

The bradykinin-forming cascade and its role in hereditary angioedema

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Objective: To review the mechanisms by which bradykinin is generated in hereditary angioedema (HAE) (C1 inhibitor deficiency), including the role of human plasma proteins and endothelial cells.

Data Sources: Published articles in reviewed journals that address (1) the fundamentals of bradykinin formation, (2) interactions between kinin-forming proteins and endothelial cells, (3) clinical evidence that bradykinin causes swelling in HAE, and (4) therapeutic options focused on inhibition of the plasma kallikrein-kinin cascade.

Study Selection: Historical articles that have made fundamental observations. Recent articles that address evolving concepts of disease pathogenesis and treatment.

Results: C1 inhibitor deficiency causes dysregulation of the plasma bradykinin-forming cascade with overproduction of bradykinin due to uninhibited effects of activated factor XII and plasma kallikrein. Swelling in HAE and production of bradykinin are localized (and may then disseminate); activation along the endothelial cell surface involves cell membrane ligands of factor XII and high-molecular-weight kininogen, release of endothelial cell heat shock protein 90, activation of the high-molecular-weight kininogen–prekallikrein complex, and endothelial cell activation at the B₂ receptor. Attacks of swelling may be terminated by treatment with a kallikrein inhibitor or B₂ receptor blockade. Replenishing C1 inhibitor can abort attacks of swelling and provide prophylaxis with intravenous administration.

Conclusions: Bradykinin is the mediator of swelling in types I and II HAE and is overproduced because of a deficiency in C1 inhibitor. Inhibition of bradykinin formation by novel agents can provide targeted therapeutic approaches that address the pathophysiologic abnormalities.

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INTRODUCTION

Hereditary angioedema (HAE) has an incidence of approximately 1 per 50,000 persons due to a mutation of 1 allele of the C1 inhibitor (C1-INH) gene^{1,2} on chromosome band 11q12-q13.³ It is inherited as an autosomal dominant disorder, yet as many as 25% of patients present with a new mutation, that is, they have no family history.⁴ Angioedema is the primary manifestation of this disorder and is defined as swelling due to extravasation of fluid into the interstitium of the deep dermis and subcutaneous tissue. The swelling typically lasts 2 to 5 days, and the tissue returns to normal between episodes.⁵ An evanescent rash reminiscent of erythema marginatum⁵ can herald an attack of angioedema in approximately 30% of patients, but there is no associated pruritus or urticaria.

Swelling can occur at virtually any site but most commonly occurs in the face (lips, cheek, and periorbital area), gastrointestinal tract, extremities, penis, and scrotum. Swelling can affect the tongue and pharynx to such a degree that secretions cannot be handled and placement of an airway becomes essential. Laryngeal edema with inspiratory stridor can progress rapidly to cause asphyxiation,⁶ and tracheostomy may be needed. Abdominal attacks lasting 2 to 4 days are present in more than 90% of patients and are characterized by severe abdominal pain that can be accompanied by nausea, vomiting, and diarrhea. The symptoms may suggest the presence of an acute abdomen, and, if the diagnosis is not recognized, unnecessary surgery may be performed. The cause of these symptoms is edema of the bowel wall,^{5,7} with partial or complete luminal obstruction, and ascites may be present.⁸

Although sudden episodes of swelling can occur seemingly spontaneously, exogenous events, including blunt trauma, infection (particularly infection with concomitant fever), menstruation, and the use of estrogen or angiotensin-converting enzyme inhibitors, may precipitate attacks.⁹ Progesterone, on the other hand, may be protective.¹⁰ The swelling seen in HAE is due to activation of the plasma bradykinin-forming cascade, a complex 3-step process in which there is activation of factor XII (Hageman factor)

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(Fig 1), conversion of prekallikrein to plasma kallikrein, and digestion of high-molecular-weight kininogen (HK) to release bradykinin. The cascade is activated *in vitro* by binding of factor XII and a complex of prekallikrein and HK to initiating surfaces (eg, silicates, endotoxin, and uric acid crystals); however, *in vivo*, trauma to the endothelial cell surface and other tissue may expose initiating surfaces (eg, negatively charged proteins) that activate factor XII and initiate factor XII-dependent cascades.

CLASSIFICATION OF HAE

HAE has been divided into 3 types, 2 of which are attributable to mutant C1-INH. Type I HAE has a very low serum level of C1-INH protein, with C1-INH function correspondingly low, and is seen in approximately 85% of patients.^{6,9} Type II HAE accounts for approximately 15% of cases. This latter group has a mutation that allows gene transcription and messenger RNA translation to proceed, but the protein produced is inactive. The total C1-INH protein level typically is normal or even elevated,¹¹ although C1-INH function is diminished.

Mutations in the C1-INH gene may be seen along the entire protein sequence.² Type I mutations are those in which the gene product is not translated into a viable protein product. The mutation may, for example, create a stop codon that leads to intracellular degradation of the protein fragment produced or sequester the protein synthesized in the cell and prevent secretion. These mutations are likely to be insertions, dele-

tions, transversions, formation of stop codons, or altered carbohydrate linkages needed for secretion.¹²⁻¹⁵ Type II disease is typified by mutations that involve single amino acid substitutions and that are more likely to lead to synthesis of a dysfunctional protein.^{16,17}

Type III HAE is familial, with dominant inheritance, and accounts for approximately 1% of cases. These patients have normal functional levels of C1-INH and are almost exclusively women,¹⁸ with an occasional exception.¹⁹ Administration of exogenous estrogen is strongly implicated in the initiation of attacks of angioedema in these patients, and the timing of symptom presentation may vary with the menstrual cycle. In type III HAE, a subgroup of patients has a mutant factor XII such that when activated to factor XIIa or factor XII fragment (factor XII_f), the specific activity is much greater than that of the normal, unmutated protein,²⁰ leading to augmentation of bradykinin formation. One effect of estrogen therapy, even in types I and II HAE, is raised blood levels of factor XII.²¹

Because HAE is an autosomal dominant disease, patients are typically heterozygous; however, 2 patients have been identified as having a rare recessive form of HAE due to a homozygous mutation in the promoter region of the gene.¹⁶ In addition, 2 patients have been described who are homozygous for mutations in the C1-INH coding region.²² In these patients, the C1-INH gene product is normal but has very low protein and functional levels, that is, type I disease.

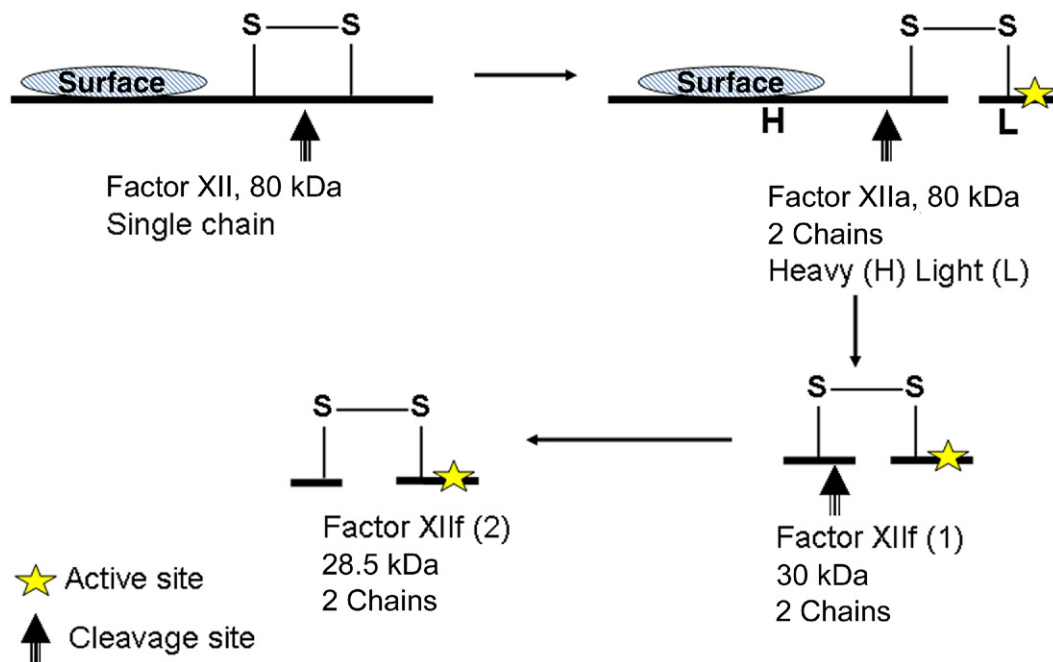


Figure 1. Diagrammatic representation of the activation of factor XII. The single-chain, 80-kDa zymogen is activated by cleavage of a single Arg-Val bond in a disulfide bridge to yield factor XIIa. A heavy chain (H) of 50 kDa is bound to the surface; the active site serine is in the light chain (L). Conversion to factor XII_f occurs by cleavage at the C-terminal end of the heavy chain; the light chain of 28 kDa is then disulfide linked to a 2-kDa chain factor XII_f (1). An additional cleavage decreases the smaller chain to just 0.5 kDa to yield factor XII_f (2) at 28.5 kDa. Both forms of factor XII_f are often seen together and appear as a doublet.

DIMINISHED C1-INH LEVELS AND ATTACKS OF SWELLING

The functional C1-INH level in patients with type I or II HAE should be approximately 50% if the one normal gene is functioning properly. Yet, blood levels are typically less than that, and, when functional levels are 30% or less, there is insufficient inhibition of bradykinin-forming enzymes, and an attack of swelling becomes likely. This reduction of expected C1-INH levels is due to multiple mechanisms. First, C1-INH is an activatable inhibitor, that is, C1-INH is cleaved by the enzyme that is slated for inhibition, then the cleaved C1-INH binds to the active site of the enzyme.²³ The enzyme is irreversibly inhibited, and that molecule of C1-INH cannot be reused. When baseline levels are low, these normal reactions further deplete C1-INH, and blood levels decrease. A second mechanism involves transinhibition,²⁴ in which the messenger RNA product of the abnormal allele inhibits translational or posttranslational steps involving the normal allele. Blood levels decrease well below the theoretical 50% level. Trauma or infection might lead to further activation of the enzymes requisite for bradykinin formation, leading to depletion of functional C1-INH levels, which initiates episodes of swelling. Patients with HAE exhibit a range of functional C1-INH levels, yet there is no evidence that C1-INH levels correlate with attack severity or frequency.⁵

CONSTITUENTS OF THE PLASMA BRADYKININ-FORMING CASCADE

The conditions requisite for the generation of bradykinin in normal human plasma are an initiating surface, factor XII, prekallikrein, and HK. Factor XII circulates as a single-chain proenzyme of 80 kDa with no detectable enzymatic activity.²⁵ It slowly autoactivates when bound to initiating, polyanionic surfaces,²⁶ with conversion to factor XIIa (molecular weight of 80 kDa) and subsequent digestion to factor XIIf (molecular weight of 30 kDa). In the presence of plasma kallikrein, there is rapid conversion of surface-bound factor XII to factor XIIa and then factor XIIf (Fig 1).²⁷⁻²⁹ Factor XIIf lacks the surface binding site and continues to activate prekallikrein at sites distant from the initial activation site. It is also the form of activated factor XII that activates the C1r subcomponent of complement (and, to a lesser degree, C1s) and, thereby, links bradykinin generation to activation of complement.³⁰

Prekallikrein circulates bound to HK in a 1:1 stoichiometric complex.³¹ Using purified proteins, it has been shown that prekallikrein has enzymatic activity even before conversion to plasma kallikrein and will cleave HK.³² Bradykinin is released in proportion to the prekallikrein input; for example, 5 mol of prekallikrein incubated with 50 mol of HK will liberate 5 mol of bradykinin. The reaction is inhibited by C1-INH. Prekallikrein is converted to plasma kallikrein by factor XIIa or factor XIIf,³³ and plasma kallikrein digests HK to release bradykinin; for example, 1 mol of plasma kallikrein incubated with 50 mol of HK will liberate 50 mol of bradykinin.

HK is a single-chain glycoprotein with an apparent molecular weight of 115 kDa. It forms a noncovalent complex with prekallikrein, as noted previously herein, and a similar complex with factor XI. During contact activation, plasma kallikrein digests HK, liberating the nonapeptide bradykinin (Arg-Pro-Gly-Phe-Ser-Pro-Phe-Arg) and leaving a heavy chain of 65-kDa disulfide linked to a light chain of 62 kDa; further cleavage of the light chain reduces it to 49 kDa.³⁴

Factor XII binds directly to initiating, polyanionic surfaces (eg, the silicate of glass), as does HK, whereas prekallikrein and factor XI are brought to the surface by virtue of their binding to HK. The binding sites of prekallikrein and factor XI overlap so that a single HK molecule binds either one but not both.³⁵ There is sufficient HK present such that 85% of prekallikrein and more than 95% of factor XI are bound.

The domain structure of HK is unusual (Fig 2). The heavy chain in the N-terminal region has 3 contiguous sequences³⁶ (domains 1-3) that are homologous to a family of small cysteine protease inhibitors (cystatins), and domains 2 and 3 exhibit cysteine protease inhibitory activity.³⁷ Bradykinin is contained in domain 4. The co-factor functions of HK all reside in the light chain portion, which is divided into 2 domains. The first is a histidine-rich region (domain 5) that binds to negatively charged surfaces that are required for factor XII autoactivation so as to start the formation of bradykinin. The second (domain 6) is an acidic carboxyterminal portion with the binding site for prekallikrein and factor XI.³⁸

ACTIVATION OF THE PLASMA BRADYKININ-FORMING CASCADE

Binding of factor XII to macromolecular surfaces does not create any active site, but the bound factor XII autoactivates.³⁹ The concentration of enzyme (factor XIIa) continuously increases, and the reaction rate accelerates until the concentration of substrate (factor XII) becomes limiting and then slows down.²⁶ This initiating step is inhibited by C1-INH (Fig 3).⁴⁰

As factor XIIa is produced, it converts prekallikrein to plasma kallikrein and also factor XI to factor XIa (Fig 3). The latter enzyme continues the intrinsic coagulation cascade. Plasma kallikrein also rapidly activates factor XII to factor XIIa^{27,28} in a positive feedback loop with a rate that is at least 2000-fold faster than the autoactivation rate (Fig 3).⁴¹ The addition of a surface to the mixture increases the catalytic efficiency of factor XII activation by 11,000-fold.⁴²

As shown in Figure 3, HK is along the arrows that depict activation of prekallikrein and factor XI by factor XIIa, indicating that HK is a cofactor that accelerates the rate of each of these reactions.^{43,44} Bradykinin formation is, therefore, a peptide cleavage product of this complex initiating step of the intrinsic coagulation cascade with factor XII, prekallikrein, and HK, all required for optimal factor XI activation. Once production of bradykinin stops, the rate of bradykinin degradation is very fast, mediated primarily by

HK domains

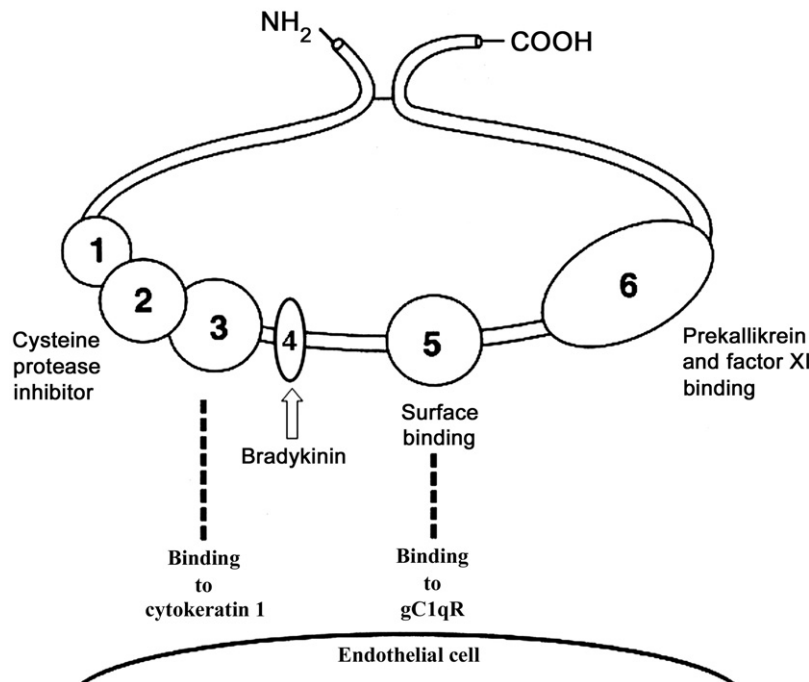


Figure 2. Domain structure of high-molecular-weight kininogen (HK). Domains 2 and 3 have cysteine protease inhibitory activity. Domain 4 contains the bradykinin sequence. Domain 5 binds to initiating negatively charged cell surfaces. Domain 6 has the binding site for prekallikrein (or factor XI). Domains 3 and 5 bind to cell surfaces via interaction with cytokeratin 1 and receptor for the globular heads of complement component C1q (gC1qR), respectively.

angiotensin-converting enzyme located along the pulmonary vascular endothelium and by plasma carboxypeptidase N.

C1-INH AS A CONTROL PROTEIN OF COMPLEMENT AND BRADYKININ FORMATION

After C1-INH levels were identified to be abnormal in patients with HAE, the action of C1-INH on enzymes other than C1 was considered. It was next shown to bind to the active site of plasma kallikrein⁴⁵ and, therefore, to block the ability of plasma kallikrein to cleave HK and to activate factor XII. Next, C1-INH was identified as the only significant plasma inhibitor for factor XIIa and factor XIIIf⁴⁶; thus, it controls virtually all of the reactions of the plasma bradykinin-forming cascade (Fig 3).

The first component of complement is unstable if C1-INH is absent and a slow autoactivation of C1r may be seen.⁴⁷ This may be the reason that C4 levels are diminished in 95% of patients, even when asymptomatic. Thus, C4 determination represents a good screening test, although results are normal in 5% of patients.^{2,6,10} During an episode of swelling, complement is further activated, C4 levels approach zero, and C2 levels diminish. The activated C1 forms a complex with any functional C1-INH that is present, which decreases levels further.⁴⁸ Factor XIIIf activation of C1r, and to a lesser degree C1s,³⁸ accounts for the changes in C4 and C2 that are coin-

cident with attacks of swelling. Levels of other complement components and anaphylatoxins are normal. The diagnosis is corroborated by determination of C1-INH protein and functional levels. Both are decreased approximately in proportion in type I HAE, whereas only functional C1-INH levels are diminished in type II disease.

BRADYKININ AS THE MEDIATOR OF HAE

The first report of an inhibitor deficiency in HAE antedates the discovery of C1-INH deficiency when Landerman et al⁴⁹ described deficiencies in "serum globulin permeability factor" and in plasma kallikrein noted when plasma was diluted. The serum globulin permeability factor turned out to be factor XIIIf.⁵⁰ The next reports of the mediator of swelling were of a trypsin-sensitive, kininlike molecule derived from C2 or, more specifically, by plasmin cleavage of C2b.⁵¹ Augmented skin reactivity of individuals with HAE or guinea pigs to activated C1^{52,53} seemed consistent with this idea.⁵²

Discovery of C1-INH inhibition of the enzymes of the bradykinin-forming cascade suggested bradykinin as the mediator of the swelling, and a link between complement activation and factor XII activation was reported.⁵⁴ Activated plasma kallikrein was demonstrated in skin blisters of patients with HAE that far exceeded that seen in controls.⁵⁵ Soon thereafter, a key study by Fields et al⁵⁶ demonstrated

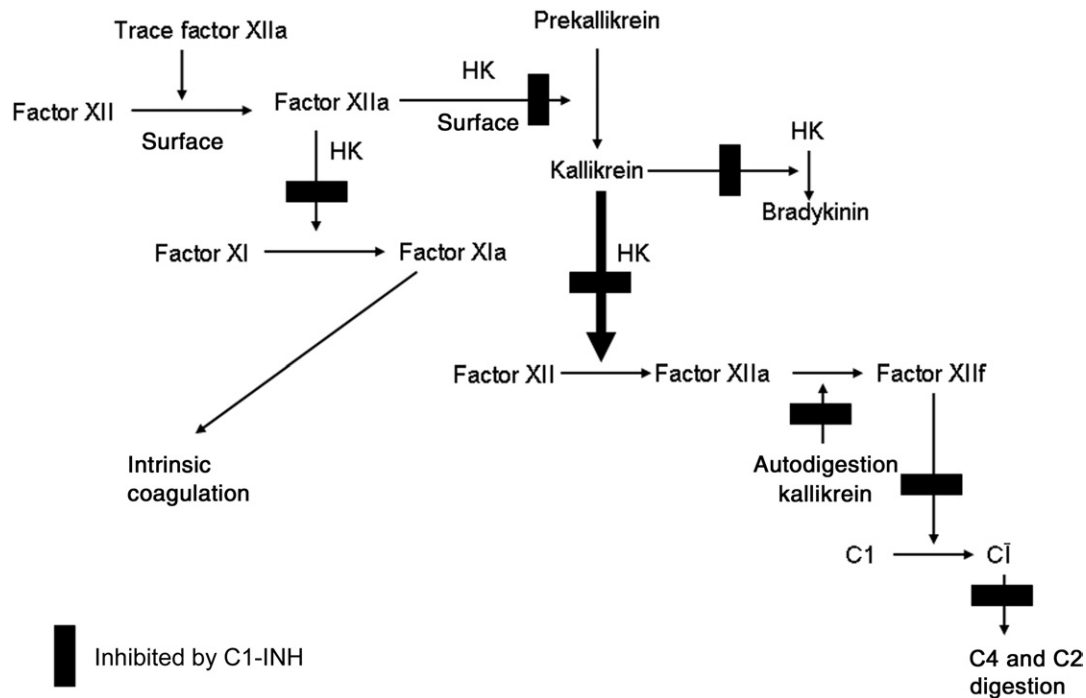


Figure 3. Steps in the plasma bradykinin-forming cascade that are inhibited by C1 inhibitor: (1) factor XII autoactivation, that is, factor XIIa cleavage of factor XII; (2) factor XIIa activation of prekallikrein and factor XI; (3) plasma kallikrein digestion of high-molecular-weight kininogen (HK), the feedback activation of factor XII by plasma kallikrein, and conversion of factor XIIa to factor XIIIf; (4) factor XIIIf activation of the C1r subcomponent of C1; and (5) activated C1 digestion of C4 and C2.

two things. First, they did not reproduce formation of a kinin by plasmin cleavage of an activation mixture containing C1, C4, and C2. Other studies⁵⁷ came to the same conclusion. Second, Fields et al⁵⁶ demonstrated instability of HAE plasma on incubation at 37°C, even in a nonactivating plastic test tube, with gradual, continuously increasing formation of bradykinin (Fig 4). Such results were reproduced 11 years later by Shoemaker et al,⁵⁸ who demonstrated that contractile and permeability-enhancing activity generated on incubation of HAE plasma was attributable to bradykinin and that C2-deficient plasma was not different from normal plasma. Recent data indicate that this formation of bradykinin might proceed in the absence of C1-INH because prekallikrein digests the HK to which it is bound³² to release bradykinin, and the addition of C1-INH stops the reaction (Fig 5). It is unknown whether the active site is present in prekallikrein as it is synthesized or whether the active site is induced conformationally as a result of binding to HK.

Additional studies, taken in aggregate, demonstrate that bradykinin is the mediator of swelling in C1-INH deficiency as follows: (1) prekallikrein and HK are depleted during attacks of swelling and HK is extensively cleaved^{59,60}; (2) bradykinin levels are elevated in the plasma of patients with HAE and in the venous effluent from a swollen arm compared with the unaffected arm⁶¹; (3) an animal model of C1-INH deficiency with swelling resembling that of patients

with HAE is effectively treated by a “knock out” of the bradykinin B₂ receptor⁶²; and (4) a family with an unusual mutation of C1-INH that is unable to inhibit activated C1 but that inhibits factor XIIa and plasma kallikrein normally has no evidence of angioedema in a large cohort.⁶³

ACTIVATION ALONG THE SURFACE OF ENDOTHELIAL CELLS

Although sampling of any random blood vessel demonstrates elevated levels of bradykinin and kinin-forming enzymes in HAE, it is clear that bradykinin formation predominantly occurs locally, at the site of swelling.⁶¹ The reasons for this localization are not yet clear but may be related to the fact that the natural initiating surface in the body seems to be endothelial cells with surface “receptors” that bind all the constituents of the bradykinin-forming cascade. The bradykinin-forming cascade is now known to assemble and activate along the surface of vascular endothelial cells. Zinc-dependent binding of HK and factor XII to human umbilical vein endothelial cells⁶⁴ was observed, and prekallikrein was bound to the HK. Factor XII binds similarly to human umbilical vein endothelial cells as to HK, and they compete for binding,⁶⁵ suggesting that they interact with the same receptor(s). The binding proteins were next purified from cell membrane preparations. The receptor for the globular heads of complement component C1q (gC1qR) binds HK⁶⁶ and factor XII⁶⁶;

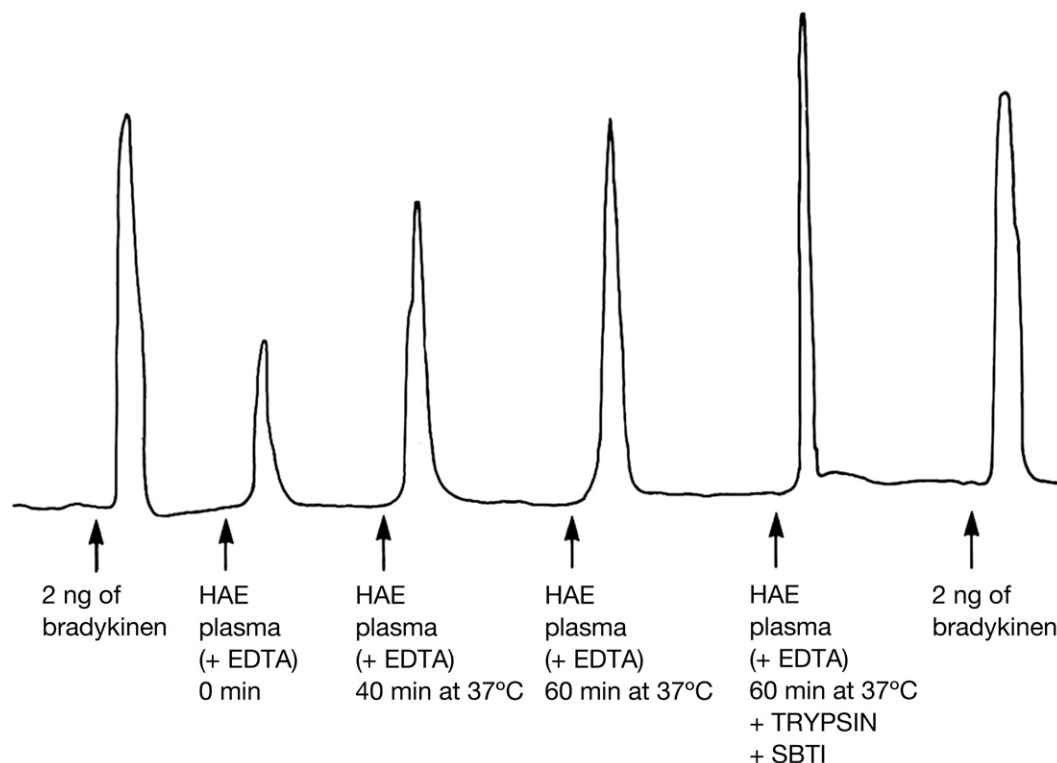


Figure 4. Detection of bradykinin in a sample of citrated plasma from a patient with hereditary angioedema (HAE) during a quiescent period as assessed by contraction of a guinea pig ileum. Standard contractions to 2 ng of bradykinin are shown at the beginning and end of the experiment, indicating no change in sensitivity during the study. Plasma is incubated at 37°C in a plastic (nonactivating) test tube. Bradykinin is detectable in the initial sample at time 0. There is a gradual increase in bradykinin throughout 1-hour incubation that is not inactivated by trypsin digestion. All the contractile activity is inhibited by carboxypeptidase B consistent with bradykinin (data not shown). Normal plasma, tested similarly, has no detectable bradykinin (data not shown). SBTI indicates soya bean trypsin inhibitor.

the urokinase plasminogen activator receptor (u-PAR) also binds HK,⁶⁷ although its interaction with factor XII is more prominent⁶⁸; and cytokeratin 1 also binds HK⁶⁹ and factor XII.⁷⁰ Thus, a confusing array of molecules was identified, and the nature of the binding and assembly in the cell membrane was pursued. Binding of HK was the most complex because its light chain (domain 5) binds to gC1qR, whereas the heavy chain (domain 3) binds to cytokeratin (Fig 2).⁷⁰⁻⁷²

Subsequent data demonstrated that these binding proteins exist in the cell membrane as 2 bimolecular complexes consisting of gC1qR–cytokeratin 1 and u-PAR–cytokeratin 1⁷³; gC1qR and u-PAR do not interact. Because factor XII binds preferentially to u-PAR,⁶⁸ it is likely that u-PAR–cytokeratin 1 binds factor XII, whereas gC1qR–cytokeratin 1 binds HK. This assembly along the endothelial cell surface has been corroborated using cutaneous vascular endothelial cells (Fig 6).⁷⁴

Endothelial cells may be activated as a result of factor XII or HK binding⁷⁵ but are more prominently activated as a result of bradykinin stimulation of B₂ receptors. Nitric oxide and prostacyclin⁷⁶ are produced, which contributes to the vascular leakage that results from bradykinin interaction with

its receptor. Endothelial cells also have been shown to secrete 2 factors that can activate the bradykinin-forming cascade, even in the absence of factor XII: heat shock protein 90 (HSP90)⁷⁷ and prolylcarboxypeptidase.⁷⁸ Both interact with the complex of prekallikrein and HK to convert prekallikrein to plasma kallikrein, but neither is a direct prekallikrein activator as are factor XIIa and factor XIIf.

HSP90 has been shown to react stoichiometrically so that if excess prekallikrein–HK is present, the conversion of prekallikrein to plasma kallikrein is equal to the molar input of HSP90. The active site for these reactions is not clearly defined. HSP90 is not known to have proteolytic activity, although it is an adenosine triphosphatase, whereas prolylcarboxypeptidase has exopeptidase activity but is not an endopeptidase. Perhaps formation of a trimolecular complex of HSP90–prekallikrein–HK allows prekallikrein to autoactivate (and autodigest). A role for HSP90 in the pathogenesis of HAE might relate to its secretion or surface expression on activation of endothelial cells followed by activation of the prekallikrein–HK complex. Infection; hypoxia; release of histamine, leukotriene, or cytokines; or possibly even psychic stress might perturb endothelial cells sufficiently to start the

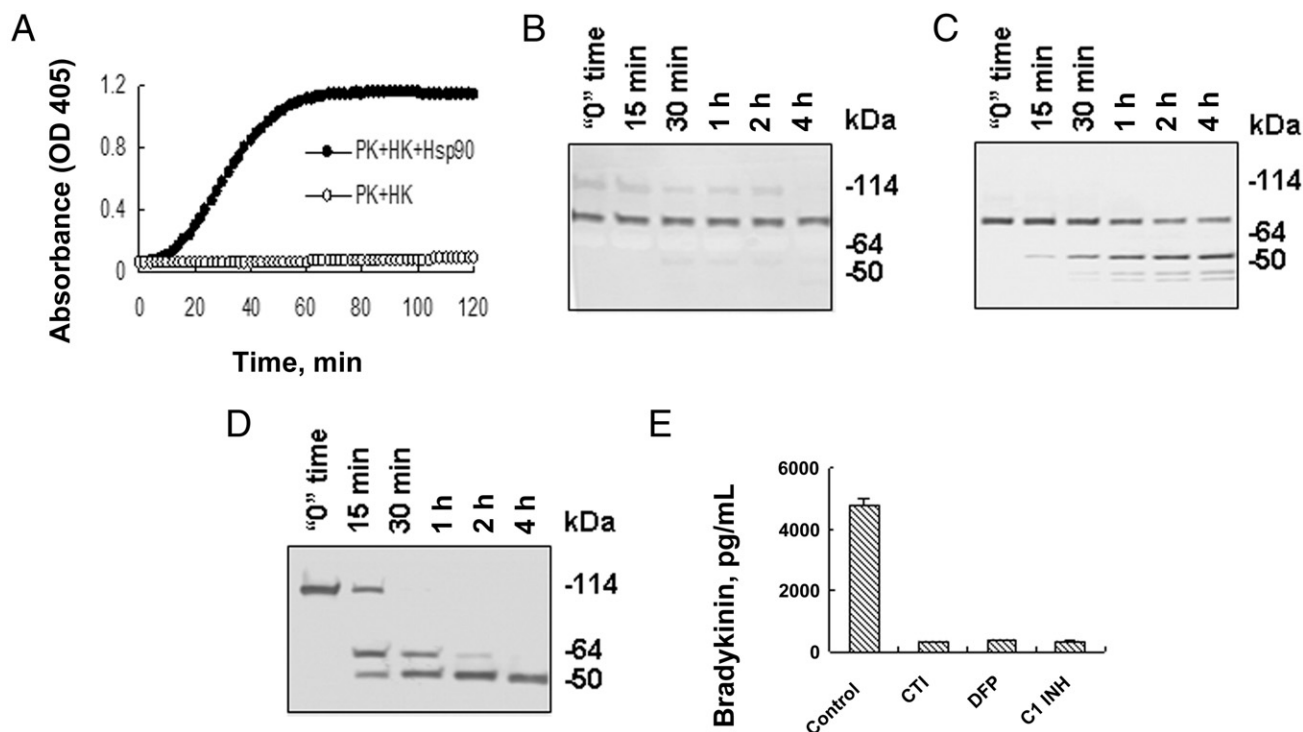


Figure 5. Digestion of high-molecular-weight kininogen (HK) by prekallikrein (PK). A, Incubation of PK and HK at 37°C and the addition of pro-phe-arg chloromethylketone yields no absorbance at 405 nm, indicating that plasma kallikrein is not present. Addition of heat shock protein 90 (HSP90) as a positive control converts PK to plasma kallikrein in 60 minutes. B, Western blot analysis of the mixture of PK and HK developed with anti-PK antibody. Sodium dodecyl sulfate gel electrophoresis is performed under reducing conditions, and antibody development reveals PK at 86 to 88 kDa, with no change during 4-hour incubation. C, Western blot analysis of PK incubated with HK plus HSP90 followed by development with anti-PK antibody demonstrates conversion of PK at 86 to 88 kDa to plasma kallikrein, with a heavy chain at 56 kDa and light chains (doublet) at 33 and 36 kDa. D, Digestion of HK by PK. Both PK and HK are incubated as in (A), but an antibody to the light chain of cleaved HK is used in this Western blot analysis, demonstrating cleavage of native HK at 114 kDa and formation of a 60-kDa intermediate and then a 50-kDa stable product. E, Inhibition of bradykinin formation. PK was incubated with HK for 2 hours at 37°C, and bradykinin formation was assayed by means of enzyme-linked immunosorbent assay. The bradykinin released is shown first as a positive control. The reaction is inhibited by incubation with corn trypsin inhibitor (CTI), which has no effect on active plasma kallikrein; by DFP, a serine protease inhibitor; and by C1 inhibitor (C1-INH), the natural plasma inhibitor of activated factor XII and plasma kallikrein. CTI distinguishes the active site of PK from that of plasma kallikrein. Error bars represent SD. Figure reproduced with permission from Joseph K, Tholanikunnel BG, Kaplan AP. Factor XII-independent cleavage of high-molecular-weight kininogen by prekallikrein and inhibition by C1 inhibitor. *J Allergy Clin Immunol* 2009;124:143-149.

process. The plasma kallikrein thus formed would convert factor XII to factor XIIa and factor XIIIf even if factor XII autoactivation is not apparent.⁷⁹⁻⁸¹

In patients with HAE, the demonstration that bradykinin levels are much higher at a site of swelling than at a comparable healthy site suggests local production at the aforementioned cell membrane-fluid phase interface. Dissemination then can occur by formation of factor XIIIf,⁸² which lacks the binding site for the surface or dissociation of plasma kallikrein from HK.⁸³ Trauma may be a localizing initiator of swelling, and endothelial cell production of HSP90 may relate to stress-induced episodes.

SPECIAL CONSIDERATION OF FIBRINOLYSIS

Plasmin is assumed to have a role in the pathogenesis of HAE because antifibrinolytic agents, such as ϵ -aminocaproic acid⁸⁴ and tranexamic acid,⁸⁵ have been shown to be effective ther-

apeutic modalities (although less so than attenuated androgens), but neither has been approved by the US Food and Drug Administration for the treatment of HAE. Plasma kallikrein can convert plasminogen to plasmin directly or indirectly³³ by activating the small amount of plasma prourokinase to urokinase. Urokinase, even in minute quantities, is a much more potent plasminogen activator than is plasma kallikrein. Once bradykinin stimulates the B₂ receptor, release of cellular tissue plasminogen activator can theoretically lead to a large burst in the conversion of plasminogen to plasmin. C1-INH does contribute to plasmin inhibition; it is also degraded by plasmin so as to further diminish functional C1-INH levels.⁸⁶

The major effect of plasmin on the proteins requisite for bradykinin formation seem to be enzymatic activation of factor XII, which augments bradykinin formation, especially when C1-INH is deficient. In fact, the original studies on the

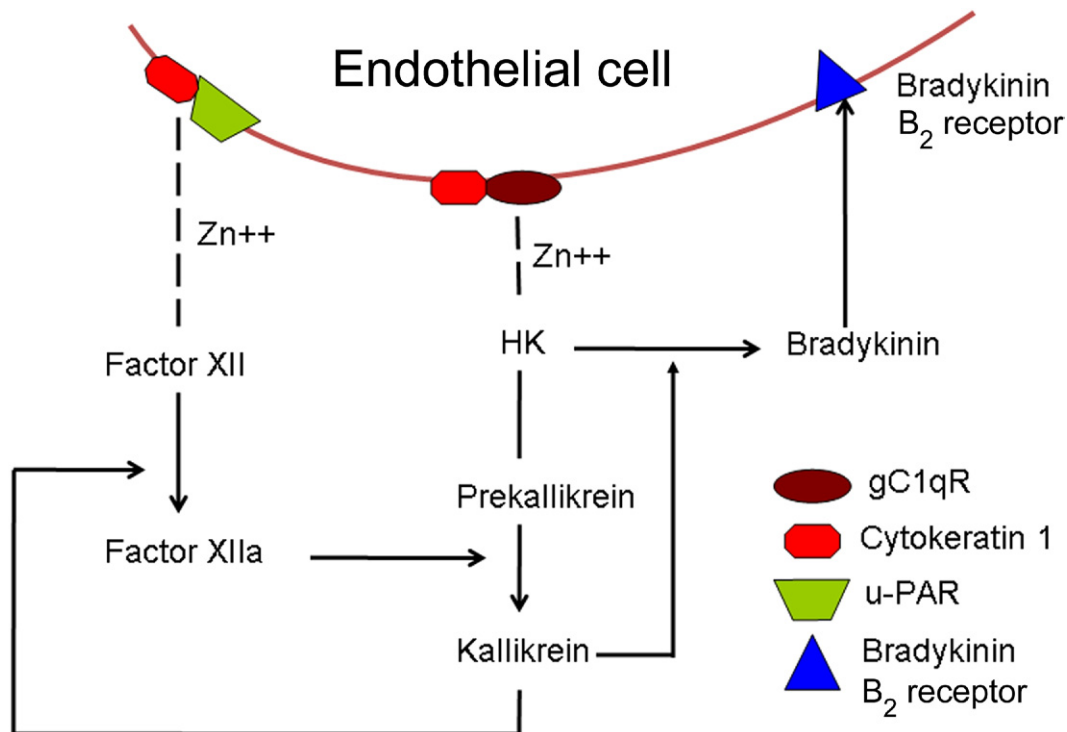


Figure 6. Assembly of the bradykinin-forming cascade along the surface of vascular endothelial cells. Zinc-dependent binding (Zn^{++}) of factor XII and high-molecular-weight kininogen (HK) to the cell surface occurs by binding to complexes of cytokeratin 1–urokinase plasminogen activator receptor (uPAR) and cytokeratin 1–receptor for the globular heads of complement component C1q (gC1qR), respectively. Prekallikrein is bound to the HK. Reciprocal activation of prekallikrein by factor XIIa and factor XII by plasma kallikrein is shown. Plasma kallikrein digests the HK to release bradykinin, and bradykinin binds to the B_2 receptor to activate the endothelial cell.

conversion of factor XII to factor XIIa and factor XII to factor XIII were conducted with plasmin, not plasma kallikrein.⁸⁷ Plasmin does not convert prekallikrein to plasma kallikrein, and any plasmin cleavage of HK to liberate bradykinin is so much less than that of plasma kallikrein that the amount of bradykinin released by this mechanism is unlikely to be clinically significant. A summary of these considerations is shown in Figure 7. Early and recent studies^{82,88} of enzyme activation during attacks of swelling in HAE emphasize the prominence of plasmin in addition to plasma kallikrein and activated factor XII.

TARGETED TREATMENT OF HAE

In the past, acute episodes of swelling were treated with supportive measures because no agent was available for this purpose.^{5,6,89} Fresh-frozen plasma administration can be effective⁹⁰; however, its use is controversial because marked exacerbation of symptoms has been reported⁶ as prekallikrein and HK are also being replenished. Because activation proceeds much faster than does inactivation (seconds vs minutes), symptoms may worsen before they get better.

Prophylactic therapy has used attenuated androgens, such as danazol and stanazolol,^{91,92} for years. These agents stimulate the secretion of C1-INH in hepatic cells, although clinical

response does not relate readily to the increment of plasma C1-INH. Nevertheless, the effect was much improved control of symptoms, but the long-term adverse effects can be substantial. Furthermore, these agents are contraindicated in children and in pregnant women.⁹³ The long-term adverse effects of attenuated androgens include weight gain, virilization, liver function abnormalities, abnormal lipids, peliosis hepatis, and hepatocellular adenomas.⁹⁴ Antifibrinolytic agents are a less effective alternative and may cause muscle cramps and increased secretion of muscle enzymes. These agents have truly been life altering for many patients, yet better alternatives for prophylaxis and the development of agents for therapy of acute attacks would represent major advances.

Replacement therapy with C1-INH has been available for prophylactic and acute therapy of HAE attacks in Europe, Australia, and several other countries for many years,⁹⁵ and now there are new agents on the horizon. A summary of the steps inhibited by these agents is shown in Figure 8. Purified and recombinant preparations of C1-INH are being tested for the treatment of acute attacks of swelling and for prophylaxis.^{6,96} A recent abstract⁹⁷ demonstrates inactivation of activated factor XII, plasma kallikrein, and plasmin on treatment of HAE attacks with a C1-INH preparation. A pasteurized, nanofiltered C1-INH preparation was approved recently for

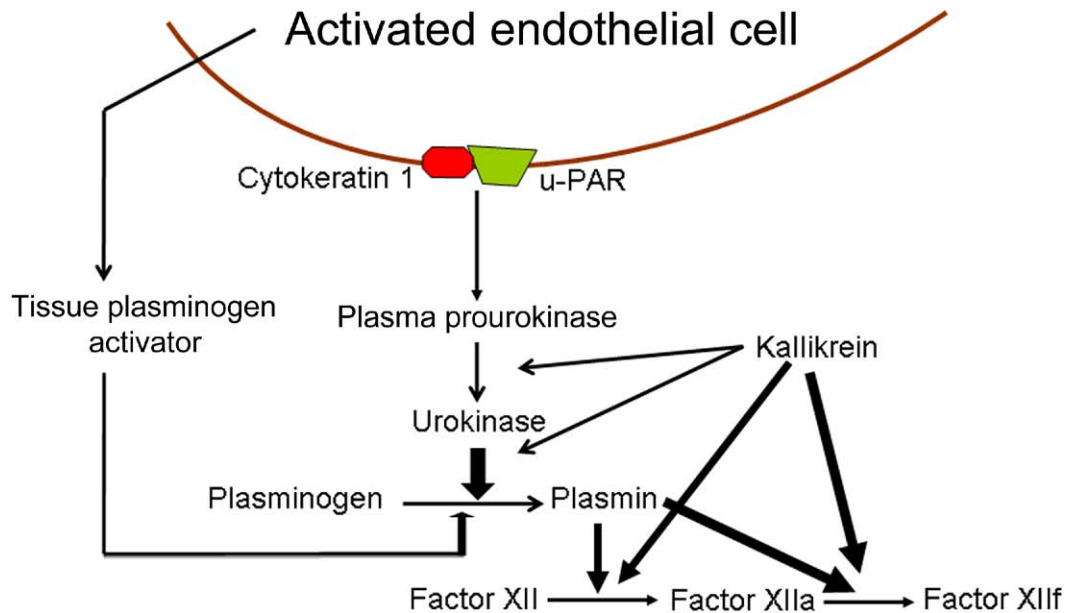


Figure 7. Proposed model for the conversion of plasminogen to plasmin in hereditary angioedema. Bradykinin activation of endothelial cells releases tissue plasminogen activator, which converts plasminogen to plasmin. In addition, plasma prourokinase binds to cytokeratin 1–urokinase plasminogen activator receptor (uPAR) (as does factor XII [see Fig 6]) and is converted to urokinase by plasma kallikrein. Urokinase converts plasminogen to plasmin, although plasma kallikrein can also do it, albeit weakly. Plasmin and plasma kallikrein activate factor XII to factor XIIa, and each enzyme performs the second cleavage to convert factor XIIa to the forms of factor XII fragment (factor XIIIf) as shown in Figure 1.

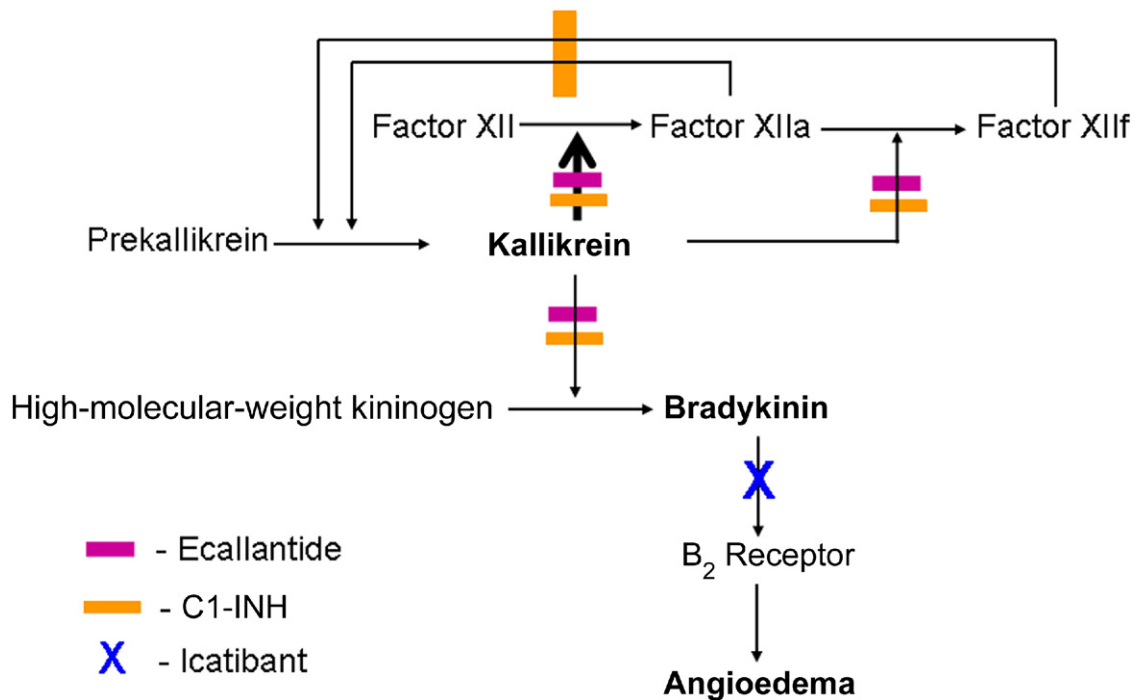


Figure 8. Inhibition of enzymes of the plasma bradykinin-forming cascade by each of the agents used to treat acute attacks of angioedema, including ecallantide, C1 inhibitor (C1-INH), and icatibant. Ecallantide inhibits all functions of plasma kallikrein; C1-INH inhibits all functions of plasma kallikrein, factor XIIa, and factor XIIIf; and icatibant blocks interaction of bradykinin with the B₂ receptor.

prophylaxis (but not acute therapy) in the United States.⁹⁸ In addition, a pasteurized C1-INH preparation was approved recently in the United States for acute treatment (but not prophylaxis) of facial and abdominal attacks.⁹⁹

Ecallantide is a specific, potent, reversible inhibitor of plasma kallikrein that has been shown to be effective for therapy of acute attacks of swelling in HAE,^{100–102} and it was approved for use in the United States in December 2009. This low-molecular-weight active-site titrant inactivates plasma kallikrein so that it no longer digests HK to produce bradykinin and blocks the feedback activation of factor XII by plasma kallikrein (Fig 8). Ecallantide is administered by subcutaneous injection.

Icatibant is a synthetic peptide resembling bradykinin that binds to the B₂ receptor and blocks bradykinin from functioning (Fig 8). This agent has also been shown to be effective for the treatment of acute attacks of swelling¹⁰³ and has been approved for this indication in Europe and Brazil but not in the United States.

Ecallantide, C1 INH, and icatibant are agents that can be used for acute therapy of episodes of swelling, and the administration of C1-INH for prophylaxis may supplant traditional approaches, such as attenuated androgens and anti-fibrinolytic agents. Additional roles for these agents might be treatment of angiotensin-converting enzyme inhibitor-induced angioedema, acquired C1-INH deficiency, and type III HAE with normal C1-INH levels.

CONCLUSIONS

Bradykinin is the mediator of the swelling of HAE. Fluid-phase interaction of factor XII, prekallikrein, and HK produces bradykinin in an uncontrolled manner when C1-INH is absent. However, new data suggest that activation along the endothelial cell surface may relate to the fact that bradykinin formation starts as a local phenomenon at the site of swelling and may then spread to become more generalized. The endothelial cell may be an active participant and not simply the site of formation of gaps through which fluid may pass. Thus, secretion of tissue plasminogen activator, nitric oxide, prostacyclin, HSP90, and prolylcarboxypeptidase, all as a result of bradykinin stimulation of the B₂ receptor, can lead to augmented fibrinolysis, enhanced vasodilatation, and activation of the prekallikrein-HK complex. Finally, the uncontrolled formation of bradykinin observed when HAE plasma is incubated in a test tube lacking any initiating surface may be due to prekallikrein activation of HK, a newly described enzymatic reaction inhibitable by C1-INH. We are beginning an era in which therapy for HAE can be targeted to the pathophysiologic changes that underlie the swelling mediated by the bradykinin-forming cascade: C1-INH blocks the enzymes responsible for bradykinin formation, ecallantide inhibits the activities of plasma kallikrein, and icatibant prevents bradykinin from interacting with the B₂ receptor.

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