Ca²⁺-INDUCED CLEAVAGE OF HUMAN PLATELET POLYPEPTIDES MEDIATED BY THE CALCIUM IONOPHORE A-23187, by Milica Jakábová and David R. Phillips, Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, Tennessee

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The effect of calcium on human platelet polypeptides was investigated. When lysed platelets were incubated with 1 mM Ca²⁺, two major intracellular polypeptides (Mr = 255,000 and 230,000) were found to rapidly disappear. A similar phenomenon was also observed when intact platelets were treated with the calcium ionophore A-23187 in the presence of mM Ca²⁺. Determinations of lactic dehydrogenase activity in supernatant fractions demonstrated that these losses occurred before platelet lysis. Investigations into the identity of the high molecular weight polypeptides revealed that one (Mr = 255,000) had similar properties to actin binding protein. The loss of the high molecular weight polypeptides was accompanied by formation of lower molecular weight polypeptides (Mr = 135,000, 93,000 and 48,000), indicating that Ca²⁺ activates a polypeptide cleavage mechanism. The Ca²⁺-activated polypeptide cleavages were rapid, with significant changes being observed within the first 0.5 min of incubation. An obvious explanation for these effects is that there is Ca²⁺-activated proteolytic activity within platelets. The Ca²⁺-activated proteolytic activity was determined by the hydrolysis of the artificial substrate azocasein. We found that more than 90% of the proteolytic activity in lysed platelets was due to Ca²⁺-activated proteases. These studies show that Ca²⁺-activated proteases may play an important role in platelet activation.

CHARACTERIZATION OF A PLASMA MEMBRANE BOUND PYRIDINE NUCLEOTIDE REDUCTASE FROM HUMAN PLATELETS, Gary Moroff, Manley McGill, and David P. Kosow, American National Red Cross, Blood Research Laboratory, Bethesda, Maryland, U.S.A.

Platelet membranes isolated by the glycerol lysis technique contain a pyridine nucleotide dependent reductase which can reduce cytochrome c. This reductase is not inhibited by superoxide dismutase. The Km (apparent) for NADH, the preferred cofactor, is 1 μM. The sulphydryl reagent, parachloromercuribenzoate sulfonate (PCMBS), inhibits but KCN does not. PCMBS inhibition is time dependent. Inhibition of the reductase is also seen with quinacrine HCl (atebrin) and salts (both monovalent and polyvalent). Cytochemical staining with nitroblue tetrazolium has also localized the reductase to the platelet membrane. ADP and collagen induced aggregation in citrated plasma is inhibited by quinacrine HCl while aggregation by the calcium ionophore A23187 is not. It has recently been reported that aggregation of human platelets by collagen or thrombin cause an increase in the non-superoxide dependent reduction of cytochrome c or nitroblue tetrazolium (Marcus, A.J., et al., J. Clin. Invest. 59, 149, 1977). The quinacrine HCl inhibition studies suggest that a pyridine nucleotide dependent reductase may be involved in one of the steps which trigger aggregation.

POWERFUL STIMULATION BY FORMALDEHYDE OF PLATELET FUNCTIONS, H. Holmsen and C.A. Dangelmaier, Specialized Center for Thrombosis Research, Temple University, Philadelphia, Pennsylvania, U.S.A.

Low concentrations of formaldehyde (HCHO) increased both the rate and the extent of thrombin-induced secretion of ATP, ADP, preabsorbed ¹⁴C-serotonin, β-N-acetyl glucosaminidase and β-galactosidase from gel-filtered human platelets (GFP). The effect was optimal (7-fold stimulation) with 0.03-0.07 u/ml of thrombin; above 0.1 u/ml there was little effect. With 0.05 u/ml of thrombin maximal stimulation was seen with 0.03-0.5 mM HCHO while the aldehyde was inhibitory above 1 mM. The degree of stimulation increased with the time of exposure of the platelets to HCHO and was maximal after 15-20 min. (37°C). Incubation of control GFP (without HCHO) at 37°C caused a progressive increase in the secretory response 2-3 fold above preincubation values. The degree of stimulation was not altered by indomethacin or aspirin suggesting that prostaglandin/thromboxane formation was not involved. HCHO (0.3 mM) caused a progressive reduction in the steady-state level of metabolic ATP which leveled off after 15-20 min. of incubation. There was no change in the adenylate energy charge, but HCHO caused a transient increase in lactate production in the presence of antimycin A. Similar results were obtained with ADP and epinephrine as inducers of serotonin and adenine nucleotide secretion while the response to collagen was abolished by these low HCHO concentrations. The primary and secondary aggregation responses to ADP and epinephrine, which had been markedly depressed by GFP maintained at 37°C for longer periods of time, was restored by exposure to HCHO. The mechanism of activation, preservation and restoration of platelet activities by HCHO is obscure.