INVITED SYMPOSIUM XVI

Platelet Receptors.

STUDIES ON RISTOCETIN INDUCED PLATELET AGGREGATION. R.L. Nachman, E.A. Jaffe and B.B. Weksler. Cornell University Medical College, New York, N.Y.

Human platelets washed and fixed in paraformaldehyde aggregate in the presence of the antibiotic ristocetin and normal plasma. This aggregation response is abolished after digestion of the fixed platelets with chymotrypsin. Antisera to fixed washed platelets were produced in rabbits and absorbed with chymotrypsin-treated, fixed washed platelets. Monovalent Fab fragments obtained from the isolated γ -globulin fractions of the antisera blocked ristocetin-induced aggregation of fixed washed platelets in buffer and normal platelets in platelet-rich plasma. By double-antibody immunoprecipitation, it was shown that the antibody which blocked the ristocetin reaction interacted with a platelet membrane surface protein of mol.wt.155,000. Partially purified preparations of membrane glycoprotein I obtained from wheat germ affinity columns block the ristocetin reaction. The results suggest that the glycoprotein I complex on the surface of the human platelet mediates ristocetin-induced von Willebrand factor-dependent platelet aggregation.

CELLULAR THROMBIN BINDING. B.M. Martin and T.C. Detwiler. S.U.N.Y. Downstate Medical Center, Brooklyn, New York, U.S.A.

The clot promoting enzyme thrombin induces cell-specific changes in washed human blood platelets and cultured chick embryo fibroblasts. In the attempt to understand the mechanism of the enzyme-cell reaction, the association of ^{125}I labeled human α -thrombin with these cells was studied. The conditions chosen for the binding studies enabled parallel investigation of the effect of thrombin on these cells. The results indicate that for both cell types studied, most of the thrombin remains free in solution, however the thrombin binding characteristics of these cells appear to be different. The binding of thrombin to platelets is rapid, with maximum binding occurring within 15 sec. Thrombin is bound reversibly to platelets and rapidly dissociates by dilution of the reaction mixture or by the addition of unlabeled thrombin. The thrombin platelet association can be described as a complex equilibrium with the apparent number of binding sites and dissociation constants dependent on the thrombin concentration. In contrast, the thrombin binding to avian fibroblasts is slow, with maximum binding occurring within 4 to 8 hours. While the enzyme is also reversibly bound to fibroblasts, it is more tightly associated with these cells since it is not easily removed by washing the cultured cells. Furthermore, the majority, 70-80 % of the bound enzyme is not removed by trypsin and appears to be inside the cell. The cell associated thrombin has the same molecular weight as native α -thrombin and retains catalytic activity. Cell fractionation studies show that the majority of the cell-associated thrombin is located in the nuclear fraction since the ^{125}I -label co-purifies with radiolabeled thymidine.