

STRUCTURE AND FUNCTION OF THE HUMAN PLATELET VON WILLEBRAND FACTOR RECEPTOR. K.J. Clemetson, H.Y. Naim and E.F. Lüscher. Theodor Kocher Institute, University of Berne, Switzerland.

The interactions between platelets and subendothelium involving von Willebrand factor play an important role in haemostasis and thrombosis. The identity of the von Willebrand factor receptor with human platelet membrane glycoprotein Ib has now been fairly conclusively established but the precise nature of the binding site and the changes induced by binding have yet to be demonstrated.

Asialoglycoprotein Ib and asialoglycocalicin have been isolated from neuraminidase treated platelets by affinity-chromatography on peanut agglutinin and compared by tryptic peptide mapping. The results clearly show that glycocalicin is closely related to the α -chain of glycoprotein Ib and is probably a proteolytic fragment of it. Unreduced and reduced glycocalicin showed minor differences in their tryptic peptide maps indicating that glycocalicin and by extension the α -chain of glycoprotein Ib probably contain at least one intramolecular disulphide bond.

Treatment of platelets with reducing agents such as dithiothreitol results in loss of response to bovine von Willebrand factor. Similar treatment of surface-labelled platelets did not release any glycoprotein subunits into the supernatant, implying that disulphide bridge-linked subunits are tightly associated with the membrane or with other subunits. Hydrophobic chromatography of reduced, purified glycoprotein Ib indicates that both α and β subunits are hydrophobic and thus probably integral membrane glycoproteins. Intact disulphide bridges seem to be a prerequisite for von Willebrand factor receptor activity.

THE RECEPTOR FOR FACTOR VIII/VWF ON HUMAN PLATELETS: ITS APPEARANCE AND DISAPPEARANCE UPON ACTION OF THROMBIN AND PROSTACYCLIN. J. Hawiger, T. Fujimoto and S. Ohara. Department of Pathology, Vanderbilt University, Nashville, Tennessee, U.S.A.

Under normal physiologic conditions the receptor for F. VIII/VWF on human platelets is not readily available unless the antibiotic, ristocetin, is added. Searching for a physiologic mechanism inducing the receptor for F. VIII/VWF on human platelets, we found that low doses of thrombin (0.01-0.05 U/ml) induce steady state binding of ^{125}I -F. VIII/VWF to human platelets. The binding is saturable and specific. Specificity was established by demonstrating that 100-fold molar excess of unlabeled F. VIII/VWF inhibited the binding while an excess of fibrinogen or fibronectin, two common contaminants of F. VIII/VWF, did not inhibit binding of ^{125}I -F. VIII/VWF. Furthermore, rabbit antibody against F. VIII inhibited the binding. Thrombin-induced binding to human platelets can be observed only with fresh, metabolically active platelets. Glutaraldehyde-treated platelets did not bind ^{125}I -F. VIII/VWF following their treatment with thrombin, although they exhibited good binding in the presence of ristocetin. Thrombin appears to induce binding by acting on platelets rather than on F. VIII/VWF itself. Prostacyclin (PGI_2 10^{-9}M) inhibited thrombin-induced binding of ^{125}I -F. VIII/VWF to human platelets, while ristocetin-induced binding remained unaffected. Strikingly, PGI_2 -induced inhibition of binding was observed only when PGI_2 was added before thrombin but also when PGI_2 (10^{-6}M) was used 30 minutes after thrombin treatment and addition of ^{125}I -F. VIII/VWF. Thus, binding of F. VIII/VWF to platelet receptor is induced by low concentrations of thrombin and is inhibited by PGI_2 . Since both agents are generated in the vicinity of injured vessel wall they may contribute to platelet-F. VIII/VWF-vessel wall interaction *in vivo* by regulating "up and down" platelet receptor for F. VIII/VWF.

ADENOSINE 5'-O-(2-THIODIPHOSPHATE) (ADP- β -S) IS A PARTIAL AGONIST AT THE ADP RECEPTOR OF HUMAN PLATELETS. N. J. Cusack and S. M. O. Hourani.* Department of Pharmacology, University of London, King's College, London, U.K.

ADP induces human platelet aggregation and inhibits the stimulation of platelet adenylate cyclase by prostaglandin E_1 (PGE_1), but analogues of ADP in which the diphosphate group is modified retain only weak aggregating activity. However, ADP- β -S, an ADP analogue in which a terminal phosphate oxygen is replaced by sulphur, is known to be equipotent with ADP as an inhibitor of PGE_1 -stimulated adenylate cyclase in purified human platelet membranes. We therefore tested ADP- β -S on intact human platelets. ADP- β -S induced human platelet aggregation and inhibited PGE_1 -stimulated adenylate cyclase, but in both cases was less potent than ADP and only achieved 75% and 50% respectively of the maximal effects of ADP. Aggregation induced by ADP- β -S was competitively inhibited by ATP ($50\text{ }\mu\text{M}$), a known ADP antagonist.

Both these actions of ADP could be inhibited by the simultaneous addition of ADP- β -S ($50\text{ }\mu\text{M}$). Aggregation induced by a stable endoperoxide analogue (11,9-epoxymethano PGH_2), which acts at a prostaglandin receptor rather than at an ADP receptor, was not inhibited by the simultaneous addition of ADP- β -S ($50\text{ }\mu\text{M}$). The behaviour of ADP- β -S towards human platelets is therefore that of a partial agonist at the ADP receptor.

SELECTIVE BINDING OF ^3H YOHIMBINE TO THE α_2 -RECEPTOR OF INTACT PLATELETS -- COMPARISON WITH ^3H DIHYDROERGOCRYPTINE BINDING. Donald E. Macfarlane and David C. Stump, Department of Internal Medicine, University of Iowa Hospitals, Iowa City, Ia. USA

Analysis of the coupling of receptors and the adenylate cyclase is facilitated by radioligand analysis of receptor occupancy. The platelet α -receptor has been characterized by ^3H dihydroergocryptine binding to membranes, but poorly reproducible results are obtained with intact platelets. We incubated washed platelets with ^3H dihydroergocryptine and measured bound counts after 6-fold dilution with plasma and centrifugation. After 10 minutes, phentolamine ($5\text{ }\mu\text{M}$) suppressible counts equivalent to 400 molecules per platelet were found with 1/2 saturation at 1.2 nM . There was considerable intra-experimental variation, and non-specific binding "space" was greater than $10\text{ ul}/10^8$ platelets. Time course of displacement with cold dihydroergocryptine, phentolamine or yohimbine was biphasic with a rapid (2 min) component amounting to 20-30% followed by a further 20% over the next 60 minutes. These results, which confirm those of others, suggest that equilibrium is not reached in a reasonable period of time and that more than one binding mechanism may be involved. In contrast, ^3H yohimbine bound with simple first order kinetics (37°) giving $k_1 = 5.25 \times 10^5\text{ M}^{-1}\text{sec}^{-1}$ and $k_{-1} = 3.33 \times 10^{-3}\text{ sec}^{-1}$. Scatchard analysis revealed $K_d = 4.8\text{ nM}$, $B_{\text{max}} = 180$ molecules per platelet. Non-specific binding "space" was less than $1\text{ ul}/10^8$ platelets. Binding was completely reversed or prevented by epinephrine, clonidine, p -aminoclonidine, phentolamine, dihydroergotamine and dihydroergocryptine in that order of potency. Prazosin, an α_1 -antagonist, was less potent than epinephrine. Yohimbine blocks the ability of epinephrine to potentiate and induce aggregation, and to inhibit the adenylate cyclase. It is concluded that this novel radioligand has clear advantages over dihydroergocryptine for analysis of receptor occupancy in intact platelets.