

Molecular mechanisms in allergy and clinical immunology

(Supported by a grant from Merck & Co, Inc, West Point, Pa)

Series editor: Lanny J. Rosenwasser, MD

Pathways for bradykinin formation and inflammatory disease

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Bradykinin is formed by the interaction of factor XII, prekallikrein, and high-molecular-weight kininogen on negatively charged inorganic surfaces (silicates, urate, and pyrophosphate) or macromolecular organic surfaces (heparin, other mucopolysaccharides, and sulfatides) or on assembly along the surface of cells. Catalysis along the cell surface requires zinc-dependent binding of factor XII and high-molecular-weight kininogen to proteins, such as the receptor for the globular heads of the C1q subcomponent of complement, cytokeratin 1, and urokinase plasminogen activator receptor. These 3 proteins complex together within the cell membrane, and initiation depends on autoactivation of factor XII on binding to gC1qR (the receptor for the globular heads of the C1q subcomponent of complement). There is also a factor XII-independent bypass mechanism requiring a cell-derived cofactor or protease that activates prekallikrein. Bradykinin is degraded by carboxypeptidase N and angiotensin-converting enzyme. Angioedema that is bradykinin dependent results from hereditary or acquired C1 inhibitor deficiencies or use of angiotensin-converting enzyme inhibitors to treat hypertension, heart failure, diabetes, or scleroderma. The role for bradykinin in allergic rhinitis, asthma, and anaphylaxis is to contribute to tissue hyperresponsiveness, local inflammation, and hypotension. Activation of the plasma cascade occurs as a result of heparin release and endothelial-cell activation and as a secondary event caused by other pathways of inflammation. (*J Allergy Clin Immunol* 2002;109:195-209.)

Key words: *Bradykinin, factor XII, prekallikrein, kininogen, kininase, angioedema*

Kinins are low-molecular-weight peptides that participate in inflammatory processes by virtue of their ability to activate endothelial cells and, as a consequence, lead to vasodilatation, increased vascular permeability, production of nitric oxide, and mobilization of arachidonic acid. Kinins also stimulate sensory nerve endings to cause a

Abbreviations used

ACE:	Angiotensin-converting enzyme
Factor XII _f :	Factor XII fragment (Hageman factor fragment)
gC1qR:	Receptor for the globular heads of the C1q subcomponent of complement
HAE:	Hereditary angioedema
HK:	High-molecular-weight kininogen
HUVEC:	Human umbilical cord endothelial cell
LK:	Low-molecular-weight kininogen
u-PAR:	Urokinase plasminogen activator receptor

burning dysaesthesia. Thus the classical parameters of inflammation (ie, redness, heat, swelling, and pain) can all result from kinin formation. Bradykinin is the best characterized of this group of vasoactive substances.

There are 2 general pathways by which bradykinin is generated. The simpler of the two has only 2 components: an enzyme tissue kallikrein¹ and a plasma substrate, low-molecular-weight kininogen (LK).^{2,3} Tissue kallikrein is secreted by many cells throughout the body; however, certain tissues produce particularly large quantities. These include glandular tissues (salivary and sweat glands and pancreatic exocrine gland) and the lung, kidney, intestine, and brain. The enzyme is processed intracellularly from a precursor, prokallikrein, to produce tissue kallikrein; however, the enzyme responsible for this conversion has not been identified. Tissue kallikrein is secreted and digests LK to yield a 10-amino-acid peptide, lysyl-bradykinin (kallidin), with the sequence lys-arg-pro-pro-gly-phe-ser-pro-phe-arg. A plasma aminopeptidase cleaves the N-terminal lys from it, and the 9-amino-acid peptide bradykinin results.

The second pathway for bradykinin formation is far more complex and is part of the initiating mechanism by which the intrinsic coagulation pathway is activated.⁴ Factor XII is the initiating protein that binds to certain negatively charged macromolecular surfaces and autoactivates (autodigests) to form factor XIIa.^{5,6} There are 2 plasma substrates of factor XIIa, namely prekallikrein⁷ and factor XI.^{8,9} and each of these circulates as a complex with high-molecular-weight kininogen (HK).^{10,11} These complexes also attach to initiating surfaces, and the major attachment sites are on 2 of the domains of HK, thereby placing both prekallikrein and factor XI in

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0091-6749/2002 \$35.00 + 0 1/10/121316

doi:10.1067/mai.2002.121316

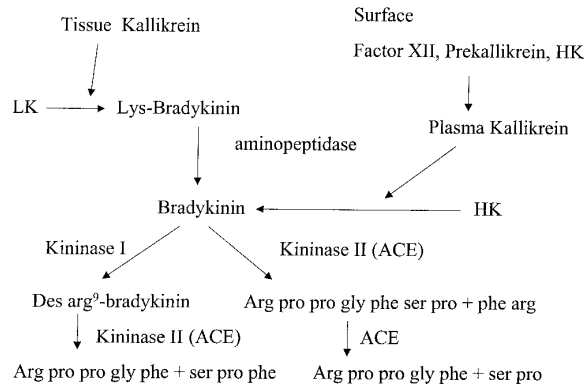


FIG 1. Pathways for formation and degradation of bradykinin.

optimal conformation for cleavage to kallikrein (plasma kallikrein) and factor XIa, respectively. It is important to note that plasma kallikrein and tissue kallikrein are separate gene products and have little amino acid sequence homology, although they have related functions (ie, cleavage of kininogens). Tissue kallikrein prefers LK but is capable of cleaving HK, whereas plasma kallikrein cleaves HK exclusively. The 2 kininogens have an identical amino acid sequence starting at the N-terminus and continuing to 12 amino acids beyond the bradykinin moiety³ but differ in C-terminal domains because of alternative splicing.^{12,13}

This scheme for production and degradation of kinins is shown in Fig 1, and further details of the plasma cascade are given in Fig 2. The enzymes that destroy bradykinin consist of kininases I and II. Kininase I is also known as plasma carboxypeptidase N,¹⁴ which removes the C-terminal arg from bradykinin or kallidin to yield des-arg⁹ bradykinin or des-arg¹⁰ kallidin, respectively.¹⁵ It is the same enzyme that cleaves the C-terminal arg from the complement anaphylatoxins C3a and C5a. Kininase II is identical to angiotensin-converting enzyme (ACE).¹⁶ Bradykinin and kallidin stimulate constitutively produced B2 receptors,¹⁷ whereas des-arg⁹-BK or des-arg¹⁰ lys-BK both stimulate B1 receptors,¹⁸ which are induced as a result of inflammation. Stimuli for B1 receptor transcription include IL-1 and TNF- α .^{19,20} Kininase II is a dipeptidase that cleaves the C-terminal phe-arg from bradykinin to yield a heptapeptide, which is cleaved once again to remove ser-pro and to leave the pentapeptide arg-pro-pro-gly-phe.^{21,22} If the C-terminal arg of bradykinin is first removed with kininase I, then ACE functions as a tripeptidase to remove ser-pro-phe and to leave the above pentapeptide.²¹

THE SURFACE

In vitro studies have typically been done with nonphysiologic substances, such as glass (negatively charged silicates activate the intrinsic coagulation cascade to clot the blood and bradykinin is formed), kaolin, and dextran sulfate. The physiologic surface, however, may be assembly of the cascade along the surface of endothelial cells, and

pathologic initiators can include proteoglycans (sulfate residues on heparan sulfate or chondroitin sulfate), endotoxins (LPS), or crystals of uric acid or pyrophosphate.²³

FACTOR XII (HAGEMAN FACTOR)

Factor XII circulates as a single-chain zymogen (proenzyme) with no detectable enzymatic activity. Its molecular weight is 80 kd on SDS gel electrophoresis. It is synthesized in the liver and circulates at a plasma concentration of 30 to 35 $\mu\text{g/mL}$. During contact activation of normal plasma, factor XII autoactivates when bound to polyanionic surfaces, with conversion of factor XII to factor XIIa.^{5,6} Once small amounts of kallikrein are formed, however, there is rapid conversion of surface-bound factor XII to factor XIIa, representing an important positive feedback in the system.²⁴⁻²⁶ Activation of factor XII by kallikrein results in the formation of a series of active enzymes formed by successive cleavages.²⁶ The first cleavage to produce factor XIIa from factor XII occurs within a disulfide bond and yields a 2-chain disulfide-linked molecule with a new amino terminal sequence of Val-Val-Gly.²⁷ This form of factor XIIa is produced either by means of autoactivation or kallikrein.²⁶ The newly formed factor XIIa has the same molecular weight as the zymogen but, when reduced, is composed of a heavy chain of 50 kd and a light chain of 28 kd.²⁸ The light chain contains the catalytic domain and is derived from the carboxyterminal region of the molecule, whereas the heavy chain contains the surface-binding region and is derived from the amino terminal region.²⁹ Further cleavage can occur in the heavy chain to produce a series of lower-molecular-weight forms of activated XII, all of which retain activity in terms of conversion of prekallikrein to kallikrein³⁰ but lose the ability to activate factor XI. The most prominent cleavage product of factor XIIa is a 30-kd fragment known as factor XII fragment (factor XIIIf).³¹ Careful examination of the bands obtained on SDS gel electrophoresis of factor XIIIf in the absence of reducing agents indicates that a doublet is present, consisting of an upper band of 30 kd, which is gradually converted to the lower band at about 28.5 kd.³² These species of factor XIIIf are made up of the light chain of factor XIIa, to which a small fragment of the original heavy chain is linked by a disulfide bond (Fig 3). The active forms of factor XII are trypsin-like serine proteases. Factor XIIIf cleaves prekallikrein in the fluid phase, but in contrast to factor XIIa, it does not readily cleave factor XI and therefore is not a procoagulant enzyme. It has only 2% to 4% of the coagulant activity of factor XIIa.³⁰ Similarly, factor XIIIf will not activate factor XII zymogen and therefore does not participate in autoactivation.⁵ The lack of activity toward factor XI and factor XII itself probably reflects the absence of the surface-binding site, which is found in the heavy chain. Therefore we can envision that during contact activation, bound factor XIIa becomes cleaved to factor XIIIf, which, free of the surface-binding domain, can diffuse away and promote kinin generation (ie, activation of prekallikrein) at sites distant from the original

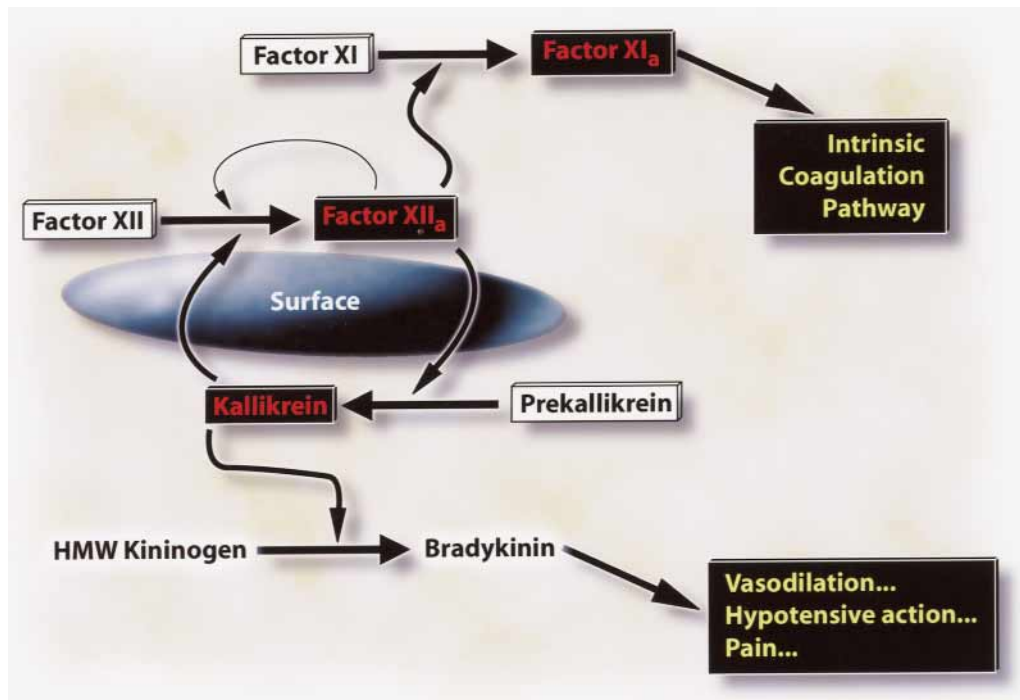


FIG 2. Contact activation pathway leading to bradykinin formation. *HMW*, High molecular weight. Reprinted with permission from *Blood, Principles and Practice*. Philadelphia: J. B. Lippincott Co; 1995. p. 1127-50.

activation site. Factor XII_f can also activate the C1r and, to a lesser degree, the C1s subunits of the first component of complement.³³ Thus massive activation and cleavage for factor XII can activate complement directly. Factor XII_f can also activate factor VII, the initiating proenzyme of the extrinsic coagulation pathway. However, it does so only at low temperatures.

The primary structure of factor XII is known from analysis of complementary DNA^{34,35} and direct protein sequence data.^{27,29} The mature protein has 596 amino acids and a molecular weight of 66.5 Kd. The carbohydrate content is 16.8%, and therefore the total predicted molecular weight is 80,427 d. The amino terminal heavy-chain region of the protein shows extensive homologies with distinct domains possessing strong homology to domains contained in fibronectin, plasminogen, plasminogen activators, and epidermal growth factor. A connecting region consisting of amino acids 279-330 connects the heavy chain with the catalytic serine protease domain; it has 17 prolines and also bears the proposed attachment sites for 6 linked carbohydrate side chains. The C-terminal residues from 354-596 make up the catalytic domain of the factor XII sequence. This light-chain region is homologous with typical serine proteases, such as trypsin, chymotrypsin, and elastase. The cleavage by kallikrein (or factor XII_a itself) to form factor XII_a occurs at Arg 353-Val 354.^{29,34} Two subsequent cleavages count for the formation of the 2 forms of factor XII_f. First cleavage at Arg 334 produces a disulfide-linked 2-chain molecule composed of the light chain of factor XII_a linked to a 19-amino-acid peptide. A second cleav-

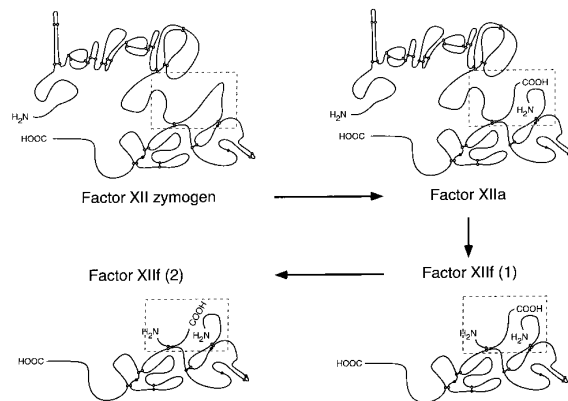


FIG 3. Diagrammatic representation depicting the sequential cleavages occurring in factor XII and leading initially to factor XII_a, followed by cleavages external to the disulfide bridge to form factor XII_f (1) and factor XII_f (2). Note that only the C-terminal portion of the heavy-chain disulfide is linked to the light chain. Reprinted with permission from *Blood, Principles and Practice*. Philadelphia: J. B. Lippincott Co; 1995. p. 1127-50.

age at Arg 343 leaves a residual 9-amino-acid peptide attached by means of the same disulfide bond. The primary surface-binding site appears to be located within the first domain of factor XII termed a fibronectin type 2 domain.³⁶ A second binding site may also be present in the adjacent fibronectin type 1 domain.³⁷ Studies with cDNA hybridization probes have shown that the gene for factor XII is located on chromosome 5 and is localized to the 5q33 qter region of the chromosome.³⁸

PREKALLIKREIN

Like factor XII, prekallikrein is a zymogen without detectable activity, which is converted to a functional serine protease during contact activation. SDS gel electrophoresis of purified prekallikrein yields 2 bands at 85 and 88 kd, regardless of the source.⁷ Activation is accomplished with either factor XIIa or factor XIIIf and results in disulfide-linked 2-chain forms composed of a heavy chain of 56 kd and a light chain bearing the catalytic center. The heterogeneity shown by the zymogen is reflected in the light chain; on reduction, a pair of bands of 33 and 36 kd is seen that contains the active-site serine. Prekallikrein binds to HK through a site on its heavy chain, with a dissociation constant of 12 to 15 nmol/L.^{39,40} This value, which is unchanged by conversion to kallikrein, is such that at plasma concentrations of HK, about 80% to 90% of the prekallikrein circulates bound in a 1:1 molar complex. Thus it is the prekallikrein-HK complex that participates in surface-induced contact activation, and the binding of prekallikrein to the surface is mediated through the HK. The dissociation of 10% to 20% of the kallikrein into the fluid phase may serve to propagate both the activation of factor XII and the generation of bradykinin.^{41,42} Kallikrein then digests HK to liberate the vasoactive peptide bradykinin. Kallikrein possesses other functions that may have a role in inflammation. It is chemotactic for human neutrophils and monocytes,^{43,44} it causes neutrophil aggregation and secretion of elastase,^{45,46} it activates factor B of the alternative complement pathway,⁴⁷ and it can convert plasma prorenin to renin and may thereby be responsible for the neutral phase of acid-induced prorenin activation.^{48,49}

The entire amino acid sequences of protein has been determined by using a combination of direct protein sequencing and amino acid sequence prediction from cDNAs isolated from a λ gt-11 expression library.⁵⁰ There is a signal peptide of 19 residues followed by the sequence of the mature plasma prekallikrein molecule, which has 619 amino acids and a calculated molecular weight of 69,710 d. It has 15% carbohydrate by weight, for a total of 79,545 d. Five asparagine residues have been identified as attachment sites for carbohydrate: 3 are in the light chain, and 2 are in the heavy chain. The molecular weight heterogeneity seen on SDS gel electrophoresis is not explained by any sequence heterogeneity and may therefore be due to variations in glycosylation. The site of cleavage that generates kallikrein from prekallikrein is Arg 371-Ile 372, generating a light chain of 248 amino acid residues with a new amino terminal sequence, IleVal-Gly. The amino acid sequence of the heavy chain is unusual, with little homology with other serine proteases of the coagulation cascade with the exception of factor XI. The heavy-chain sequence has 4 tandem repeats, each of which contains 90 to 91 amino acids. The presence of 6 conserved half cysteines per repeat suggests a repeating structure with 3 disulfide loops. Thus a gene segment coding for the ancestor of the repeat sequence is duplicated, and then the entire segment is duplicated again to give the present structure.

FACTOR XI

Like factor XII and prekallikrein, factor XI is a zymogen that is activated to a serine protease during contact activation. Factor XI is unique among the clotting factors in that the circulating zymogen consists of 2 identical subunits linked by a disulfide bond.^{8,9} The dimer has an apparent molecular weight of 160 kd on SDS gel electrophoresis. The major activator of factor XI is factor XIIa; however, thrombin also serves as an activator, particularly when factor XI is incorporated into a fibrin clot.^{51,52} The activation of factor XI follows the familiar pattern of cleavage of a peptide bond within a disulfide bridge to yield an amino terminal heavy chain and a disulfide-linked light chain. Because both precursor subunits can be cleaved and each resulting light chain bears a functional active site, factor XIa is a 4-chain protein with 2 identical active sites. Factor XI circulates with a plasma concentration of only 4 to 6 μ g/mL. The heavy chain of factor XIa, like that of kallikrein, binds to the light chain of HK. Thus factor XI and HK also circulate as a complex.¹¹ The dissociation constant is 70 nmol/L, which is high enough to ensure that over 90% of the factor XI is complexed. The molar ratio of the complex can vary from 1 to 2 molecules of HK per factor XI because of the dimeric nature of factor XI. The binding site for HK on the factor XI heavy chain has been localized to the first (amino terminal) tandem repeat. Although prekallikrein can be activated in the fluid phase by factor XIIIf, whether it is or is not bound to HK, factor XI activation requires interaction with the surface, with HK as an obligate cofactor.^{53,54} Thus both factor XII and HK interact with the initiating surface, and HK facilitates factor XI cleavage by factor XIIa. The amino acid sequence of factor XI has been determined by translation of a cDNA insert obtained from a λ gt-11 cDNA library prepared from human liver poly(A) RNA.⁵⁵ An 18-amino-acid leader sequence is followed by a 607-amino-acid sequence for each of the 2 subunits of the mature protein. The calculated molecular weight of the 2 polypeptide chains totals 135,979 d. With the addition of 5% carbohydrate, the total molecular weight would be about 140 kd. Five potential N-glycosylation sites have been identified in each subunit: 3 in the heavy-chain portions and 2 in the light-chain portions of the sequence. Conversion of factor XI to factor XIa occurs by means of cleavage between Arg 369 and Ile 370, resulting in a light chain of 238 amino acids with a new N-terminal sequence of Ile-Val-Gly-Gly. The light chain has the characteristic features of a trypsin-like serine protease. The sequence of the heavy chain of factor XIa, like that of kallikrein, contains 4 tandem repeats of about 90 amino acids, including 6 conserved half cysteines. This suggests that each repeat forms a domain containing 3 internal disulfide bridges. However, unpaired half cysteines in the first and fourth repeat are postulated to form the interchain disulfide bridges to form the factor XI homodimer. The amino acid sequence of factor XI shows a striking homology with that of prekallikrein. The com-

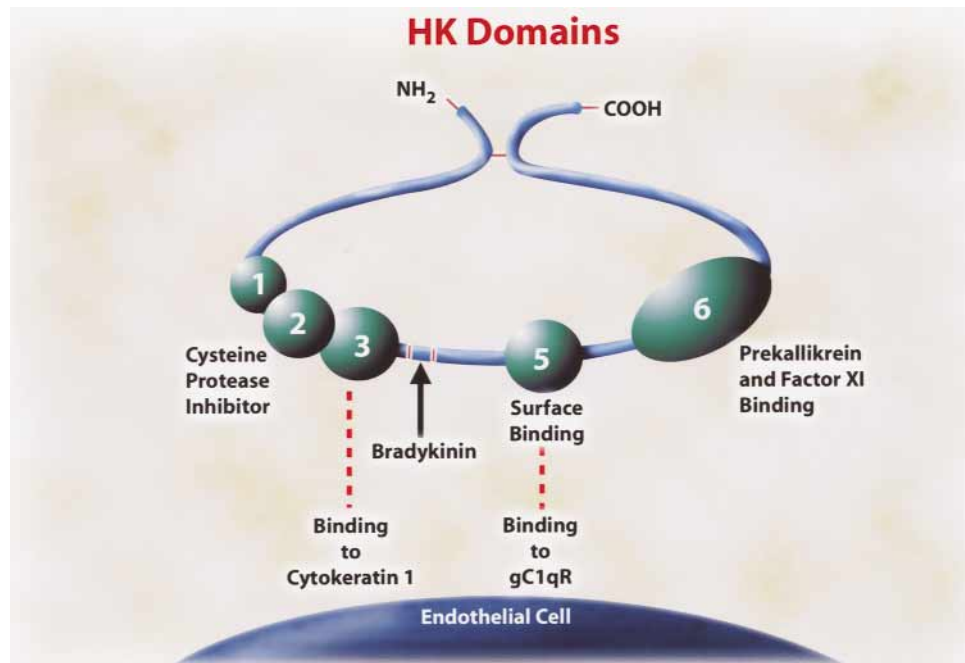


FIG 4. Domain structure of HK depicting attachment to an endothelial cell by domains 3 and 5 interacting with cytokeratin 1 and gC1qR, respectively.

plete sequence of factor XI has a 58% identity with that of prekallikrein, and if homologous amino acids are included, the degree of homology is 67%. The carboxy terminal sequence of 88 amino acids, which includes the reactive-site serine and the substrate binding pocket, has 81% identity. Thus both proteins are likely to have evolved from a common ancestor.

HIGH-MOLECULAR-WEIGHT KININOGEN

HK circulates as a single-chain glycoprotein with an apparent molecular weight of 200 kd by means of gel filtration and 115 kd by means of SDS gel electrophoresis under reducing conditions.^{10,56} It circulates at a plasma concentration of 70 to 90 µg/mL and forms a noncovalent complex with prekallikrein and a similar complex with factor XI.^{10,11} During contact activation, plasma kallikrein digests HK, leaving a disulfide-linked heterodimer consisting of an amino terminal heavy chain (65 kd) and initially a carboxy terminal light chain of 56 to 62 kd. A subsequent cleavage reduces the light chain to 45 to 49 kd.⁵⁷⁻⁶⁰ HK can also be cleaved by tissue kallikrein to form the heterodimer of the heavy chain and the 56- to 62-kd light chain; the additional cleavage made by plasma kallikrein in the light chain is not made by the tissue enzyme.⁶⁰ The complexes of HK with prekallikrein and factor XI are formed with the light-chain region.^{40,61,62} Kallikrein first cleaves HK at the carboxy terminal portion of the bradykinin sequence, which is an Arg-Ser bond, leaving the bradykinin attached to the carboxy terminal end of the heavy chain.⁵⁹ The sequence leu-met-lys-arg is cleaved at the lys-arg bond to liberate bradykinin from the

heavy chain. HK binds to negatively charged surfaces through the histidine region of the light chain, which corresponds to domain 5. The ability to bind to a surface and simultaneously complex factor XI or prekallikrein is responsible for its cofactor activity in contact activation.⁴¹ The complete amino acid sequence of HK has been determined from cDNA,^{12,13} as well direct sequence analysis of the purified protein.^{63,64} HK contains a total of 626-amino-acid residues with a calculated molecular weight of 69,896 d. Together with 40% carbohydrate, the molecular weight approaches that observed at 115 kd. The heavy chain of 362 residues, which is derived from N-terminus, is followed by the 9-residue bradykinin sequence and then by the light-chain structure of 255 residues. The mature protein has a blocked amino terminal end with pyroglutamic acid. Three glycosyltic cleavage sites are on the heavy chain, and 9 are on the light chain. All of the glycosyltic bonds on the heavy chain are N-linked, and those on the light chain are O-linked. The relevance of this unique glycosyltic linkage distribution is unknown. A diagrammatic representation of the domain structure of HK is given in Fig 4. The heavy chain contains 3 contiguous sequences that are homologous to each other. Domain 1 comprises residues 1-116, domain 2 comprises residues 117-238, and domain 3 comprises residues 239-360. Of the 17 half cysteines, one is near the N-terminal and forms a disulfide bond with the light chain; the others form a linear arrangement of 8 consecutive disulfide loops aligned with the sequence repeats. These 3 domains share considerable homology with a family of small (12-13 kd) cysteine protease inhibitors called cystatins. Domains 2 and 3, but not domain 1, exhibit cysteine protease inhibitor activ-

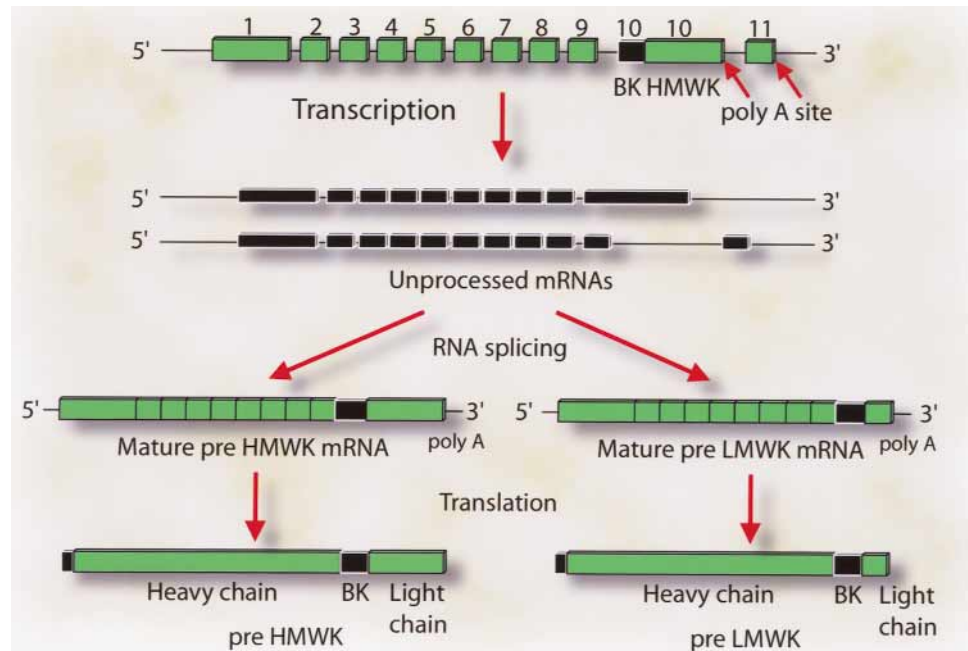


FIG 5. The gene for HK. The mature mRNAs are assembled by means of alternative splicing events in which the light-chain sequences are attached to the 3' end of the 12-amino-acid common sequence C-terminal to bradykinin. *HMWK*, High-molecular-weight kininogen; *LMWK*, low-molecular-weight kininogen. Reprinted with permission from *Blood, Principles and Practice*. Philadelphia: J. B. Lippincott Co; 1995. p. 1127-50.

ity. It has been shown by means of limited proteolytic digestion that protease-sensitive sites on the heavy chain map to its interdomain junction. Possibly cleavage at these sites may occur in plasma to release the inhibitory domains under certain pathologic conditions. The isolated heavy chain has greater cysteine protease inhibitor activity than HK and is able to bind to molecules of a cysteine protease, such as papain.⁶⁵⁻⁶⁷

The procoagulant properties of HK all reside in the light-chain portion.^{57,61} The light chain is divided into a basic amino acid sequence portion that binds to activating surfaces and an acidic carboxy terminal portion that bears the binding sites for prekallikrein and factor XI. The surface-binding regions spanning residues 91-121 of the light chain is called the histidine-rich region because 24 of 91 residues are histidines, and an additional 11 residues are lysines. The histidine-rich region also has 3 internal homologous sequences of about 30 residues each, which in turn contains further internally homologous sequences. The sole half cysteine found in the light chain forms a disulfide bond with the heavy chain. No homology with any protein except for other kininogens has been recognized. The prekallikrein binding site of HK maps to residues 194-224 of the light chain. Factor XI is bound to the same region, but in this case optimal binding requires a 58-amino-acid peptide spanning residues 185-242 of the light chain.^{62,68}

The second kininogen, namely LK, is digested by tissue kallikrein to convert a single-chain precursor to a 2-chain form to which bradykinin has been excised. This digestion yields a 65-kd amino terminal heavy chain disulfide linked to a light chain of 4 kd. In contrast to HK,

LK is not cleaved by plasma kallikrein. The sequences of the 2 kininogens are identical, starting from the amino terminus through the bradykinin sequence and the subsequent 12 amino acids. Studies of the cDNAs show that the identity persists through the 18-residue signal peptide and the 5' untranslated region of the mRNA. Both HK and LK are produced from a single gene that is thought to have originated as a result of 2 successive duplications of a primordial kininogen gene.¹² As schematically represented, this gene consists of 11 exons, the first 9 of which encode the heavy chain. Each of the 3 domains indicated by the protein sequences is encoded by a set of 3 exons. The 10th exon codes for bradykinin and the light chain of HK, and the light chain of LK is coded for by exon 11. The mRNAs for HK and LK are produced from the single gene by means of alternative splicing and thereby provides the 2 kininogens with separate light-chain moieties. This is summarized in Fig 5.

INTERACTION OF PROTEINS AND SURFACES IN INITIATION OF CONTACT ACTIVATION

Surfaces that initiate activation of the plasma bradykinin-forming pathway include glass, dextran sulfate, sulfatides, endotoxin, and naturally occurring proteoglycans containing highly sulfated mucopolysaccharides such as heparan sulfate, chondroitin sulfate E, or mast-cell heparin.^{69,70} The requirement for a high density of strong negative charges is suggested by the finding of a relationship between the rate of factor XII autoactivation and the sulfate content of a series of dextran sulfate

preparations. A striking feature of contact activation is the cyclic or reciprocal interactions, in which the activator of prekallikrein is factor XIIa and the activator of factor XII is kallikrein (Fig 2). This positive feedback ensures an accelerating reaction once initiation has occurred. It has been proven that binding of factor XII (or other component) to surfaces does not induce an active site by means of a conformational change. Enzymatic cleavage is required; thus the origin of the initiating active site is an enigma. It has been considered that native, uncleaved factor XII might have some infinitesimal activity that cannot as yet be measured, but the presence in plasma of trace amounts of factor XIIa seems to be more likely.⁷¹ There may be low-level continuous activation of the pathway, perhaps along cell surfaces, to generate minute amounts of active enzyme that are not inhibited by plasma C1 inhibitor. Binding creates a local milieu in the fluid-phase along the surface, where local concentrations of zymogens and active enzymes are much increased, thereby increasing the rate of their interactions. Also, factor XII, when bound, undergoes a conformational change, rendering it much more susceptible to cleavage by kallikrein.⁷² C1 inhibitor does not bind, and therefore there is some local protection against inactivation. Thus the balance of activation versus inhibition is profoundly changed at the fluid-surface interface.

It has been estimated that the activation of prekallikrein by factor XIIa is augmented 70-fold by the surface,⁶ whereas prekallikrein activation by factor XII_f, which lacks the surface-binding site, is not influenced. On the other hand, activation of surface-bound factor XII by kallikrein in the reciprocal reaction is enhanced by the surface 3000- to 12,000-fold, and this rate is at least 2000-fold faster than the rate of factor XIIa autoactivation.⁶ Thus although factor XII is the initiator, most factor XIIa is formed by kallikrein. This is consistent with the fact that factor XII-deficient plasma will not activate when surfaces are added, but prekallikrein-deficient plasma will activate slowly, presumably as a result of factor XII autoactivation.^{25,73}

HK is a cofactor for the reciprocal interactions of prekallikrein and factor XII and the interaction of factor XII with factor XI.^{53,74} It is a cofactor for factor XIIa activation of both prekallikrein and factor XI; that is, binding of prekallikrein and factor XI to HK renders them more sensitive to cleavage by factor XIIa.^{41,53} Attachment of the HK-prekallikrein complex and the HK-factor XI complex to the surface is mediated by domain 5 of the light chain of HK. In fact, if factor XI and prekallikrein are bound to surfaces in the absence of HK, their activation is inhibited. HK also has a prominent augmenting role in the activation of surface-bound factor XII by kallikrein,⁵³ which is difficult to explain because binding to HK does not render kallikrein to be a more effective enzyme. Its effects are 3-fold and indirect.⁴¹ First, it is required to create kallikrein (ie, conversion of prekallikrein to kallikrein). Second, the binding constant for kallikrein binding to HK is such that about 10% is always free. Thus kallikrein dissociates from HK and can disseminate factor XII activation along the surface. As a result, the effective

concentration ratio of kallikrein/factor XII is increased when kininogen is present.⁴¹ Last, HK in plasma can displace other competing adhesive glycoproteins, such as fibrinogen, from binding to the surface and occupying critical binding sites.⁷⁵ Plasma that lacks HK⁷⁶⁻⁷⁸ is almost as abnormal as factor XII-deficient plasma; that is, that rate of clotting on addition of a surface is markedly diminished because it is a cofactor for factor XI activation, and the factor XIIa needed is dependent almost exclusively on autoactivation. The formation of kallikrein and the activation of factor XII by kallikrein are virtually nil. Like factor XII- or prekallikrein-deficient plasma, no bradykinin is made because the substrate from which bradykinin is derived is absent. Bradykinin can be generated in all of these deficiencies by means of tissue kallikrein interaction with LK.

BINDING TO CELL SURFACES

Schmaier et al⁷⁹ and Van Iwaarden et al⁸⁰ first demonstrated that HK binds to endothelial cells and platelets in a zinc-dependent reaction. These data were soon confirmed, and both heavy and light chains of HK were shown to be responsible for binding.⁸¹ Ultimately, the site of binding was shown to be within domains 3 and 5 of HK (ie, domain 3 is the site of heavy-chain binding and domain 5 the site of light-chain binding).^{82,83} The amino acid sequence of the peptides within the domains were determined and are known as LDC 27 (heavy chain) and HKH 20 (light chain) peptides.

The binding of HK to the cell surface was not only zinc dependent but was saturable, reversible, and had a dissociation constant of 15 nmol/L, indicating high-affinity binding. Negligible amounts are internalized,⁸⁴ and bound HK can be cleaved by kallikrein to liberate bradykinin.⁸⁵ Also, factor XII was shown to bind to endothelial cells with a very similar dissociation constant, the binding again was zinc dependent, and HK and factor XII could displace each other from the cell surface (ie, they appeared to compete for binding to the same receptor or docking sites).⁸⁶

We next sought to identify the endothelial-cell proteins that might mediate this binding. To do so, we prepared solubilized cell-membrane fractions and, using an HK affinity column, passed the cell-membrane preparation over the column and, after extensive washing, eluted bound proteins with dilute acid. A number of bands were seen, but the most striking was at 34 kd. Therefore we transferred this band to nitrocellulose from which it was sequenced. The N-terminal amino acids were identical to the published amino acid sequence of the receptor for the globular heads of the C1q subcomponent of complement (gC1qR). We then demonstrated binding of both HK and factor XII to gC1qR; each was zinc dependent, and they could compete with each other for binding. The light chain of HK bound to gC1qR, but the heavy chain did not. C1q, however, did not compete for binding with HK or factor XII because C1q binds to the C-terminus of gC1qR, whereas HK and factor XII compete for a com-

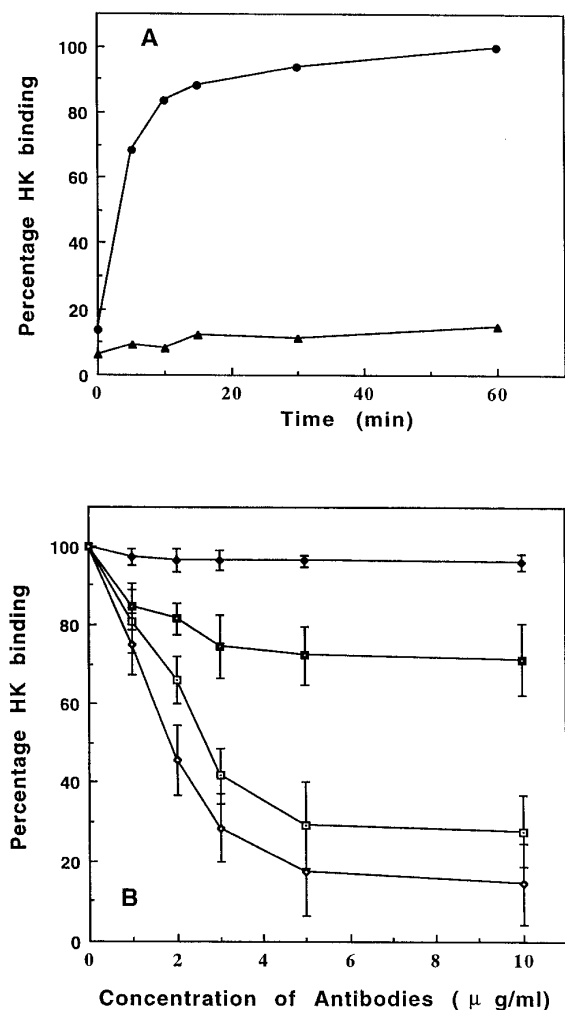


FIG 6. Iodine 125-labeled HK binding to HUVECs and its inhibition by mAbs. **A**, HUVECs were incubated with iodine 125-labeled HK (20 nmol/L) in the presence (filled circles) or absence (filled triangles) of 50 µmol/L zinc. **B**, For inhibition studies, cells were preincubated with mAbs or nonimmune mouse IgG for 30 minutes. The lines represent HK-binding values after treatment with an mAb to gC1qR (open squares), an mAb to u-PAR (filled triangles), an mAb to cytokeratin 1 (filled squares), a combination of mAbs to gC1qR and cytokeratin 1 (filled diamonds), and a control mouse IgG (filled circles). Each point is a mean of 3 different experiments performed in triplicate.

mon site at the N-terminus, as demonstrated by means of site-specific mAbs.⁸⁷ Herwald et al⁸⁸ came to the same conclusion, but they⁸⁹ and others⁹⁰ questioned whether sufficient quantities of gC1qR are expressed at the cell surface to mediate binding because gC1qR is a prominent intracellular molecule associated with mitochondria.

Meanwhile, we sought to identify the cell-binding site for the HK heavy chain, and to eliminate any possible light-chain binding to gC1qR, we coupled the peptide LDC 27 to prepare the affinity column that was used to fractionate endothelial-cell membranes. We used the same protocol as used in the isolation of gC1qR. A major protein was eluted at 68 kd, which was identified as cytokeratin 1.⁹¹ Cytoker-

atin 1 had previously been identified as one of the cell-binding proteins for HK,⁹² and a peptide derived from the N-terminal domain of cytokeratin 1 was shown to mediate binding.⁹³ We were then able to demonstrate that antibody to gC1qR inhibits HK binding to human umbilical cord endothelial cells (HUVECs) by 72%, that antibody to cytokeratin 1 inhibits binding by 30%, and that the combination of both antisera inhibits binding by 85% (Fig 6). These data support the expression of both proteins at the cell surface, despite the fact that neither of them has either a transmembrane domain or evidence of glycosylphosphatidylinositol linkage to membrane lipids. Subsequently, gC1qR has been demonstrated to be within the cell membrane by means of immunoelectron microscopy,⁹⁴ and it has been shown to be a receptor to which *Listeria monocytogenes* binds during cell infection⁹⁵; hepatitis C virus binds to it as well.⁹⁶

A third protein has been thought to mediate HK binding to HUVECs, as demonstrated by antibody inhibition,⁹⁷ but it has not been isolated from cell-membrane preparations. That protein is the urokinase plasminogen activator receptor (u-PAR), and a complex of u-PAR with cytokeratin 1 and gC1qR was proposed.⁹⁸ U-PAR does insert itself into cell membranes by means of glycosylphosphatidylinositol linkage, and immunoelectron microscopy has indeed demonstrated that u-PAR and cytokeratin 1 are in close proximity to each other⁹⁴; however, the number of gC1qR sites was far greater. This is perhaps consistent with estimates of 72,000 sites per cell for cytokeratin 1,⁹⁴ 250,000 sites per cell for u-PAR,⁹⁹ and over 1 million sites per cell for gC1qR.¹⁰⁰ We have since demonstrated that cytokeratin 1 can bind to both gC1qR and u-PAR,¹⁰¹ but gC1qR and u-PAR do not bind to each other. Thus cytokeratin 1 may be the link molecule that holds the other 2 together in complex formation, while u-PAR may provide a membrane linkage to anchor the complex. It would appear, however, that gC1qR is present in considerable excess, which is consistent with our observations that gC1qR accounts for a major percentage of the total binding of HK seen. If all 3 truly complex within the cell membrane, it is possible that as a result of binding to the complex or to gC1qR alone, a signal might be transduced to the cell, and studies to test this are in progress. A hypothetical diagram depicting the attachment of the kinin-forming cascade along the surface of endothelial cells is given in Fig 7.

The total zinc-dependent HK-binding site numbers on HUVECs are very large and have been variously extended to range from 1 to 10 million.^{79-82,102} Our own value was 3 million when estimated at 37°C. Thus other binding moieties are possible, and an additional one reported recently is cell-surface proteoglycan, with heparan sulfate side chains as the binding sites.¹⁰³ Proteoglycan can account, theoretically, for millions of sites, whereas protein receptors are usually in the tens or hundreds of thousands. But gC1qR may be an exception. How proteoglycan-binding sites (which are also zinc dependent and bind to HK domains 3 and 5) relate to the aforementioned docking proteins is not known.

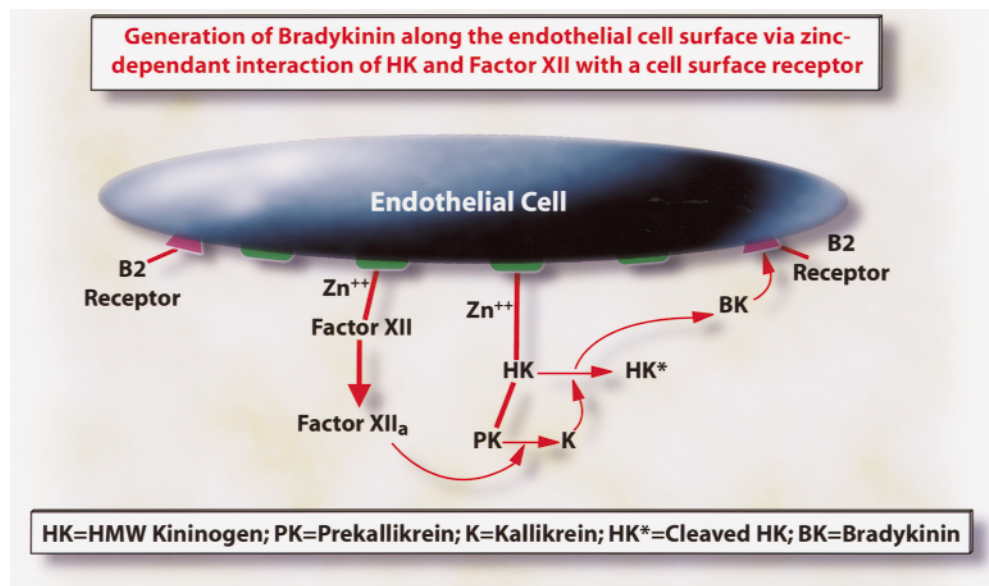


FIG 7. Generation of bradykinin along the endothelial-cell surface through zinc-dependent interaction of HK and factor XII with a cell-surface receptor (representing gC1qR, cytokerin 1, and u-PAR, perhaps as a complex).

ACTIVATION ALONG THE CELL SURFACE

The addition of factor XII, HK, and prekallikrein to HUVECs leads to activation of all of the components (ie, conversion to factor XII_a, kallikrein, and generation of bradykinin). Thus it would appear to be similar to traditional contact activation, although much slower.⁸⁶ We could demonstrate autoactivation of factor XII on binding to cloned gC1qR,¹⁰⁴ thus providing an initiation mechanism, and sulfated proteoglycans could behave similarly. When we tested whole plasma rather than purified components, we could demonstrate activation of normal plasma, as assessed by conversion of prekallikrein to kallikrein but not plasma deficient in factor XII (presumably lacking the initiator), prekallikrein, or HK (which is needed to bind to the cell surface as the prekallikrein-HK complex). The activation was inhibited with antisera to gC1qR and cytokerin 1 (Fig 8).¹⁰⁴ These data are most consistent with factor XII-dependent activation of the bradykinin-forming cascade on a cell surface. Motta et al,¹⁰⁵ however, have demonstrated that it is possible to activate the bradykinin-forming pathway by incubating HUVECs with prekallikrein and HK in the absence of factor XII, thus proposing a factor XII bypass dependent, perhaps, on a cell-surface enzyme or a secreted enzyme.^{106,107} It was theorized that HK interaction with the cell leads to the expression of this activity because activation was zinc dependent and HK needed to be added. Addition of prekallikrein alone did not lead to conversion to kallikrein, as would occur if factor XII_a were present. We have corroborated the existence of this bypass by extending the previous experiment in which HUVECs were incubated with normal plasma and various deficient plasmas for many hours.¹⁰⁸ The factor XII-deficient plasma did eventually activate, but the HK-deficient plasma did not. Furthermore, we have demonstrated that a cytosol fraction prepared for HUVECs

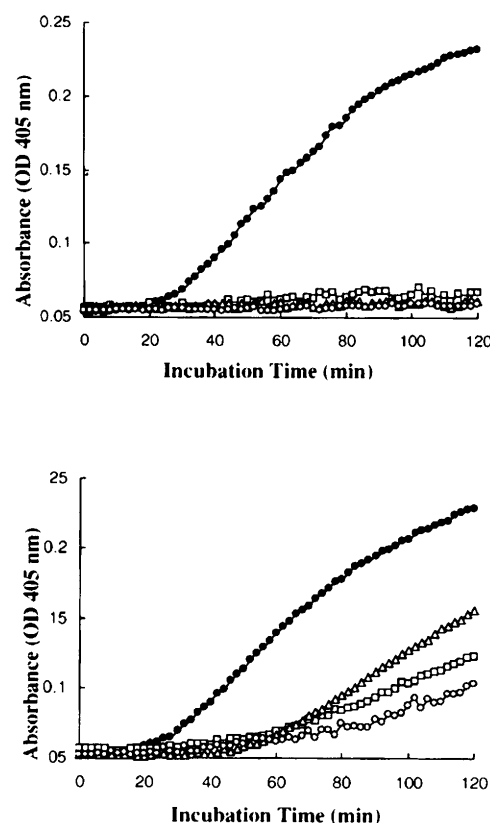


FIG 8. Prekallikrein activation on endothelial cells. **A**, Endothelial cells were incubated with normal (filled circles), prekallikrein-deficient (open circles), factor XII-deficient (open squares), or HK-deficient (open triangles) plasmas for 1 hour at 37°C and washed with zinc-containing buffer, and prekallikrein activation was monitored. **B**, Endothelial cells were preincubated with antibodies to cytokerin 1 (open triangles), gC1qR (open squares), or a combination of both (open circles) for 30 minutes before addition of normal plasma.

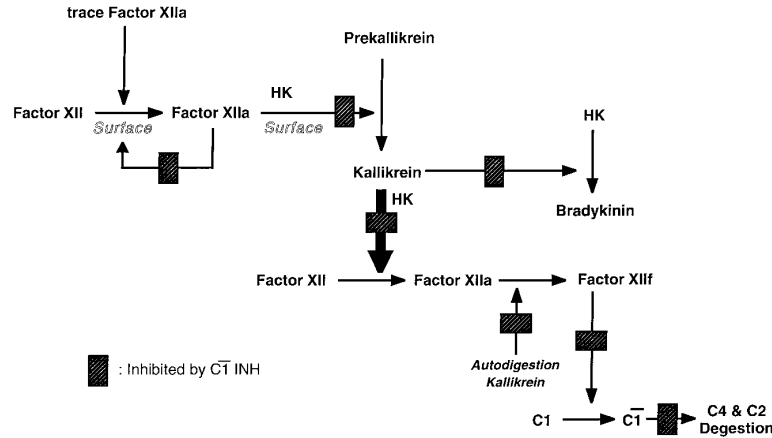


FIG 9. Pathway for formation of bradykinin, indicating all steps inhibitable by C1 inhibitor, as well as complement activation by means of factor XII.

contains a factor that converts prekallikrein to kallikrein but only if HK and zinc are present. However, there is no HK interaction with any cell membrane. Thus the new factor (? enzyme) digests prekallikrein only when it is complexed with HK in the presence of zinc ion, and HK and zinc are not required for binding to a cell surface but rather are cofactors for the enzymatic activity to be expressed. Purification of this protein is in progress.

It is of interest that if prourokinase is bound to u-PAR along endothelial cells and HK and prekallikrein is added, with or without factor XII, the kallikrein formed activates prourokinase to urokinase. If plasminogen is provided, it is converted to plasmin.¹⁰⁹ Thus fibrinolysis can be activated, along with the generation of bradykinin.

KININS AND C1 INHIBITOR DEFICIENCY

C1 inhibitor is the sole plasma inhibitor of factor XIIa and factor XIIf,^{110,111} and it is one of the major inhibitors of kallikrein,¹¹² as well as factor XIa.¹¹³ Thus in the absence of C1 inhibitor, stimuli that activate the kinin-forming pathway will do so in a markedly augmented fashion; the amount of active enzyme and the duration of action of the enzymes are prolonged. C1 inhibitor deficiency can be familial, in which there is a mutant C1 inhibitor gene, or it can be acquired. Both the hereditary and acquired disorders have 2 subtypes. For the hereditary disorder, type I hereditary angioedema (HAE; 85%) is an autosomal dominant disorder with a mutant gene (often with duplication, deletions, or frame shifts) leading to markedly suppressed C1 inhibitor protein levels as a result of abnormal secretion or intracellular degradation.¹¹⁴ Type 2 HAE (15%) is also a dominantly inherited disorder, typically with a point (missense) mutation leading to synthesis of a dysfunctional protein.¹¹⁵ The C1 inhibitor protein level may be normal or even elevated, and a functional assay is needed to assess activity. The acquired disorder also has 2 forms, although they may overlap in some patients. One group is associated with B-cell lymphoma¹¹⁶⁻¹¹⁸ or connective tissue disease,¹¹⁹ in which there is consumption of C1 inhibitor. Examples

are systemic lupus erythematosus and cryoglobulinemia, in which complement activation is prominent or immune complexes formed by anti-idiotypic antibodies to monoclonal immunoglobulin expressed by B-cell lymphomas.¹²⁰ The second form is an autoimmune disorder with a circulating IgG antibody to C1 inhibitor itself.¹²¹⁻¹²³ Acquired types have depressed C1q levels, whereas hereditary types do not, and depressed C4 levels characterize all forms of C1 inhibitor deficiency. The acquired autoimmune subgroup has a circulating 95-kd cleavage product of C1 inhibitor because the antibody depresses C1 inhibitor function yet allows cleavage by enzymes with which it usually interacts.¹²³⁻¹²⁵

It is now clear that depletion of C4 and C2 during episodes of swelling^{126,127} is a marker of complement activation but does not lead to release of a vasoactive peptide responsible for the swelling. Bradykinin is, in fact, the mediator of the swelling,¹²⁸⁻¹³⁰ and the evidence in support of this conclusion is summarized below. Patients with HAE are hyperresponsive to cutaneous injection of kallikrein.¹³¹ They have elevated bradykinin levels, and low prekallikrein and HK levels during attacks of swelling.¹³²⁻¹³⁴ The augmentation in complement activation seen at those times may be due to activation of C1r and C1s by factor XIIIf.³³ The presence of kallikrein-like activity in induced blisters of patients with HAE also supports this notion,¹³⁵ as does the progressive generation of bradykinin on incubation of HAE plasma in plastic (non-contact-activated) tubes,^{128,129} as well as the presence of activated factor XII and cleaved HK levels seen during attacks.¹³⁶ One unique family has been described in which there is a point mutation in the C1 inhibitor (A1a⁴⁴³ → Val) leading to an inability to inhibit complement but normal inhibition of factor XIIIa and kallikrein.^{137,138} No family member of this type II mutation has had angioedema,¹³⁷ although complement activation is present. In recent studies plasma bradykinin levels have been shown to be elevated during attacks of swelling in both hereditary and acquired C1 inhibitor deficiency,¹³³ and local bradykinin generation has been documented at the sites of swelling.¹³⁹ It is not known

TABLE I. Bradykinin in allergic diseases

Disease	Abnormality	Manifestation
HAE	(1) C1 inhibitor deficiency, genetic abnormality; (2) bradykinin overproduction caused by lack of enzyme inhibition	Angioedema
Acquired C1 inhibitor deficiency	(1) C1 inhibitor depletion caused by immune complexes (tumor antigen or autoantigen) or anti-C1 inhibitor; (2) bradykinin overproduction caused by lack of enzyme inhibition	Angioedema
Treatment with ACE inhibitors	Accumulation of bradykinin caused by inhibition of degradation	Cough, angioedema
Anaphylaxis	Massive enzymatic cleavage of HMW kininogen-direct bradykinin release, multiple enzymes	Hypotension
Rhinitis, asthma	Activation of bradykinin-forming pathways by sulfated mucopolysaccharides, endothelial-cell activation	Local edema, tissue hyperreactivity (nasal and airways), additional cytokine release

HMW, High molecular weight.

whether bradykinin generation is predominantly during the fluid phase, occurs along cell (endothelial) surfaces, or both. Fig 9 includes all the steps in the bradykinin-forming cascade that are inhibitable by C1 inhibitor.

KININS AND ACE INHIBITION

Severe angioedema often involving the face, tongue, or both is seen as a complication of the use of ACE inhibitors. It appears that this swelling is also a consequence of elevated levels of bradykinin¹³³; however, the accumulation of bradykinin is due to a defect in degradation rather than an excessive production. ACE, being identical to kininase II, is the major enzyme responsible for bradykinin degradation (Fig 1), and although it is present in plasma, the vascular endothelium of the lung appears to be its major site of action.¹⁴⁰ The action of ACE always leads to the formation of degradation products with no activity, whereas kininase I alone yields the des-Arg products, which are capable of stimulating B1 receptors.

The excessive accumulation of bradykinin implies that production is ongoing or that some event leads to activation of the plasma cascade or release of tissue kallikrein, and then faulty inactivation leads to swelling. Continuous turnover of the cascade is implied by data demonstrating activation along the surface of cells and cellular expression or secretion of a prekallikrein activator other than factor XII.

KININS AND ALLERGIC RHINITIS

Early studies suggested activation of the plasma bradykinin-forming cascade in allergic rhinitis on the basis of the finding of tosylarginine methyl ester esterase activity in the secretions, which is indicative of production of plasma kallikrein.¹⁴¹ Assessment of kinins by means of HPLC demonstrated the presence of both lysyl bradykinin and bradykinin during both the immediate phase and the late phase of allergen-induced rhinitis.^{142,143} The presence of lysyl bradykinin indicated release of tissue kallikrein, whereas bradykinin can be derived from lysyl bradykinin by the action of plasma aminopeptidase or it can be produced directly by plasma kallikrein. Both HK and LK were present, and therefore the preferred substrate for each

type of kallikrein was present.¹⁴⁴ Chromatographic assessment of the secretions demonstrated that both tissue kallikrein and plasma kallikrein are produced.^{145,146} Bradykinin can produce hyperemia, rhinorrhea, and nasal congestion; however, it has not been possible to assess its contribution to symptoms because a potent and selective bradykinin receptor antagonist that can be administered in vivo has not been available.

BRADYKININ AND ASTHMA

Kinins are present in bronchoalveolar lavage fluid. Early studies demonstrated the presence of tissue kallikrein in the bronchoalveolar lavage fluid,¹⁴⁷ but there has been no definitive assessment of the plasma bradykinin-forming cascade in bronchoalveolar lavage fluids or within lung parenchyma. Bradykinin challenge of asthmatic subjects leads to symptomatic and physiologic changes similar to those seen in the natural disease,¹⁴⁸ and allergen challenge of asthmatic subjects leads to an increase in kinin levels in conjunction with histamine release during both the early and late-phase time intervals.^{149,150} Of particular interest is that Icatibant (HOE 140), a B2 receptor antagonist, inhibited histamine-induced bronchial hyperresponsiveness induced by allergen in allergic rhinitis, and more prolonged treatment improves pulmonary function in asthmatic subjects followed for a 1-month interval.^{151,152} In rats a B1 receptor antagonist diminished airway hyperresponsiveness, suggesting induction of B1 receptors as a result of the inflammatory process.¹⁵³ A role for kinins in general is suspected as a mediator of bronchial hyperreactivity, but these need not be restricted to bradykinin or lysyl bradykinin and may involve neurokinins secreted from type C sensory nerve fibers, such as substance P, neurokinin A, or vasoactive intestinal polypeptide. Bradykinin may be a stimulus for secretion of these neuropeptides.¹⁵⁴

BRADYKININ AND ANAPHYLAXIS

The hypotension of anaphylaxis is clearly multifactorial, including the effects of multiple vasoactive mediators leading to hypovolemia and edematous tissues.

Bradykinin is certainly one of those capable of causing hypotension, and one study of insect-sting anaphylaxis demonstrated 100% cleavage of HK concomitant with changes in blood coagulation and fibrinolytic parameters indicative of disseminated intravascular coagulation.¹⁵⁵ Thus massive release of bradykinin seems likely, although kinin levels were not measured directly. It is of interest that in a rhesus monkey model of endotoxic shock, infusion of an mAb to factor XII leads to a 50% improvement in blood pressure, although disseminated intravascular coagulation was unaffected.

BRADYKININ FORMATION

The mechanism of bradykinin formation in any IgE-mediated reaction is multifactorial, including not only tissue kallikrein secretion, as noted above, but direct activation of the plasma cascade by secreted heparin,¹⁵⁶⁻¹⁵⁸ activation along the surface of endothelial cells, or exposure to connective tissue proteoglycans. Any local dilution of plasma constituents decreases the effect of protease inhibitors and leads to an increased rate of the enzymatic reactions to augment kinin formation. Plasma leakage can initially be the result of other permeability factor (eg, histamine or leukotriene C₄/D₄), which is then augmented as bradykinin is produced. A summary of the disorders discussed herein in which bradykinin is produced and represents either the direct cause of the clinical syndrome or a contributor to it is given in Table I.

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