ADP BINDING PROTEINS IN THROMBASTHENIC PLATELET MEMBRANES. Joel S. Bennett, Schlomo Friedman, Roberta F. Colman, and Robert W. Colman. Depts. of Med. and Peds., Univ. of Pennsylvania, Philadelphia, PA. and Dept. of Chem., Univ. of Delaware, Newark, DE.

Thrombasthenia is a severe congenital platelet disorder characterized by absence of ADP-induced platelet aggregation, diminished clot retraction and abnormalities of platelet membrane glycoproteins. The finding of normal ADP-induced shape change suggests that initial ADP binding might be normal. We have previously shown that the synthetic ADP analogue 5'-p-fluorosulfonylbenzoyladenosine (5'FSBA) inhibits ADP-induced platelet shape change and covalently binds to specific proteins in normal platelet membranes. This affinity label was thus used to identify ADP binding proteins in platelet membranes from a patient with thrombasthenia. Isolated platelet membranes were incubated with ^{3}H-5'-FSBA (0.1mM) at 37^{o}C for 1 hr. then dissolved in 19% SDS, 8M urea, and 0.2M dithiothreitol, and subjected to SDS disc gel electrophoresis. The radiochromatograms of disc gels from 16 normal individuals demonstrated consistent labelling of polypeptides of 200, 120, 100, and 43 x 10^{3} daltons. The covalent binding was almost completely prevented by simultaneous incubation with a 100-fold excess of ADP or ATP, only partially prevented by AMP, adenosine and GDP, and was unaffected by UDP, thrombin, and epinephrine. When 5'FSBA was incubated with membranes from thrombasthenic platelets, the incorporation was identical to that found in normal membranes. Further the SDS-disc gel pattern showed the same 4 radiolabelled polypeptides despite the characteristic thrombasthenic membrane protein abnormalities. These experiments demonstrate that the ADP binding proteins of thrombasthenic platelet membranes are similar to those of normals and support the concept that the defect in thrombasthenia occurs subsequent to initial ADP binding.

SPECIFIC INHIBITION OF ADP-INDUCED PLATELET RESPONSES BY 2-n-AMYLTHIO AMP.
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"Primary" aggregation responses to ADP are blocked by 2-n-amylthio AMP (nAmSAMP)*, apparently competitively ($K_i \approx 10$ μM). Shape change is inhibited by higher concentrations (> 0.1 mM). nAmSAMP has a modest inhibitory effect on platelet responses to ionophore Lilly A23187 and a greater effect on responses to collagen and blocks secretion and secondary aggregation induced by ADP, adrenaline, arachidonic acid, PGG_2, and synthetic analogues of PGE_2 and PGH_2. nAmSAMP is a much less potent inhibitor than adenosine against all stimulants apart from ADP and is qualitatively unlike adenosine in the following respects: 1. primary aggregation responses to the above agents (except ADP) and to serotonin and vasopressin are unaffected; 2. inhibition is not increased by preincubation; 3. inhibition is not decreased by an inhibitor of adenylate cyclase, SQ22536 (9-[tetrahydro-2-furyl]-adenine); 4. basal levels of platelet cyclic AMP are unaffected. We conclude that, unlike adenosine, nAmSAMP does not inhibit platelet responses by stimulating adenylate cyclase. nAmSAMP appears to be a "specific" competitive antagonist of ADP and should therefore be useful in clarifying the role of ADP in platelet reactions.