The Endocytosis Mediated by Proteoglycans Controls Plasma Kallikrein/Kinin System Activity

Guacyara Motta and Ivarne L. S. Tersariol

Dep de Bioquímica, Escola Paulista de Medicina, UNIFESP, SP, Brasil

Our studies have focused on the influence of heparan sulfate proteoglycans (HSPG) interaction and activation of human plasma proteins high molecular weight kininogen (H-kininogen) and prekallikrein on cell surface. The cell lines used were CHO-K1 (wild type) or CHO-745 (mutant deficient in xylosiltransferase). The binding studies were performed using biotin-H-kininogen; H-kininogen or prekallikrein intracellular localization were analyzed by confocal fluorescence microscopy; both prekallikrein and H-kininogen structures were analyzed by immunoblotting and bradykinin release was also measured by radioimmunoassay. The interaction between biotin-H-kininogen and CHO cells was a temperature and energy dependent process and was due to an increase in more binding sites on the cell surface of living cells at 37ºC. In CHO-K1 the biotin-H-kininogen interaction was inhibited strongly by heparin (82%) and heparan sulfate (78%) comparing to chondroitin 4-sulfate inhibition (47%). The H-kininogen internalized in CHO-K1 (2,719.00 pixels/cells), but not CHO-745 (225.00 pixels/cell) and colocalized with LysoTracker in endosomal acidic vesicles, which was inhibited by sodium chlorate, chloroquine, FCCP and 2-deoxy-D-glucose. The endocytosis process was lipid raft-mediated dependent on caveolae but independent on clathrin. Both CHO cells did not internalize bradykinin-free H-kininogen. The H-kininogen endocytosis in CHO-K1 was dependent on exogenously added zinc. Prekallikrein colocalized with LysoTracker in CHO-K1, independent of exogenously applied H-kininogen, and no prekallikrein internalization was observed in CHO-745. The prekallikrein cleavage/activation was independent of glycosaminoglycans but kallikrein formation is more specific in the presence of H-kininogen assembled on the cell surface through glycosaminoglycans. At pH 7.35 bradykinin release from H-kininogen on surface of CHO-K1 involved either serine or cysteine proteases; nevertheless, in CHO-745 only serine proteases released bradykinin. The lysate fractions prepared from CHO-K1 and purified on antipain-Sepharose hydrolyzed intact H-kininogen indicating the presence of different endogenous kininogenases. The endocytosis mediated by HSPG is an important mechanism in cell biology activities of plasma kallikrein/kinin system.

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