Role of Calcium in Platelet Reactions

Waterloo Room

Time

14.00 **0184** POSSIBLE ROLE OF A MEMBRANE PHOSPHOPOLYPEPTIDE IN THE INHIBITION OF PLATELET FUNCTION BY CYCLIC AMP

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The inhibition of platelet function by PGE_1, which is mediated by cyclic AMP, is associated with an increased phosphorylation of a 24,000 dalton platelet polypeptide (P24) (Haslam et al. (1979) Biochem J. 178, 397). The significance of this phosphorylation reaction has been investigated. Washed human platelets that had been labelled with ^{32}P were incubated with 2 μM PGE_1 for 2 min, sonicated and separated into subcellular fractions by differential centrifugation. Analysis of the phosphopolypeptides present in these fractions by SDS-polyacrylamide gel electrophoresis showed that P24 was enriched in a 19,000 90,000 g fraction that contained both plasma and intracellular membranes and accumulated $^{45}Ca^{2+}$ by an ATP-dependent, oxalate-stimulated process. Uptake of $^{45}Ca^{2+}$ by membranes from PGE_1-treated platelets was significantly greater (approx. 50%) than by membranes from control platelets. When the former membranes were loaded with $^{45}Ca^{2+}$ in the presence of oxalate and centrifuged through a discontinuous sucrose density gradient, three membrane fractions were obtained. Enrichment of both $^{45}Ca^{2+}$ and P24 was greatest in the most dense of these. Since platelet responses to aggregating agents are believed to be mediated by Ca^{2+} ions, we suggest that PGE_1 may inhibit these processes by causing the cyclic AMP-dependent phosphorylation of the membrane-bound P24 which then stimulates the active transport of Ca^{2+} ions out of the platelet cytosol.

14.15 **0185** MODULATOR BINDING PROTEIN IN PLATELETS

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In a previous paper (Muszbek et al. FEBS Letters 1977, 80, 308) the existence of a troponin C-like protein has been revealed in platelets. This protein was isolated, characterized and identified as the multifunctional Ca^{2+} dependent regulator protein (modulator). Here we show that there is a platelet protein which in 6-8 M urea if Ca^{2+} is present can form a complex with modulator protein and with skeletal muscle troponin C, too. It withstands aceton treatment and can be extracted from platelet aceton powder by 1 M KCl. Further purification could be achieved by affinity chromatography on an Affi-Gel 10-troponin C column. Modulator binding protein also copurified with platelet actomyosin though there was no detectable amount of modulator protein in this preparation. Since modulator protein appears to be responsible for the Ca^{2+} regulation of purified platelet myosin light chain kinase (Dabrowska and Harsthorne BBRC 1978, 85, 1352) the low Ca^{2+} sensitivity of platelet actomyosin may be due to the virtual absence of modulator and/or to the presence of modulator binding protein in thrombosthenin.