Assembly of the Human Plasma Kinin-Forming Cascade along the Surface of Vascular Endothelial Cells

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Key Words

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Abstract

The generation of bradykinin by contact activation requires autoactivation of factor XII (Hageman factor) upon initiating surfaces, conversion of prekallikrein to kallikrein, and digestion of high-molecular-weight (HMW) kininogen. Endothelial cells have a high-affinity receptor that binds either HMW kininogen or factor XII in a zinc-dependent interaction, and activation of factor XII can occur along this surface to initiate kinin formation. Tissue injury, exposure of proteoglycans, or release of mast cell heparin will markedly accelerate these reactions. The bradykinin released binds to endothelial cell B-2 receptors along the inner surface of blood vessels which results in dilatation and increased vascular permeability.

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The generation of bradykinin in human plasma is dependent upon interaction of three plasma problems, namely, factor XII (Hageman factor), prekallikrein, and high-molecular-weight kininogen (HK). Factor XII autoactivates upon negatively charged initiating macromolecules such as proteoglycans. Factor XII then converts prekallikrein to kallikrein and kallikrein digests HK to release the vasoactive peptide bradykinin [1]. Bradykinin then acts upon B-2 receptors of vascular endothelial cells which results in vaso-dilatation and increased vascular permeability [2].

Since endothelial cells are the targets of the resultant peptide, we have examined the interaction of each of the plasma proteins required for kinin formation with endothelial cells, and have characterized a receptor that binds either factor XII or HK in a zinc-dependent reaction [3-5].

There is no specific receptor for prekallikrein; however, prekallikrein circulates bound to HK [6] as a bimolecular complex and is brought to the cell surface by virtue of HK binding.

The interaction of HK with human umbilical vein endothelial cells (HUVECs) was first described by Schmaier et al. [7] and van Iwaarden et al. [8] in which a high-affinity receptor was described which required 15-50 μ M zinc for specific binding. We have further characterized this interaction and demonstrated that binding is saturable, reversible, with 1×10^6 - 10^7 sites/cell and a dissociation constant of 40-50 $\times 10^{-6}$ M. A similar interaction with platelets has been observed that is also zinc dependent, of comparable affinity, but with 50-fold fewer sites.

The structure of HK is complex. The N terminus consists of three sequential domains homologous to cysteine protease inhibitors such as cystatins. The second and third domains function in this manner and inhibit certain intracellular cathepsins. These three domains comprise the heavy chain. It is followed by the bradykinin sequence and then the light chain. The latter portion of HK has a surface binding site at its N terminus followed by a binding site for either prekallikrein or coagulation factor XI. By cleaving kininogen with kallikrein to release bradykinin followed by reduction and alkylation, the heavy and light chains can be isolated and tested individually for binding to endothelial cells. To our surprise, either chain could bind to endothelial cells and each one could displace or compete with binding of in-

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tact HK whereas bradykinin could not. The binding of each chain was zinc dependent and of similar affinity, however, the number of calculated light-chain sites exceeded that for the heavy chain; the light-chain interaction may be primary. Since only a single high-affinity site for HK is found after Scatchard binding analysis, we propose that subsites exist that interact with each chain.

Berrettini et al. [9] reported binding of prekallikrein or factor XI to HUVECs that had been saturated with HK. Thus, as in plasma [6], prekallikrein can attach to a surface by virtue of its interaction with HK. When we tested ¹²⁵I-prekallikrein for direct binding to HUVECs, binding was observed, but it was neither suitable nor specific and therefore not consistent with a receptor-dependent interaction.

We next studied factor XII interaction with HUVECs, and observed binding that was zinc dependent, saturable, and reversible with a K_d of 140 nM, and 107 binding sites/cell. These data were strikingly similar to those obtained with HK. We questioned whether each protein binds to the same receptor or to separate receptors and determined that if ¹²⁵I-factor XII was first bound to HUVECs, it could be displaced with a 100-fold molar excess of factor XII or, if ¹²⁵I-HK was bound first, it could be displaced by excess factor XII [9]. These data are consistent with competition for binding to the same receptor. Given the plasma concentration of each protein and the number and affinity of the binding sites, HUVECs would normally be saturated with factor XII or the prekallikrein-HK complex. Thus all the components required for bradykinin formation are present. These interactions are shown in figure 1.

Finally, we have isolated the heparin proteoglycan from the Furth murine mastocytoma and tested it for the ability to

HK≈HMW Kininogen; PK≈ekall¹/₈rein; K=Kallikrein; HK*≈Cleaved HK; BK=Bradykinin

Fig. 1. Generation of bradykinin along the endothelial cell surface via zinc-dependent interaction of HK and factor XII with a cell surface receptor.

activate a mixture of factor XII and prekallikrein. Conversion to kallikrein is observed as the concentration of mast cell heparin is increased. The activity is inhibited by heparinase I or II but not by heparitinase or chondroitinase ABC. Of the glycosaminoglycans tested, heparin, dermatan sulfate, keratin polysulfate, and chondroitin sulfate C are positive, in that order of potency, while

heparan sulfate and chondroitin sulfate A are negative. Collagen types I, III, IV, and V, laminin, fibronectin, and vitronectin are also negative. These observations suggest that one mechanism by which bradykinin may be generated in allergic reactions is via secretion of mast cell heparin or related glycosaminoglycans from perivascular mast cells. Fluid-phase augmentation will occur as permeability factors such as histamine, platelet-activating factor, or leukotriene C4 recruit plasma factor XII and the PK-HK complex such that activation may be sustained or augmented during the late-phase reaction [10].

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