Patho-Physiology of Kallikrein System

ROBERT W. COLMAN, M.D.

Thrombosis Center and Hematology/Oncology,
Section of the Department of Medicine,
Temple University School of Medicine,
Philadelphia, PA 19140

ABSTRACT

The properties of the contact factors, factor XII, high molecular weight kininogen and prekallikrein are described as well as the abnormalities in the hereditary deficiencies of these proteins. The interactions of each of these proteins with the other as well as their regulation by plasma proteolytic inhibitors such as Cl inhibitor and antithrombin III are delineated. Biochemical techniques for measuring this system are discussed. Conditions associated with abnormal synthesis of these proteins are described. Diseases in which increased kinin formation has been documented as well as disorders where there is strong evidence for the activation of kallikrein are presented. Further knowledge of this system should increase our understanding of its pathophysiological alterations.

Introduction

The plasma proteolytic enzyme systems of coagulation, fibrinolysis and kinin formation have been categorized as a "tangled web." The three interlocking networks are linked together in two ways. First, the initiating pathways are common and factor XII (Hageman) factor is a protein intimately involved in all. Second, at least four inhibitors with defined specificity each have the capacity to regulate more than one system. The current understanding of the three interlocking networks that mediate the vascular responses necessary for hemostasis, fibrinolysis and vasodilation (kinin forming system) are displayed in figure 1. The working of these enzymes illustrates the various control mechanisms possible in proteolysis, including activation of inactive precursors, positive feedback, stochiometric inhibition, multistep amplification and enzymatic degradation of active products.

Factor XII (a beta globulin), molecular weight 90,000, is first converted to an active derivative of the same size, factor XIIa, upon exposure to negatively charged foreign surfaces which in vivo is the subendothelial basement membrane. The importance of factor XII for initiation of the various plasma proteolytic systems is emphasized by the fact that Hageman factor deficient plasma not only has a very prolonged partial thromboplastin time (PTT), which measures the steps to the
formation of thrombin, but it also fails to support surface initiated fibrinolysis as well as the generation of bradykinin. These pathways have been shown to be mediated by the various Hageman factor substrates.

**Activation of Proteins**

Activation of other proteins is required for the coagulation pathway to proceed. Hageman factor activation of prekallikrein generates the enzyme kallikrein which digests kininogen to liberate the vasoactive peptide bradykinin. However, factor XIIa, though a potent coagulant, has relatively less activity in initiating kallikrein or plasmin formation. To perform these latter functions effectively, factor XIIa must be proteolysed to Hageman factor fragments (XII\(_{f}\)). The formation of these activators are a site for control since the eventual products of the reaction exert a positive feedback in their own formation.

Kallikrein can in the fluid phase cleave factor XII to Hageman factor fragments (molecular weight 30,000) which are much more potent in prekallikrein activation than the intact molecule.\(^{1}\) High molecular weight (HMW) kininogen has a central place in the scheme. It is the preferred substrate from which kallikrein releases bradykinin. It also potentiates the conversion of Hageman factor to activated Hageman factor on a surface\(^{22}\) and to Hageman factor fragments in the fluid phase.\(^{10}\) In addition, it potentiates the action of activated Hageman factor on clotting factor XI and Hageman factor fragments on the reciprocal activation of kallikrein.\(^{14}\) Kallikrein also directly activates plasminogen to plasmin, the enzyme responsible for fibrinolysis.\(^{4}\) Recently, it has been demonstrated by us that Hageman factor fragments can activate factor VII.\(^{21}\) Thus, a link is created between the intrinsic and extrinsic pathways. In the dissection of the system, patients with congenital abnormalities have disclosed new functions for old proteins.

**Thromboplastin Factor**

In 1965, Fletcher trait deficiency\(^{11}\) was described as an autosomal recessive disorder characterized by a diminished rate of surface mediated coagulation. This plasma possessed an unusual abnormality in that the partial thromboplastin time, a measure of overall intrinsic coagulation, returned to normal as the time of incubation with surface (kaolin) was increased. The factor in normal plasma which corrected the deficiency was partially purified. In 1972, the corrective protein was identified as prekallikrein.\(^{24}\) The Fletcher trait plasma also possessed an abnormality in the rate of surface mediated fibrinolysis and generated no bradykinin upon incubation with kaolin. This last abnormality is expected if the plasma contained no prekallikrein, but one would not have readily predicted an effect upon the coagulation and fibrinolytic pathways.

The presence of both of these abnormalities suggested an effect upon Hage-
man factor, one protein common to each pathway. Correction of both the coagulation and fibrinolytic defects of Fletcher trait plasma by activated Hageman factor in the absence of a surface demonstrated that the abnormality was a diminished rate of formation of activated Hageman factor. This positive feedback of kallikrein may account for the decrease of surface initiated fibrinolysis and coagulation in Fletcher factor trait.

An additional protein was found to be required for surface activation of Hageman factor as a result of investigation of three patients in different laboratories. In 1974, a 64-year-old woman Mrs. Williams, was referred, owing to an abnormal intrinsic coagulation system in which all known factors were normal. The results of further investigation of her coagulation defect revealed that the abnormal partial thromboplastin time test was corrected by one-quarter volume of Hageman trait or Fletcher trait plasma, indicating that these plasmas contained adequate concentrations of the missing factor in William's trait plasma. Unlike Fletcher trait plasma, prolonged incubation with kaolin failed to correct the coagulation abnormality of William's trait.3

A profound defect was also found in the fibrinolytic system of William's plasma. The addition of kaolin markedly increased the fibrinolytic potential of normal plasma shown as an increase of the reciprocal of the euglobulin lysis time (figure 2). As previously reported, Fletcher plasma showed a decreased rate of activation by kaolin. In contrast, William's plasma showed virtually no increase of fibrinolytic activity after exposure to kaolin. A mixture of equal volumes of William's and Fletcher plasma completely corrected the defect. Similar defects were found in the conversion of prekallikrein to kallikrein.

When kaolin was added to William's plasma, no bradykinin was detected by bioassay. Although this might have been due to the failure of prekallikrein activation, the addition of Hageman factor fragments or even purified plasma kallikrein did not initiate kinin formation indicating complete lack of kininogen. This result was confirmed by demonstrating that William's plasma did not react with an antiserum against kininogen. However, low molecular weight kininogen did not cor-
rect the fibrinolytic, coagulation and prekallikrein activation defects in William's plasma. About 15 percent of kininogen is in a high molecular weight form.

To determine whether or not the HMW kininogen is identical to William's factor, HMW kininogen was purified with the use of an immunoabsorbent column of monospecific kininogen antiserum. HMW kininogen ran as a single peak on disc electrophoresis. Elution of an unstained gel showed that the kininogen antigen and William's factor, as measured by a coagulant assay, were in the same position (figure 3). Thus, the major coagulant factor missing in William's plasma appears to be similar or identical to HMW kininogen which by itself corrects all the defects.

Evaluation of Abnormalities

In evaluating the abnormalities in deficient plasma, it became clear that prekallikrein and HMW kininogen are coagulation factors, both of which are required for Hageman factor activation and function. HMW kininogen has no known enzymatic activity, but it facilitates the generation of a proteolytic site upon surface bond Hageman factor. The Hageman factor activity generated is proportional to the HMW kininogen input. Upon the surface, HMW kininogen enhances the function of Hageman factor upon its substrates—prekallikrein to form kallikrein (bradykinin release) and factor XI to accelerate the PTT. The effect of kallikrein upon Hageman factor is presumed to be a direct one however since HMW kininogen augments this interaction.

In this system HMW kininogen could accelerate the formation of activated factor XI and/or enhance its activity on prekallikrein. One way to separate these two effects is to use Hageman factor fragments already active in the fluid phase. Figure 4 shows the dependence of the initial rate prekallikrein activation on kininogen concentration. The potentiation of Hageman factor fragments to convert prekallikrein to kallikrein is a function of the concentration of HMW kininogen. The maximum effect is seen at about 6 percent of the high molecular weight kininogen in normal plasma as determined by a clotting assay using William's plasma as a substrate. At this concentration more than a three fold augmentation of the initial rate is observed.

The characteristics of a group of proteins which are mainly serine proteases and their cofactors have been discussed. It should be no surprise that the most effective inhibitors of these pathways are protease inhibitors. Seven naturally occurring plasma inhibitors of proteolytic enzymes have been described. Two of these proteins, $\alpha_2$ macroglobulin and $\alpha_1$ antitrypsin, make up about 10 percent of total plasma proteins by weight. All of these proteins are globulins of widely varying molecular weight. In considering the role...
Figure 5. Inhibition of kallikrein's esterolytic activity by antithrombin-heparin cofactor. Kallikrein and antithrombin-heparin cofactor were incubated at 25°C in the presence (Δ) and absence (○) of heparin. The buffer used for these experiments was 0.15 M sodium chloride in 0.01 M sodium phosphate (pH 7.6). All reactants were extensively diluted in or dialyzed against this buffer. The final concentrations of enzyme, inhibitor and acidic mucopolysaccharide (if employed) were 250 μg per ml (2.5 μmol of TAME hydrolyzed min⁻¹ ml⁻¹), 75 μg per ml (75 units per ml) and 1 unit per ml, respectively. At appropriate times, aliquots were withdrawn and residual kallikrein activity was determined by esterolytic assay as mentioned in Evaluation section. During the time course of these experiments, the esterolytic activity of kallikrein remained stable when incubated at 25°C with buffer. The results presented are representative of five separate experiments and are expressed as percentages of zero time activity prior to the incubation of kallikrein with antithrombin-heparin cofactor.

One way to approach this problem is to compare the effect of plasma congenitally deficient in a single inhibitor with the inhibitory capacity of normal plasma. A second is to study the interaction of purified inhibitors with the enzyme in question. To demonstrate the stoichiometry of the reaction of kallikrein with C1 inhibitor, 1600 units of purified human inhibitor were incubated with increasing concentrations of kallikrein for 30 minutes. The residual kallikrein and C1 inhibitor were then measured. The exact parallelism demonstrates stoichiometric interaction between kallikrein and C1 inhibitor.

Upon incubation with plasma kallikrein, the C1 inhibitor is depleted (as measured in a hemolytic assay) and the esterolytic activity of kallikrein is suppressed. Similar results were shown for kallikrein release of bradykinin. Studies of the inhibition of purified kallikrein by plasma from normal patients and patients with hereditary angioneurotic edema showed that the ability of plasma to inhibit kallikrein paralleled the C1 inhibitor concentrations and was markedly decreased in hereditary angioedema.

The effect of another major inhibitor anti-thrombin III with kallikrein has been examined by us. It is demonstrated in figure 5 that purified anti-thrombin III inactivates the esterolytic activity of purified kallikrein. The process with inhibitor in excess obeys pseudo first order kinetics. Heparin (lower curve) appears to accelerate the rate of inactivation. Similar results are obtained when the ability of kallikrein to release bradykinin measured in a radioimmunoassay is measured indicating that the anti-thrombin III affects the proteolytic activity of kallikrein as well as its esterolytic activity.

Once again, the accelerating effect of heparin was seen. In normal plasma, antithrombin III apparently plays little role in inhibiting kallikrein. Thus, the degree of inhibition by normal plasma is not altered by addition of as much as 50 units per ml heparin. In contrast, plasma from patients with hereditary angioedema had little inhibitory capacity measured by esterase or bradykinin release. This inhibitory capacity is markedly increased by adding...
PATHO-PHYSIOLOGY OF KALLIKREIN SYSTEM

I. Deficiency of Proteins Necessary for Kinin Formation

A. Hereditary
   1. Factor XII deficiency (Hageman trait)
   2. Prekallikrein deficiency (Fletcher trait)
   3. High molecular weight kininogen deficiency (Williams, Fitzgerald and Flaujeac traits)

B. Acquired
   1. Dengue hemorrhagic fever
   2. Cirrhosis of the liver

II. Acquired

A. Dengue hemorrhagic fever
B. Cirrhosis of the liver

TABLE I

<table>
<thead>
<tr>
<th>Therapeutic concentrations of heparin. These observations suggest a potential role for heparin in treating the acute manifestations of the disease.</th>
</tr>
</thead>
<tbody>
<tr>
<td>With this background, the role of the kallikrein system can be surveyed in human disease. In table I are summarized conditions which are associated with abnormal synthesis of one or more of the proteins involved in this system. The three hereditary syndromes have been previously discussed. In dengue hemorrhagic fever, a selective deficiency of plasma prekallikrein occurs probably owing to the abnormal liver function studies. In cirrhosis of the liver, the mean prekallikrein levels are depressed to about 25 percent of normal and in the several cases, concentrations of 0 to 10 percent are usual. In contrast, factor XII is decreased to about 40 percent of normal, and HMW kininogen is reported reduced to a similar level.</td>
</tr>
<tr>
<td>Of greater interest are the conditions in which increased kinin formation has been documented (table II). In carcinoid syndrome and postgastrectomy dumping syndrome, the vasomotor symptoms are related to bradykinin release by either tissue or plasma kallikrein. Septic shock is accompanied in man by decreased prekallikrein and kallikrein inhibitory activity, formation of active kallikrein and elevated kinin levels. The latter may contribute to the early phases of reversible hypotension. Elevated bradykinin levels in pancreatitis are probably due to release of trypsin and pancreatic kallikrein.</td>
</tr>
</tbody>
</table>

TABLE II

Disorders Leading to Increased Kinin Formation

<table>
<thead>
<tr>
<th>I. Hereditary</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Hereditary angioedema</td>
</tr>
<tr>
<td>II. Acquired (documented)</td>
</tr>
<tr>
<td>A. Carcinoid syndrome</td>
</tr>
<tr>
<td>B. Postgastrectomy dumping syndrome</td>
</tr>
<tr>
<td>C. Septic stock</td>
</tr>
<tr>
<td>D. Hemorrhagic pancreatitis</td>
</tr>
</tbody>
</table>

TABLE III

Disorders Leading to Kallikrein Activation

<table>
<thead>
<tr>
<th>I. Acquired (suspected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Coronary artery disease</td>
</tr>
<tr>
<td>B. Intravascular coagulation</td>
</tr>
<tr>
<td>C. Hyperlipoproteinemia</td>
</tr>
<tr>
<td>D. Transfusion reactions</td>
</tr>
<tr>
<td>E. Typhoid fever</td>
</tr>
<tr>
<td>F. Nephrotic syndrome</td>
</tr>
</tbody>
</table>

Kinin formation not documented.

TABLE IV

Disorders in Which Kallikrein-Kallikrein Interactions Have Been Suggested

<table>
<thead>
<tr>
<th>I. Acquired</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Thermal injury</td>
</tr>
<tr>
<td>B. Arthritis</td>
</tr>
<tr>
<td>C. Allergic disorders</td>
</tr>
<tr>
<td>D. Cystic fibrosis</td>
</tr>
<tr>
<td>E. Migraine syndrome</td>
</tr>
</tbody>
</table>
levels lead to kallikrein factor XI and plasminogen activation.\textsuperscript{13}

Finally, kallikrein activation has been suggested to occur in various conditions but none of these have been shown definitely (table IV). Further investigation is important to appreciate the role of this defensive system in inflammatory disorders.

References


