

REVIEW ARTICLE

New topics in bradykinin research

M. Maurer¹, M. Bader², M. Bas³, F. Bossi⁴, M. Cicardi⁵, M. Cugno⁶, P. Howarth⁷, A. Kaplan⁸, G. Kojda⁹, F. Leeb-Lundberg¹⁰, J. Lötvald¹¹ & M. Magerl¹

¹Department of Dermatology and Allergy, Allergie-Centrum-Charité, Charité – Universitätsmedizin, Berlin, Germany; ²Max-Delbrück-Center for Molecular Medicine, Berlin-Buch, Germany; ³Hals-, Nasen- und Ohrenklinik, Technische Universität München, München, Germany; ⁴Department of Life Sciences, University of Trieste, Trieste, Italy; ⁵Dipartimento di Scienze Cliniche, Università degli Studi di Milano, Ospedale L. Sacco, Milan, Italy; ⁶Dipartimento di Medicina Interna, Università degli Studi di Milano, Fondazione IRCCS Ospedale Maggiore Policlinico, Milan, Italy; ⁷Infection, Inflammation and Immunity Research Division, Faculty of Medicine, University of Southampton, Southampton, UK; ⁸Department of Medicine, Medical University of South Carolina, Charleston, SC, USA; ⁹Institut für Pharmakologie und Klinische Pharmakologie, Universitätsklinikum Düsseldorf, Düsseldorf, Germany; ¹⁰Division of Cellular and Molecular Pharmacology, Department of Experimental Medical Science, Lund University, Lund, Sweden; ¹¹Department of Internal Medicine, Krefting Research Centre, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

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Correspondence

Marcus Maurer, MD, Department of Dermatology and Allergy, Allergie-Centrum-Charité, Charité – Universitätsmedizin, Charitéplatz 1, 10117 Berlin, Germany
Tel.: +49 30 450 518 043;
Fax: +49 30 450 518 972;
E-mail: Marcus.Maurer@charite.de

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Abstract

Bradykinin has been implicated to contribute to allergic inflammation and the pathogenesis of allergic conditions. It binds to endothelial B₁ and B₂ receptors and exerts potent pharmacological and physiological effects, notably, decreased blood pressure, increased vascular permeability and the promotion of classical symptoms of inflammation such as vasodilation, hyperthermia, oedema and pain. Towards potential clinical benefit, bradykinin has also been shown to exert potent antithrombogenic, antiproliferative and antifibrogenic effects. The development of pharmacologically active substances, such as bradykinin receptor blockers, opens up new therapeutic options that require further research into bradykinin. This review presents current understanding surrounding the role of bradykinin in nonallergic angioedema and other conditions seen by allergists and emergency physicians, and its potential role as a therapeutic target.

The kinins are a family of vasoactive peptides including bradykinin, kallidin and methionyl-lysyl-bradykinin, the latter two compounds being converted very rapidly to bradykinin by aminopeptidases. Bradykinin is a low molecular weight (1060.21 Da; C₅₀H₇₃N₁₅O₁₁) nonapeptide, which is rapidly metabolized by endogenous metalloproteases including angiotensin-converting enzyme (ACE or kininase II), neutral endopeptidase (NEP or neprilysin), carboxypeptidase N (CPN or kininase I) and aminopeptidase P (1). Bradykinin has only a short plasma half-life of ~15 s, and circulating levels are usually relatively low (0.2–7.1 pM) (2, 3).

Much progress was made in understanding the physiological role of kinins with the development of selective antagonists for endothelial B₁ and B₂ receptors (4) and of C1 esterase inhibitor (C1-INH)- and B₂-receptor-transgenic

mice (5, 6). Bradykinin binds these receptors, exerting potent effects in different pathophysiological states (7) (Fig. 1). For example, bradykinin exerts potent antihypertensive, antithrombogenic, antiproliferative and antifibrogenic effects (8) as summarised in Table 1. Bradykinin also participates in inflammatory processes by activating endothelial cells to promote vasodilation and increased vascular permeability, producing classical symptoms of inflammation such as redness, heat, swelling and pain (1, 9). Specifically, bradykinin contributes to tissue hyper-responsiveness and local inflammation in allergic rhinitis, asthma and anaphylaxis, while bradykinin-dependent angioedema can result from hereditary or acquired C1-INH deficiency or the use of ACE inhibitors (ACEi) to treat hypertension, heart failure, diabetes or scleroderma.

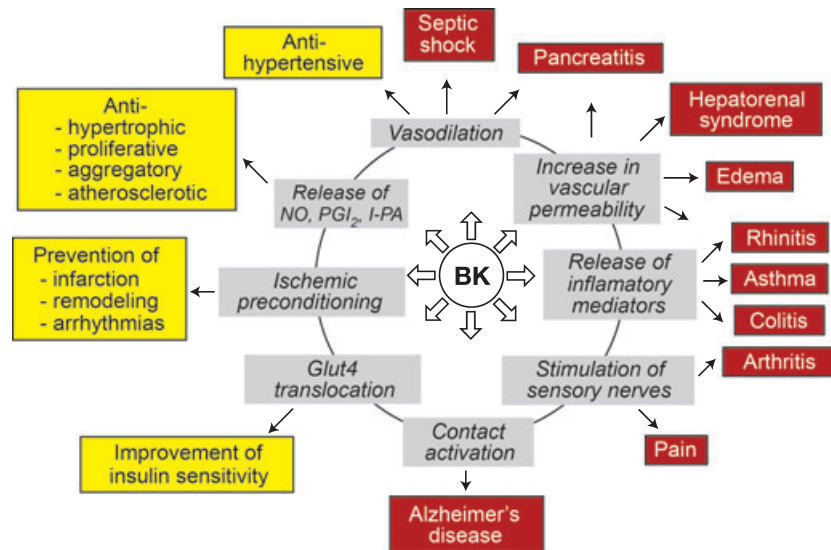


Figure 1 Effects of bradykinin in different pathophysiological states. Reprinted with permission from Heitsch (7).

Table 1 Pharmacological and physiological functions of bradykinin.

System	Pharmacological/physiological response(s)	Pathophysiological state(s)
Central nervous system	Excitation of primary sensory neurons Release of substance P, neurokinin A	Acute algnesia Pain of chronic inflammation
Immune	Production of IL-1 and TNF α Increase vascular permeability	Inflammation, oedema, rhinitis 'Wheal and flare' response
Respiratory	Bronchospasm, bronchoconstriction	Asthma, rhinitis
Cardiovascular system	Release of prostacyclin, NO, EDHF Vasodilation, vascular permeability	Transient hypotension, reflex tachycardia
Renal	Inhibition of sodium reabsorption at the cortical collecting ducts	Hyponatremia (?)
Other	Stimulation of uterine contractility Disrupts blood–brain barrier	

EDHF, endothelium-derived hyperpolarizing factor; IL-1, interleukin 1; NO, nitric oxide; TNF α , tumour necrosis factor α .

Bradykinin biology

How is bradykinin formed?

The bradykinin-forming cascade is initiated by factor XII autoactivation, cleavage of surface-bound factor XII by factor XIIa and feedback activation by generated kallikrein. Although plasmin activates factor XII, this is not thought to contribute significantly unless plasma is deficient in C1-INH (10). *In vitro*, the cascade is activated by the binding of factor XII and a complex of high molecular weight kininogen (HK) and prekallikrein to a negatively charged surface (e.g. silicate or glass); prekallikrein binds indirectly to the surface via HK. Factor XII is subsequently autoactivated (albeit slowly) to factor XIIa, which converts the proenzyme prekallikrein into plasma kallikrein; HK is then digested by kallikrein, releasing bradykinin (Fig. 2). Notably, even a modest degree of factor XII autoactivation can promote exponential kallikrein formation (10).

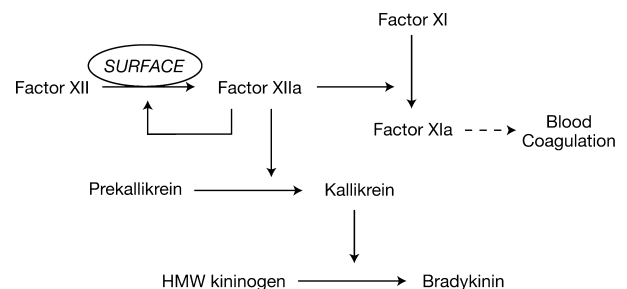


Figure 2 Formation of bradykinin *in vitro*. Reprinted with permission from Kaplan & Ghebrehiwet (10).

These bradykinin-forming cascade proteins also attach to cultured endothelial cells, where autoactivation of factor XII to factor XIIa is believed to occur, thus initiating the cas-

cade, most likely on binding to endothelial cell surface gC1qR (10). In the presence of zinc, HK and factor XII each bind overlapping sites on human umbilical vein endothelial cells (HUVECs). Factor XII binds primarily to a duplex of endothelial membrane proteins, namely uPAR (urokinase plasminogen activator receptor) and cytokeratin 1, while HK binds primarily to gC1qR complexed to cytokeratin 1. In addition, the unusual structure of HK is integral to HK endothelial binding and bradykinin formation; HK consists of six domains. Domain 5 (HK light chain) and domain 3 bind to the endothelial membrane proteins gC1qR and cytokeratin 1, respectively. Bradykinin is situated in domain 4. At domain 6, prekallikrein binds to HK, the site requisite for prekallikrein cleavage.

Until recently, prekallikrein was assumed to be inactive, but recent studies involving incubation of prekallikrein with HK showed stoichiometric bradykinin formation, thus prekallikrein possesses enzymatic activity. Moreover, prekallikrein activation at endothelial cells occurs on incubation with factor XII-deficient plasma, but only slowly (> 100 min). If prekallikrein, HK and zinc are all added to cell cultures (in the absence of inhibitors), prekallikrein activation occurs rapidly, resulting in bradykinin formation. Notably, Joseph et al. (11) demonstrated that factor XII may be bypassed via heat-shock protein (HSP)-90 binding to prekallikrein-HK, resulting in a stoichiometric formation of kallikrein. Kallikrein thus formed can activate factor XII to factor XIIa (12); thus, the cascade need not be initiated by factor XII. This positive feedback mechanism is also seen with factor XII-initiated bradykinin production. This may explain the localized onset of hereditary angioedema (HAE) attacks after trauma (e.g. during surgery or dentistry), while direct disruption of the endothelium may expose surfaces (e.g. negatively charged proteins) to initiate the bradykinin-forming cascade through HSP-90 or factor XII. Indeed, incubation of pure gC1qR (i.e. the 'surface') with factor XII (plus zinc) resulted in dose-dependent autoactivation of factor XII, suggesting subtle activation when cascade proteins bind to the endothelial cell surface. Additionally, HSP-90-stimulated formation of kallikrein occurs quickly (seconds) *in vivo*, whereas this process is relatively slow (hours) *in vitro* (12); evidence suggests the presence of an accelerator *in vivo*, possibly positive feedback via kallikrein for the formation of factor XIIa.

How does bradykinin mediate its effects?

Receptor signalling

The bradykinin B₁ (inducible, slow agonist desensitization) and B₂ (constitutively expressed, rapid agonist desensitization) receptors are G protein-coupled receptors (GPCR) that interact primarily via the G proteins G_{α_{q/11}} and G_{α_{i/o}} and also independently of G proteins through intracellular effectors (13). For example, bradykinin binding to endothelial B₂ receptors leads to nitric oxide (NO) production, prostacyclin formation, elevated intracellular Ca²⁺ and the formation of hyperpolarizing factor, triggering vasodilation and increased vascular permeability.

Bradykinin receptor subtypes are discriminated by their natural ligands: B₁ is preferentially bound by the carboxypeptidase M and N degradation product des-Arg⁹-bradykinin and B₂ by bradykinin (13). Using NPC17731 (a B₂-selective peptide antagonist), Fathy et al. (14) showed that a C-terminal Arg residue in the peptide ligand dictated which receptor subtype is selected.

In many cell types, the unoccupied B₂ receptor exhibits typical GPCR behaviour, remaining at the cell surface without any significant agonist-independent activity (15, 16). Once bradykinin binds, the receptor mediates a rapidly desensitizing response: a transient increase in intracellular Ca²⁺ within seconds of bradykinin binding (17), followed by rapid receptor endocytosis and recycling (16). Specifically, bradykinin-stimulated B₂ receptors rapidly become phosphorylated by a GPCR kinase and recruit β-arrestin, which leads to receptor endocytosis, β-arrestin dissociation and receptor recycling to the cell surface (16, 18). In contrast, the B₁ receptor is expressed at the cell surface primarily in response to injury and inflammation (19). The unoccupied receptor, which shows some spontaneous activity (15), is endocytosed and degraded. Binding by des-Arg⁹-bradykinin interrupts this recycling process, so the B₁ receptor is stabilized at the cell surface. The agonist response is slow to desensitize as shown by persistent oscillations in Ca²⁺ levels that last for minutes (17). Hence, optimal B₂ receptor antagonists should compete with bradykinin to prevent receptor signalling. Conversely, as well as competitive binding, optimal B₁ receptor antagonists must destabilize the receptor by promoting endocytosis and receptor degradation to prevent spontaneous signalling.

Physiological response

Studies in genetically altered animal models, primarily B₁ and B₂ receptor knockout (ko) mice, have provided strong evidence that bradykinin may act indirectly on the brain and/or sympathetic nervous system and support for the various roles of B₁ and B₂ receptors in important physiological conditions when kinins are overexpressed (20–22).

In sepsis and inflammation, BP decreased markedly in wild-type (wt) mice and ko-B₂ mice, and moderately in ko-B₁ mice, after lipopolysaccharide (LPS) injection, yet BP remained unchanged in ko-B₁/B₂ mice (20, 23). Although no difference in mortality was evident between wt and ko-B₁/B₂ mice, kinins and their receptors are pivotal in hypotension induced by endotoxemia (20). Confirmation of endothelial B₁ receptor involvement in BP homeostasis was gained in another sepsis model: rats overexpressing B₁ receptors [TGR(Tie2B1)] localized to the endothelium. Administration of des-Arg⁹-bradykinin provided a dose-dependent BP reduction in the TGRB1 rat, with hypotension being more pronounced following exposure to LPS in TGRB1 rats *vs* controls; in this model, endothelial B₁ receptors thus influence BP with hypotension exacerbated by sepsis (24). The B₁ receptor is the key towards initiating inflammatory responses; injection of carrageenan into ko-B₁ mice resulted in downregulation of neutrophil infiltration (23). Likewise, cytokine-mediated neutrophil infiltration of

the lung is apparent in wt mice following intestinal ischaemia-reperfusion, but abrogated in ko-B₁ counterparts, demonstrating reduced inflammation in ko-B₁ animals (25). In a murine model of renal fibrosis, B₁ receptor expression was upregulated, induced by urethral obstruction. Consequently, all markers of fibrosis (collagen, macrophages and myofibroblasts) were improved in ko-B₁ animals relative to wt. These findings could be reproduced using a B₁ agonist, demonstrating pharmacotherapeutic potential, whereas ko-B₂, or pharmacological B₂ antagonism in wt animals, was associated with elevated renal fibrosis, indicating that B₁ receptors aggravate while B₂ receptors protect against renal fibrosis (26, 27). Other studies suggest the B₁ receptor may adversely regulate energy balance by influencing leptin signalling; B₁ receptor ko animals administered a high-fat diet showed inhibition of weight gain, improved lipid oxidation, reduced food intake and increased leptin sensitivity. Pharmacological ablation of B₁ receptors confirmed these findings (28).

In contrast, following ischaemia induced by severing the femoral artery, blood flow was restored after 3 weeks in wt but not in ko-B₁ mice, the latter showing a high incidence of limb necrosis suggesting the B₁ receptor as integral towards promoting neovascularization and angiogenesis (29, 30). In another murine model, apolipoprotein (Apo) E and B₁ double ko animals showed a greater incidence of atherosclerotic plaque development and aneurysm than animals with ApoE ko alone, supporting further an antiatherogenic role for kinin B₁ receptors (31). B₁ receptors are expressed on T-cells in multiple sclerosis lesions and have been implicated in immune cell entry into the central nervous system (CNS). Observations that ko-B₁ mice with experimentally induced autoimmune encephalitis show more pronounced T-cell entry into the CNS, and present with a worse pathological outcome than wt mice, indicate the B₁ receptor activity conveys neuroprotection in multiple sclerosis (32).

Studies in ko-B₁ and ko-B₂ mice also implicate bradykinin receptors as potential therapeutic targets for neuro-pathological conditions such as stroke. Following brain trauma, ko-B₁ animals showed lower lesion volume, while ko-B₂ animals showed less contusion, brain infarction and oedema, compared with wt animals (33, 34); bradykinin receptor ablation would therefore appear neuroprotective. Moreover, fewer microglial cells were detected in brain lesions of ko-B₁ mice compared with wt, suggesting bradykinin may influence neurological pathology via microglial migration; however, whether microglia are neuroprotective or neurodestructive appears to depend on the overall pathological milieu (35, 36). Thus, depending on the pathological condition, bradykinin B₁ and B₂ receptor activity can confer either a protective or an aggravating effect and thus represent potential pharmacotherapeutic targets. The relatively complex nature of the B₁ receptor system has made it difficult to develop a B₁ antagonist that accelerates receptor endocytosis and also prevents agonist binding. This does not apply to the B₂ receptor as, for example, the B₂ receptor antagonist icatibant provides an effective treatment for acute HAE attacks (37).

What are the pathological effects of bradykinin?

Elevated vascular permeability contributes to various pathological conditions including inflammation, trauma, sepsis, ischaemia-reperfusion, diabetes, atherosclerosis, tumour development and metastasis. Proteins and fluids cross the vascular endothelium in transendothelial vesicles (active transport) or via interendothelial junctions (IEJs) (38). Bradykinin is considered the key mediator among many in vascular leakage by disrupting IEJs and integrin-extracellular matrix complexes (39). For example, bradykinin elicits many of the features of asthma because of epithelial damage possibly associated with reduced activity of epithelial NEP (40). Using a guinea pig model, Lötvall et al. (41) studied plasma exudation in the lung following bradykinin inhalation in the presence of phosphoramidon (NEP inhibitor) or captopril (ACEi). Bradykinin led to dose-dependent increases in plasma exudation (associated with changes in lung function changes) that were exacerbated by both NEP inhibition and captopril as these agents reduce bradykinin degradation, thus enhancing bronchoconstriction (41, 42).

Bradykinin and HAE

Factors leading to vascular permeability and oedema have become better understood recently, and the contact (bradykinin-forming) cascade appears key (10). Hereditary angioedema represents an ideal *in vivo* model for investigating the bradykinin-forming cascade and the role of bradykinin in disease processes. Bradykinin levels are high during acute HAE attacks, particularly at oedematous areas; an early experiment implicating bradykinin in HAE involved the addition of HAE plasma to guinea pig ileum, which induced contractions of comparable amplitude to bradykinin-mediated contractility. Furthermore, a suspect kinin derived from C2 was disproven (43). Patients with HAE are deficient in functional C1-INH. Notably, C1-INH inhibits the plasma bradykinin-forming cascade and, because of shared components, also affects the complement and intrinsic coagulation pathways. For instance, while bradykinin release and factor XII activation are promoted by kallikrein, C1-INH binds the active site of kallikrein, inhibiting the generation of bradykinin from HK, while also suppressing the production of factor XIIa and, further downstream, factor XII fragment (XII_f). C1 esterase inhibitor is the sole inhibitor of factor XIIa and factor XII_f. It is also the only physiological inhibitor of the esterase and proteolytic activity of complement components C1_r and C1_s, which would otherwise digest C4, rendering a reduced C4 level as diagnostic of HAE.

Icatibant reduces HAE symptoms (37), implying a role of the bradykinin B₂ receptor in this condition. But an inherent delay before symptom relief suggests additional molecular mechanisms are involved in maintaining angioedema. It remains unclear which other receptors may be responsible. Bossi and co-workers (44) studied B₁ and gC1q receptors using *in vitro* and *in vivo* models of vascular leakage induced by plasma [remission phase (RPP) or attack phase (APP)] from patients with C1-INH deficiency. In isolated HUVECs and human adult dermal microvascular endothelial cells,

bradykinin increased endothelial leakage [evaluated by fluorescent bovine serum albumin (BSA) quantification] after only 5 min, which was comparable with the effects of APP but not RPP.

The *in vivo* effects of APP, RPP and bradykinin were assessed via topical application on rat mesenteric microvessels. APP promptly (after 10 min) induced a significant increase in vascular leakage, while RPP had no effect (44). Abolition of any permeabilizing effect of APP following incubation of cultured endothelial cells with a monoclonal antibody against the gC1q receptor confirmed the involvement of gC1q receptors in vascular leakage (44). Icatibant induced a partial reduction in APP-induced BSA leakage, as did R715 and R954 (B_1 receptor antagonists) (44). Combined B_1 and B_2 receptor antagonists completely inhibited increased permeability, virtually comparable to RPP. The use of brefeldin-A (protein trafficking inhibitor) confirmed that a newly synthesized protein, most likely the B_1 receptor, mediated the IL-1 β -induced increase in vascular leakage.

Together, these experiments suggest that, besides B_2 receptors, B_1 and gC1q are involved in angioedema attacks; thus, the blockade of both B_1 and B_2 receptors, or of gC1q, may provide therapeutic benefits towards angioedema.

Clinical relevance of bradykinin

Angioedema

Angioedema most frequently affects the skin, bowel and upper aero-digestive tract and is generally mediated by histamine. Many other compounds can cause angioedema by affecting endothelial and smooth muscle cell function. In other forms of nonhistaminergic angioedema, i.e. not prevented by antihistamines [acquired or hereditary C1-INH deficiency (AAE or HAE, respectively), ACE-induced angioedema (ACEi-AE) and possibly nonhistaminergic idiopathic angioedema (nh-IAE)], there is increasing evidence that bradykinin has a pivotal role as such patients demonstrate elevated plasma bradykinin during attacks as compared with periods of remission (39, 45, 46). Observations in humans that inhaled bradykinin challenge causes bronchial plasma exudation (40), and in C1-INH ko mice that enhanced vascular permeability can be reversed by specifically inhibiting the release of or receptor binding by bradykinin (6) provide further evidence for the role of bradykinin.

HAE

Much is known about the pathogenesis of HAE and its association with mutations in the C1-INH gene (47). Elevated kinin activity seen in the plasma of patients with HAE during acute oedema is now considered because of bradykinin rather than complement activation and formation of a kinin-like C2 peptide (43, 48), a finding strongly supported by evidence showing that bradykinin promotes vascular permeability in C1-INH deficiency (46, 49).

Relatively little is known, however, about factors that initiate contact system activation *in vivo* and cause angioedema or about molecular mechanisms governing the highly variable

clinical phenotypes of HAE. This variability does not segregate with C1-INH defect or with the multiple associated C1-INH genetic mutations (47). Recent studies comparing patients having HAE attacks with those in remission identified prothrombin F1 + 2 and D-dimer as biochemical markers that can distinguish attack status (50); these parameters, along with kallikrein activity and degree of conversion of HK, may contribute towards distinguishing patients with severe vs mild disease (unpublished observations).

AAE

The aetiology of AAE associated with C1-INH deficiency remains unclear but thought to involve C1-INH consumption via the activation of C1q by immune complexes, the activation of complement or the development of autoantibodies against C1-INH (51, 52). Perhaps unsurprisingly therefore, C1-INH concentrate (up to 2000 U) is not reliably effective in AAE (53, 54). Very recently however, new drugs that specifically block either bradykinin generation (ecallantide) or block bradykinin action (icatibant) have been shown to rapidly alleviate symptoms associated with C1-INH deficiency, including AAE (55–57). This clearly supports the role of bradykinin in the pathogenesis of AAE associated with C1-INH deficiency.

ACEi-AE

The time to the first manifestation of angioedema after initiating ACEi treatment has been variably reported from 1 day to 11 years. In our experience, ACEi-AE typically occurs at least 1 year after starting treatment (58). Such a substantial delay may impede the recognition of angioedema as a drug-related adverse effect.

Antihypertensive treatment with ACEi might inhibit the degradation of bradykinin and elevate bradykinin levels, which could trigger angioedema, particularly in the upper airway (59, 60). In 101 patients followed up for ≥ 12 months after withdrawal of the ACEi, relapse was seen in 48% of patients (M. Cicardi, unpublished data). Why angioedema tends to recur in these patients is unclear but may be due to an abnormality in bradykinin degradation – as demonstrated for an APP variant (61) – which becomes clinically relevant when ACE is pharmacologically blocked. Triggering factors could include inflammation, injury or surgery, all of which are associated with an increase in acute phase proteins (e.g. C-reactive protein and fibrinogen) (58). Fibrinogen has induced concentration-dependent vascular dilatation in human mammary and porcine coronary arteries and increased bradykinin-dependent dilatation in the same tissues (62), but a recent study of 65 patients with ACEi-AE found no association with either B_2 receptor or ACE insertion/deletion polymorphism (63). No medication is currently approved for ACEi-AE, but in a small retrospective analysis in patients with ACEi-AE (including laryngopharyngeal) treated with either icatibant ($n = 8$) or standard care (cortisone/antihistamine, $n = 47$), time to complete symptom relief was 4 h and 33 h, respectively (64). Thus, if ACEi-AE is suspected, particularly in an emergency situation, bradykinin should be considered the key mediator and an agent such as icatibant

administered alongside usual care. Currently, a double-blind, placebo-controlled, randomized trial with icatibant *vs* the previous standard therapy (steroids and antihistamines) is ongoing (ClinicalTrials.gov identifier: NCT01154361). The results could lead to approval of icatibant to treat patients with ACEi-AE.

nh-IAE

Hereditary *nh-IAE* with normal C1-INH is frequently referred to as HAE type III. For one X-linked form that affects women only, angioedema recurrence is strongly associated with high oestrogen levels (both naturally and pharmacologically induced). Another form is oestrogen and sex independent. In some families, HAE with normal C1-INH segregates with two missense mutations in the factor XII gene, leading to increased activity of factor XII. Sporadic angioedema that is unresponsive to high-dose antihistamines and is not linked to C1-INH deficiency (i.e. acquired *nh-IAE*) is clinically reminiscent of bradykinin-mediated angioedema (65). Such patients show markedly increased bradykinin levels at the site of oedema, which is further proof of a link between angioedema development and bradykinin (66). Tranexamic acid resolved symptoms in fifteen of these patients who experienced severe and frequent recurrences (67).

Bradykinin involvement in other conditions

Bradykinin levels may be elevated sufficiently to be a pathological mediator in many clinical conditions including allergic rhinitis, liver cirrhosis, heart disease, brain trauma, Alzheimer's disease, asthma, pancreatitis and sepsis. Direct measurement of bradykinin in human plasma is extremely difficult owing to very low bradykinin concentration (pM), its short half-life (seconds), its ready enzymatic generation and degradation during sampling and handling procedures and interference by precursor molecules, bradykinin catabolic products and other kinin peptides. Recently, reliable data towards plasma bradykinin measurement have been derived using (i) blockade of protease and peptidase activity during sampling, (ii) liquid-phase extraction to eliminate proteins and high molecular weight molecules, (iii) high-performance liquid chromatography to eliminate precursor molecules and catabolic peptides of bradykinin, and (iv) high-sensitivity radioimmunoassay (45). Indirect evaluation of bradykinin may be derived by assessing the cleavage of HK (68). These methods, along with functional and molecular approaches, have helped improve the definition of disease pathogenesis.

Allergic rhinitis is an inflammatory nasal condition associated with mast cell activation and inflammatory mediator release. The nose, which contains B₁ and B₂ receptors, is an easily accessible section of the respiratory tract and ideal for performing challenge studies. Bradykinin is approximately sevenfold more potent than histamine in promoting symptoms of allergic rhinitis (69), and bradykinin nasal insufflation has been shown to accurately reproduce the features of allergic rhinitis (70–72). Importantly, an increase in nasal obstruction, rhinorrhea and plasma protein exudation occurs following nasal challenge with the B₂ ligands bradykinin and

kallidin, but not with the B₁ ligand des-Arg⁹-bradykinin (73). Furthermore, icatibant blocks activating protein-1 (AP-1) activation by bradykinin reduces allergen-induced eosinophil (inflammatory cell) recruitment and increases expression of epithelial IL-8 receptor (CXCR1 and CXCR2) (74, 75), further implicating B₂-receptor activation by bradykinin in allergic rhinitis. Intranasal administration of icatibant may help alleviate B₂ receptor-mediated allergic rhinitis.

Elevated endotoxin levels in cirrhosis stimulate the contact system to produce bradykinin. High molecular weight kininogen cleavage and bradykinin formation appear to be involved in ascites formation via arterial vasodilation and increased capillary permeability (76).

Bradykinin appears to be cardioprotective in mild heart failure (via contact activation) (77) but not so as the disease progresses (78). The contact system is also activated following thrombolytic therapy for acute myocardial infarction, possibly because of bradykinin production via upregulated factor XIIa and HK cleavage (79). During cardiopulmonary bypass (CPB), plasma bradykinin levels may be elevated and lead to proinflammatory and haemodynamic responses. Measurement of plasma bradykinin in both artificial and natural lungs (particularly rich in ACE) has shown that the CPB-associated bradykinin increase is partially attributed to the activation of the contact system by artificial surfaces and to reduced bradykinin catabolism as a consequence of shunting the natural lung (80).

In summary, bradykinin may be increased in certain conditions as a result of increased formation or reduced catabolism; this may be either protective (heart failure and thrombolytic treatment in MI) or detrimental (allergic rhinitis, liver cirrhosis and CPB).

Pharmacological targeting of the kallikrein-kinin system

Endothelial B₁ and B₂ receptors and elevated bradykinin are implicated in many clinical situations including angioedema. It appears feasible, therefore, that inhibitors of bradykinin production (kallikrein inhibitors) or receptor binding (B₁ and B₂ antagonists) might deliver clinical benefit.

A number of agents have been developed for the treatment of HAE including recombinant C1-INH to correct the inherent defect, a kallikrein inhibitor (ecallantide) to restrict bradykinin generation and a selective B₂ receptor antagonist (icatibant) to block bradykinin binding (81) (Fig. 3). Icatibant is the only agent to directly target the action of bradykinin (60) and was recently shown to be effective in patients with HAE (37) (Fig. 4). Additionally, bradykinin-induced retinal vascular permeability (RVP) was shown to involve both B₁ and B₂ receptors and to mediate proliferative diabetic retinopathy and diabetic macular oedema. Icatibant (B₂ antagonist) or des-Arg¹⁰-icatibant (B₁ antagonist) reduced induction of RVP by 67% and 45%, respectively, possibly preventing sight loss (82). B₁ receptor antagonists have also been shown to reduce glomerular filtration (27) and increase tumour permeability (gliomas), which could feasibly inhibit the metabolism and clearance of cytostatic agents allowing greater entry of cytostatic agent into tumours.

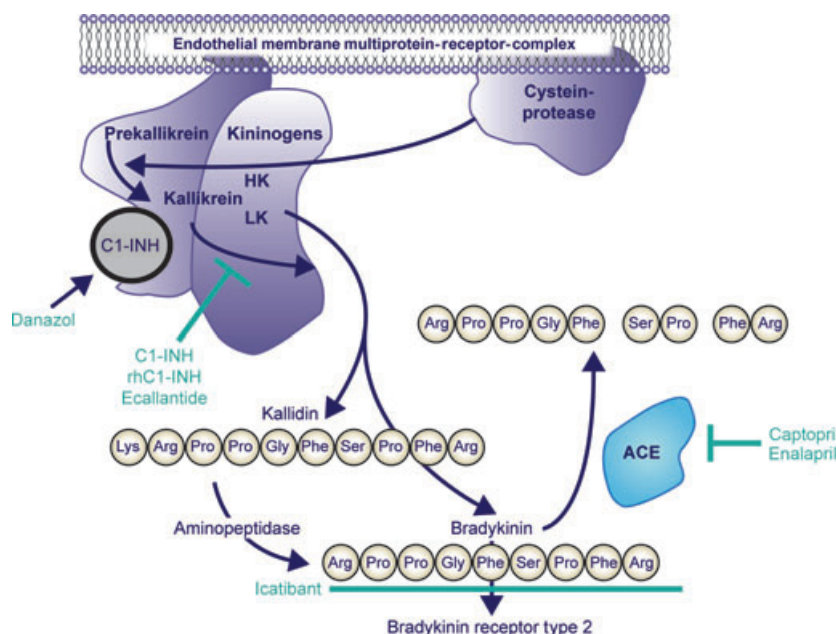
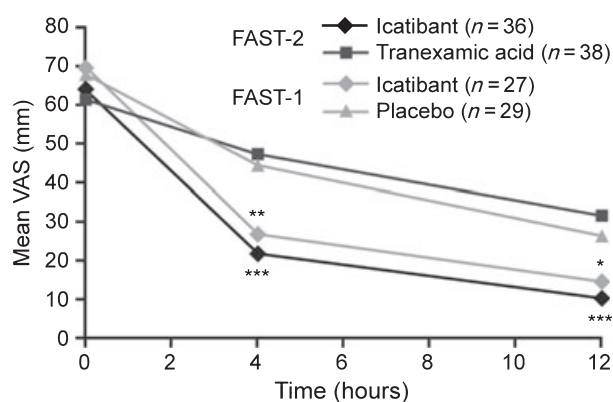


Figure 3 Simplified scheme showing molecular targets of drugs that interfere with the generation and metabolism of bradykinin. While many of these drugs are used to treat the symptoms of bradykinin-induced angioedema occurring via activation of bradyki-

nin receptor type 2, ACEi and AT-1 receptor blockers (sartans, not shown) are known to induce this form of angioedema. Reprinted from Bas & Kojda (81). Copyright with permission from Editor, Prof. Georg Kojda.



* $P=0.03$; ** $P=0.002$; *** $P<0.001$ (P -values versus respective comparator)

Figure 4 Mean visual analogue scale (VAS) scores post-treatment and at 4-h and 12-h post-treatment in the For Angioedema Subcutaneous Treatment (FAST) trials. Adapted from Cicardi et al. (37), Supplementary Appendix Table 3, available at <http://www.NEJM.org>.

B_2 receptor stimulation by bradykinin may promote NO-induced vasoprotective effects, including thrombin inhibition and contributing to preconditioning to protect against ischaemia. B_2 receptor antagonism may thus be inappropriate for long-term therapy. Moreover, B_2 receptor expression is upregulated in endothelial NO synthase (eNOS)-ko mice possibly to compensate for loss of vascular

NO generation, demonstrating the importance of B_2 receptors for vascular reactivity and organ perfusion (83). Studies of aortic rings from transgenic mice that overexpress endothelium-specific B_2 receptors on a C57Bl/6 background showed that bradykinin can induce vasoconstriction that is completely dependent on intact endothelium and B_2 receptors; this effect was blunted in the presence of icatibant and diclofenac (cyclooxygenase inhibitor) and increased after the inhibition of eNOS (G. Kojda, unpublished observations).

In summary, drugs targeting the kallikrein-kinin system include ACEi, DPP-IV inhibitors, B_1 and B_2 receptor agonists, B_1 and B_2 receptor antagonists and potentially AT-1 blockers. As with ACEi, although less frequently, AT-1 blockers may inhibit bradykinin degradation, thereby promoting angioedema (82). Retinal capillary leakage is mediated by the activation of both B_1 and B_2 receptors, and B_1 receptor agonists may aid the action of cytostatic agents. B_2 receptor antagonism by icatibant represents a new therapeutic principle that is effective in HAE.

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Conflict of interest

M. Maurer is, or recently was, a Speaker and/or Advisor for Almirall Hermal, Bayer Schering Pharma, Biofrontera, Essex Pharma, Genentech, JADO Technologies, Shire Human Genetic Therapies Inc. and Jerini AG, Merckle Recordati, Novartis, Sanofi Aventis, Schering-Plough, Leo, MSD,

Merck, Symbiopharm, UCB and Uriach. M. Bas, M. Cicardi, A. Kaplan and M. Magerl have received consulting fees and honoraria from Shire Human Genetic Therapies Inc. and Jerini AG. M. Bader, F. Bossi, M. Cugno, P. Howarth, G. Kojda, F. Leeb-Lundberg and J. Lötvall have no conflicts of interest to declare.

References

- Kaplan AP, Joseph K, Silverberg M. Pathways for bradykinin formation and inflammatory disease. *J Allergy Clin Immunol* 2002;**109**:195–209.
- Nussberger J, Cugno M, Cicardi M, Agostoni A. Local bradykinin generation in hereditary angioedema. *J Allergy Clin Immunol* 1999;**104**:1321–1322.
- Moreau ME, Garbacki N, Molinaro G, Brown NJ, Marceau F, Adam A. The kallikrein-kinin system: current and future pharmacological targets. *J Pharmacol Sci* 2005;**99**:6–38.
- Regoli D, Barabe J. Pharmacology of bradykinin and related kinins. *Pharmacol Rev* 1980;**32**:1–46.
- Madeddu P, Emanuelli C, Gaspa L, Salis B, Milia AF, Chao L et al. Role of the bradykinin B₂ receptor in the maturation of blood pressure phenotype: lesson from transgenic and knockout mice. *Immunopharmacology* 1999;**44**:9–13.
- Han ED, MacFarlane RC, Mulligan AN, Scafidi J, Davis AE 3rd. Increased vascular permeability in C1 inhibitor-deficient mice mediated by the bradykinin type 2 receptor. *J Clin Invest* 2002;**109**:1057–1063.
- Heitsch H. Bradykinin B₂ receptor as a potential therapeutic target. *Drug News Perspect* 2000;**13**:213–225.
- Skidgel RA, Erdös EG. Histamine, bradykinin and their antagonists. In: Hardman JG, Limbird LE, Gilman AG, editors. *The Pharmacological Basis of Therapeutics*, 11th edn. New York: McGraw-Hill, 2006:629–651.
- Marceau F, Regoli D. Bradykinin receptor ligands: therapeutic perspectives. *Nat Rev Drug Discov* 2004;**3**:845–852.
- Kaplan AP, Ghebrehiet W. The plasma bradykinin-forming pathways and its interrelationships with complement. *Mol Immunol* 2010;**47**:2161–2169.
- Joseph K, Tholanikunnel BG, Kaplan AP. Heat shock protein 90 catalyzes activation of the Prekallikrein–Kininogen Complex in the absence of factor XII. *Proc Natl Acad Sci USA* 2002;**99**:896–900.
- Silverberg M, Dunn JT, Garen L, Kaplan AP. Autoactivation of human Hageman factor: demonstration utilising a synthetic substrate. *J Biol Chem* 1980;**255**:7281–7286.
- Leeb-Lundberg LM, Marceau F, Müller-Esterl W, Pettibone DJ, Zuraw BL. International Union of Pharmacology. XLV. Classification of the kinin receptor family: from molecular mechanisms to pathophysiological consequences. *Pharmacol Rev* 2005;**57**:27–77.
- Fathy DB, Leeb T, Mathis SA, Leeb-Lundberg LM. A single position in the third transmembrane domains of the human B₁ and B₂ bradykinin receptors is adjacent to and discriminates between the C-terminal residues of subtype-selective ligands. *J Biol Chem* 1998;**273**:12210–12218.
- Leeb-Lundberg LM, Kang DS, Lamb ME, Fathy DB. The human B₁ bradykinin receptor exhibits high ligand-independent, constitutive activity. Roles of residues in the fourth intracellular and third transmembrane domains. *J Biol Chem* 2001;**276**:8785–8792.
- Enquist J, Skräder C, Whistler JL, Leeb-Lundberg LM. Kinins promote B₂ receptor endocytosis and delay constitutive B₁ receptor endocytosis. *Mol Pharmacol* 2007;**71**:494–507.
- Mathis SA, Criscimagna NL, Leeb-Lundberg LM. B₁ and B₂ kinin receptors mediate distinct patterns of intracellular Ca²⁺ signaling in single cultured vascular smooth muscle cells. *Mol Pharmacol* 1996;**50**:128–139.
- Blaukat A, Pizard A, Breit A, Wernstedt C, Alhenc-Gelas F, Müller-Esterl W et al. Determination of bradykinin B₂ receptor in vivo phosphorylation sites and their role in receptor function. *J Biol Chem* 2001;**276**:40431–40440.
- Marceau F, Hess JF, Bachvarov DR. The B₁ receptors for kinins. *Pharmacol Rev* 1998;**50**:357–386.
- Cayla C, Todiras M, Iliescu R, Saul VV, Gross V, Pilz B et al. Mice deficient for both kinin receptors are normotensive and protected from endotoxin-induced hypotension. *FASEB J* 2007;**21**:1689–1698.
- Silva JA Jr, Araujo RC, Baltatu O, Oliveira SM, Tschöpe C, Fink E et al. Reduced cardiac hypertrophy and altered blood pressure control in transgenic rats with the human tissue kallikrein gene. *FASEB J* 2000;**14**:1858–1860.
- Fischer R, Barbosa ME, Baptista HA, Pesquero JB, Bader M. Transgenic overexpression of bradykinin B₂ receptors reduces arrhythmias in a rat model of cardiac hypertrophy. *Hypertension* 2009;**54**:E54.
- Pesquero JB, Araujo RC, Heppenstall PA, Stucky CL, Silva JA Jr, Walther T et al. Hypoalgesia and altered inflammatory responses in mice lacking kinin B₁ receptors. *Proc Natl Acad Sci USA* 2000;**97**:8140–8145.
- Merino VF, Todiras M, Campos LA, Saul V, Popova E, Baltatu OC et al. Increased susceptibility to endotoxic shock in transgenic rats with endothelial overexpression of kinin B(1) receptors. *J Mol Med* 2008;**86**:791–798.
- Souza DG, Lomez ES, Pinho V, Pesquero JB, Bader M, Pesquero JL et al. Role of bradykinin B₂ and B₁ receptors in the local, remote, and systemic inflammatory responses that follow intestinal ischemia and reperfusion injury. *J Immunol* 2004;**172**:2542–2548.
- Schanstra JP, Neau E, Drogoz P, Arevalo Gomez MA, Lopez Novoa JM, Calise D et al. In vivo bradykinin B₂ receptor activation reduces renal fibrosis. *J Clin Invest* 2002;**110**:371–379.
- Klein J, Gonzalez J, Duchene J, Esposito L, Pradère JP, Neau E et al. Delayed blockade of the kinin B₁ receptor reduces renal inflammation and fibrosis in obstructive nephropathy. *FASEB J* 2009;**23**:134–142.
- Mori MA, Araújo RC, Reis FC, Sgai DG, Fonseca RG, Barros CC et al. Kinin B₁ receptor deficiency leads to leptin hypersensitivity and resistance to obesity. *Diabetes* 2008;**57**:1491–1500.
- Emanuelli C, Bonaria Salis M, Stacca T, Pinus G, Kirchmair R, Isner JM et al. Targeting kinin B(1) receptor for therapeutic neovascularization. *Circulation* 2002;**105**:360–366.
- Bader M. Kallikrein-kinin system in neovascularization. *Arterioscler Thromb Vasc Biol* 2009;**29**:617–619.
- Merino VF, Todiras M, Mori MA, Sales VM, Fonseca RG, Saul V et al. Predisposition to atherosclerosis and aortic aneurysms in mice deficient in kinin B₁ receptor and apolipoprotein E. *J Mol Med* 2009;**87**:953–963.
- Schulze-Toppoff U, Prat A, Prozorovski T, Siffrin V, Paterka M, Herz J et al. Activation of kinin receptor B₁ limits encephalogenic T lymphocyte recruitment to the central nervous system. *Nat Med* 2009;**15**:788–793.

33. Raslan F, Schwarz T, Meuth SG, Austinat M, Bader M, Renné T et al. Inhibition of bradykinin receptor B1 protects mice from focal brain injury by reducing blood-brain barrier leakage and inflammation. *J Cereb Blood Flow Metab* 2010;**30**:1477–1486.
34. Trabold R, Erös C, Zweckberger K, Relton J, Beck H, Nussberger J et al. The role of bradykinin B(1) and B(2) receptors for secondary brain damage after traumatic brain injury in mice. *J Cereb Blood Flow Metab* 2010;**30**:130–139.
35. Ifuku M, Färber K, Okuno Y, Yamakawa Y, Miyamoto T, Nolte C et al. Bradykinin-induced microglial migration mediated by B1-bradykinin receptors depends on Ca^{2+} influx via reverse-mode activity of the $\text{Na}^{+}/\text{Ca}^{2+}$ exchanger. *J Neurosci* 2007;**27**:13065–13073.
36. Pocock JM, Kettenmann H. Neurotransmitter receptors on microglia. *Trends Neurosci* 2007;**30**:527–535.
37. Cicardi M, Banerji A, Bracho F, Malbrán A, Rosenkranz B, Riedl M et al. Icatibant, a new bradykinin-receptor antagonist, in hereditary angioedema. *N Engl J Med* 2010;**363**:532–541.
38. Mehta D, Malik AB. Signaling mechanisms regulating endothelial permeability. *Physiol Rev* 2006;**86**:279–367.
39. Nussberger J, Cugno M, Cicardi M. Bradykinin-mediated angioedema. *N Engl J Med* 2002;**347**:621–622.
40. Arvidsson P, Löfdahl CG, Skoogh BE, Lötvall J. Exudation of plasma and production of thromboxane in human bronchi after local bradykinin challenge. *Respir Med* 2001;**95**:313–318.
41. Lötvall JO, Tokuyama K, Barnes PJ, Chung KF. Bradykinin-induced airway microvascular leakage is potentiated by captopril and phosphoramidon. *Eur J Pharmacol* 1991;**200**:211–217.
42. Cui ZH, Arakawa H, Kawikova I, Skoogh BE, Lötvall J. Relationship between systemic blood pressure, airway blood flow and plasma exudation in guinea-pig. *Acta Physiol Scand* 1999;**165**:121–127.
43. Fields T, Ghebrehiet W, Kaplan AP. Kinin formation in hereditary angioedema plasma: evidence against kinin derivation for C2 and in support of “spontaneous” formation of bradykinin. *J Allergy Clin Immunol* 1983;**72**:54–60.
44. Bossi F, Fischetti F, Regoli D, Durigutto P, Frossi B, Gobeil J Jr et al. Novel pathogenic mechanism and therapeutic approaches to angioedema associated with C1 inhibitor deficiency. *J Allergy Clin Immunol* 2009;**124**:1303–1310.
45. Nussberger J, Cugno M, Amstutz C, Cicardi M, Pellacani A, Agostoni A. Plasma bradykinin in angio-oedema. *Lancet* 1998;**351**:1693–1697.
46. Cugno M, Zanichelli A, Fioeni F, Caccia S, Cicardi M. C1-inhibitor deficiency and angioedema: molecular mechanisms and clinical progress. *Trends Mol Med* 2009;**15**:69–78.
47. Pappalardo E, Caccia S, Suffritti C, Tordai A, Zingale LC, Cicardi M. Mutation screening of C1 inhibitor gene in 108 unrelated families with hereditary angioedema: functional and structural correlates. *Mol Immunol* 2008;**45**:3536–3544.
48. Donaldson VH, Rosen FS, Bing DH. Role of the second component of complement (C2) and plasmin in kinin release in hereditary angioneurotic edema (H.A.N.E.) plasma. *Trans Assoc Am Physicians* 1977;**90**:174–183.
49. Davis AE 3rd. C1 inhibitor and hereditary angioneurotic edema. *Annu Rev Immunol* 1988;**6**:595–628.
50. Cugno M, Zanichelli A, Bellatorre AG, Griffini S, Cicardi M. Plasma biomarkers of acute attacks in patients with angioedema due to C1-inhibitor deficiency. *Allergy* 2009;**64**:254–257.
51. Kim JS, Pongracic JA. Hereditary and acquired angioedema. *Allergy Asthma Proc* 2004;**25**:S47–S49.
52. Cugno M, Castelli R, Cicardi M. Angioedema due to acquired C1-inhibitor deficiency: a bridging condition between autoimmunity and lymphoproliferation. *Autoimmun Rev* 2008;**8**:156–159.
53. Agostoni A, Aygören-Pürsün E, Binkley KE, Blanch A, Bork K, Bouillet L et al. Hereditary and acquired angioedema: problems and progress: proceedings of the third C1 esterase inhibitor deficiency workshop and beyond. *J Allergy Clin Immunol* 2004;**114**:S51–S131.
54. Sánchez-Cano D, Callejas-Rubio JL, Lara-Jiménez MA, López-Trascasa M, Cicardi M, Ortego-Centeno N. Successful use of rituximab in acquired C1 inhibitor deficiency secondary to Sjögren's syndrome. *Lupus* 2008;**17**:228–229.
55. Schneider L, Lumry W, Vegh A, Williams AH, Schmalbach T. Critical role of kallikrein in hereditary angioedema pathogenesis: a clinical trial of ecallantide, a novel kallikrein inhibitor. *J Allergy Clin Immunol* 2007;**120**:416–422.
56. Bork K, Frank J, Grundt B, Schlattmann P, Nussberger J, Kreuz W. Treatment of acute edema attacks in hereditary angioedema with a bradykinin receptor-2 antagonist (Icatibant). *J Allergy Clin Immunol* 2007;**119**:1497–1503.
57. Weller K, Magerl M, Maurer M. Successful treatment of an acute attack of acquired angioedema with the bradykinin-B2-receptor antagonist icatibant. *J Eur Acad Dermatol Venereol* 2011;**25**:119–120.
58. Bas M, Hoffmann TK, Bier H, Kojda G. Increased C-reactive protein in ACE-inhibitor-induced angioedema. *Br J Clin Pharmacol* 2005;**59**:233–238.
59. Bas M, Hoffmann TK, Kojda G, Bier H. ACE-inhibitor induced angioedema. *Laryngorhinootologie* 2007;**86**:804–808.
60. Bas M, Adams V, Suvorava T, Niehues T, Hoffmann TK, Kojda G. Nonallergic angioedema: role of bradykinin. *Allergy* 2007;**62**:842–856.
61. Duan QL, Nikpoor B, Dube MP, Molinaro G, Meijer IA, Dion P et al. A variant in XPNPEP2 is associated with angioedema induced by angiotensin I-converting enzyme inhibitors. *Am J Hum Genet* 2005;**77**:617–626.
62. Bas M, Kirchhartz N, Hochfeld J, Tüllmann C, Kumpf S, Suvorava T et al. Potential role of vasomotor effects of fibrinogen in bradykinin-induced angioedema. *J Allergy Clin Immunol* 2008;**121**:969.e2–975.e2.
63. Bas M, Hoffmann TK, Tiemann B, Dao VT, Bantis C, Balz V et al. Potential genetic risk factors in angiotensin-converting enzyme-inhibitor-induced angio-oedema. *Br J Clin Pharmacol* 2010;**69**:179–186.
64. Bas M, Greve J, Stelter K, Bier H, Stark T, Hoffmann TK et al. Therapeutic efficacy of icatibant in angioedema induced by angiotensin-converting enzyme inhibitors: a case series. *Ann Emerg Med* 2010;**56**:278–282.
65. Zingale LC, Beltrami L, Zanichelli A, Maggioni L, Pappalardo E, Cicardi B et al. Angioedema without urticaria: a large clinical survey. *CMAJ* 2006;**174**:175.
66. Cugno M, Nussberger J, Cicardi M, Agostoni A. Bradykinin and the pathophysiology of angioedema. *Int Immunopharmacol* 2003;**3**:311–317.
67. Cicardi M, Bergamaschini L, Zingale LC, Gioffré D, Agostoni A. Idiopathic nonhistaminergic angioedema. *Am J Med* 1999;**106**:650–654.
68. Cugno M, Cicardi M, Bottasso B, Coppola R, Paonessa R, Mannucci PM et al. Activation of the coagulation cascade in C1-inhibitor deficiencies. *Blood* 1997;**89**:3213–3218.
69. Rajakulasingam K, Polosa R, Lau LC, Church MK, Holgate ST, Howarth PH. Comparative nasal effects of bradykinin and histamine: influence on nasal airways resistance and plasma protein exudation. *Thorax* 1993;**48**:324–329.
70. Proud D, Togias A, Naclerio RM, Crush SA, Norman PS, Lichtenstein LM. Kinins are generated in vivo following nasal airway challenge of allergic individuals with allergen. *J Clin Invest* 1983;**72**:1678–1685.
71. Naclerio RM, Proud D, Kagey-Sobotka A, Freidhoff L, Norman PS, Lichtenstein LM.

- The effect of cetirizine on early allergic response. *Laryngoscope* 1989;**99**:596–599.
72. Shirasaki H, Kanaizumi E, Himi T. Immunohistochemical localization of the bradykinin B₁ and B₂ receptors in human nasal mucosa. *Mediators Inflamm* 2009;**2009**:102406.
 73. Christiansen SC, Eddleston J, Woessner KM, Chambers SS, Ye R, Pan ZK et al. Up-regulation of functional kinin B1 receptors in allergic airway inflammation. *J Immunol* 2002;**169**:2054–2060.
 74. Turner P, Dear J, Scadding G, Foreman JC. Role of kinins in seasonal allergic rhinitis: icatibant, a bradykinin B₂ receptor antagonist, abolishes the hyper-responsiveness and nasal eosinophilia induced by antigen. *J Allergy Clin Immunol* 2001;**107**:105–113.
 75. Eddleston J, Christiansen SC, Jenkins GR, Koziol JA, Zuraw BL. Bradykinin increases the *in vivo* expression of the CXC chemokine receptors CXCR1 and CXCR2 in patients with allergic rhinitis. *J Allergy Clin Immunol* 2003;**111**:106–112.
 76. Cugno M, Nussberger J, Biglioli P, Alamanini F, Coppola R, Agostoni A. Increase of bradykinin in plasma of patients undergoing cardiopulmonary bypass: the importance of lung exclusion. *Chest* 2001;**120**:1776–1782.
 77. Cugno M, Agostoni P, Mari D, Meroni PL, Gregorini L, Bussotti M et al. Impaired bradykinin response to ischaemia and exercise in patients with mild congestive heart failure during angiotensin-converting enzyme treatment. Relationships with endothelial function, coagulation and inflammation. *Br J Haematol* 2005;**130**:113–120.
 78. Cugno M, Agostoni P, Brunner HR, Gardinali M, Agostoni A, Nussberger J. Plasma bradykinin levels in human chronic congestive heart failure. *Clin Sci (Lond)* 2000;**99**:461–466.
 79. Merlini PA, Cugno M, Rossi ML, Agricola P, Repetto A, Fetiveau R et al. Activation of the contact system and inflammation after thrombolytic therapy in patients with acute myocardial infarction. *Am J Cardiol* 2004;**93**:822–825.
 80. Cugno M, Salerno F, Nussberger J, Bottasso B, Lorenzano E, Agostoni A. Bradykinin in the ascitic fluid of patients with liver cirrhosis. *Clin Sci (Lond)* 2001;**101**:651–657.
 81. Bas M, Kojda G. Das nicht-allergische Angioödem. *Fortbildungstelegramm Pharmazie* 2008;**2**:85–100.
 82. Gao BB, Clermont A, Rook S, Fonda SJ, Srinivasan VJ, Wojtkowski M et al. Extracellular carbonic anhydrase mediates hemorrhagic retinal and cerebral vascular permeability through prekallikrein activation. *Nat Med* 2007;**13**:181–188.
 83. Kocgirli O, Valcaccia S, Dao VT, Suvorava T, Kumpf S, Floeren M et al. Effects of NO on endothelial vascular bradykinin type-2 receptor. EuroPRevent 2010 (European Society of Cardiology), Abstract P155.