as well as those challenged in various disease models, suggest that the cardiovascular safety profile of B_1 receptor antagonists in a long-term treatment setting may be better than that of COX-2 inhibitors or B_2 receptor antagonists. In our view, there is a good chance that in a diseased population with some inflammatory condition a B_1 receptor antagonist might exhibit a cardiovascular risk-reducing effect. However, in conditions with serious damage, where healing via a proliferative process, e.g., fibrosis or revascularization, may be needed, avoiding treatment with B_1 receptor antagonists might be prudent. Ultimately, cardiovascular event outcome data from large clinical trials with B_1 receptor antagonists should give us definite answers.

Role in inflammation

Inflammation occurs as a response to infection or tissue injury and involves a highly complex interplay between multiple factors at the humoral, cellular and neuronal level. During inflammation, besides activation of the kallikrein–kinin system, other inflammatory mediators, e.g., cytokines, prostanoids, histamine, neuropeptides and NO, are released from various sources and comprise the "inflammatory soup". The released mediators generate the symptoms of inflammation, i.e., vasodilation, extravasation and pain, as well as attract inflammatory cells to aid in elimination of microbes and tissue debris, and also to potentiate tissue repair. Kinins have been implicated in the release of all the above-mentioned mediators, but there are certainly redundant proinflammatory pathways. Due to the inducible nature of the B_1 receptor, the B_1 receptor agonist kinins are involved in positive feedback loops, such as cytokine > B_1 receptor induction and stimulation > chemotaxis and cellular response > cytokine and prostanoid release. Therefore, B_1 receptor antagonists can attenuate but do not completely block the inflammatory response. Both BK and DABK can increase the permeability of blood vessels and rapidly cause edema via stimulation of B_2 and B_1 receptors, respectively (164). Increased spontaneous and B_1 receptor agonist-induced extravasation is present, for example, in streptozotocin diabetic rats, and this can be almost completely reversed by chronic treatment with the B_1 receptor antagonists R-715 and R-954 (165, 166). Stimulation by B_1 receptors of fibroblasts leads to increased collagen synthesis (85) and B_1 receptor ablation prevents pathological fibrosis (145, 146, 148). Although fibrosis may be a reparative process after serious tissue injury, it is also an adverse consequence of chronic inflammation, for example, in the lung (167). In conclusion, mitigation of inflammation with B_1 receptor antagonists is a potential therapeutic opportunity for several indications, such as inflammatory bowel disease (168, 169). However, more mechanisms and opportunities related to potential antiinflammatory use of B_1 receptor antagonists are discussed elsewhere (54).

Role in pain transmission

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage (170). Pain sensation is normally transmitted and processed by pain pathways, which include various receptors ("nociceptors") sensitive to mechanical, chemical and thermal stimulus modalities, afferent nerve fibers with their cell bodies in the sensory ganglia (e.g., dorsal root or trigeminal ganglia), spinal cord neurons, and a complex network of spinal and supraspinal connections ("the pain matrix") that processes and regulates the sensory and emotional experience and effector responses. Pain is a physiological process that serves the obvious function of avoiding damage. However, under pathological conditions, such as inflammation or nerve injury, the pain often outlives its usefulness as a warning system and instead becomes chronic and debilitating. This transition to a chronic phase involves changes within the spinal cord and brain, but there is also remarkable modulation where pain messages are initiated, i.e., at the level of the primary sensory neurons and their most distal structures, the nociceptors (171). Thus, pathological dysfunction of the system leads to peripheral and/or central sensitization, which manifests as spontaneous pain sensation, i.e., pain without stimuli, and exaggerated sensitivity to normally nonpainful innocuous stimuli, called "allodynia", or to painful stimuli, called "hyperalgesia". It is a serious gap between pain states in humans and pain research in animal models that a good measure of pain sensation does not exist and we usually rely on motor responses, i.e., nocifensive behaviors such as paw flinching or licking, withdrawal responses or vocalizations. In preclinical pain studies, depending on the method of stimulation, thermal hyperalgesia/allodynia (warm/hot stimuli), mechanical or tactile hyperalgesia/allodynia, cold allodynia or chemically induced nocifensive behaviors are investigated.

Bradykinin has long been known as an algogenic substance in humans when applied onto exposed tissues ("blister base") or injected or microdialyzed into tissues (115, 117, 172, 173). This effect can be antagonized by a B_2 receptor antagonist (115). Experimental results indicate the same in animals, i.e., injection of B_2 receptor agonists (BK or [Tyr8]-BK) into the paw elicits nocifensive behaviors in naive mice (42) or rats (104). On the contrary, the B_1 receptor agonists LysDABK, microdialyzed into the skin in humans (173), or DABK, injected in rodents (42, 104, 174, 175), do not elicit significant pain or nocifensive behaviors in normal subjects. However, ample evidence suggests that after inflammatory stimuli and various neuropathic conditions, B_1 receptors are upregulated in the affected skin, afferent nerves, dorsal root ganglion (DRG) neurons, and a strong algogenic response appears upon application of B_1 receptor agonist peptide(s) in the form of nocifensive behaviors and hyperalgesia in animals (42, 104, 174, 175) and increased pain scoring in humans (173). The DABK-induced pain can be abolished by B_1 receptor antagonists applied either topically (175) or systemically (104). These functional pharmacodynamic results, in terms of pain perception, are consistent with the notion that B_2 receptors are constitutively present in healthy tissues, whereas B_1 receptors are inducible. On the other hand, when injected intrathecally, both BK and DABK elicited hyperalgesia (176, 177), a finding which might be consistent with constitutive expression of B_1 receptors in the spinal cord of naive animals (39). However, it should be noted that an intrathecally administered substance can also reach the dorsal root ganglia (178), where constitutive expression has also been demonstrated (37, 38, 41, 78, 177).

Genetic ablation of bradykinin B_1 receptors in mice causes slight hypoalgesia, as detected by increased latencies either in hot plate (110) or tail immersion flick (101) tests, but only in a limited stimulating temperature range (52-55.5 °C), i.e., mildly painful thermal stimulation. Testing thermal hyperalgesia by radiant heat or tactile

hyperalgesia by withdrawal threshold testing with von Frey filaments did not show any analgesic effect (101). This mild hypoalgesic effect in mice, to the best of our knowledge, has not been demonstrated with any B_1 receptor antagonist. However, in rats, in a hot plate test at 52 °C, one study demonstrated increased latency after administration of the reputedly CNS-penetrating B_1 receptor antagonist LF-22-0542 (101). Bradykinin B_1 receptor knockout mice exhibit attenuated nocifensive behavior compared to wild-type controls in the formalin test modeling tonic pain (101, 110). The pain induced by plantar injection of formalin has two characteristic phases, with an immediate appearance of nocifensive behavior (first phase: 0-5 minutes post-injection) followed by an apparent relief (around 10-15 minutes), and a second exacerbation of pain (15-40 minutes) (179). Apparently, both the first and second phases of formalin-induced pain were attenuated by approximately 50% in B_1 receptor-ablated mice (110). Studies with antagonists in rats and mice, including DALBK administered topically, systemically or intrathecally (102, 176, 180, 181), as well as SSR-240612 and LF-22-0542 administered systemically (99, 101), confirm a role for B_1 receptors in mediating formalin-induced pain. Interestingly, intrathecally administered DALBK attenuated only the second phase but not the first phase in mice (176). Genetic ablation or pharmacological blockade of B_1 receptors in mice by LF-22-0542 also attenuated the nocifensive behavior in the i.p. acetic acid-induced writhing test (101). The mild thermal hypoalgesia, the first-phase formalin-inhibitory effects and inhibition of writhing by B_1 receptor ablation suggest some role for B_1 receptors in acute nociception, which is independent from B_1 receptor induction. Whether this role is associated with the low level of constitutive expression of B_1 receptors in sensory neurons or in other cells releasing algogenic substances, or centrally located B_1 receptors play a role, remains elusive. However, these effects on acute nociception do not yield a strong analgesic action in acute pain.

The efficacy of B_1 receptor antagonists in inflammatory pain and hyperalgesia is well established. Several studies indicated mitigation of the role of B_2 receptors and evolvement of that of B_1 receptors in conditions associated with inflammation (176, 182-185). B_1 receptor antagonists inhibit the hyperalgesia following acute inflammation induced by carrageenan (89, 101, 102, 186), which usually resolves within 24-48 hours, as well as the more chronic inflammation caused by CFA (109, 114, 176, 177, 184), which –depending on dose– lasts from 3 days to several weeks. The role of B_1 receptors in these inflammatory hyperalgesic states is supported by B_1 receptor knockout mouse data with measurement of either mechanical or thermal hyperalgesia (101, 114, 184). Bradykinin B_1 receptor antagonists are also effective pain relievers after several other inflammatory stimuli, such as injection of cytokines or zymosan, with similar efficacy to the NSAIDs diclofenac or indomethacin (103, 175). Accordingly, B_1 receptor antagonists hold promise as therapeutics for the treatment of chronic inflammatory conditions, particularly arthritis (54, 185, 187).

Nevertheless, until recently, very few studies investigated the efficacy of a B_1 receptor antagonist in animal models of genuine arthritis, i.e., monoarthritis elicited by CFA injection into the ankle or knee joint (102, 185, 188), not simply into the paw, or polyarthritis models, such as peptidoglycan-induced polyarthritis (189). Furthermore, only the effect of the partial agonist DALBK was tested in rats, which has its limitations, as discussed above. However, we recently published findings showing that the novel nonpeptide peripherally acting B_1 receptor antagonist RGH-478 (0.3-3 mg/kg p.o. b.i.d.) effectively alleviated CFA-induced monoarthritic pain in both rats and rabbits, as assessed by the incapacitance test measuring the weight-bearing propensity of the affected leg (107). The antagonist was administered in an established subacute or chronic state of arthritis 3 and 11 days after CFA injection to rats and rabbits, respectively. The alleviation occurred immediately after the first dose and sustained pain relief developed in both species upon repeated dosing, which was equal or nonsignificantly superior compared to relevant doses of diclofenac and COX-2 inhibitors. The CFA model is considered to be a model of rheumatoid arthritis (RA). Although, osteoarthritis (OA) is also a plausible indication for B_1 receptor antagonists, we could not find any data in the public domain testing the effect of a B_1 receptor antagonist or genetic B_1 receptor ablation in an animal model of osteoarthritis. On the contrary, the efficacy of an intra-articularly administered B_2 receptor antagonist (MEN-16132) has been demonstrated in the monosodium iodoacteate-induced model of OA (132). The kallikrein–kinin system is activated and produces increased amounts of kinins in arthritis patients with various etiologies, including RA, OA, psoriatic arthritis and gout (30, 190, 191). Both B_1 and B_2 receptors are expressed in synoviocytes and endothelial cells in the synovial membrane of normal subjects and RA patients. Furthermore, in RA patients both B_1 and B_2 receptors are markedly upregulated in circulating neutrophil granulocytes, and even more extensively in neutrophils in the synovial fluid (71, 192). Therefore, the involvement of stimulation of both B_1 and B_2 receptors in arthritic inflammation and pain is likely not only in animal models, but also in human arthritis.

The peptide antagonist DALBK and the nonpeptide antagonist LF-22-0542 were shown to attenuate almost equally the nocifensive behaviors of mice in an experimental model of bone cancer pain (193). From the efficacy of the peptide it might be concluded that this analgesic effect is mediated by peripheral receptors. A caveat to this conclusion is that the dose of DALBK was rather high (10 µmol/kg s.c.), and thus its specificity as a selective antagonist of peripheral B_1 receptors may be challenged.

Painful neuropathies may result from nerve injury or damage of nerves elicited either by metabolic disorders, first of all diabetes, or by chronic treatment with neurotoxic drugs. The most widely used animal models of nerve injury include chronic constriction injury (CCI) (194) or partial nerve ligation (PNL) (195) of the sciatic nerve and the spinal nerve ligation (SNL) (196) model. Pharmacological or genetic ablation of B_1 receptors has analgesic effects on some aspects of injury-induced neuropathic pain. However, published results are controversial, probably due to differences in model conditions and pain modalities tested, as well as the involvement of peripheral neurogenic inflammatory components in the different models. Genetic ablation of B_1 receptors did not have any effect on the development of mechanical hyperalgesia in PNL (114) or tactile allodynia in the SNL model of neuropathic pain in mice (101). However, thermal hyperalgesia was alleviated by the genetic ablation of B_1 receptors in mice, and these findings, i.e., lack of effect on tactile allodynia but efficacy against thermal hyperalgesia, were further supported by pharmacological inhibition with LF-22-0542 in the SNL model in rats (101). In contrast, complete abolishment of both thermal hyperalgesia and tactile allodynia in B_1 receptor knockout mice was reported in the PNL model (174). However, in a rat PNL model, again, efficacy for LF-22-

0542 on thermal hyperalgesia, but not on mechanical and cold allodynia, was reported (41). Perhaps a species difference in the regulation of the expression of bradykinin receptors may account for this latter discrepancy. Namely, opposite changes –downregulation and upregulation– of B_2 receptors were observed in sensory neurons of mice (42) and rats (41), respectively, with simultaneous upregulation of B_1 receptors in both species following PNL surgery. To further the confusion, partial reversal of both cold allodynia and thermal hyperalgesia with relevant low doses of DALBK (with a bell-shaped dose–response relationship peaking at 100 nmol/kg i.p.) were observed in a rat SNL model. A small but statistically significant effect on mechanical allodynia was also observed in this study. In this rat model, upregulation of both B_1 and B_2 receptors was also observed in the skin and spinal nerves (104). There are at least three studies suggesting efficacy for B_1 receptor antagonists in the CCI model of neuropathic pain in rats. The nonpeptide B_1 receptor antagonist SSR-240612 caused dose-dependent reversal of thermal hyperalgesia in CCI rats. However, the doses needed for efficacy (20 and 30 mg/kg p.o.) in the CCI model were higher than those needed for maximum efficacy in inflammatory models or DABK-induced edema (3-10 mg/kg p.o.). Although the relevance of a study showing reversal of thermal hyperalgesia with extremely high single doses of DALBK (1-100 µmol/kg i.p.) may be questioned (197), another study with chronic (7-day) infusion of low doses (10 nmol/kg/day i.v.) of DALBK (198) strengthens the notion that B_1 receptor antagonists are also effective in alleviating neuropathic pain in the CCI model. In this latter study, both thermal hyperalgesia and tactile allodynia were completely reversed after 7 days of treatment. The results summarized above are quite consistent in that B_1 receptors, which are upregulated in the skin, nerves, sensory ganglia and spinal cord, are strongly involved in mediating thermal hyperalgesia following nerve injuries, but their involvement in other hyperalgesia modality domains is more controversial. The studies with genetic ablation of B_1 receptors do not differentiate between peripheral or central sites of involvement. The two nonpeptide B_1 receptor antagonists SSR-240612 and LF-22-0542 have been proposed to penetrate into the CNS. However, the peptide antagonist DALBK is believed not to enter the brain and spinal cord. Therefore, its anti-hyperalgesic efficacy could be an argument for the involvement of peripheral B_1 receptors in these states. On the other hand, since it is a partial agonist, its stimulating effect may also interfere with the hyperalgesia tests. Therefore, further studies with pure peripheral full B_1 receptor antagonists, such as R-715, in rodents would be needed to clarify the contribution of peripheral and central B_1 receptors to hyperalgesia in neuropathic pain conditions.

In a recent study, the involvement of different sites in a novel neuropathic pain model, the brachial plexus avulsion (ABP) test, was investigated and the effects of genetic ablation and pharmacological blockade of B_1 and B_2 receptors were tested (199). An interesting feature of this model is that the injured nerve innervates the forelimbs, but the thermal hyperalgesia and tactile allodynia ("hyperexcitability") are measured from the ipsilateral hindlimbs, indicating involvement of at least long intersegmental and/or supraspinal integration. The tactile allodynia develops rapidly within 4 days and remains sustained for more than 80 days after surgery, whereas the thermal hyperalgesia lasts for about 20 days. Studies with knockout mice showed complete prevention of the allodynia and hyperalgesia by B_1 but not by B_2 receptor ablation compared to wild-type animals. Studies with the B_1 receptor antagonists SSR-240612 and R-715 administered by local (surgery site), systemic (low doses: 0.3 and 0.5 mg/kg i.p., respectively), intrathecal and i.c.v. routes showed that, at the early stages (0-10 days post-surgery), local, systemic or lumbar intrathecal administration of either antagonist effectively alleviated hyperalgesia/allodynia, suggesting involvement of B_1 receptors at peripheral, DRG and/or spinal cord sites, whereas the smaller effect after i.c.v. administration suggested less involvement of supraspinal (brain) B_1 receptors. However, at a later stage, 30 days post-surgery, only i.c.v. administered antagonists proved effective, suggesting that at this stage only central supraspinal B_1 receptors are involved in the maintenance of hyperexcitability. These data also suggest that SSR-240612 at the low systemic dose applied, which is effective in inflammatory pain (99), does not provide sufficient central B_1 receptor blockade to alleviate this chronic pain state.

The B_1 receptor antagonist DALBK, although at a high dose of 1 µmol/kg i.p. in rats, was found to attenuate orofacial thermal hyperalgesia in a trigeminal pain model (200). To sum up, a large body of evidence suggests analgesic efficacy for B_1 receptor antagonists against some aspects of nerve injury-induced neuropathic pain. The results are more unequivocal for thermal hyperalgesia, but controversial for mechanical and cold allodynia/hyperalgesia. Probably some effects are mediated by peripheral B_1 receptors, but centrally acting B_1 receptor antagonists might have a broader analgesic profile affecting some aspects of central sensitization.

The potential efficacy of B_1 receptor antagonists in painful diabetic neuropathy is a special issue. Diabetes is associated with vascular permeability changes leading to many complications, including nephropathy, retinopathy, hypertension, hyperalgesia and neuropathy. A multitude of data has demonstrated the induction of B_1 receptors in various tissues and cell types, including macrophages, fibroblasts, blood vessels, kidneys, spinal cord and brain, either by morphological or functional pharmacological approaches in rodent models of diabetes (164, 201-207). Various B_1 receptor antagonists, including the probably only peripherally acting R-715, were found to alleviate thermal hyperalgesia and tactile and cold allodynia in rats and mice (98, 204, 208, 209). Most studies relate to the streptozotocin (STZ)-induced model of type 1 diabetes, which involves inflammation and causes hyperalgesia, and which is partly independent of hyperglycemia (210). However, the development of hyperalgesia, the involvement of B_1 receptors and the effectiveness of B_1 receptor antagonists were shown to be present also in non-STZ models of diabetes, such as the rat glucose feeding model of type 2 diabetes with insulin resistance (208), or genetic models, such as Zucker diabetic fatty rats (type 2) (210) and nonobese diabetic mice (type 1) (211). Bradykinin B_1 receptor antagonists either alleviated the developed hyperalgesia by acute administration (208, 210, 211), or attenuated/prevented the development of hyperalgesia by chronic administration (212, 213). These findings are also supported by B_1 receptor knockout mouse data (214). Hence, a great body of evidence suggests the potential utility of B_1 receptor antagonist drugs in the treatment of diabetic neuropathic pain, and in this case, peripherally acting B_1 receptor antagonists will probably be sufficiently effective. What is more, other complications, such as oxidative stress (213), vasculopathy and increased plasma extravasation

(150, 151), cardiomyopathy (148) and retinopathy (152, 166), are also thought to be alleviated by treatment with B_1 receptor antagonists, according to preclinical data. Therefore, painful diabetic neuropathy and other dreadful complications of this devastating disease are among the most promising indications for B_1 receptor antagonists.

COMPETITION AND PROGRESS IN B_1 RECEPTOR ANTAGONIST DRUG RESEARCH

The promise of potential utility in chronic pain and in a large number of other indications with an unmet medical need has compelled numerous pharmaceutical companies to engage in the difficult task of producing orally active nonpeptide B_1 receptor antagonists. Details of the medicinal chemistry of these efforts have been summarized in recent reviews (138, 215, 216). However, here we briefly summarize the competition in B_1 receptor antagonist research, presenting a representative set of compounds from published scientific and patent literature (Figs. 1 and 2). For most patent references, the reader is advised to access the above three medicinal chemistry reviews. Researchers at Merck, Novartis and Fournier have played a pioneering role in establishing the toolbox of bradykinin receptor research. However, the first patent on nonpeptide B_1 receptor antagonists was filed by sanofi in 1996 (217). Further optimization of that series led to the discovery of the dipeptidomimetic SSR-240612 (99, 218). Although according to a medicinal chemist's perspective it does not appear to be a "drug-like" compound, it proved to be orally active in pharmacodynamic tests (99). It is characterized by high molecular weight (757 Da), high lipophilicity and probably possesses significant metabolic liabilities associated with the naphthalene moiety. However, pharmacokinetic data for SSR-240612 have not been published. The compound reached phase II clinical development by the end of 2005 according to portfolio information released by sanofi-aventis, but development was halted for undisclosed reasons and the compound was removed from portfolio reports by 2008. Thus, SSR-240612 became a good "pharmacological tool". Furthermore, sanofi-aventis in conjunction with the acquired Fovea Pharmaceuticals is developing FOV-2304 (structure undisclosed) (152), a potent antagonist of bradykinin B_1 receptors, active as eye drops for the treatment of diabetic macular edema (Press Release, October 1, 2009). Sanofi's entrance with an aryl-sulfonamide scaffold was followed by a wave of 46 patent applications between 1999 and 2005 (priority dates) claiming nonpeptide B_1 receptor antagonists. Competitor companies in this race included Novartis, Pharmacopeia, Bayer, Merck (MSD), Fournier, Elan, Amgen and Boehringer. However, of these efforts, apparently only two compounds (MK-0686 and AMG-379) entered clinical development. A second wave of patenting activity emerged from 2006, with 39 disclosed patents filed by Boehringer, Neurogen, Grünenthal, Evotec, Jerini and Gedeon Richter between 2006 and 2009. Of these, a compound from Gedeon Richter is in clinical development, RGH-478 (structure undisclosed), which was optimized for oral efficacy in arthritis (107). It is very likely that more clinical entries will be revealed in the near future from this second wave of efforts.

Most of the patents were covering compounds with a similar aryl-sulfonamide-containing pharmacophore (Fig. 1). However, Pharmacopeia, Elan, Merck and Neurogen have also invented nonsulfon compounds that resemble the sulfonamide family (e.g., top four structures in Fig. 2). Within this family, the molecular weights of the compounds that retained specificity remained high, mostly above 500 Da, which makes it difficult, although not impossible, to optimize a viable clinical candidate with good drug disposition and metabolic properties. Merck was the most dominant player in the field of B_1 receptor antagonists with 21 patent applications. In addition, Merck scientists invented a completely new type of structure, the biaryls, with potent B_1 receptor antagonist effects, a class that was recently followed by Jerini (bottom six structures in Figure 2).

As can be concluded from both patent literature and publications, Merck's priority driving the optimization was to find a brain-penetrating clinical candidate. For this reason, they were fine-tuning potency, selectivity, molecular size, physicochemical and pharmacokinetic properties, and, in addition to oral bioavailability, they particularly focused on eliminating efflux enzyme (P-glycoprotein) liability to enable brain penetration. The first fruit of these efforts was MK-0686 (see Fig. 2; molecular weight 487 Da), which reached phase II clinical trials. According to information on www.clinicaltrials.gov, a postoperative dental pain study (NCT00533403) was started and completed in June 2004 with MK-0686. Then, an osteoarthritis study (NCT00296569) was started in September 2005 and completed in May 2006. The third study was for post-herpetic neuralgia (NCT00282763) and started in December 2005, but was prematurely terminated by August 2006. From this timing, it might be speculated that some adverse features may have emerged, since lack of efficacy in one indication probably would not have affected expectations in another. The development of MK-0686 was halted but the reason was not disclosed. Nevertheless, MK-0686 did not seem to be an ideal drug candidate. Its in vivo characterization in preclinical tests was limited by the fact that it has potent activity at human B_1 receptors (K_i = 0.4 nM), but very weak activity at rat B_1 receptors (K_i = 1646 nM). However, the oral bioavailability of MK-0686 in rats was acceptable (34%). The only published pharmacodynamic characterization for analgesic efficacy was a study of CFA-induced allodynia in human B_1 receptor knock-in mice. In this study, MK-0686 (cited as Compound 13b) had an ED_{50} value of 9.8 mg/kg p.o., with maximum efficacy at around 60 mg/kg (109), which represents a surprisingly low in vivo potency for a compound with subnanomolar affinity. Although brain penetration in mice was much lower due to mouse P-glycoprotein liability than in monkeys (109), and presumably in man, this probably does not explain the low in vivo potency in an inflammatory pain test, where the efficacy may be mediated via peripheral B_1 receptors. Another issue with MK-0686 was its affinity for pregnane X receptors (NR1I2, PXR) and associated cytochrome P450 metabolic enzyme-inducing property (219, 220). The goal of further improvements in brain penetration and elimination of PXR liability gave rise to further optimization efforts (109, 219, 221-224) and claims about selection of further clinical candidate compounds (222, 224). However, the developmental fate of these Merck compounds remains elusive.

Disclosed information is scarce about the fate of efforts of other companies. However, these reflect the struggling with optimization of such peptidomimetic compounds also afflicted by species specificity. Amgen had a development candidate, AMG-379 (structure not disclosed), which was in phase I clinical trials in 2007 according to an internet-published agenda of a New Zealand ethics committee, but only as parenteral (intravenous and subcutaneous) administra-

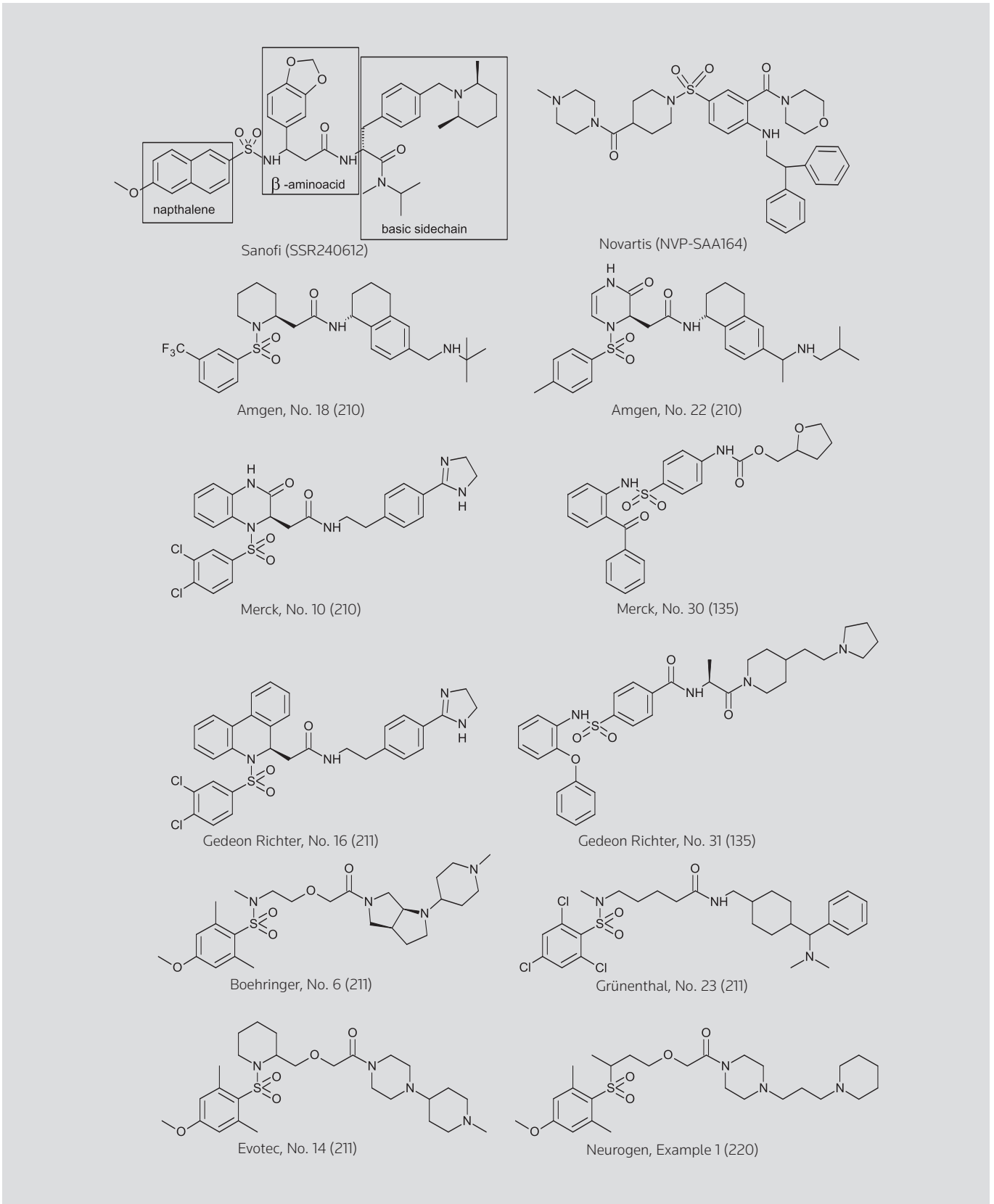
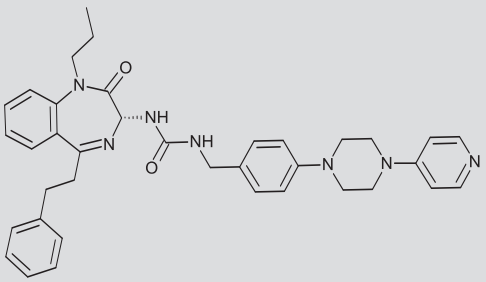
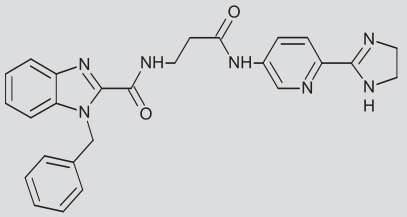


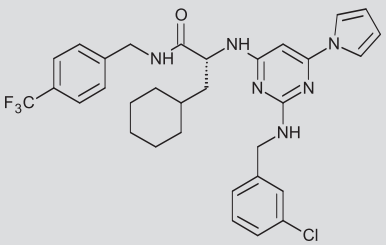
Figure 1. Representative B_1 antagonist arylsulfon(amide) structures from different companies.



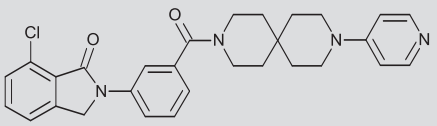
Merck, No. 33 (135)



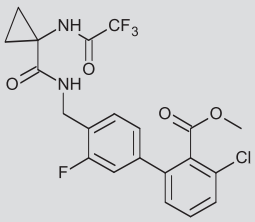
Neurogen, No. 34 (135)



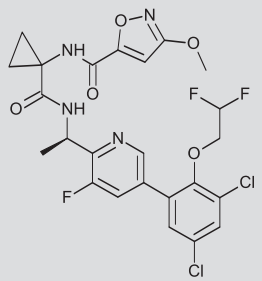
Pharmacopeia, No. 32 (210)



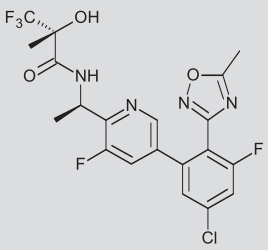
Elan, ELN441958



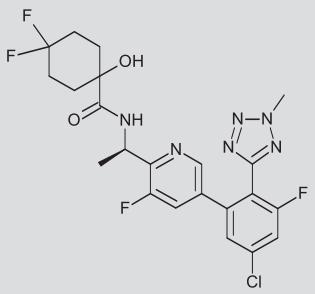
Merck, MK-0686



Merck, No. 7g (217)



Merck, No. 21 (219)



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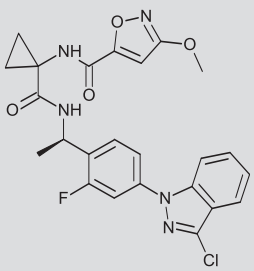

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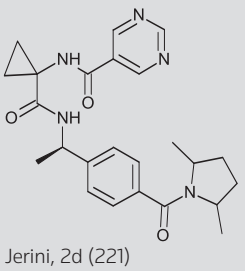

Jerini, 2d (221)

Figure 2. Representative B_1 antagonist non-sulfonamide structures from different companies.

tion, suggesting that oral bioavailability was an issue. However, AMG-379 was dropped from Amgen's portfolio in 2008. Fournier apparently ended up with the research tool compound LF-22-0542 (see Table II), also with lack of sufficient oral bioavailability (101). Novartis also quit the field without a compound going to clinic but publishing favorable scientific results using B_1 receptor knockout and human B_1 receptor knock-in mice using a compound, NVP-SAA164, that was potent at human (K_i = 8 nM) but ineffective at rat and rabbit B_1 receptors (114). Elan researchers have also discovered and described a very interesting compound, but not free from issues. ELN-441958 had good oral bioavailability in both rats and monkeys and was highly potent at primate B_1 receptors (K_b = 0.12 and 0.24 nM, respectively, for human and monkey receptors) and also considerably potent at rodent receptors (K_b = 1.5 and 14 nM, respectively, for rat and mouse receptors). Nevertheless, analgesic effects were not demonstrated in rodents. Instead, a carrageenan-induced thermal hyperalgesia test was established in rhesus monkeys, which indicated analgesic effects only at surprisingly high doses (3-10 mg/kg s.c.) and plasma concentrations (ca. 1-5 μM). Furthermore, at 10 mg/kg, side effects, such as facial flushing, paling and sedation, were observed and the compound probably caused histamine release, which may also have confounded the analgesic profiling (89).

These findings point to the great challenge of optimizing nonpeptide B_1 receptor antagonists and that fulfilling crude pharmacokinetic criteria and in vitro potencies and liability panels may be insufficient for good candidate selection.

CONCLUDING REMARKS

Preclinical data with some privileged B_1 receptor antagonists in concert with B_1 receptor knockout animals suggest that the B_1 receptor is an attractive therapeutic target for both inflammatory and neuropathic pain. However, this has to be confirmed in clinical studies with suitable clinical candidate compounds. The selection of good clinical candidates must also be supported by good in vivo pharmacodynamic results, which warrant proper drug disposition properties and high safety margins, even if this is hindered by species differences. The low success rate may be attributed to multiple factors, such as the species discrepancies in early animal studies, poor ADME properties of some compounds, or unexpected toxicity in clinical studies (138). The only two compounds known to have reached the clinical stage beyond phase I (SSR-240612 and MK-0686) were probably suboptimal. However, it is strongly hoped that some new clinical candidates will emerge from the second wave of medicinal chemistry efforts, which will be suitable for clinical proof-of-concept studies.

Another critical issue is: Do we need a brain-penetrating compound? On the one hand, it appears that for several indications, e.g., inflammatory pain or diabetic complications, there is no need for brain penetration. Adding brain penetration to the wish list with high priority may necessitate compromise in other aspects of this complicated multifactorial optimization. Furthermore, our knowledge on the function of central B_1 receptors is still very scarce. Therefore, it might be prudent to go to the clinic first with compounds optimized for efficacy in peripheral indications/disease models, such as arthritis or diabetic complications, to exploit at least this kind of promise of B_1 receptor antagonists. On the other hand, there is no doubt that appropriately distributing centrally acting B_1 receptor antagonists might have a broader analgesic profile, particularly in neuropathic pain. Nevertheless, it is also apparent that B_1 receptor antagonists and knockout animals address only some aspects of neuropathic pain. Therefore, to aim for centrally acting B_1 receptor antagonists may be a secondary approach that may carry more risks but may also hold more promise.

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DISCLOSURES

The authors state no conflicts of interest.

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