Survey Review

The Kallikrein-Kinin System: Current and Future Pharmacological Targets

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Abstract. The kallikrein-kinin system is an endogenous metabolic cascade, triggering of which results in the release of vasoactive kinins (bradykinin-related peptides). This complex system includes the precursors of kinins known as kininogens and mainly tissue and plasma kallikreins. The pharmacologically active kinins, which are often considered as either proinflammatory or cardioprotective, are implicated in many physiological and pathological processes. The interest of the various components of this multi-protein system is explained in part by the multiplicity of its pharmacological activities, mediated not only by kinins and their receptors, but also by their precursors and their activators and the metallopeptidases and the antiproteases that limit their activities. The regulation of this system by serpins and the wide distribution of the different constituents add to the complexity of this system, as well as its multiple relationships with other important metabolic pathways such as the renin-angiotensin, coagulation, or complement pathways. The purpose of this review is to summarize the main properties of this kallikrein-kinin system and to address the multiple pharmacological interventions that modulate the functions of this system, restraining its proinflammatory effects or potentiating its cardiovascular properties.

Keywords: kallikrein-kinin system, B₁ and B₂ receptors, metallopeptidase, pharmacological agent

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Introduction

The existence of the kallikrein-kinin system was first discovered almost one century ago when Abelous and Bardier, in 1909, showed the hypotensive effect of human urine (1). Since that time, this system has been and continues to be the subject of intensive research. In fact, more than 30,000 papers are referenced for kallikrein-kinin in Medline for the last 50 years.

Such a scientific interest is explained in part because of the duality and the complexity of this system. Bradykinin (BK) is at the center of this system; however, it is not the only pharmacologically active kinin. The kinins

(BK-related peptides) are generated from two types of kininogens, mainly by two types of activators: tissue and plasma kallikreins. In fact, two classes of kinin receptor ligands are now recognized corresponding to each receptor subtype (*B₁* and *B₂* receptors). The expression of these receptors is regulated by specific mechanisms. The duality also exists as to the pharmacological activity of kinins, which are often considered as either pro-inflammatory or protective (namely for heart, kidney function, angiogenesis-promoting) depending on the experimental approach, or scientific interest. This system is also complex in its distribution, as the different constituents have been shown to be present in plasma,

but also on blood cells, in various tissues or their exocrine secretions. The autocrine or paracrine activity of kinins is regulated by several metallopeptidases, the relative importance of which varies from one biological medium to the other. Finally, the regulation of this system by serpins adds to the complexity of the system, as well as its multiple relationships with other important metabolic pathways such as the renin-angiotensin, coagulation or complement pathways.

An understanding of the multifaceted aspects of the different constituents of this system is necessary to grasp the complexity of its multiple pharmacological activities, mediated not only by kinins and their receptors, but also by their precursors and their activators, and the metallopeptidases and the antiproteases that limit their activities.

The purpose of this review is, first, to summarize the main properties of the various constituents of this complex but fascinating system. We will secondly address the multiple pharmacological interventions that modulate the functions of the kallikrein-kinin system and their clinical applications.

Part I: The Kallikrein-Kinin System

The kallikrein-kinin system represents a metabolic cascade that, when activated, triggers the release of vasoactive kinins. This complex multi-protein system includes the serine proteases tissue and plasma kallikreins, which liberate kinins from high- and low-molecular-weight kininogen (HK and LK). Kinins exert their pharmacological activities by binding specific receptors, before being metabolized by various peptidases.

I- Kinins

In humans and in most mammals, the term “kinin” refers to the nonapeptide, BK (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg), the decapeptide kallidin (KD: Lys-BK), and their carboxy-terminal des-Arg metabolites. Other kinins, like T-kinin (Ile-Ser-BK) and Met-T-kinin have only been reported in the rat (2).

Kinins are implicated in many physiological and pathological processes. By virtue of their ability to activate endothelial cells, leading to vasodilation, increased vascular permeability, tissue-type plasminogen activator (t-PA) release, production of nitric oxide (NO), and mobilization of arachidonic acid, they participate in physiological (regulation of blood pressure, renal and cardiac functions) and pathological processes like inflammation.

1. The kininogens: precursors of kinins

HK and LK, the precursors of kinins (BK and KD), are produced from a structural gene localized to chromosome 3q26 → qter that is thought to have originated as a result of two successive duplications of a primordial kininogen gene (3, 4). This gene consists of 11 exons. The first 9 exons encode the heavy chain. The 10th exon codes for BK and the light chain of HK; the light chain of LK is coded by exon 11.

Both HK and LK have an identical aminoacid sequence starting at the N-terminus (heavy chain) and continuing to 12 aminoacids beyond the BK moiety but differ at the C-terminal because of alternative splicing, thereby providing the two kininogens with different light-chain moieties (3, 4). In fact, both native proteins are produced as single chain polypeptides, and the light or heavy chain nomenclature refers to their disulfide bond-assembled structure after activation by kallikrein cleavage.

1.1 High-molecular-weight kininogen (HK)

HK, an α -globulin, circulates in plasma as an 88- to 120-kDa single-chain glycoprotein at a concentration of 70 to 90 $\mu\text{g/mL}$ (5). This kininogen is a multifunctional protein composed of six domains. Each domain is thought to have distinct functions. HK heavy chain (64 kDa) contains domains 1, 2, and 3. The light chain (45 to 58 kDa) is comprised of domains 5 and 6. The heavy chain and light chain are linked by domain 4, which contains the BK sequence. Domain 1 has a low affinity calcium-binding site (6), domains 2 and 3 have specific sequences (Gln-Val-Val-Ala-Gly) that inhibit cysteine proteases (7), and domain 3 has platelet- and endothelial cell-binding activity (8). Domain 5 has cell-binding sites, antiangiogenic properties, and sequences for heparin binding (9–11). HK binds to negatively charged surfaces through the histidine region of the light chain corresponding to domain 5. Domain 6 has prekallikrein and factor XI-binding sites (12). The ability to bind to a surface (domain 5) and simultaneously complex factor XI or prekallikrein (domain 6) is responsible for the cofactor activity of HK in contact activation of plasma (13).

1.2 Low-molecular-weight kininogen (LK)

LK is a β -globulin present in human plasma at a concentration between 170 and 220 $\mu\text{g/mL}$ (5). It has a molecular mass ranging from 50 to 68 kDa (5, 14). LK has the same basic structure as HK: the same amino-terminal heavy chain of 50 to 60 kDa linked to a carboxy-terminal light chain by the kinin segment (14). The light chain of LK is only 4 to 5 kDa and lacks contact activation and prekallikrein-binding sites. The

contact system of plasma, consists of 3 serine pro-enzymes (factor XII or Hageman factor, factor XI, and prekallikrein) and the kinin precursor HK.

2.1.1 Plasma prekallikrein

Chromosomal localization of the human plasma kallikrein gene was mapped to the q34 – q35 region of the long arm of chromosome 4 (15). Plasma kallikrein (EC 3.4.21.34), a serine protease, is encoded by a single gene, *KLKB1*, and synthesised in the liver. It is predominantly secreted by hepatocytes as an inactive molecule called prekallikrein that circulates in plasma as a heterodimer complex bound to HK with 1:1 molar stoichiometry (16, 17). Prekallikrein is a single chain α -globulin that is present in the plasma of humans and of other animal species at a concentration of 35 – 50 $\mu\text{g/mL}$. About 80 – 90% of prekallikrein is normally complexed to HK (16, 18).

2.1.2 Contact system activation of plasma

Contact of plasma with a negatively charged surface leads to the binding and autoactivation of factor XII (Hageman factor) to factor XIIa, activation of prekallikrein to kallikrein by factor XIIa, and cleavage of HK by kallikrein to release BK (19). Factor XII activation is not only a first step in the initiation of the intrinsic clotting cascade and the generation of kinins, but it also leads to the activation of the complement pathway (20).

Plasma kallikrein cleaves human HK in a two-step process. First, HK is cleaved at the Arg³⁸⁹-Ser³⁹⁰ bond of the carboxy-terminal portion of the BK sequence, leaving the BK attached to the carboxy-terminal end of the heavy chain, and the sequence Leu³⁷⁸-Met-Lys-Arg³⁸¹ is cleaved at the Lys³⁸⁰-Arg³⁸¹ bond to liberate BK from the heavy chain (23). As a result of domain rearrangement, HKa acquires new properties. Recent observations suggest that HKa inhibits endothelial cell

Fig. 1. The kinin-forming systems. The kallikrein-kinin system and its interactions with both intrinsic and extrinsic coagulation cascades and fibrinolysis. Solid lines are established pathways, whereas dashed lines are speculative or experimental activation pathways. TF: tissue factor; PKK: prekallikrein; HK: high-molecular-weight kininogen; LK: low-molecular-weight kininogen; BK: bradykinin; CPN: carboxypeptidase; t-PA: tissue plasminogen activator; u-PA: urokinase plasminogen activator.

proliferation and neovascularisation due to antiapoptotic properties. This property contrasts with the angiogenic effect of HK and LK, due to the release of BK (24).

2.2 Endothelial cells and kinin forming activity

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 as leukocytes, platelets, endothelial cells, and myocytes (25).

HK specifically binds to platelets, granulocytes, and endothelial cells in a zinc-dependent, saturable and reversible reaction (26, 27). The dissociation constant equals 15 nM, indicating high affinity binding (21). This binding involves both the heavy (domain 3) and light (domain 5) chains of HK (28), which could be considered as a receptor for prekallikrein on endothelial cells (29–31). Binding of HK to endothelial cells leads to activation of prekallikrein to kallikrein (11, 25, 31) and presumably a release of BK from HK (31, 32).

The interaction of HK with endothelial cell membranes involves a multiprotein receptor complex comprising at least cytokeratin 1, gC1qR, and the urokinase plasminogen activator receptor (u-PAR) (33–38). These three proteins co-localize on the endothelial cell membrane (39). The same three proteins form a receptor complex for factor XII, but binding of factor XII in vivo is likely limited both by the low plasma concentration of free Zn^{2+} , which is below the requirement for factor XII binding, and by the much higher plasma concentration of HK (40).

2.3 Tissue kallikrein-kinin system

Tissue (glandular) kallikrein (EC 3.4.21.35) is an acid glycoprotein which differs from plasma kallikrein. This tissue serine protease is encoded by one of the kallikrein gene family, *KLK1* gene, located on chromosome 19 locus q13.2–q13.4 (41, 42). Tissue kallikrein is widely distributed (kidney, blood vessels, central nervous system (CNS), pancreas, gut, salivary and sweat glands, spleen, adrenal, and neutrophils) (17, 42), and this wide distribution suggests a paracrine function (43). The origin of tissue kallikrein detected in plasma has been suggested to be the exocrine glands. Tissue kallikrein is synthesized as a proenzyme, pro-kallikrein, which is inefficiently activated by plasmin or plasma kallikrein (44).

Tissue kallikrein releases KD from LK (42), cleaving the Met³⁷⁹-Lys³⁸⁰ and Arg³⁸⁹-Ser³⁹⁰ bonds (45). Although LK is considered to be the main substrate of tissue kallikrein, tissue kallikrein is also capable of cleaving HK.

2.4 Other kinin forming enzymes

In addition to tissue and plasma kallikrein, other serum and tissue proteases have a kinin forming capacity (46). Plasmin, which is responsible for lysis of the fibrin clot, releases not only BK but also des-Arg⁹-BK from HK (47), circulates in plasma as an inactive zymogen, plasminogen. The presence of plasminogen activators and their inhibitors is essential in controlling fibrinolysis (48).

The major activator of plasminogen in vivo is tissue plasminogen activator (t-PA), a serine protease synthesized and secreted by endothelial cells as a single chain active enzyme (46). In the absence of fibrin, t-PA is an inefficient activator of plasminogen but the binding of t-PA to fibrin greatly accelerates the activation of plasminogen. Urokinase or urinary type plasminogen activator (u-PA) can also activate plasminogen and plays an important role in the degradation of the extracellular matrix. This facilitates the migration of cells which is important in wound healing and in tumour invasion and metastasis. u-PA can be of primary importance for cell-mediated activation of plasminogen in tissues, whereas t-PA, possessing a high affinity for fibrin, can be of primary importance for the lysis of fibrin clots in the circulation (49).

Factor XIIa, XIa, and kallikrein are also capable of converting plasminogen to plasmin in vitro. The contribution of these enzymes to the activation of plasminogen in vivo is uncertain; in fact, deficiencies in these proteins do not appear to lead to pathological states that could be explained by an impaired fibrinolysis (48).

3. Regulation of the kininogenase activity

Protease inhibitors regulate the contact activation of plasma. The serpins of plasma are namely C1-inhibitor (C1INH), antithrombin III, α_2 -macroglobulin, α_1 -protease inhibitor, and α_2 -antiplasmin (19, 50). However, C1INH is the major regulator of the intrinsic system, interfering with the activities of factor XIIa and of kallikrein (51). Both C1INH and α_2 -macroglobulin account for more than 90% of the kallikrein inhibitory activity of plasma.

The regulatory mechanism of tissue kallikrein remains partly unknown. Kallistatin and aprotinin are an example of serine protease inhibitors. Unlike many other serpins that are present only in plasma, kallistatin can be detected in various tissues, cells, and fluids, where it regulates the activity of tissue kallikrein (52, 53).

Plasmin, t-PA, and u-PA are also regulated by inhibitors in the serpin family. The primary inhibitor of active plasmin is α_2 -antiplasmin. Similarly, antithrombin, α_1 -antitrypsin, and C1INH have been shown to inhibit plasmin in vitro but have a minimal physio-

logical effect in blood. Inhibitors of t-PA play an important role in regulating fibrinolysis. Currently, four distinct types of plasminogen activator inhibitors (PAI) have been described: PAI-1, PAI-2, PAI-3, and protease nexin. Of these, PAI-1 is the most important in inhibiting t-PA in plasma. PAI-1 is present in molar excess over t-PA; hence the majority of t-PA circulates bound to PAI-1, thereby preventing its interaction with plasminogen and thus a premature lysis of fibrin and a systemic fibrinolysis (54).

II- Metabolism of Kinins

The nature and properties of the various peptidases capable of metabolizing kinins have been extensively reviewed (55). We have experimental evidences that 4 metallopeptidases are mainly responsible for the metabolism of BK. These are angiotensin I-converting enzyme (ACE), aminopeptidase P (APP), neutral endopeptidase 24.11 (NEP, neprilysin), and carboxypeptidases M and N (CPM, CPN). The importance of these peptidases depends on the animal species, the analytical approach, the biological milieu, and the pathophysiological context. Interestingly, these peptidases are zinc metallopeptidases, that is, they all require zinc in their catalytic site to hydrolyse substrates, and are membrane-bound glycoproteins, except CPN, which is a soluble tetrameric glycoprotein. However, they are all present in a soluble form in biologic fluids.

1. Angiotensin I-Converting Enzyme (ACE)

1.1 Definition

ACE (EC 3.4.15.1) is a well-characterized type I ectoenzyme membrane anchored Zn^{2+} -dependent dipeptidyl carboxypeptidase that regulates bioactivities of vasoactive peptides such as angiotensin I (Ang I) and BK, responsible for the control of blood pressure (56).

Two distinct forms of ACE are expressed in humans, a larger one (150–180 kDa) usually referred to as somatic ACE that is composed of approximately 1300 aminoacids and is present in most tissues (vascular endothelial surface of the lungs and on brush-border membranes of kidney, intestine, placenta, and choroid plexus) and a smaller isoenzyme referred to as the germinal form, testicular ACE (100–110 kDa), with only 730 aminoacids and is found exclusively in the testicles and appears to be involved in male fertility (56, 57).

The somatic form has two homologous metalloproteinase domains (N- and C-terminal domains) with an overall 60% homology in both nucleotide and aminoacid sequence, each containing a canonical Zn^{2+} -binding sequence motif: HEXXH (His-Glu-X-X-His)

and bearing a functional active site (58, 59). The stoichiometry value of 1:1 for the complete inhibition of the enzyme indicates that both active sites would work in a cooperative manner (60).

The testicular ACE has a single domain corresponding to the C-terminal domain of somatic ACE (61) together with the hydrophobic membrane-anchoring domain and a small N-terminal region that has multiple *O*-linked oligosaccharides (62).

Although ACE is primarily a membrane-bound protein, a soluble form does exist in many body fluids and is the result of post-translational proteolytic cleavage in the juxtamembrane stalk by a membrane protein secretase or sheddase, itself a zinc metallopeptidase (63, 64). Although the ACE secretase has not yet been identified, studies with a range of hydroxamic acid-based inhibitors have shown that it has a remarkably similar inhibition profile to the amyloid precursor protein α -secretase, leading to the conclusion that the two secretases are, at the very least, closely related (65). Human plasma ACE originates from endothelial cells, while in other body fluids ACE originates from epithelial, endothelial, or germinal cells (66).

A number of proteins with sequences related to ACE have been described, but the only mammalian relative to be found is ACE2. Similar to ACE, ACE2 is also a type I integral membrane glycoprotein found on the surface of endothelial and epithelial cells, although it has a more limited tissue (testis, heart, kidney) distribution than ACE. ACE2 consists of a single active site domain that, by sequence comparison, more closely resembles the N-domain than the C-domain of somatic ACE. However, ACE2 differs from ACE in that it acts as a carboxypeptidase removing single aminoacid residues from its substrates, which include angiotensin II (Ang II) with high catalytic efficiency ($k_{\text{cat}}/K_m = 1.9 \times 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}$) (67). ACE2 efficiently hydrolyzes des-Arg⁹-BK ($k_{\text{cat}}/K_m = 1.3 \times 10^5 \text{ M}^{-1} \cdot \text{s}^{-1}$), but fails to hydrolyze BK itself (68). Also, ACE2 is not inhibited by some inhibitors of ACE. Since the discovery of ACE2, some authors have begun to refer to ACE as ACE1 (69, 70).

1.2 Synthesis, regulation, and localization

Somatic and germinal ACE are transcribed from the same gene (17q23) using alternative promoters (71). Cloning of the gene that encodes ACE revealed the relationship between the two isoforms. Besides their different tissue specificity, expression of the two ACE transcripts is regulated by different developmental and hormonal controls; for example, the endothelial enzyme is induced by glucocorticoids, whereas the testicular form is stimulated by androgens (71).

1.3 Properties

1.3.1 Angiotensinase vs kininase

ACE catalyzes the conversion of the inactive decapeptide Ang I to the potent vasopressor octapeptide Ang II by the removal of the C-terminal dipeptide His⁹-Leu¹⁰.

Ang I is originally considered the main physiological substrate for ACE (K_m , approximately 16 μ M). Because of its higher affinity (K_m , approximately 0.18 μ M) for BK, ACE could also be now considered as a kininase (kininase II) (72, 73). As a peptidyl dipeptidase, ACE inactivates BK by hydrolyzing two separate bonds on its C-terminal end. It removes sequentially the dipeptide Phe⁸-Arg⁹ and next cleaves the Phe⁵-Ser⁶ bond to generate the second dipeptide Ser⁶-Pro⁷, transforming BK into its inactive final BK_[1-5] product (74, 75). ACE also metabolizes des-Arg⁹-BK by removing the carboxy-terminal tripeptide Ser⁶-Pro⁷-Phe⁸, yielding the same final pentapeptide.

1.3.2 ACE GPIase activity

Recently, Kondoh et al. reported that ACE has a GPIase activity which allows the release of a membrane glycosylphosphatidylinositol (GPI)-anchored protein. They have found that ACE-specific inhibitors, such as captopril and lisinopril, which bind to the catalytic center and completely inhibit the peptidase activity, had only a minor inhibitory effect on this GPIase activity which does not require the zinc ion (76). This new activity of ACE which is different from the GPIase activity of GPI-specific phospholipase D (GPI-PLD) (76, 77) and of phosphatidylinositol-specific phospholipase C (PI-PLC)-like could be of physiological importance in fertilization.

1.3.3 ACE: a signal transduction molecule

By its short cytoplasmic domain (29 aminoacids), ACE could also play a role as a signal transduction molecule. In fact, the cytoplasmic tail of ACE is phosphorylated in endothelial cells (78). ACE inhibitors as well as BK elicit outside-in signalling in these cells. They enhance the activity of ACE-associated protein kinase CK2, increasing its phosphorylation of ACE, and lead to the activation of c-Jun N-terminal kinase (JNK) as well as the accumulation of phosphorylated c-Jun in the nucleus (79).

1.3.4 ACE insertion/deletion polymorphism

Population studies indicated that the large inter-individual variability in plasma ACE levels is genetically determined. Regulation, as well as tissue ACE activity are under strong genetic control. An insertion/deletion (I/D) polymorphism located in the noncoding

region of the gene is associated with differences in the level of ACE in plasma and cells. The insertion that gives rise to the I allele is an *alu* repeat sequence (287 bp) in intron 16 of the ACE gene; the D allele results from the absence of the above insertion. There is a relationship between the ACE I/D polymorphism and circulating ACE activity, such that ACE activity is highest in individuals homozygous for the D allele, lowest in those homozygous for the I allele and intermediate in heterozygotes.

Mean serum ACE level in DD homozygotes are nearly twice the value measured in II plasma, while DI heterozygous have intermediate activities (80–82). Although some association between the I/D polymorphism with the incidence of cardiovascular and Alzheimer diseases, the results remain inconsistent (83–87). However, ACE genotype determines BK degradation and suggests another mechanism whereby the ACE D allele could be associated with deleterious cardiovascular effects (82).

2. Neprilysin (NEP)

2.1 Definition

NEP (EC 3.4.24.11, neprilysin) is a type II surface protein with a short membrane-proximal stalk region that is not sensitive to proteolytic activity of any particular secretase (88). It is the prototype zinc peptidase of the M13 membrane metalloendopeptidase (MME) family. This family includes other peptidases of potential pathophysiological interest: ECE-1 and ECE-2, which generate the vasoconstrictor endothelins from big endothelin; the erythrocyte cell-surface antigen KELL; PHEX, associated with congenital X-linked hypophosphatemic ricket; ECEL1, present in CNS; SEP (MML1 or NL1) present in testis; and MMEL2 (89–91).

2.2 Synthesis, localization, and properties

Human NEP gene (*MME*) is localized to 3q21–q27 (92). First identified in the brush border of the kidney epithelial cells, where it represents 4–5% of the protein content, its immunoreactivity or activity has also been detected in cells or tissues as different as the CNS, the endothelium, testis, lungs, salivary glands, and bone marrow (93). NEP preferentially cleaves bonds on the amino-side of hydrophobic aminoacids residues. However, the physiological role of NEP depends on its tissue localization and the presence of the substrate.

Like ACE, NEP inactivates BK by sequentially removing dipeptide Phe⁸-Arg⁹ and tripeptide Phe⁵-Ser⁶-Pro⁷ to yield BK_[1-4], an inactive metabolite. However, a prolonged incubation also results in the hydrolysis of the Gly⁴-Phe⁵ bond (94). In contrast to ACE, it is likely that NEP cleaves the Gly⁴-Phe⁵ bond of des-Arg⁹-BK

to generate the same BK_[1-4]-inactive peptides. NEP is the main enzyme responsible for the metabolism of kinins in the kidney and plays an important role in the metabolism of BK at the endothelium. In plasma, unlike ACE, NEP does not play a significant role in the metabolism of kinins (95).

Besides kinins, NEP also inactivates other peptides like enkephalins, neurokinins, and amyloid- β peptide, a marker for Alzheimer disease of the CNS (96). In the kidney, it regulates the degradation of natriuretic peptides (97).

3. Aminopeptidase P (APP)

3.1 Definition

Human APP (X-prolyl aminopeptidase, EC 3.4.11.9) exists at least in two forms: a soluble cytosolic (cAPP) and membrane-bound (mAPP) APP forms. Both forms have an X-prolyl aminopeptidase activity (98). cAPP and mAPP share 43% of homology. cAPP is a homodimer of 70 kDa subunits and the mature form APP has a predicted subunit molecular weight of 71 kDa (99, 100). mAPP is a heavily N-glycosylated, Zn²⁺-containing, peptidase and contains a C-terminally attached GPI membrane anchor that increases the overall subunit molecular weight to approximately 90 kDa. A secreted form of mAPP has recently been produced and characterized in vitro in a transfected HEK-293 cell line. This protein exhibits a molecular mass of 85 kDa (101).

The effects of divalent metal ions on the activity of mammalian APPs appear to differ between the cytosolic and membrane-bound form of the enzyme (102). Indeed, whereas mAPP uses Zn²⁺ at the active site, human cAPP contains Mn²⁺ and is activated in vitro by the presence of Mn²⁺, but not Zn²⁺, which is inhibitory (99, 103).

There is evidence for the existence of another form of APP in mammals. The third isoform is hypothetical and has been identified on the basis of sequence homology. The chromosomal location of this isoform is 22q13.31 – q13.33. This isoform is predicted to have a subunit molecular weight of 57 kDa and is therefore smaller than cAPP and mAPP (104).

3.2 Synthesis, regulation, and localization

The chromosomal location of the human cAPP gene (*XPNPEPL*) is 10q25.3 (105) and the *XPNPEP2* gene of mAPP localizes to chromosome Xq25-26.1 (106).

mAPP is located on the external side of the plasma membrane of vascular endothelial cells and on brush border membranes of epithelial cells in the intestine and the renal proximal tubule (107 – 112).

3.3 Properties

cAPP exhibits broad substrate specificity and can

cleave X-Pro dipeptides as well as longer peptides of the form X-Pro-Y-, where X and Y are of the common aminoacids (113 – 115). Compared to cAPP, mAPP has much more restricted substrate specificity; it fails to hydrolyze X-Pro dipeptides and cleaves X-Pro-Y-peptides poorly when X is Pro or Gly or when Y is an aminoacid with a bulky side chain (116 – 118).

Kinins are the best substrates for mAPP. In fact, the secreted form of mAPP exhibits a K_m of $75 \pm 15 \mu M$ for BK and a K_m of $56 \pm 13 \mu M$ for des-Arg⁹-BK. In human plasma, APP transforms BK and des-Arg⁹-BK into the inactive peptide BK_[2-9] and BK_[2-8], respectively, and is the major inactivating pathway for des-Arg⁹-BK in plasma (55, 95, 119).

4. Carboxypeptidase N and M (CPN, CPM)

Currently known as kininase I, both CPN and CPM are zinc metallopeptidases that exhibit a 41% sequence identity (120).

CPN (kininase I, EC 3.4.17.3) is a tetrameric protein synthesized in the liver and secreted in blood, although CPM (membrane-bound kininase I) is a GPI peptidase anchored at the membrane of lung and kidney epithelial cells. They cleave a variety of peptides containing a carboxy-terminal Arg or Lys. CPN inactivates complement anaphylatoxins (C3a, C4a, and C5a) by cleaving their carboxy-terminal Arg residue (121). Although likely, definitive evidence for such a metabolic role does not exist for CPM. Both carboxypeptidases transform BK and KD into des-Arg⁹-BK and des-Arg¹⁰-KD, which are active metabolites via B₁ receptor (B1R). This kininase I activity constitutes a minor metabolic pathway unless ACE is inhibited (55, 121).

5. Other peptidases

The properties of dipeptidyl peptidase IV (DPP IV) have been extensively reviewed (122). DPP IV (CD26; EC 3.4.14.5) is a 110 kDa plasma membrane glycoprotein ectopeptidase (119, 122). The most important physiological actions of DPP IV is on regulatory peptides in mammals. DPP IV degrades the inactive metabolite BK_[2-9] generated by APP and leads to the final BK_[4-9] product (122). Substance P is another example of a regulatory peptide for which DPP IV plays an additional role in the inactivation (123).

Aminopeptidase N (APN, CD13) is an ectoenzyme (EC 3.4.11.2) of the superfamily of zinc metalloproteases widely used as a marker of myelomonocytic cells in the diagnosis of hematopoietic, malignant disorders (88, 124). Because of its ectopeptidase activity, APN has also been implicated in the regulation of vasoactive peptides, neuropeptide hormones, and immunomodulating peptides such as interleukin-6 and -8 (IL-6

and IL-8, respectively) (125, 126). Its importance in the field of kinins is related to its ability to hydrolyze the N-terminal Lys residue in KD and des-Arg¹⁰-KD; this reaction is functionally significant for the latter peptide which is the optimal agonist of the B1R in humans and other species. Indeed, the reaction product des-Arg⁹-BK has a much lower affinity at the human, rabbit, porcine, and bovine B1R. APN mediates the major inactivation pathway for des-Arg¹⁰-KD in the human isolated umbilical artery (127) and rabbit aorta (F. Marceau and A. Adam, unpublished observations). APN blockade with amastatin potentiates the hypotensive effect of the B1R agonist des-Arg¹⁰-KD in the LPS-pretreated rabbit (128).

III- Kinin Receptors

Two types of G-protein-coupled receptors (GPCRs) mediate the cellular effects of kinins, the B1R and B₂ receptor (B2R) (129, 130). The various pharmacological effects of the BK-related peptides, such as vasodilatation, increased vascular permeability, stimulation of sensorial and sympathetic nervous connections, and smooth muscle contraction, derive from the presence of these receptors on various cell types such as the vascular endothelium, primary sensory afferent neurons, vascular and nonvascular smooth muscle, epithelial cells, and perhaps some types of leukocytes. The two types of receptors were initially defined using pharmacological criteria, before their molecular characterization.

1. Pharmacological classification

The potency order of agonists and the affinity of antagonists and their properties are summarized in Table 1 for B1R and Table 2 for B2R.

1.1 Potency order of agonists

The B2R has a high affinity for “native” kinins (generated by either plasma or tissue kallikreins), BK, and KD in all mammalian species. None of the fragments of BK retain a significant affinity for the B2R. KD has also a significant activity for the human and rabbit B1R (e.g., in radioligand binding assays), but in complex bioassay systems, BK or KD effects at B1R are often mediated by their des-Arg metabolites generated *in situ* (131). Indeed, the B1R is specialized across species to respond to kinin metabolites generated by arginine carboxypeptidases (either des-Arg⁹-BK, Lys-des-Arg⁹-BK or des-Arg¹⁰-KD) (129, 130). Des-Arg⁹-BK is a highly selective agonist of the mammalian B1R, but of high affinity (nanomolar) only in rodents (the rat and mouse); the only natural kinin sequence with a subnanomolar affinity for the human, rabbit,

porcine, and bovine B1R is Lys-des-Arg⁹-BK, suggesting that the B1R works in concert with the tissue kallikrein that generates its parent native peptide, KD (130).

1.2 Affinity of antagonists

Antagonists for the B1R were discovered almost 10 years before the antagonists for the B2R; thus, the receptor nomenclature is justified by the fact that it was the first to be pharmacologically fully defined. The first series of compounds capable of antagonizing BK and des-Arg⁹-BK with specificity for B1R included the prototype [Leu⁸]des-Arg⁹-BK (132). However, this prototype exhibits fairly high partial agonist behavior in some species, especially in the rat and mouse (130). This has practical implications because one of the emerging therapeutic applications of B1R antagonists, analgesia, is commonly evaluated using models involving these species.

The first generation of B2R antagonists was based on [D-Phe⁷]-BK (133), but these early peptidic compounds showed an antagonist/partial agonist activity and a low potency. This problem was progressively solved with the second generation of B2R antagonists in which rigidity was added to the peptide backbone by introducing non-natural aminoacid residues; thus, the spatial orientation of the C-terminal region of the peptide molecule critical for antagonism was more precise. Optimal peptide B2R antagonists retain both Arg¹ and Arg⁹ residues and B1R agonists or antagonists typically lack Arg⁹ (as in [Leu⁸]des-Arg⁹-BK), just like agonists; furthermore, the N-terminal Lys residue present in KD also confers high affinity to peptide B1R antagonists in human, rabbit, and other species (as in Lys-[Leu⁸]des-Arg⁹-BK).

HOE 140 (D-Arg-[Hyp³, Thi⁵, D-Tic⁷, Oic⁸]-BK, Icatibant) is a representative of the second generation of peptide B2R antagonists (134) and has been exploited in more than 700 research papers and several clinical studies. Significant species-related differences in potency and competitive behavior are typically seen with kinin receptor antagonists; thus HOE 140 is potent and competitive at the human B2R, but insurmountable and atypically induces a slow B2R endocytosis of the rabbit B2R (135). The selectivity of HOE 140 for the human B2R is fair, but not complete; the fragment des-Arg¹⁰-HOE 140 is predominantly a B1R antagonist (130). Second generation B1R antagonists include the N-terminal Lys residue or its analog Orn that affords high affinity for the human B1R (as in B-9858 = Lys-Lys-[Hyp³, Igl⁵, D-Igl⁷, Oic⁸]des-Arg⁹-BK or R-954 = Ac-Orn-[Oic², α-MePhe⁵, D-βNal⁷, Ile⁸]des-Arg⁹-BK) (136). The presence of D-Arg instead of Lys, as in des-Arg¹⁰-

Table 1. Pharmacological and clinical application of kinin B₁-receptor ligands

Ligands	Application	Studies	References
Agonists			
R-838 (Sar-[D-Phe ⁸]des-Arg ⁹ -BK)	Metabolically stable	Rabbit	128
	High affinity and selectivity Hypertension Stimulation of vasculature formation (following ischemia)	Rodent	130, 281
Antagonists			
[Leu ⁸]des-Arg ⁹ -BK	Pain	Rat	132
		Mice	302, 317
	Ischemic vascular disease	Mice	130, 281
Lys-[Leu ⁸]des-Arg ⁹ -BK	Optimal B1R antagonist	Human B1R	130
Ac-Lys-[MeAla ⁶ , Leu ⁸]des-Arg ⁹ -BK	Metabolically stable (not very potent compared with the affinity of the reference compound Lys-[Leu ⁸]des-Arg ⁹ -BK)	Rabbit	318
R-715 (Ac-Lys-[βD-Nal ⁷ , Ile ⁸]des-Arg ⁹ -BK)	High affinity	Human and rabbit B1R	130
	Allergic lung inflammation	Mice	319
B9858 (Lys-Lys-[Hyp ³ , Igl ⁵ , D-Igl ⁷ , Oic ⁸]des-Arg ⁹ -BK)	Fairly high selectivity for B1R due to Lys ⁹ Metabolically stable residue	Human and rabbit B1R	320
des-Arg ¹⁰ -HOE 140	Residual antagonistic effects on B2R	Rabbit jugular vein, guinea pig ileum, rabbit aorta	321
	Moderate affinity		
B9430 (D-Arg-[Hyp ³ , Igl ⁵ , D-Igl ⁷ , Oic ⁸]-BK)	Mixed B1R and B2R antagonist even if desArg ⁹ fragment has substantial selectivity for B1R	Demonstration of compatibility of B1R and B2R structure by the accommodation of a single antagonist pharmacophore	322
R-954 (Ac-Orn-[Oic ² , α-MePhe ⁵ , D-βNal ⁷ , Ile ⁸]des-Arg ⁹ -BK)	Allergic lung inflammation	Mice	319, 323
	Airway allergy	Rat model, speculative on human	324
PS020990	Potent and competitive B1R antagonist High affinity	Human receptor (no in vivo data)	139
Compound 12 (benzodiazepine-based structure)	Selective antagonist	Human and rat B1R in vitro (equal activity)	140
Benzo-sulfonylamide compounds			
Compound 12	Hyperalgesia	Rat, dog, orally bioavailable	142
Compound 11	Speculative on pain, inflammation and sepsis	Rabbit aortic preparations, rabbit jugular vein	141
SSR240612	Inflammation and hyperalgesia	Mice, rat, oral activity	143

HOE 140 and D-Arg-[Hyp³, Igl⁵, D-Igl⁷, Oic⁸]-BK, is associated with a strong decrease in affinity in human and rabbit, but not in the mouse B1R, corroborating the previous interpretation.

Efforts to create a third generation of kinin receptor antagonists have evolved towards conventional non-peptide drug development programs, with oral bio-availability, higher lipophilicity, and lower molecular weight increasingly represented. Various chemical subclasses of nonpeptide B2R antagonists have been discovered: phosphonium compounds, as in WIN64338, WIN62318 (136 – 138); quinoline and imidazol [1,2-*a*]

pyridine family, such as FR165649, FR167344, FR173657, and FR184280; compound 38; substituted 1,4-dihydropyridines; CP2522; and CP0597 (138). Several nonpeptide B1R antagonists have also been synthesized: PS020990 (139), benzodiazepine-based compounds, such as compound 11 and 12 (140); powerful oxo-sulfonyl agents (141 – 143).

Some kinin antagonists have also been discovered as natural compounds: martinelline, a pyrroloquinoline alkaloid isolated from the plant *Martinella iquitosensis*, is the most remarkable example (144).

Table 2. Pharmacological and clinical application of kinin B₂-receptor ligands

Ligands	Application	Studies	References
Agonists			
Labradimil ([Hyp ³ , Thi ⁵ , 4-Me-Tyr ⁸ Ψ (CH ₂ -NH)Arg ⁹]-BK)	Vascular permeability (blood brain barrier): adjuvant to chemotherapy of brain tumors	In vivo rodent models Human: phase II studies on glioma	284
FR190997	Hypertension	Rat	283
Antagonists			
First generation			
[D-Phe ⁷]-BK	Low potency, Antagonist/partial agonist activity	Rat uterus, guinea pig ileum Rat	133
[Thi ^{5,8} , D-Phe ⁷]-BK	Potent antagonist, no agonist activity	Rat uterus, guinea pig ileum	133
Second generation			
HOE 140 (Icatibant; D-Arg-[Hyp ³ , Thi ⁵ , D-Tic ⁷ , Oic ⁸]-BK)	High affinity, long-lasting, competitive activity in but measurable affinity for B1R No residual agonist effects Resistance to peptidases Acute rhinitis Asthma Early stage of inflammation Persistent inflammatory pain	Animal models (high affinity for the human, rabbit, and guinea pig B2R) Human, nasal treatment Human Rat	134 325 269 317
Third generation – Nonpeptide compounds			
Phosphonium family:			
WIN64338	Inactive Limited affinity	On human tissues For guinea pig B2R	137 136
WIN62318	Micromolar binding affinity to human B2R	Identification of the absolute requirement for B2R binding affinity: presence of two positive charges at a distance of about 10 Å separated by a lipophilic residue, playing the role of Phe ⁸ side chain in the native ligand	138
Quinoline and imidazol [1,2- α]pyridine family:			
FR165649, FR173657, FR184280	High B2R affinity and selectivity versus B1R Oral activity on hyperalgesia and inflammation	Oral activity at doses ranging between 1 and 30 mg/kg in different tests and species Rat, mice	282, 326 138
FR167344	Selective and high potent binding activity Bronchoconstriction	Guinea pig ileum, human A-431 cells Guinea pigs (oral activity) Designed as clinical candidate to treat inflammatory diseases	327
Compound 38	High affinity	Human B2R	328
CP2522	High affinity Modeled on CP0597 by replacing β -turn conformation of the peptide by a rigid 1,4-piperazine ring	Human B2R	138
Substituted 1,4-dihydropyridines	B2R antagonist at the nanomolar range	Human B2R	138
Bradyzide	Hypertension Inflammation	Rodent, orally active, less potent in human B2R	292
Natural compounds			
Pyrroloquinoline alkaloid: Martinelline	Affinity for both B1R and B2R at the micromolar range but not selective	Alkaloid isolated from the South American tropical plant <i>Martinella iquitosensis</i>	144
L-755807	Inhibition of BK binding to cloned human B2R at micromolar range	Complex metabolite isolated from a culture of the mould <i>Microsphaeropsis</i> sp. No further pharmacological data	329

2. Molecular classification

Distinct B1R and B2R genes coexist in the human genome and, most probably, in the genome of most if not all mammals (129). It is interesting to note that a fish and a bird (the chicken) possess only one type of receptor for kinin homologs and that it is most related to the mammalian B2R, which appears to be ancestral to the B1R (129). Furthermore, the Human Genome project revealed that the human kinin receptor genes are clustered in tandem in the same locus of chromosome 14 (14q32.1 – q32.2), with less than 20 kb of genomic DNA separating them. This has profound implications for the study of genetic polymorphisms, as a genetic marker in one of the two genes may point out a functional alteration of the other, as this genetic distance is very small and as both genes are likely to be transmitted together vertically (see below).

The molecular definition of the kinin receptor was initiated by the expression cloning of the rat B2R (145) followed by that of the human B1R (146). The degree of aminoacid identity is not very high between the human B1R and B2R sequences (36%), but they are the most highly related pair, followed by the receptors for angiotensin. The determination of the pharmacological profile of cloned receptors from various species has ended much speculation about the existence of multiple additional receptor subtypes, as it was recognized that the rather large differences in their affinity to agonists and antagonists were a species-related issue.

2.1 Organization and structure of the receptor genes

The B1R protein (40.4 kDa) exhibits a seven transmembrane structure typical for GPCR ($G\alpha_q$ and $G\alpha_i$) and possesses three consensus sites for N-linked glycosylation in extracellular domains, DRY (Asp-Arg-Tyr) and NPXXY (Asn-Pro-XX-Tyr) motifs, and putative sites for phosphorylation and acylation. B1R is not expressed in significant levels in normal tissues (130). The expression of B1R is inducible rather than constitutive. However, exceptions occur in mammals concerning the inducible behavior of B1R. For example, dogs and cats constitutively express the receptor (130).

The human B1R gene (*BDKRB1*) is located on chromosome 14q32.1 – q32.2. The gene product consists of 353 aminoacids and approximately 70% of its overall genomic sequence is homologous to the mouse and the rat *BDKRB1* genes. The three-exon structure of the human B1R gene has been determined with the protein sequence being encoded by exon 3 exclusively (147, 148). Critical receptor epitopes for G-protein binding and activation are located on multiple intracellular domains, which are thought to act in concert to form a binding site (149).

As B1R, the B2R protein structure is typical of that of a GPCR consisting of a single polypeptide chain that spans the membrane seven times, with the amino terminus being extracellular (N-terminal domain) and the carboxyterminus (C-terminal domain) being intracellular and with three extracellular loops (EL1 – 3) and three intracellular loops (IL1 – 3). Three consensus sites for N-linked glycosylation are found in extracellular domains. Moreover, the protein contains motifs such as DRY and NPXXY partially embedded in cytosolic receptor domains that are common to most rhodopsin family GPCRs (150), and the C-terminal tail contains serine and threonine residues that are putative phosphorylation sites and cysteines that are putative sites for acylation.

The B2R has been identified in most tissues and is particularly present on endothelial cells, smooth muscular cells, fibroblasts, mesengial cells, some neurons, astrocytes, and polynuclear neutrophils (151). Its gene expression level is constitutive. The human *BDKRB2* gene product consists of 391 aminoacids and the three-exon structure of the gene is also located on chromosome 14 (152) but about 12 kb upstream from *BDKRB1* (153). About 80% of the human *BDKRB2* gene is homologous to the mouse and rat *BDKRB2* gene. The human *BDKRB1* and *BDKRB2* are also similar to each other with 36% genomic sequence homology. The mRNA coding for the B2R (4 kb) is large compared with that of B1R (1.4 kb).

2.2 Receptors expression and regulatory elements in gene promoters

Expression of the B1R is up-regulated following some types of tissue injury, and its relationship to pathology is therefore obvious. Exposure to bacterial endotoxins or cytokines, tissue trauma, inflammation, anoxia (130), and myocardial infarction (154) are examples of inducing stimuli. IL-1 β and tumor necrosis factor (TNF)- α induce the expression of the B1R in vitro (155, 156) and in vivo (157, 158).

Cytokine-induced B1R expression is mediated by specific MAP-kinase pathways (notably, p38 and JNK) and nuclear factor- κ B (NF- κ B) (159 – 162). Inflammatory induction of B1R expression correlates with NF- κ B stimulation in various systems (163, 164). Studies on the human embryonic IMR-90 cell line led to the hypothesis of autoregulation of the B1R; stimulation of either the B1R or B2R in these cells led to the increased expression of the B1R (mRNA, protein) (165, 166). However, this model may not be generally applicable, as these permissive cells produce autocrine IL-1 β in response to the stimulation of various GPCRs (167) and as kinin receptor stimulation does not up-regulate

B1R expression in primary vascular cells (cultured smooth muscle cells) or *in vivo*, following the activation of the contact system in rabbits using dextran sulfate (168). Evidence of the role of the transcription factor AP-1 in B1R expression has been derived from a construction composed of the 1.8 kb core promoter, exon 1, and 1.5 kb of intron 1, exon 2, intron 2, and a luciferase reporter (169).

The expression of B2R is up-regulated not only by BK but also by cAMP and phorbol esters (170). Multiple potential binding sites have been identified recently in the B2R promoter (171): GATA-1, CCAAT displacement protein, E2F, Egr2, IL-6 activator protein, NF- κ B, p53, estrogens. IL-1 β increases both the number of B2R as well as the level of B2R mRNA through a prostanoid and cAMP dependent pathway, which may lead to protein kinase A activation of the transcription factor cAMP response element-binding protein (172).

2.3 Second messengers

Signal transduction of kinin receptors activates several second messenger systems, depending on cellular type, via the activation of G-proteins (Fig. 2).

Fig. 2. Kinin receptors and their signaling pathways. Schematic representation of B₁ and B₂ receptors and the second messengers released by their activation. PLC: phospholipase C; ER: endothelial reticulum; DAG: diacylglycerol; IP₃: inositol 1,4,5-triphosphate; PLA₂: phospholipase A₂; NO: nitric oxide; eNOS: endothelial NO synthase.

Activation of adenylyl cyclase or guanylyl cyclase is a transduction mechanism that leads to the production of cAMP and to cGMP, an efficient vasodilatory mechanism in vascular smooth muscle. However, the kinin receptors, coupled to G_q and G_i proteins, are only indirectly linked to cyclic nucleotide signaling: via the endothelial production of NO, itself capable of activating the soluble guanylate cyclase in neighboring cell types or via the production of prostaglandins (PGs) I₂ or E₂ that possess G_s-protein-coupled receptors. The activation of ionic channels and of PLs A₂, C, and B also play a role in kinin receptor signaling. PLC products, inositol 1,4,5-triphosphate and diacylglycerol (DAG) (173), are respectively responsible for transporting calcium into the cytosol from intracellular stores and for protein kinase C translocation from the cytosol to the plasma membrane. Ca²⁺ mediates the activation of endothelial NO synthase (eNOS) and ultimately the production of NO in endothelial cells (174) and stimulation of PLA₂ (129). Arachidonic acid could also be liberated from cellular membrane phospholipids by the action of PLA₂ and lead to PG production. In addition to these classical pathways, B2R can activate signaling proteins that possess cytoplasmic tyrosine kinase activity (175). The activated B2R also transiently promotes tyrosine phosphorylation of MAP-kinase (176), PLC (177), and Hsp90 (178) and has a direct interaction with neuronal and eNOS allowing the control of NOS phosphorylation and NO production (179, 180). Furthermore, BK activates caveolae-associated Janus-activated kinase/signal transducers and activators of the transcription (JAK/STAT) pathway in endothelial cells (181). Tyrosine kinases of the JAK family phosphorylate STAT proteins that directly regulate transcription of specific genes.

B1R also interacts directly with G α_q and G α_i proteins and recruits essentially the same signaling pathways as the B2R. Although the B1R and B2R seem to couple to similar cellular signal transduction pathways, the patterns of signaling are different in terms of variation of Ca²⁺ concentration (in duration and in intensity, see below). B1R is principally associated with PLC activation and with the phosphoinositol pathway, but also acts through PLA₂ and the MAP-kinase (130).

2.4 Receptor desensitization

Besides their pharmacological differences, B1R and B2R also display important differences in their susceptibility to desensitization. When activated by an agonist, B2R undergoes a rapid desensitization (182), involving phosphorylation of specific Ser and Tyr residues in the receptor large C-terminal domain (129).

Another mechanism that may participate in B2R

desensitization involves BK-promoted transient association of $G\alpha_q$ and $G\alpha_i$ with in caveolae (183). B2R activation leads to functional desensitization which is associated with receptor phosphorylation/dephosphorylation and endocytosis/surface re-expression cycles (184). Cys³²⁴ in the cytoplasmic carboxyl terminus of the human B2R appears to play a role in agonist-induced internalization. It is true that B2R down-regulation is observed in some forms of intense and chronic inflammation, but the mechanism is unknown; proteases present in the extracellular fluid have been shown to destroy a form of recombinant B2R, thus providing a possible mechanisms for inflammatory B2R down-regulation (185).

The B1R differs from the B2R in that it is desensitized only to a very limited degree and human B1R is not phosphorylated to any significant degree either in the absence or presence of agonist (186). The receptor lacks any Ser and Tyr residues in the C-terminal tail. This lack of regulation can contribute to the constitutive activity of the receptor. Its agonist-induced translocation to caveolae-related rafts without internalization has been proposed (129, 187). This type of reversible translocation may be of interest for a subset of the signaling pathways activated by kinin receptors, as rafts are rich in signaling molecules.

Part II: The Kallikrein-Kinin System: Pathophysiology and Pharmacological Target

I- The Kinin Forming System in Plasma

1. Genetic defects

1.1 Defects of the contact system components

Genetic deficiencies have been reported for HK (William trait), PKK (Fletcher trait), and factor XII (Hageman trait) (188). These defects do not lead to bleeding tendencies. Deficiencies of factor XII, forever associated with thromboembolic events, suggest a relationship between depressed factor XII-dependent fibrinolysis and cardiovascular diseases. Quantitative and qualitative defects of plasminogen have also been associated with thromboembolism diseases (19, 189).

1.2 Defect in the control of the contact system: C1 inhibitor (C1INH)

1.2.1 Definition

Patients who present a genetic deficiency in C1INH suffer from hereditary angioedema (HAE). HAE attacks involve the activation of two pathways controlled by this serpin: the classical complement and the contact system pathways. The latter is responsible for the release of vasoactive BK (190), which is probably the main

but not the sole mediator responsible for the increased vascular permeability that results in angioedema (AE) (191–194). The activation of other pathways could also be involved in the pathogenesis of HAE. In fact, Cugno et al. (1993) reported that generation of BK is associated with activation of fibrinolysis during acute attacks of HAE (51).

The prevalence of HAE is believed to be between 1/10,000 and 1/50,000 people worldwide (195). HAE is traditionally described as Type I (HAE-I, 85% of patients), which is characterized by a defective or absence in gene production of C1INH and Type II (HAE-II, 15% of patients), which is characterized by a functionally impaired C1INH (196). C1INH deficiency is heterogeneous at the gene level and is caused by subtle changes affecting one or several nucleotides, large deletions or duplications. These modifications have been discussed recently by Agostoni (190). In either case, HAE is associated with low functional activity of C1INH, low levels of C4, and normal levels of C3.

1.2.2 Pathophysiology

A murine model of HAE contributed to support the hypothesis that BK mediates HAE. In this model, mice heterozygous and homozygous for a gene coding for C1INH demonstrated increased permeability and depletion of HK. When treated with a specific plasma kallikrein inhibitor or a B2R antagonist, the increased vascular permeability was completely reversed (197).

Besides HAE, acquired forms of angioedema (AAE) have been described. These AAE, characterized by normal immunoreactive and functional C1INH levels, are associated with drug therapy, such as with estrogens and metalloproteinase inhibitors (ACE inhibitors (ACEi) and vasoactive peptide inhibitors (VPI), see below) (198). They also occur during immunoproliferative and autoimmune diseases. Finally, idiopathic forms of AAE have also been reported (190).

1.2.3 Treatment of HAE

The purpose of this treatment is to inhibit the release of vasoactive peptides among which is BK or to block their proinflammatory effects (Fig. 3).

1.2.3.1 Serine proteases inhibitors: C1INH and aprotinin are two serpins used in the treatment of HAE (199).

A) Aprotinin and aprotinin-like drugs: Aprotinin is a naturally occurring 58 amino acid serpin isolated from bovine lung that inhibits serine proteases with a particularly high affinity for plasma kallikrein and plasmin (200). Inhibition of plasma kallikrein, which triggers the release of BK during the contact system activation,

2. Acquired diseases

2.1 Sepsis

Although the pathophysiology of sepsis remains largely unknown, experimental and clinical data point to a role of kinins in sepsis. First, in vitro, different bacterial strains responsible for sepsis interact with the contact system of plasma leading to the release of BK. In vivo, the factors of the contact system are consumed in plasma of patients suffering from sepsis. Recently, a significant increase of plasma BK was also measured in plasma of patients suffering from *S. aureus* sepsis while simultaneously treated with an ACEi (210). On the other hand, the injection of LPS from *E. Coli* into the dorsal skin of rats caused a dose-dependent increase in vascular permeability and this increase caused by LPS was attenuated by pretreatment with the B2R antagonist HOE 140 (211). These observations could open a new area for the clinical application of B2R antagonists. However, and until now, the behavior of B1R agonists (des-Arg⁹-BK and des-Arg¹⁰-KD) in septic patients and the effect of sepsis on the kinin forming capacity of fibrinolysis have not been documented.

Another therapeutic approach of sepsis could be the use of the pentapeptide Arg-Pro-Pro-Gly-Phe, the stable metabolite of BK (212). In fact, recent studies have shown that this pentapeptide is able to increase significantly the survival of rats treated with LPS (213) and to inhibit the thrombin-induced platelet aggregation (214).

2.2 Anaphylactoid and severe hypotensive reactions

Contact system activation is also responsible for the anaphylactoid reaction (AR) in hemodialysis and severe hypotensive reaction (SHR) during blood product transfusion in patients simultaneously treated with an ACEi (see below) (215, 216).

3. Antithrombotic treatment and kinins

A complex and dual relationship exists between the fibrinolysis and the kinin system.

The application of recombinant technology has allowed large-scale production of t-PA and its use for thrombolytic therapy. Wide use of recombinant t-PA (rt-PA) for thrombolysis in patients with myocardial infarction stimulated intensive studies of structure function relationships of t-PA and development of mutant forms of t-PA with improved pharmacokinetic properties, that is, with prolonged lifetime in circulation and increased resistance to inhibitors and to cleavage by plasmin (46). However, by stimulating the plasmin formation, thrombolytic drugs not only dissolve the clot but also activate factor XII, the complement cascade and the kinin system (20, 217). In vitro, rt-PA at a therapeutic concentration generates significant quantities of

BK and des-Arg⁹-BK from human plasma, and this kinin-forming activity depends on the activation of plasmin which hydrolyzes HK, independently of the activation of factor XII and PKK (47).

In addition, BK stimulates the exocytosis of t-PA from the vascular endothelium. Studies using the B2R antagonist HOE 140 indicate that ACEi also increase endogenous t-PA release through a B2R-dependent pathway (218). This potentiating effect of ACEi on fibrinolysis constitutes a new aspect of the cardio-protection by ACEi.

II- The Metabolism of Kinins

Until now, among the kinin metabolizing enzymes, only ACE has been a clinically exploited pharmacological target. However, the effectiveness of this class of metallopeptidase inhibitors has opened research in the development for more potent inhibitors that inhibit two metallopeptidases, namely ACE and NEP.

1. ACE: pathophysiology

The broad spectrum of substrates for ACE and its wide distribution throughout the body indicates that this enzyme, in addition to an important role in cardiovascular homeostasis, may be involved in additional physiologic processes such as neovascularization, fertilization, atherosclerosis, kidney and lung fibrosis, myocardial hypertrophy, inflammation, and wound healing (66). The deleterious effects of ACE on the cardiovascular system were initially thought to be a consequence of the formation of Ang II, which initiates a cascade of events involving increased free radical production and vascular smooth muscle cell proliferation (79). However, as BK is much more readily hydrolyzed by ACE than Ang I, the hydrolysis of BK may also contribute to this phenomenon (219).

ACE2 has been implicated in cardiovascular pathology, and the generation of ACE2-knockout mice revealed that the enzyme is an essential regulator of heart function (67, 220).

2. ACE inhibitors (ACEi)

The inhibitors of ACE have emerged as a first-line therapy for a range of cardiovascular and renal diseases, including hypertension, congestive heart failure, myocardial infarction, and diabetic nephropathy. The first clinically used ACEi was captopril ($K_i = 1700$ pM). Numerous other more potent dipeptide and tripeptide inhibitors, which bind to both catalytic sites in somatic ACE have been synthesized subsequently for clinical use in humans (61).

The inhibition of ACE activity is reported to improve

endothelial function and to stimulate vascular remodeling, as well as attenuate the progression of arteriosclerosis and the occurrence of cardiovascular events in humans (221, 222). The identification of ACE as a signalling molecule that can be activated by the binding of ACEi may account for some of the beneficial effects of this class of compounds on the cardiovascular system.

Since their discovery, a series of large, multicentre clinical randomized trials have definitively established the very important role of ACEi in cardiovascular medicine (223).

2.1 Multicenter clinical randomized trials

Numerous clinical trials have established the role of ACEi in ischemic heart disease, particularly after acute myocardial infarction (MI). Early trials (SAVE, TRACE, AIRE) focused on patients with left ventricular dysfunction (224–226). More recent trials (HOPE, PROGRESS, EUROPA) have demonstrated a benefit of ACEi in preventing cardiovascular events even in patients with normal ejection fraction.

The HOPE study, an international randomized trial, showed that ramipril ($K_i = 7$ pM) was beneficial in a broad range of patients without evidence of left ventricular systolic dysfunction or heart failure who are at high risk for cardiovascular events (222). The EUROPA study extended the findings of HOPE to a group of lower risk patients with coronary artery disease frequently seen in clinical practice. PROGRESS demonstrated that lowering blood pressure with an ACEi and diuretic reduced strokes and cardiovascular events in stroke patients. While some of the effect of ACEi may be attributable to blood pressure lowering, the mortality benefit is unequivocal. In addition, ACEi prevents the progression of renal injury in diabetes (227) and a number of diseases and reduces death due to MI in diabetic population (228). Taken together these studies present strong evidence that patients with evidence of stable coronary heart disease, vascular disease, and/or diabetes (plus one further risk factor), regardless of left ventricular function, should be treated with an ACEi. The challenge now is to translate this evidence-based therapeutic management into cardiological practice (229).

The mechanism by which ACEi reduce cardiovascular mortality is the subject of intense investigation. ACEi have been shown to improve endothelial function, a marker of future cardiovascular events. While some investigators have suggested that ACEi with higher tissue affinity for somatic ACE may have a greater impact on cardiovascular mortality, no comparative trials have been conducted.

2.2 Role of BK in the cardiovascular effects of ACEi

The evidence for a contribution of kinins (mainly BK) in the cardiovascular effects of ACEi is essentially of pharmacological nature. These results have been extensively reviewed elsewhere (230). In various experimental models, in vitro, ex vivo, and in vivo, ACEi mimic and potentiate the pharmacological effects of BK, which can be suppressed by a B2R antagonism, mainly by HOE 140 (Icatibant). These observations have been made using cell models, where an ACEi stimulates the NO and prostacyclin (PGI₂) production triggered by BK. In vivo, Icatibant has been shown to suppress the antihypertensive, antihypertrophic, and antiproliferative effects of ACEi to a variable extent, depending on the experimental model. The effect on the antihypertensive effects of ACEi is more controversial and depends on the experimental model. In hypertensive subjects, a short-term hypotensive effect of captopril was abolished at least in part by Icatibant (231). Several but not all studies indicate that BK contributes to the effects of chronic ACEi in patients with congestive heart failure.

The contribution of endogenous kinins in these pharmacological effects is more controversial, and it is still not clear, at the present time, which part is due to the B1R and B2R agonists in the pharmacological effects. In fact, depending on the animal model, ACEi potentiate either the concentration of BK or des-Arg⁹-BK. These apparent discrepancies could be the result of varying importance of other metallopeptidases responsible of the metabolism of kinins (72).

3. Vasopeptidase inhibitors (VPI)

The VPIs possess the ability to inhibit simultaneously two membrane-bound zinc metalloproteases, ACE and NEP, with similar nanomolar inhibitory constants (232). Omapatrilat was the first of this new class of drugs. This dual inhibitor has been evaluated clinically for the treatment of hypertension, heart failure, and renal disease.

3.1 Multicenter clinical randomized trials

Preclinical (233) and early clinical studies conducted with omapatrilat were very promising. Indeed, omapatrilat appeared to be a potent antihypertensive agent with favorable effects on cardiac function in heart failure patients (234, 235). In contrast to these early studies, the large clinical trials were more disappointing.

The results of the Omapatrilat Cardiovascular Treatment Versus Enalapril (OCTAVE) trial suggested that use of a more efficacious monotherapy such as omapatrilat can result in greater blood pressure reductions, but at the price of an AE rate more than threefold

higher than that of an ACEi in the overall population (see below) (236).

In The Omapatrilat Versus Enalapril Randomized Trial of Utility in Reducing Events (OVERTURE), the patients with chronic heart failure and hypertension treated with omapatrilat had a reduced morbidity and mortality when compared with enalapril. AE was also more common with omapatrilat (236 – 238).

3.2 Role of kinins in the cardiovascular effects of VPi

The protective effect of omapatrilat on BK degradation on both cardiomyocytes and endothelium, two target sites for metallopeptidase inhibitors, has also been compared to that of ACEi (239 – 241). These studies have shown the complexity of the different metabolic pathways, whose importance varies according to the tissue but also the nature of the pathophysiological background. As an example, Fig. 4 illustrates the influence of insulin on the protective effect of an ACEi and a VPi on the cardiac metabolism of BK in experimental type I diabetes. Hence, BK may also be an important mediator of this new class of drugs. The effects of a single inhibitor of both ACE and NEP, resulting in an indirect activation of the B2R, could be responsible for additional beneficial therapeutic effects

of VPi such as omapatrilat (242, 243).

4. Kinins and side effects of metallopeptidase inhibitors

Despite their clinical effectiveness, ACEi can cause chronic and acute side effects. The best known chronic side effect is a non-productive cough. Although some experimental and clinical evidences suggest a role of BK in the pathophysiology of this effect, no definitive arguments exist for such a role.

The nature of acute side effects of ACEi depends on the clinical context. Angioedema has been reported in hypertension and heart failure, but also in stroke patients during fibrinolysis with rt-PA (244). AR occur in patients dialysed with a negatively charged membrane (119). Finally, SHR have been described during blood product transfusion (216). In these cases, the majority of the reactions occurred during transfusion of blood components administered through a negatively charged bedside leukocyte reduction filter. A similar reaction also occurs during LDL and plasmapheresis (245).

The incidence of these acute side effects has traditionally been around 0.1% to 0.2%. However, this incidence must be revised in light of the OCTAVE study (236, 246). This study has shown an incidence of AE with enalapril equal to 0.68% in Caucasian hypertensive patients. This incidence was still higher in African Americans (1.62%) and in smokers (0.81%), whereas patients with diabetes had lower rates of AE. This study showed an increased incidence of AE associated with omapatrilat in Caucasians (2.17%), African Americans (5.54%), and smokers (3.93%). These results are important because they highlight 3 factors: the presence of inflammation (smokers), the genetic aspect (African Americans), and the nature of the metallopeptidase inhibitor that inhibits one or two peptidases, specifically or non-specifically.

In fact, acute side effects of ACEi and, by extension, of other metallopeptidase inhibitors (VPi) could result in the meeting of at least 3 different factors. The first one is the presence of a drug that inhibits specifically ACE (or ACE plus NEP) but could also non-specifically inhibit other enzymes involved in the metabolism of kinins, such as APP or CPN. The second factor may be a pathophysiological or physicochemical trigger for the release of kinins. It has been identified as the negatively charged surface in AR and as the negatively charged filter in SHR. The triggering factor remains unknown in AE but could be linked to the inflammatory status, as suggested by the higher incidence in smokers. The third factor could be intra- or inter-individual genetic or environmental factors that effect non-ACE inhibitor mediated pathways metabolizing vasoactive peptides such as the kinins.

Fig. 4. Influence of insulin on the cardiac metabolism of exogenous BK in experimental type I diabetes. The half-life of exogenous BK incubated with heart membranes from Sprague-Dawley control rats (CTL), streptozotocin (STZ)-treated rats 8 weeks post-injection and STZ-treated rats before and after (7 days) insulin administration (+INS-1 week). Membranes were incubated with exogenous BK without enzymatic inhibitor (shade) or in presence of enalaprilat (grey) or omapatrilat (black). Values are means $\pm$ S.E.M. * P <0.05 versus without inhibitor; † P <0.05 versus enalaprilat. (Reproduced with permission from P. Leclair, MSc Thesis, p. 79, Université de Montréal, 2000)

We have investigated the metabolism of endogenous kinins from the plasma of patients who developed AE while being treated with an ACEi. While we found no abnormality in the metabolism of BK, Caucasian patients with a history of AE exhibited slower degradation of des-Arg⁹-BK, correlating with a decreased activity of plasma APP (247, 248). This anomaly could also be observed in patients who presented an AR (119) or a SHR (245, 249). We hypothesized that the accumulation of the B1R agonist could be responsible for the symptoms that characterize AR and SHR, but our observations cannot be related to the local inflammatory reaction that characterizes AE.

As no inhibitor of APP is present in plasma (119), we investigated the genetic aspect of AE. A recent report provides evidence that variable APP activity in humans is partially regulated by genetic factors. From data ascertained from 8 Caucasian kindreds whose proband suffered either an AE whilst being treated with an ACEi, we estimated that 34% of the phenotypic variation results from genetic differences in the linkage analysis. We described two sequence variations, a nonsense mutation resulting in a truncated protein and a potentially regulatory single nucleotide polymorphism (SNP) that segregated with reduced plasma APP activity, suggesting an increased susceptibility to ACEi associated acute side-effect (250). These findings do not mean that this active metabolite, des-Arg⁹-BK, is necessarily the only mediator of AE; a local release of neurokinins or a decrease in DPP IV activity (substance P-degrading enzyme) seems to be associated with this potentially life-threatening side effect (251), particularly in African Americans. The precise mechanism responsible for ACEi-associated AE is not known.

III- Kinin Receptors

1. Receptor polymorphisms and pathology

The potential role of kinin receptors in human disease is derived from studies evaluating kinin receptor polymorphisms, measurement of differences in kinin receptor expression level, and detection in differences in receptor functions. However, reports concerning a correlation between SNP in kinin receptor genes and complex pathologies should be carefully interpreted. Usual analysis involves a statistical comparison between case and control populations. Nevertheless, this method does not always take in account the influence of a nearby gene rather than the gene of interest or the possibility of a true relationship between a SNP with a disease which were not at first directly correlated. The specific situation of the kinin receptor locus is particularly problematic in this respect: as both

kinin receptor genes lay very close to each other in the same locus, a genetic marker in one of them may point out to a functional alteration of the other; there is some experimental support for this idea (252). Further pharmacological studies, appropriate family-based designs, or case-control designs analyzing haplotypes rather than single SNP (253) should also be used to demonstrate the predicted interrelation.

Many polymorphisms of B2R and B1R have been described (254). Some of them have been related to pathophysiological events.

1.1 Polymorphism of B2Rs

Left ventricular hypertrophy and cardiomyopathy are some of the adverse effects of hypertension. These pathologies have been linked to kinin receptors (255).

1.1.1 +9/−9, exon 1 polymorphism

A +9/−9 exon 1 polymorphism of the B2R was strongly associated with the left ventricular growth response among normotensive white males undergoing a ten-week physical training program. Individuals with the lowest levels of B2R (+9/+9 genotype) and the highest levels of ACE (DD genotype) had the greatest increase in left ventricular mass (256). Subjects with B2R +9/+9 genotype showed less left ventricular mass regression compared with other genotypes. B2R (+9/−9) polymorphism was also shown to be significantly associated with higher skeletal muscle metabolic efficiency and with endurance in athletic performance (257).

The (−9/−9) genotype of B2R exon 1 was also examined for the contractile response of human umbilical veins to stimulation with kinins (252). This polymorphism was associated with increased contractile efficiency of the B1R agonist, suggesting that the B2R gene may be in linkage disequilibrium with the B1R gene.

Cardiovascular risk and increase in blood pressure associated with hypertension are also influenced by the presence of B2R (+9) allele, an effect not identified in individuals homozygous for B2R (−9) (258). This suggests a role of B2R in human coronary vascular disease.

B2R (+9/−9) polymorphism was significantly associated with diabetic nephropathy (259).

The B2R (−9) allele has been associated with the most symptomatic cases of C1INH deficiency (hereditary angioedema with angioedema crises) and thus is proposed to modulate in a dominant manner the phenotype of the basic genetic defect of this disorder (130). However, this polymorphism did not predict the incidence of the most common side effect of ACEi, the non-productive cough (260).

1.1.2 ($C^{-58} \rightarrow T$), promoter region polymorphism

Several studies have detected a significant association between the B2R ($C^{-58} \rightarrow T$) polymorphism and hypertension. A consistent finding that the C^{-58} allele has higher frequency in hypertensive individuals compared to normotensive individuals has been reported in various populations (261–263). The allele frequencies are similar in Caucasians and Afro-American populations, whereas the T allele is slightly more frequent in the Japanese.

The genotypic and allelic frequencies of ($C^{-58} \rightarrow T$) polymorphism of general hypertensive subject were analyzed (264). The frequencies of the TT genotype and the T allele of ($C^{-58} \rightarrow T$) are higher in the subjects with cough than in subjects without cough. Moreover, T^{-58} was found to have a higher transcription rate than that of C^{-58} . The high transcriptional activity of the B2R promoter might be involved in the occurrence of ACEi-related cough.

1.1.3 ($C^{181} \rightarrow T$), exon 2 polymorphism

Presence of the nonsynonymous coding region B2R exon 2 ($C^{181} \rightarrow T$) was associated with increased contractile potency in response to stimulation with BK consistent with the enhanced in vitro potency of BK at the $\text{Arg}^{14} \rightarrow \text{Cys}$ mutant receptor (252) and with significantly lower systolic and diastolic blood pressure (265).

1.2 Polymorphisms of B1Rs: ($G^{-699} \rightarrow C$) substitution

Cardiovascular risk and increase in blood pressure associated with hypertension are influenced by the presence of B1R (G^{-699} substitution) allele, an effect not identified in the presence of the homozygous B1R (C^{-699} substitution) allele (258). This suggests a role of B1R in human coronary vascular disease.

The B1R polymorphism consisting of a single base substitution ($G^{-699} \rightarrow C$) in a positive control region of the promoter exhibits an altered frequency in patients with end-stage renal failure (266). A deficit of the C allele was observed among diseased patients and in some etiologic groups (polycystic kidneys, pyelonephritis, and interstitial nephritis) compared with healthy volunteers. This polymorphism may be a marker of prognostic significance for the renal function in diseased individuals. Profound alteration of allele frequencies were found in patients with inflammatory bowel disease such as ulcerative colitis or Crohn's disease in a small case-control study (267). Thus, the B1R promoter gene does not have a specific etiologic influence but rather modifies a downstream inflammatory pathway common to these disorders.

2. Kinin receptors as pharmacological targets

Roles for the kallikrein-kinin system in inflammation have been investigated and reviewed extensively (268). As a great number of disease states such as chronic inflammatory pain, edema, asthma, and sepsis have their basis in the inflammatory response, the development of novel antagonist drugs targeted at the B1R and B2R provides a novel therapeutic opportunity. The clinical development of these drugs is at an early stage, with few human clinical studies reported until now and mainly based on peptide compounds (269–272). These charged and rather large molecules may not show the full therapeutic effect of kinin receptor blockade due to limitations in oral bioavailability, distribution, and stability. The potential therapeutic applications of kinin receptor ligands (not always antagonists) include cardiovascular and renal disorders, inflammation, pain, diabetes, asthma, and perhaps cancer.

2.1 Cardiovascular and renal diseases

BK is known for its multiple effects on the cardiovascular system and particularly by its vasodilatation and plasma extravasation properties (273), leading to an inflammatory response. Vasodilatation is normally mediated by B2R (274), but under inflammatory conditions, B1R up-regulation mediates kinin-induced vasodilatation and hypotension (275, 276). BK-related peptides act as vasodilators through endothelial cells from which secondary mediators are released to affect the vascular smooth muscle. In humans, cardiovascular actions of kinins are mainly correlated to preformed B2R stimulation (leading to NO and PGI_2 formation) and the contribution of the B1R is not detectable (130). NO is derived from L-arginine by eNOS. NO diffuses from the endothelium to the smooth muscle where it activates guanylate cyclase. NO-independent ion channels are also suspected to mediate endothelial-dependent vasorelaxation. Prostacyclin is also released by kinins from the endothelial cells, probably via the cytosolic Ca^{2+} -sensitive isoforms of PLA_2 and stimulates cAMP production in smooth muscle cells (277). These physiological effects of kinins are potentially useful to treat hypertension and ischemic disorders and to maintain renal function (as the kallikrein-kinin system plays a role in handling salt excess).

The local generation of kinins or the inhibition of their degradation and the resulting B2R stimulation could be of interest to reduce blood pressure or to promote cardioprotective effects (231, 278, 279).

On the other hand, B1R activation has been shown to exert a protective effect after cardiac ischemia in mice (280). The peptidase-resistant B1R agonist $\text{Sar}[\text{D-Phe}^8]\text{des-Arg}^9\text{-BK}$ has been used to further stimulate

the formation of compensatory vasculature in a rodent ischemia model after occlusion of the femoral artery (281). FR190997 is a nonpeptide B2R agonist (or partial agonist) derived chemically from a series of B2R antagonists (282). Speculatively, it has been proposed that this drug could be exploited as an antihypertensive or cardioprotective agent, but clinical testing remains to be performed (283). Much experimental and some clinical works on the effect of ACEi naturally suggests that kinin receptor agonists could be used as vasoactive agents, but the inflammatory properties of these compounds probably cannot be dissociated from their vasodilator/angiogenic effects (273). One B2R agonist that has been developed up to a certain point, labradimil (RMP-7), was a peptidase-resistant BK analog used to transiently open the blood-brain barrier, thus deliberately exploiting an inflammatory effect (284).

B1R may not be responsible for the cardiovascular effect of kinins in healthy humans but in the case of tissue injury, the receptor expression is induced, as in the cardiac endothelium of patients with heart failure (285), and B2R expression remains usually unchanged. However infusion of a pharmacologic concentration of the BK agonist des-Arg⁹-BK does not cause vasodilation in the forearm of congestive heart failure patients treated chronically with ACEi.

2.2 Inflammation

Injections of substances known to activate the contact system, such as carrageenan, induce a swelling that reaches a maximal response at 3 h post-injection (286). BK release is related to the intensity of an acute inflammation reaction, based on the inhibitory effect of a B2R antagonist, but repeated injections of the irritant produced a reaction that becomes responsive to a B1R antagonist (Fig. 5). This type of observation was also made using an antigen-induced model of chronic inflammation where the B1R antagonist [Leu⁸]des-Arg⁹-BK was shown to become more potent to inhibit plasma extravasation of joint inflammation as the model progressed (287).

BK administration reproduces two of the cardinal signs of inflammation (*rubor*, *calor*) through the activation of B2R that causes vasodilatation due to NOS and PLA₂ stimulation leading to NO and PGI₂ production by vascular endothelial cells. The ensuing exudation of protein-rich fluid from the circulation, facilitated by kinins, is largely determined by the rise of vascular permeability, particularly at the level of post-capillary venules via endothelial cells contraction. This is the essentially vasogenic mechanism of a third cardinal sign of inflammation, swelling (*tumor*).

Models of chronic inflammation provide the involve-

Fig. 5. Effects of kinin receptor antagonists on carrageenan-induced paw edema. Effects of a B₁ (Lys[Leu⁸]des-Arg⁹-BK, 32.5 nmol/paw) and B₂ (HOE 140, 3.25 nmol/paw) receptor antagonist on carrageenan-induced paw edema. The anti-edema potential of the B1R antagonist is evidenced only at the second challenge, consistent with B1R absence in the uninflamed tissue. Carrageenan was injected twice, at 24-h intervals. Carrageenan 0.5% (black circle), carrageenan 0.5% with B1R antagonist (grey square), or with B1R and B2R antagonists (white triangle). (Reproduced with permission from A. Décarie, PhD Thesis, p. 156, Université de Montréal, 1997)

ment of the B1R (151). The role of infiltrating leukocytes is relevant in these models as they may supply cytokines (IL-1 β , TNF- α) levels required to induce B1R expression. Conversely, B1R seems to be of importance in neutrophil accumulation in inflamed tissues, as the ablation of the corresponding gene in mice is associated with a significant defect of this process (mechanism not fully elucidated) (288).

2.3 Pain and neurological applications

The specific role of B1R and B2R in inflammatory pain perception (*dolor*, the 4th cardinal sign of inflammation) is correlated to the amplitude and the kinetics of their expression. B2R are generally constitutively expressed on primary non-myelinated sensory neurons and BK activates these nociceptors to contribute to the acute pain response (289) through the release of DAG (173) and the protein kinase C activation (290).

One of the most promising applications of kinin receptor antagonists is analgesia. A number of animal models of inflammatory pain have been exploited to show that B1R or B2R antagonists exert analgesia (151). Although the interest for a B1R antagonist is currently

strong, due to their efficacy in the later of persistent phases of inflammatory pain, the analgesic effect of recent nonpeptide antagonists of the B2R is surprisingly good (LF 16-0687, bradyzide) (291, 292). However, the fact that peptide antagonists of either receptor subtype are analgesic may support a peripheral mode of action, as these agents are likely to be excluded from the CNS. A peripheral site of action is further supported by the localized expression of the B1R mRNA in a zymosan-induced inflammatory pain model applied to the rat (291).

On the other hand, the role of the CNS expression of kinin receptors in pain perception is of great interest. The occurrence of “wind up” (facilitation) to repeated dorsal root stimulation *in vitro* is reduced by about 50% in the B1R gene knockout mice (288), suggesting the presence of a constitutive B1R population in the pain neurosensory pathways in this species. B1R mRNA is expressed in rat and human dorsal spinal cords (293, 294) and throughout the rostral-caudal portion of monkey brain (295). However, whether the presence of a background mRNA concentration predicts the presence of mature receptors is not clear: there is published evidence showing that the B1R mRNA is detectable in the control rat spinal dorsal cord, but not the corresponding binding sites; however, both increase as a function of the pathology (streptozotocin-induced diabetes) (296).

Abundant preclinical evidence shows that post-traumatic brain edema is reduced by B2R pharmacological blockade (297, 298); this is likely to be a vasogenic response. The nonpeptide drug LF 16-0687 is currently being evaluated for this indication in humans. Cultured human brain microvascular endothelial cells express the B1R (299). The biological significance of B1R activation during inflammation can be considered in the context of both blood-brain barrier permeability and chemoattraction. The activation of B1R reduces IL-8 secretion by these cells, suggesting dissociation between permeability of fluids and leukocyte trafficking. By this mechanism, activation of B1R would produce a perivascular infiltrate enriched for high molecular weight molecules with a relative paucity of immune cells. Moreover, a correlation has been observed between B1R activity and its expression on peripheral T lymphocytes in multiple sclerosis patients (300). Nevertheless, B1R activation prevents the infiltration of T lymphocytes through an artificial blood-brain barrier, suggesting a protective role of B1Rs in this disease via an inhibition of IL-8 secretion.

B2R is widely distributed in the rat brain, but following kindling-induced epilepsy, the relatively low levels of B1R expression, in normal rodents, are functionally detectable in several brain areas (potentiation of electri-

cally evoked glutamate overflow) (301).

Local inflammation of viscera, such as small intestine or bladder, and related pain are mediated by endogenous kinins via a viscerovisceral hyper-reflexia. Kinin receptor antagonists were shown to inhibit this hyper-reflexia, but a temporal shift of mediation is seen from B2Rs to B1Rs (302). BK-receptor expression in inflammatory bowel disease, namely, ulcerative colitis and Crohn's disease, may be altered in intestinal inflammation. Increased B1R gene and protein expression in active inflammatory bowel disease provides a structural basis for the important role of kinins in chronic inflammation (303).

Clinical trials for the analgesic effects of B1R antagonists are currently being conducted.

2.4 Diabetes

Some experimental data suggest that diabetes is another pathological condition that could induce B1R expression. Insulino-dependent diabetes (type I diabetes) derives from an auto-immune response (insulinitis) implicating an overproduction of cytokines such as IL-1 β and TNF- α . Moreover, hyperglycemia and oxidative stress can also activate NF- κ B. The addition of cytokines overproduction and hyperglycemia could then induce B1R expression through NF- κ B (304, 305). The peptide B1R antagonist [Leu⁸]des-Arg⁹-BK prevents the progression of streptozotocin-induced insulin-dependent diabetes in rodents (306), but not treatment with Icatibant, suggesting that the B1R has a selective role in the insulinitis that precedes diabetes in this model.

Furthermore, established streptozotocin-induced diabetes in rodents is associated with prominent B1R expression (307, 308) and the associated hyperalgesia (309). However, these observations were made on insulin-dependent diabetes without insulin treatment, a wasting disease with high blood cytokine levels, and have an uncertain significance for the complications of diabetes in present day human patients. Another line of investigation suggests that kinins exert protective effects in diabetes models in rodents as inhibitors of kininases improved the sensitivity to insulin and as Icatibant significantly reduced the effect of these kininase inhibitors. These observations support that these metabolic effects are mediated by endogenous BK and B2R (310, 311).

2.5 Renal disease

Malignant hypertension is observed when B2R knockout mice are overloaded with dietary sodium (312). This confirmed the long time suspected role of the renal kallikrein-kinin system in handling sodium and water metabolism, in parallel to the renin-angio-

tensin-aldosterone endocrinal axis (313).

Specific polymorphisms of the human kinin receptor locus have been associated with end-stage renal failure (mixed aetiology), hypertension, and nephropathy in diabetics (259, 265, 266); all these findings are perfectible efforts to link the transmission of this locus to human pathology. The protective effect of kinins in renal disease has been extended to the B1R, as various aspects of the chronic inflammatory response seem to be limited by endogenous ligands of this receptor subtypes in animal models (314).

2.6 Airway disease

Icatibant (HOE 140), a widely used peptide B2R antagonist, has been found to significantly improve the ventilatory function in humans with asthma when administered in an aerosol form (269). The mode of action of this drug was not related to an acute bronchodilator action, but rather to a long-term anti-inflammatory effect.

Persistent dry cough is a side effect related to the use of ACEi in a relatively large number of patients. The cause and the mechanism of dry cough is not fully understood but can be attributed to a possible local accumulation of BK that may lead to activation of proinflammatory peptides and a local release of histamine, inducing a cough reflex hypersensitivity (264). Another explanation implicates a BK-mediated increase in lung PGs which produce cough and sensitize bronchial contractility (315, 316). Several polymorphisms of the human B2R gene may be involved in ACEi-related cough. The genotypic and allelic frequencies of the -58 thymine/cytosine (-58T/C) polymorphism of patients with essential hypertension were analyzed (264). The frequencies of the TT genotype and the T allele of -58T/C are higher in the subjects with cough than in subjects without cough. Moreover, -58T was found to have a higher transcription rate than that of -58C. The higher expression of the B2R might be involved in the occurrence of ACEi-related cough.

2.7 Angioedema (AE)

An exon 1 polymorphism in the human B2R gene, in which alleles differ by a 9 bp deletion, appears to confer a higher level of expression, probably due to more stability against the action of RNases. The B2R(-) allele was always present in the most symptomatic cases of HAE in a small series of patients and thus is proposed to modulate in a dominant manner the phenotype of the basic genetic defect of this disorder (130). However, this polymorphism did not predict the incidence of the most common side effect of ACEi, the non-productive cough (260). ACE-induced angioedema is correlated to

a decreased ability to degrade des-Arg⁹-BK in hypertensive patients (248). This biological marker has been later attributed to a largely transmissible variability of the expression of membrane APP (250). Encouraging results in the clinical trial of the B2R antagonist Icatibant support that kinin receptor blockade is of therapeutic interest in HAE (209). It remains to be seen whether B1R blockade could be as or more effective in this and other forms of AE.

Conclusion

In this review, we tried to show that the complexity of the kallikrein-kinin system results from the interaction of its multiple components. We have exposed both faces of this system, where the *ying* is often the shadow of the *yang*. We have also stressed that pathophysiological models and pharmacological approaches validated in animals (potentiation of the cardiovascular effects of kinins, suppression of their proinflammatory effects) are sometimes difficult to apply in humans. Notwithstanding these difficulties, several groups of drugs are now available or being developed in human medicine for the treatment of either the side effects resulting from the chaotic activation of the contact system or the potentiation of the cardioprotective effects of kinins.

Recent experimental works open new pharmacological venues, as is the case for the antiangiogenic property of the heavy chain of HK. It is particularly true for des-Arg⁹-kinins and their B1R, the molecular target of many novel antagonists. The nonpeptide B1R antagonists are now in development. They look particularly promising in the treatment of inflammatory pain where the roles of the B1R and its agonists have been shown in animals. A number of other applications are awaiting clear clinical conclusions about the applicability of kinin receptor antagonists: inflammation (e.g., inflammatory bowel disease), asthma and allergy, brain edema and wasting states, sepsis, congenital, or drug-induced angioedema.

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We dedicate this review to Dr. J. Damas, for his retirement. Dr. Damas discovered the Brown Norway rat, the prototype of transgenic animals in kinin research.

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