PLATELET AGGREGATION AND SECRETION: EFFECTS OF ANTICOAGULANTS AND DIVALENT CATION CHELATORS.
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The aggregation and secretion responses of human platelets to ADP and epinephrine have been studied in citrated platelet-rich plasma (cPRP) and in heparinized platelet-rich plasma (hPRP) containing varying concentrations of divalent cation chelating agents. ADP induced a biphasic aggregation in hPRP while E induced biphasic aggregation in both hPRP and cPRP. Secretion of ^3H -serotonin (5HT*) occurred in both hPRP and cPRP in response to ADP and E, but generally to a lesser extent in hPRP. The extent of 5HT* secretion in hPRP, relative to that in cPRP, decreased with increasing ADP concentration but remained essentially constant over the same range of E concentration. Addition of citrate or EDTA to hPRP produced a clear maximum in ADP-induced 5HT* secretion at levels of 5-20mM citrate or 2mM EDTA and, in some cases, was associated with the appearance of a biphasic aggregation response. With citrate, but not with EDTA, the chelator concentration corresponding to maximum secretion varied directly with the concentration of ADP. These results indicate that ADP-induced secretion is a function of divalent cation concentration but does not occur only at the low levels of divalent cation present in cPRP and that divalent cation may be more directly associated with ADP-platelet interactions than in interactions of platelets and E.

A POTENTIOMETRIC METHOD FOR STUDYING THE PLATELET RELEASE REACTION. L. S. Robblee, L. B. Kornstein and D. Shepro, Boston University Dept. of Biology and School of Medicine, Boston, Massachusetts, U. S. A.

A potentiometric method which permits the continuous recording of small changes in extracellular calcium (Ca) concentration (1-10 nmoles/ml) is used to study the thrombin-induced release of Ca from human platelets. The instrumentation consists of a Radiometer Ca ion electrode and pH meter, and a chart recorder. Platelets are suspended in Tris-buffered saline, pH 7.4, containing 50 μM Ca. The electrode is placed in a 2 ml aliquot of the platelet suspension in a beaker, thrombin is added, and the change in extracellular Ca (in mV) accompanying the release of Ca is recorded. Recorder tracings of the release reaction show three phases: 1) an initial lag period inversely related to thrombin concentration; 2) an exponential phase during which most of the Ca release occurs; and 3) a final linear phase during which a slow release of Ca is observed. The amount of Ca released is a direct function of both platelet and thrombin concentration. Because an open system is used and release is recorded continuously, considerable flexibility in experimental design is possible. Platelet and thrombin concentrations can be varied over a wide range; thrombin can be added in single or multiple increments and at different time intervals; fresh platelets can be introduced to a completed release reaction; and inhibitors can be added at various times relative to thrombin. Results obtained by using some of these variations suggest that platelets undergo a partial release reaction and become refractory to repeated low level stimuli. The method is both sensitive and inexpensive, and provides a rapid, non-traumatic means of studying temporal and quantitative aspects of the release reaction, and the effects of pathology, drugs or storage on this aspect of platelet function.

COLLAGEN-INDUCED PLATELET AGGREGATION: INVOLVEMENT OF ENDOGENOUS PHOSPHORYLATION. T.M. Chiang, L.E. King, A.H. Kang, and D.R. Phillips, VA Hospital and St. Jude Children's Research Hospital, UT Center for the Health Sciences, Memphis, Tennessee.

It has previously been shown that the aggregating agents, collagen, the $\alpha 1$ chain and the $\alpha 1$ -CB5 glycopeptide cause a decrease in the activity of adenylate cyclase and of the cAMP content, and an increase in the activity of guanylate cyclase and of the cGMP content in platelets. The present study was performed to investigate the effect of exogenous cyclic nucleotides, DBcGMP and DBcAMP, and the above aggregating agents on protein kinase(s) and phosphorylation of platelets.

Platelets were loaded with radio-labelled inorganic pyrophosphate, washed, resuspended in autologous platelet-poor plasma or 0.02M Tris/0.15M NaCl/0.001M EDTA, pH 7.4, and reacted with various test agents. The phosphorylated components were separated by SDS-slab gel electrophoresis and analyzed by radioautography and densitometry.

The results showed that DBcGMP and the aggregating agents stimulated endogenous phosphorylation in platelets of two components separable by SDS-electrophoresis. The phosphorylated components were protein in nature with apparent molecular weights of 42,000 and 20,000. The activity of cGMP-dependent protein kinase(s) was substantially higher than that of cAMP-dependent enzyme. Analyses of the time-course following exposure to the aggregating agents revealed that the phosphorylation of the two protein components preceded visible aggregation detectable by aggregometry. These data suggest the possibility that the aggregating effects of collagen and the derived peptides may be mediated by protein kinase(s).