Tuesday, July 14, 1981

Poster Presentations

Platelets – X

Membrane Glycoproteins

11:00–12:30 h

Kenora Room Boards 113–124

0325

THE ROLE OF GLYCOPROTEIN V (GPV) IN THROMBIN ACTIVATION OF HUMAN PLATELETS. K. Fujimura, S. Maehama and A. Kuramoto. Department of Medicine, Research Institute for Nuclear Medicine and Biology, Hiroshima University, Hiroshima, JAPAN

The analysis of platelet membrane glycoproteins and platelet functions was conducted to disclose the role of GPI and V in the thrombin activation of platelet. Our previous study proved that native and HNB thrombin hydrolyzed GPV(M.W.8-9 x 10^4) selectively and released new glycoprotein fragment (M.W. 6.2-6.8 x 10^4) of GPV, resulting in the development of ^{14}C-5HT release reaction and platelet MDA production. But DIP thrombin could not induce these phenomena.

Membrane surface proteins of intact platelets were labeled with Na[^{3}H]BH_4 by neuraminidase and galactose oxidase method and analyzed by fluorography after SDS-PAGE.

The high molecular weight glycoproteins,GPI,GPIII and GPV were diminished by trypsin treatment in correlation with the concentration and incubation time. In correspond to the diminution of these membrane glycoproteins, platelet release reaction was increased.Chymotrypsin treatment in various concentrations, release reaction and MDA production were not induced in spite of long incubation times. But the ristocetin aggregation was decreased in chymotrypsin treated platelets whose membrane glycoproteins did not change significantly. The chymotrypsin treated platelets whose GPI was modified functionally, showed normal release reaction and MDA production by thrombin stimulation. On the other hand, the thrombin treated platelets in low concentration previously whose GPV was hydrolyzed partially, demonstrated little release reaction and MDA production by thrombin or trypsin stimulation. From these results, the GPV was hydrolyzed specifically by thrombin and nonspecifically by trypsin but was not hydrolyzed by chymotrypsin. It was concluded that the thrombin binds to the GPI and hydrolyzed GPV specifically, and hydrolysis of GPV might act as a signal to induce the platelet release reaction and prostaglandin metabolism.

0326

FURTHER OBSERVATIONS ON THE POSSIBLE ASSOCIATION OF HUMAN PLATELET MEMBRANE GLYCOPROTEINS IIb and IIIa. J-P Rosa, D.Pidard, T. Kunicki and A.T. Nurden. U150 INSERM,Hopital Lariboisière, Paris, France.

Studies are described which represent a continuation of our investigation into the role of membrane glycoproteins (GP's) IIb and IIIa during human platelet aggregation. The surface proteins of washed platelets were labelled with ^{125}I by the lactoperoxidase-catalysed method prior to membrane isolation by the glycerol lysis procedure. Solubilisation of the membrane proteins by triton X-100 was followed by their analysis by crossed immunoelectrophoresis (CIE) using a rabbit antibody prepared against normal human platelets. In the absence of divalent cation chelation GP IIb and IIIa were contained within a single ^{125}I-labelled immunoprecipitate. When the isolated membranes were solubilised by triton X-100 in the presence of 5mM EDTA,GP IIb and IIIa formed distinctand separate immunoprecipitates during CIE. In order to further investigate this finding ^{125}I-labelled membrane proteins solubilised by triton X-100 in the presence or absence of EDTA were subjected to centrifugation for 18 h at 100,000 g over a 10-40% sucrose gradient containing the nonionic detergent. The results confirmed that in the presence of divalent cations IIb and IIIa were associated in a complex, and that this complex is dissociated by EDTA. The IgG..L is an alloantibody isolated from a polytransfused thrombasthenic patient that has been shown in previous studies to inhibit ADP-induced platelet aggregation and the binding of ^{125}I-fibrinogen to normal human platelets in the presence of ADP. When the IgG..L was incorporated in an intermediate gel during CIE it was shown to precipitate the complex containing IIb/IIIa but under identical conditions it did not precipitate the individual glycoproteins dissociated by EDTA. Divalent cation-mediated changes in the orientation of IIb and IIIa in the platelet membrane should be considered in assessing the role of these GP's in platelet function.

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.