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EFFECTS OF SOME ORGANIC SOLVENTS ON GP Ib AND ACTIN-BINDING PROTEIN IN BLOOD PLATELETS.

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Effects on filamentous proteins appear to be a central phenomenon in the neuronal toxic effects of organic solvents. We have therefore compared the effects of some organic solvents (particularly isopropylalcohol, IPA) to the previously observed effects of dibucaine (DBC) on platelet cytoskeletal proteins. Incubation of platelets with 6% IPA at 37° C, like DBC, initiates a degradation of actin-binding protein (ABP) as substrate for a calcium activated protease (CAP), shown by SDS-PAGE. IPA leads to an increase followed by a decrease in bovine von Willebrand factor-induced agglutination. The decrease is accompanied by a release of glycocalicin from the GP Ib α-chain. The process was also studied using CIE of Triton X-100 extracts of platelets against antiserum to glycocalicin. Incubation of platelets with IPA before extraction in the presence of 4.2 mM leupeptin leads to a time-dependent transformation of GP Ib-related immunoprecipitates from that of the slow-migrating peak III complex (probably between ABP and GP Ib) to the faster migrating GP Ib-precipitate. Our working hypothesis is that IPA induces an activation of the CAP by mobilizing calcium. This leads to degradation of ABP and liberation of GP Ib from the cytoskeleton accompanied by an increased tendency for agglutination. The following decrease is explained by degradation of the glycocalicin part of the GP Ib α-chain which contains the binding-site for von Willebrand factor. We conclude that IPA has a similar effect on GP Ib and ABP as DBC. Preliminary studies with 1% DMSO and 0,005% toluene at 37° C revealed that these organic solvents have some similar effects on platelets as described for IPA. Possibly the described effects are characteristic of certain cells at an early stage in a process ultimately leading to cell lysis.

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THE FUNCTIONAL DOMAIN OF PLATELET MEMBRANE GLYCOPROTEIN Ib FOR VON WILLEBRAND FACTOR AND THROMBIN-BINDING.

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In this paper, we have examined the functional domain of platelet membrane glycoprotein Ib (GPIb) by using elastase and a monoclonal antibody against GPIb which specific inhibits both von Willebrand factor (vWF) and thrombin interaction with platelets. Elastase was purified from human granulocytes by using affinity column chromatography according to the method of Okada et al.. A monoclonal antibody against platelet membrane GPIb (56-2) which inhibits both vWF and thrombin-binding to platelets was used for this study. Platelet surface glycoproteins were labelled with ^{3}H by the method of Nurden et al.. Purified GPIb was obtained by a modification of the method of Coller et al. and labelled with ^{125}I by using chloramine-T method. Either ^{3}H-labelled platelets or ^{125}I-labelled GPIb was treated with elastase for various time periods. Elastase-treated ^{125}I-GPIb was subjected to immunoaffinity chromatography using 56-2 antibody to determine the functional site of GPIb. Elastase inhibited platelet aggregation or 5-HT release by thrombin, ristocetin-induced platelet agglutination and vWF-binding to platelets in the presence of ristocetin in a dose- and time dependent manner. A fluorogram of SDS-PAGE of ^{3}H-labelled platelets treated with elastase revealed that GPIb band was reduced gradually, and fragments with MW of 97, 70, 60, 47, 44, 37, 25 and 15 KD were released from the platelets. The 47 KD fragment was initially cleaved from the platelets, and subsequently other fragments were digested. Similar results were obtained when purified ^{125}I-GPIb was digested by elastase. When the fragments from purified ^{125}I-GPIb were reacted with 56-2 antibody, only three fragments with MW of 47, 44 and 25 KD were immunoisolated. The electrophoretic mobility of all these three bands was altered under reduced conditions, indicating that all these fragments contain disulfide bonds in their molecules. The 25 KD band showed a much fainter in ^{3}H-labelling than in ^{125}I-labelling.

These results suggest that the functional domains of GPIb for both vWF and thrombin-binding may be located in a less glycosylated fragment with a MW of 25 KD on the distal portion of the GPIb molecule, which should contain at least one intramolecular disulfide bond.

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FAB FRAGMENTS OF MONOCLONAL ANTIBODIES SPECIFIC FOR PORCINE PLATELET MEMBRANE GLYCOPROTEINS GP IB AND GP IIB/IIIA.

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For further study of the porcine hemostatic mechanism, we have prepared murine monoclonal antibodies, and $F(ab')_2$ and Fab fragments, specific for porcine platelet membrane glycoproteins GP Ib and GP IIb/IIIa. To avoid production of antibodies to von Willebrand factor (vWF), mice were immunized with platelets obtained from pigs with severe von Willebrand's disease. One monoclonal antibody (PP3-4C), of IgG_1 subclass, caused 85% inhibition of Ristocetin-induced platelet binding of ^{125}I-vWF (porcine) at ≥12 μg IgG/ml. PP3-4C did not affect ADP or collagen-induced platelet aggregation nor inhibit ^{125}I-fibrinogen (porcine) binding. Pepsin and papain digestion, respectively, were used to prepare PP3-4C $F(ab')_2$ and Fab fragments. PP3-4C $F(ab')_2$ at concentrations ≥12 μg/ml caused 80% inhibition of washed platelet agglutination in the presence of vWF and Ristocetin, whereas Fab fragments at concentrations ≥10 μg/ml caused 60% inhibition. Another monoclonal antibody (PP3-3A), of IgG_1 subclass, completely inhibited ADP or collagen-induced platelet aggregation at an IgG concentration of 6 μg/ml. At 10 μg IgG/ml PP3-3A completely inhibited binding either of ^{125}I-fibrinogen or of ^{125}I-vWF to ADP-stimulated porcine platelets. PP3-3A did not affect vWF-dependent Ristocetin-induced platelet agglutination, nor ^{125}I-vWF binding to platelets in the presence of Ristocetin. PP3-3A did not bind to platelets which were treated with 10 mM EDTA at 37°C for 60 min. $F(ab')_2$ and Fab fragments were isolated from PP3-3A pepsin or papain digests. Both types of PP3-3A fragments caused 100% inhibition of ADP-induced platelet aggregation, at concentrations ≥6 μg/ml. Immunoprecipitation of surface-radiolabeled porcine platelets and subsequent SDS-PAGE demonstrated that PP3-4C recognized a glycoprotein with molecular weight of 140,000 (under reducing conditions), and 165,000 (non-reduced). PP3-3A recognized glycoproteins with molecular weights of 115,000 and 100,000 (reduced), and 130,000 and 80,000 (non-reduced). Neither monoclonal antibody bound to human platelets. These monoclonal antibodies to porcine platelet membrane glycoproteins which are analogues of human GP Ib and GP IIb/IIIa will be useful for in vitro and in vivo studies to further understanding of mammalian hemostatic mechanisms.

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PLATELET ACTIVATION INDUCED BY A MONOCLONAL ANTIBODY AGAINST THE PLATELET GP IIb/IIIa COMPLEX.

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A receptor for fibrinogen on the platelet GP IIa/IIIb complex is induced by ADP, thrombin and other agonists. To study functional domains on GP IIb/IIIa, the effects of anti-GP IIb/IIIa monoclonal antibodies (Mab's) on platelet function were determined. One of these Mab's, 6C9, induced platelet aggregation. The antibody binds to the intact GP IIb/IIIa complex only, not to free GP IIb or free GP IIIa. Its epitope is different from that of C17, a Mab that inhibits ADP-induced fibrinogen binding and platelet aggregation. 6C9 induces fibrinogen-mediated aggregation rather than agglutination since 6C9-induced platelet interactions were blocked by treatments that also inhibited the effects of ADP etc., without inhibiting binding of 6C9 itself. 6C9 induces binding of ^{125}I-fibrinogen (39.000 ± 7.300 molecules/platelet, Kd = 1.3 ± 0.4 μM) to unstirred platelets. Binding of fibrinogen was 60 to 80% inhibited by apyrase, which indicates that 6C9-induced fibrinogen binding is largely mediated via ADP released from platelets. In addition, 6C9 induced aggregation of platelets in the absence of extracellular fibrinogen. Mediation of this process by platelet fibrinogen or other α-granule proteins, released upon activation by 6C9, was implicated by the finding that aggregation of washed platelets, but not of platelets to which fibrinogen was added, could be blocked by PGI_2. Platelet release was also assessed directly by measuring β-thromboglobulin (α-granules) and (^{14}C) serotonin (dense granules) in the medium of unstirred platelets incubated with 6C9. $F(ab')_2$ fragments of 6C9 only aggregated platelets in the presence of fibrinogen and did not release (^{14}C) serotonin. Moreover, release induced by intact 6C9 was inhibited by anti-GP IIb/IIIa Mab C17 but not by C17 $F(ab')_2$, although the latter inhibited ADP-induced platelet aggregation. These data indicate that binding of antibodies to specific sites on GP IIb/IIIa may induce Fc-dependent platelet activation.

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