

ATTEMPTS TO DEFINE A PLATELET ADP RECEPTOR WITH 203Hg-p-MERCURIBENZENE SULPHONATE (MBS). D.C.B. Mills and D.E. Macfarlane. Specialized Center for Thrombosis Research, Temple University Hospital, Philadelphia, Pennsylvania U.S.A.

ADP inhibits stimulation of platelet adenylate cyclase through a receptor with specificity and affinity indistinguishable from those of the ADP receptor mediating aggregation. The former, but not the latter effect is blocked by MBS (0.3-0.6 mM in PRP) indicating involvement of an externally oriented thiol. To remove effects of plasma thiols, aspirin treated platelets, labelled with 14C-adenine, were washed successively by centrifugation into albumin and passage through sepharose 2B into Ca-free Tyrode's solution; this preserved their responses to PGE1 and ADP. Platelets incubated with 203Hg-MBS were pelleted through a layer of radiographic contrast medium. MBS binding showed a saturable high affinity component (ca. 250,000 sites/cell: 1/2 saturated at 8-10 μ M). Excess cysteine reduced but did not eliminate non-saturable binding. Treatment of the platelets with Ellmans reagent (DTNB) reduced the apparent number of high affinity sites to ca. 100,000 and increased the apparent affinity of binding to ca. 2.5 μ M. Effects of MBS on adenylyl cyclase, monitored in the same experiment, correlated closely with high affinity binding. SDS electrophoresis of unreduced platelets on gradient polyacrylamide gel slabs showed preferential labelling of several proteins by MBS, but the results suggested some redistribution of the label during extraction and separation. The number of high affinity sites for MBS found indicates the maximum number of thiols involved in ADP effects on adenylate cyclase. The true number is probably much smaller as MBS will label all externally available thiols.

INTERACTION OF HUMAN PLATELETS WITH THE 2',3'-DIALDEHYDE DERIVATIVES OF ADP (o-ADP) AND ATP (o-ATP) AS POTENTIAL AFFINITY LABELS FOR THE ADP RECEPTOR. P.H. Pearce and M.C. Scrutton, University of London King's College, London, U.K.

In order to characterise the receptor responsible for mediating platelet aggregation and secretion induced by ADP we have synthesised oADP and oATP by periodate oxidation of ADP and ATP. oADP induces platelet shape change but inhibits aggregation competitively with respect to ADP. The properties of inhibition of aggregation induced by adrenaline, collagen or thrombin are also consistent with the conclusion that the effects of oADP result from interaction with the ADP receptor(s). The effects of oATP generally resemble those of oADP except that oATP inhibits, rather than induces, shape change and causes this effect at a concentration in excess of that required for inhibition of aggregation.

Brief exposure of intact platelets to [3 H]-oADP or oATP causes stable incorporation of [3 H] into the membrane proteins. Analysis of [3 H] distribution by SDS gel electrophoresis after prolonged dialysis against SDS/mercaptoethanol shows predominant incorporation of [3 H]-oATP into a fraction at 28000 daltons over a concentration range consistent with that required for inhibition of aggregation. When [3 H]-oADP is used, incorporation occurs predominantly into 4 fractions at 28000, 43000 (probably actin), 65000 and 220000 daltons. The [3 H] incorporation/[oADP] relationship and the time course of incorporation suggest that the 28000 and 65000 dalton fractions are likely candidates for identification as ADP receptors. Our data suggest a tentative identification of the 28000 dalton fraction as the receptor responsible for mediating aggregation induced by ADP and also indicate the possibility that different receptors may be involved in induction of shape change and aggregation by this agonist.

CHARACTERIZATION OF THE BINDING OF ADENOSINE DIPHOSPHATE TO HUMAN PLATELET MEMBRANES. C. Legrand, B. Bauvois and J.P. Caen. Hôpital Lariboisière, Paris, France

ADP-mediated platelet aggregation is a routinely employed test but its mechanism is poorly understood. The aim of this study was to compare the binding of ADP to plasma membranes isolated from normal platelets and thrombasthenic platelets (which do not aggregate with ADP). Binding of ADP to isolated membranes was assayed by incubation with 14C-ADP followed by Millipore filtration. In standard conditions, 14C-ADP was not transformed and non specific binding represented less than 3 % of the total binding. Using 1 μ M 14C-ADP, the binding has been shown to be a rapid ($t_{1/2} = 2$ mn 30 sec.), saturable and reversible phenomenon at 37° C. The existence of a major population of binding sites, with an affinity constant $K_a = 0.43 (+ 0.1) \times 10^6 M^{-1}$, has been demonstrated. The kinetics of the binding was normal with membranes isolated from the platelets of 4 thrombasthenic patients and the affinity constant, when determined, was in the normal range. Dissociation of the membrane-bound 14C-ADP occurred rapidly at 37° C ($t_{1/2} \approx 3$ mn) when samples were diluted enough (dilution 1 : 100 was currently employed) to avoid rebinding of the radioligand. Accelerated dissociation ($t_{1/2} \approx 1$ mn) was observed when the dilution was performed in the presence of an excess of unlabeled ADP, suggesting the existence of negatively cooperative site-site interactions among the ADP binding sites. This effect was only observed at high concentrations of ADP ($> 10^{-5} M$) and its eventual role in vivo remains to be established. Two thrombasthenic membrane preparations studied in the same way dissociated as did the control membranes.