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# Assembly, activation, and physiologic influence of the plasma kallikrein/kinin system

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**Abstract**

The plasma kallikrein/kinin system that consists of the proteins factor XII, prekallikrein, and high molecular weight kininogen was first recognized as a surface-activated coagulation system arising when blood or plasma interacts with artificial surfaces. Although surface-activated contact activation occurs *in vivo* when various negatively charged surfaces become exposed, including a developing platelet thrombus, a physiologic, non-injury mechanism for activation, regulation, and function of this system has been elusive. Recent investigations have shown that there is a physiologic pathway for assembly and activation of this system independent of factor XII. Gene deficient mice of the bradykinin B2 receptor and factor XII have been recognized to have reduced risk for arterial thrombosis. This plasma proteolytic system influences arterial thrombosis independent of influencing hemostasis. Thus, the plasma kallikrein/kinin system has two mechanisms for its activation: one that is dependent and another independent of factor XII. Better understanding of this system may lead to insight into mechanisms for arterial thrombosis, independent of hemostasis.


There have been several major advances in understanding on the plasma kallikrein/kinin system not completely addressed in recent reviews [1,2]. These include 1) a proposal of a model for a multiprotein receptor complex that mediates assembly and activation of the zymogens of this system, 2) identification of a specific prekallikrein (PK) activator that provides a physiologic basis for the initiation of activation of factor XII (FXII) independent of autoactivation, and 3) several animal models have been established and their influence on thrombosis risk has been determined.

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## 1. Intravascular assembly of the plasma kallikrein/kinin system

It was recognized that high molecular weight kininogen (HK), FXII, and PK specifically, saturably, and reversibly bind to cultured endothelial cells, platelets, and granulocytes [3–8]. Efforts have identified specific binding sites, putative receptors, for HK and FXII. HK serves as the major binding site for PK and FXI, although both proteins can bind to endothelial cells independent of HK [3,9]. Several membrane proteins have also been recognized as HK binding proteins. These include gC1qR, urokinase plasminogen activator receptor (uPAR), and cytokeratin 1 [10–13]. When HK is

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proteolyzed by plasma kallikrein or other proteases to form HKa, membrane tropomyosin also functions as a binding site, putative receptor, uniquely for this form of kininogen [14]. FXII also has been shown to bind to gC1qR, uPAR, and CK1 [11,15]. Heparan sulfate proteoglycans, a negatively charged surface, has also been proposed as additional cell membrane sites for HK binding, although there is disagreement as to the degree it contributes to HK expression on cells [16–18]. PK binds to HK on cell membranes; PK binding sites other than HK have not been identified to date even though it has been demonstrated to specifically bind to endothelial cells independent of HK [3,19]. Factor XI also binds to HK on cell membranes, but unlike PK, also binds to prothrombin and the glycoprotein Ibα-IX-V complex on platelets as well [19–21]. Since both PK and factor XI circulate in plasma almost completely bound to HK, PK binding to endothelial cells predominates over factor XI binding [19,22]. The reasons for this finding are as follows: 1) the concentration of PK (450 nM) is more than 10-fold greater than that of factor XI (30 nM) in plasma and 2) the free $Zn^{2+}$concentration required for PK binding is only 0.3 μM, while that for factor XI binding is 7 μM [19]. In order to have factor XI binding to cells in the intravascular compartment or activated platelets, cellular disruption is necessary to provide a sufficiently high local zinc ion concentration in their releasate or lysates to support this interaction [19].

## 2. Activation of the plasma kallikrein/kinin system

Although the above investigations indicate a mechanism for physiologic assembly on cultured endothelial cells, they do not indicate a pathway for activation of the system. Insight into an activation mechanism arose out of serendipity while performing investigations to examine the mechanism(s) by which single chain urokinase becomes activated by plasma kallikrein [23]. When HK and PK were assembled on endothelial cells, plasma kallikrein activity arose independent of added FXIIa and was able to occur in the presence of neutralizing antibody to FXII and FXII-deficient plasma, but not PK-deficient plasma [3]. These results were not expected since a mechanism for PK activation independent of activated FXII had not been previously identified. Similar results occurred when HK and PK were assembled on endothelial cell matrix [24]. Formed plasma kallikrein results in kinetically favorable single chain urokinase activation in this system [3,25]. Plasma kallikrein forms when HK and PK assemble on endothelial cells and results in kinetically favorable factor XII activation [26]. These data present an alternative hypothesis for factor XII activation independent of autoactivation on a negatively charged artificial or biologic surface. The increased requirements for free $Zn^{2+}$ for FXII binding to endothelial cells suggest that FXII's association and activation on endothelial cells follows HK and PK assembly and activation [15,19]. Two recent animal knockouts suggest that this proposed mechanism for PK activation *in vivo* may occur constitutively. First, C1 INH knockout mice have constitutive vascular leakage that is blocked by a bradykinin B2 receptor (B2R) antagonist or by mating C1 INH and B2R knockout mice [27]. Since plasma bradykinin only arises from plasma kallikrein formation, bradykinin must be constantly formed *in vivo* to give the constitutive paw edema seen in the C1 INH deletion mice [27]. Bradykinin formation can only occur if plasma kallikrein is formed *in vivo*. Second, factor XII knockout mice still have formed bradykinin in their plasma indicating that at least one other mechanism for bradykinin formation exists in the intravascular compartment [28].

Proof that there is a PK activator on endothelial cells required that the activator be identified. After establishing an assay to measure the PK activator, the entity was purified from endothelial cell cultures [4]. On amino acids sequencing, the isolated PK activator was identified to be the serine protease prolylcarboxypeptidase (PRCP) [4]. PRCP was first recognized as a degrading enzyme for bradykinin and angiotensin II [29,30]. This enzyme inactivates bradykinin and angiotensin II with a $K_i \simeq 1$ and 0.15 mM, respectively, by cleaving Pro-X bonds on the carboxy-terminus of the protein [30,31]. Interestingly, des-$Arg^9$-bradykinin (RPPGFSPF) is not a substrate for PRCP indicating that it is not just a simple exopeptidase cleaving after Pro-X bonds [32]. Both purified and recombinant PRCP activate PK with a $K_m$ ~9–17 nM making it an endoprotease [4,31]. PRCP also was associated with endothelial cell matrix [32]. Although originally purified from lysosomes, PRCP is a membrane protein since it is constitutively expressed on endothelial cell membranes where it is functional and upon gene trap targeting it was interrupted along with other membrane proteins by homing with a CD4 membrane signal [3,31,33–35]. More recent cell biology studies since PRCP was first described as a lysosomal protease suggest that lysosomal compartments in all eukaryotic cells are the endomembrane system that is intimately involved in the export of internal constituents [36]. PRCP is a risk factor for metabolic syndrome in men and a PRCP polymorphism is associated with preeclampsia in women [37,38]. Further conformation that PRCP is a PK activator was performed by over expressing PRCP in CHO cells [39]. CHO cells with over-expressed PRCP have increased PK activating activity over control; treatment of these cells with siRNA ameliorates the PK activation on these cells and control cells. Last, transfected CHO mostly express PRCP on their membrane. These combined studies indicate that there is a constitutive, physiologic mechanism for PK activation on endothelial cells in the intravascular compartment.

## 3. Thrombosis risk

There is emerging information that the plasma kallikrein/kinin system influences thrombosis risk. This influence on thrombosis risk is independent of any influence on hemostasis. Although patients with factor XII, prekallikrein, and high molecular weight kininogen deficiency do not have bleeding disorders, all of these deficiencies are exceedingly rare and characterization of the phenotypes of these individuals is not been possible due to the dispersed small patient population. FXII deficiency is most common. Clinical investigations for venous thrombosis risk or on polymorphisms of FXII and their influence on cardiovascular disease have been conflicting (see below). The clearest information on thrombosis risk or risk amelioration shown to date has been derived from mouse models of this system, in which