CROSS LINKING OF HUMAN PLATELET MEMBRANE AMINOPHOSPHOLIPIDS: CHANGES INDUCED BY THROMBIN.

P.K. Schick and P. Arosteguy. Medical College of Pennsylvania, Philadelphia, Pennsylvania, USA.

The proximity of the aminophospholipids, phosphatidylethanolamine (PE) and phosphatidylserine (PS), in platelet membranes was probed with difluorodinitrobenzene (D) which reacts with and cross-links aminophospholipids when they are less than $5 \text{ \AA}$ apart. D can penetrate intact cells and labels lipids on either side of the membrane bilayer. The incubation of mixtures of pure PE and PS can result in the formation of the following derivatives: PE-D-PE, PE-D-PS, PE-D, PS-D-PS, PS-D. Following incubation of washed platelets with D, lipids and derivatives were extracted, separated by thin-layer chromatography, and quantitated by lipid phosphorus. The incubation of platelets with D ($125 \mu\text{M}$) for 2 hours resulted in derivatization of 49% PE and 34.6% PS. When platelets were treated with thrombin (0.1 U/ml) for 5 minutes prior to incubation with D, 71.4% PE and 53.8% PS reacted. Results are shown in Table; %PE(PS) = % total platelet PE(PS).

	PE-D-DE	PE-D-PS	PS-D-PS	PE-D	PS-D
Control Platelets	29.6% PE	5.1% PE; 19% PS	Trace	14.4% PE	15.6% PS
Thrombin-Platelets	29.1% PE	5.3% PE; 19.6% PS	11% PS	37.0% PE	23.2% PS

In controls significant amounts of only PE-D-PE and PE-D-PS were detected and thus are intimately associated within platelet membranes. In thrombin-treated platelets significant amounts of PS-D-PS were formed which did not occur in controls. However, there was no increase in PE-D-PE and PE-D-PS. Also, in thrombin-treated platelets additional PE and PS reacted but were not close enough to be cross-linked. The study indicates that incubation of platelets with thrombin results in the unmasking or rearrangement of specific compartments of PE and PS within platelet membranes which may be critical for platelet hemostatic activities.

EFFECTS OF MODIFICATION OF THE PLATELET SURFACE ON RECEPTORS FOR AGGREGATED IgG AND RISTOCETIN-VON WILLEBRAND FACTOR. S.L. Pfueller, C.S.P. Jenkins and E.F. Lüscher. Theodor Kocher Institute, Berne, Switzerland.

Modification of the platelet surface was used to study the receptors for ristocetin-von Willebrand factor and aggregated IgG (an Fc receptor). Expression of these receptors was measured by agglutination of platelets to ristocetin in the presence of von Willebrand factor and by binding of aggregated IgG coupled to $[^3\text{H}]$ labelled diazobenzene. Under conditions where the release reaction was inhibited, treatment of platelets with chymotrypsin, trypsin, pronase or papain caused loss of the expression of the receptor for ristocetin-von Willebrand factor and an enhancement of that for aggregated IgG. Membrane alterations produced by ADP or ionophore A23187 also abolished agglutination to ristocetin-von Willebrand factor but did not affect the receptor for aggregated IgG. Treatment of formaldehyde-fixed platelets with trypsin, pronase or papain destroyed their ability to agglutinate to ristocetin-von Willebrand factor but did not alter binding of aggregated IgG. Therefore the disappearance of receptor activity for ristocetin-von Willebrand factor following proteolysis appears due to removal of protein and glycoprotein rather than to alterations in membrane configuration. The Fc receptor is not sensitive to proteolysis but is partially masked by protease-sensitive material.

REDUCTION OF SURFACE NEGATIVE CHARGE IN HUMAN PLATELETS BY RISTOCETIN. H. Muraki, K. Watanabe, Y. Ando, K. Toyama and M. Hasegawa. School of Medicine, Keio University, Tokyo, Japan.

The effect of ristocetin on the platelet surface charge was examined. One part of citrated PRP or platelet suspensions was mixed with nine parts of 10 mM Tris-buffered saline (pH 7.4) and the mixtures were incubated at 25°C with or without ristocetin. These mixtures were applied for a cell electrophoresis microscope to determine the electrophoretic mobility (EM), from which the platelet surface charge was estimated. A significant reduction in surface negative charge of platelets was observed when ristocetin was added to the mixtures. EM of non-treated and ristocetin (1 mg/ml)-treated platelets was -1.269 ± 0.045 and $-0.870 \pm 0.040 \text{ } \mu\text{sec/V/cm}$, respectively. A dose-response relationship was observed between platelet EM change and ristocetin concentration (0.1 – 1.2 mg/ml). The change in EM reached to the maximum within seconds. Similar reduction of EM was observed when formalin-fixed platelets were incubated with ristocetin. Heparin at concentrations (10 – 100 U/ml) inhibited, but protamine sulfate (0.05 mg/ml) enhanced the change of EM and platelet aggregation by ristocetin. Both the ristocetin-induced aggregation and the reduction of EM were optimally observed at pH 7.3 and were inhibited at pH above 8.0. The presence of plasma component was found to be essential in the reaction mixture for this reduction of EM. EM was decreased in the presence of ristocetin proportionally with the increase in per cent of plasma in the mixture up to $10 (\text{v/v})\%$. The ristocetin-induced reduction in EM of platelets was not observed when normal platelets were suspended in plasma from a patient with Willebrand's disease ($\text{EM} = -1.278 \pm 0.070 \text{ } \mu\text{sec/V/cm}$, with ristocetin, 1 mg/ml).

These results strongly suggest the involvement of decreased surface negative charge in the mechanism of platelet aggregation induced by ristocetin.