

CONVULXIN, AN ADP AND THROMBOXANE-INDEPENDENT PLATELET-ACTIVATING GLYCOPROTEIN FROM THE VENOM OF CROTALUS DURISSUS CASCABELLA. G. Marlas, D. Joseph, J.P. Franceschi, J.Lefort, M. Chignard, F. Fouque and B.B. Vargaftig. Institut Pasteur, Paris 15, France.

Convulxin(Cx), a high molecular weight glycoprotein which was purified by Sephadex G75 and Sepharose 4B chromatography, aggregates platelets of guinea-pigs, rabbits and humans (thresholds of 20-100 pico M for 400,000 platelets/ μ l). Aggregation and release reaction are plasma-independent, and do not require DFP-fibrinogen (DFP-fib), which increases the platelet response. Cx is not lytic for platelets. Neither ADP scavengers nor aspirin inhibit 2-4 supra-threshold concentrations of Cx. Bivalent metal chelation and PGI_2 antagonize Cx. "Thrombinized" platelets lose granular ADP, and still respond to Cx in absence of DFP-fib. Cx-treated platelets are aggregated by ADP, thrombin (T) and arachidonic acid (AA), but are refractory to Cx and to collagen. Cx triggers release of ^{14}C -AA metabolites from rabbit and human platelets, which was inhibited by phospholipase A_2 inhibitors. "T-sized" platelets took ^{14}C -AA and failed to release it if stimulated with T, but did so with Cx. Cx1.v.induces thrombocytopenia in rabbits and guinea-pigs, and bronchoconstriction in the latter, which is not blocked by aspirin. Cx is a very effective platelet-stimulating agent, free from proteolytic, amidolytic, esterase, phospholipase and clotting activities. It probably interacts with T, ADP and thromboxane-independent receptors, and may share a component or a route with the mechanism triggered by collagen.

INDUCTION AND POTENTIATION OF PLATELET AGGREGATION BY CLONIDINE AND ρ -AMINOCLOMIDINE, PARTIAL AGONISTS OF THE α_2 -RECEPTOR WHICH REGULATES PLATELET ADENYLATE CYCLASE. David C. Stump and Donald E. Macfarlane, Department of Internal Medicine, University of Iowa Hospitals, Iowa City, Ia. USA

Epinephrine induces platelet aggregation, potentiates aggregation by other agents, and blocks the stimulation of the adenylate cyclase by prostaglandins. Synthetic α -adrenergic agents have not been shown to induce aggregation. The effects of clonidine, an α_2 -agonist, and ρ -aminoclonidine on platelets were examined. Clonidine potentiated aggregation induced by 0.5 μM ADP by 1.4-fold (1/2 max 0.5 μM). It did not induce significant aggregation itself, and it inhibited aggregation induced by 5 μM epinephrine (1/2 max 1 μM). It inhibited cyclic AMP accumulation induced by PGE_1 by a maximum of 25% (1/2 max 0.1 μM) and it blocked inhibition by epinephrine. No significant specific binding of [^3H] clonidine was observed to intact platelets. ρ -Aminoclonidine induced aggregation with delayed second phase (1/2 max 0.2 μM), and potentiated ADP aggregation by 2-fold (1/2 max 0.2 μM). Aggregation induced by epinephrine was more rapid, and was partially inhibited by ρ -aminoclonidine. It inhibited cyclic AMP accumulation by 50% max (1/2 max 0.1 μM) and attenuated epinephrine's effect to the same level. The direct effects of ρ -aminoclonidine were blocked by 1 μM yohimbine, a selective α_2 -antagonist. Both clonidine and ρ -aminoclonidine blocked the specific binding of [^3H] yohimbine (1/2 max 0.5 μM). These results suggest that the platelet bears an α_2 -receptor with affinity for epinephrine, ρ -aminoclonidine and clonidine as agonists but that these agents display differing intrinsic activity and/or receptor reserve.

IS PHOSPHORYLATION OF ADP REQUIRED FOR THE INDUCTION OF SHAPE CHANGE AND AGGREGATION? D.C.B.MILLS AND C.LIPSON. Thrombosis Research Center, Temple University Hospital, Philadelphia, PA 19140 USA.

Observations on the activity of nucleoside diphosphokinase on the external surface of washed rabbit platelets have suggested that phosphorylation of ADP at the expense of intracellular ATP occurs in association with aggregation. We have studied aggregation and shape change of washed human platelets labelled in vitro with ^{32}P i, using ADP or an analogue, 2-methylthio ADP, to induce aggregation in the presence of fibrinogen. EDTA was added during (30sec) or after (2min) aggregation, and the platelets were removed gently by centrifugation through silicone oil. Nucleotides were concentrated from the medium by absorption on, and elution from charcoal-coated beads, and were analysed by paper electrophoresis and HPLC. ATP (50-100 μM) was added to some samples to trap any labelled ATP formed. This reduced the rate of aggregation but did not stop it. Less than 0.1% of the intracellular radioactivity was recovered as extracellular ATP and this amount was not significantly increased by ADP. Under these conditions less than 2000 molecules of ADP were phosphorylated for the aggregation of each platelet. This suggests that phosphorylation of ADP is not required for aggregation or for shape change.

LIPOPROTEIN PLATELET INTERACTION: THE EFFECT ON PLATELET CHOLESTEROL CONTENT AND PLATELET FUNCTION. M. Aviram and J.G. Brook. Lipid Research Unit, Rambam Medical Center, Faculty of Medicine, Technion, Haifa, Israel.

Intracellular cholesterol metabolism is regulated by membrane receptors which selectively bind plasma low density lipoproteins (LDL), the major extracellular source of cholesterol. Human platelets, unlike other cells, are unable to synthesize cholesterol, but bind LDL with specificity. We have shown that very low density lipoproteins (VLDL) and LDL, both of which contain apolipoprotein B compete with ^{125}I -LDL for the platelet binding sites, while high density lipoproteins (HDL) is only able to compete to a limit extent and this by virtue of its apolipoprotein E content. Platelet uptake of both LDL and HDL is saturable at physiologic concentrations of these lipoproteins. The lipoproteins are internalized by the platelet but degradation occurs only to a limited extent. Incubation of LDL AND HDL with gel-filtered platelet results in significant changes in the platelet cholesterol content. LDL (1 mg protein/ml) increases cholesterol content by 15% whereas the same concentration of HDL causes a 5% reduction. In the presence of thrombin LDL enhances platelet aggregation by 300% whereas HDL decreases aggregation by 50%. We have thus shown that the lipoprotein platelet interaction affects both platelet cholesterol content and also platelet aggregation. LDL and HDL have opposing effects and this again highlights their different roles in the atherogenic process.