

EFFECT OF FACTOR XIII ON PLATELET AGGREGATION. STUDY OF PLATELET FUNCTION AND FIBRIN STABILIZATION INHIBITORS. M. Maamer*, O. Demay*, M. Aourousseau. *Centre de Recherches In-thera, Arcueil, France. Faculté de Pharmacie, Reims, France

There is little information on the participation of Factor XIII in platelet aggregation. Using BORN's photometric method to study platelet aggregation induced by ADP in vitro on platelet rich plasma (PRP) of rabbit; clot solubility in 1% monochloroacetic acid and incorporation of dansylcadaverin into casein (LORAND L. et al.) to measure plasma F.XIII concentration; we showed that addition of activated F.XIII (F.XIIIa) to a PRP, aggregating power of platelets was significantly increased (+30.4%, $p < 0.001$). Addition of inactive F.XIII or thrombin + Ca^{++} in concentrations used to activate F.XIII, had no significant effect on platelet aggregation induced by ADP.

When F.XIIIa was added to plasma in presence of F.XIII inhibitors as 3178 AQ (a new synthetic benzothienone ketone derivative) or monodansylcadaverin (DC) in concentrations of (3.27×10^{-4} M and 9.31×10^{-4} M respectively), the platelet aggregation was significantly inhibited (-48.8% and -35.4% respectively, $p < 0.001$). This inhibitory effect was not seen when dipyridamole or Acetylsalicylic Acid (ASA) in concentrations of (6.18×10^{-4} M and 17.3×10^{-4} M respectively) were added in PRP in presence of F.XIIIa.

When platelet aggregation was performed without addition of F.XIIIa the inhibitory effect of 3178 AQ and DC was respectively (-76.6% and -65.1%, $p < 0.001$), dipyridamole (-37.6%, $p < 0.001$) and ASA (-4.1%, no significant).

These results suggest that F.XIIIa increased the platelet aggregation induced by ADP and compounds which are both inhibitors of platelet aggregation and F.XIII would be more potent antithrombotic by acting on platelets and fibrin stabilization, than drugs which are inhibitors of platelet aggregation only.

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THROMBOXANE A₂-STIMULATED HUMAN PLATELET AGGREGATION IS POTENTIATED BY EPINEPHRINE ACTING VIA ALPHA ADRENERGIC RECEPTORS. G.J. Johnson, G.H.R. Rao and J.G. White. Veterans Administration Medical Center and University of Minnesota Medical School, Minneapolis, U.S.A.

Epinephrine (E) potentiates arachidonate (A)-induced aggregation of human platelets. A-insensitive dog platelets (AIP), that form thromboxane A₂ (T) but do not aggregate when stirred with A alone, aggregate when exposed to E + A. Therefore, we studied the effect of E on T-stimulated human platelet aggregation. AIP stirred with A formed T which was confirmed by TLC. 1/100 to 1/200 volume of AIP was removed 30 sec. after A, and transferred to gel-filtered, aspirin-incubated human platelets. Recipient platelet aggregation was proportional to the volume of AIP transferred. The addition of the thromboxane synthetase inhibitor, Azo Analog I, abolished the aggregating activity of AIP. Transfer of an aliquot of AIP that was inadequate to aggregate human gel-filtered, aspirin-incubated platelets resulted in irreversible aggregation in the presence of >0.5 nM E. E potentiated aggregation when added 3 min. before but not 3 min. after aliquot transfer. T-stimulated aggregation was abolished by the T-antagonist, 13 azaprostenoic acid (APA), but E added after APA and before T restored aggregation. E potentiation of T-stimulated aggregation was abolished by prior exposure to equimolar yohimbine, dihydroergocryptine and phentolamine, agents that bind to alpha₂ adrenergic receptors, but not by prazosin an alpha₁ antagonist. Higher concentrations of E reversed the inhibitory effects of the alpha₂ adrenergic agents. All of these agents in higher concentrations (1-100 μ M) also blocked aggregation induced by T alone. Therefore T-induced platelet aggregation is potentiated by E, in concentrations attained in vivo, by a mechanism linked to platelet alpha adrenergic receptors. Platelet alpha₂ receptors have a close functional relationship to the postulated T receptor. E may initiate platelet aggregation in vivo when T is formed in quantities inadequate to alone induce aggregation.

THE REGULATORY ROLE OF cAMP IN INDUCTION OF HUMAN PLATELET RECEPTOR FOR FIBRINOGEN. S. E. Graber and J. Hawiger. Departments of Medicine and Pathology, Vanderbilt University and VA Medical Center, Nashville, Tennessee, U.S.A.

Membrane receptor for fibrinogen plays an essential role in adhesion and aggregation of human platelets by allowing fibrinogen to bridge two or more platelets together. Whereas in normal, unstimulated platelets fibrinogen receptor is not available, it becomes mobilized upon stimulation of platelets with thrombin, ADP, and other stimuli. The mechanism(s) regulating availability of membrane receptor for fibrinogen remains unknown. Following our recent demonstration that prostacyclin (PGI₂) prevents mobilization of fibrinogen receptor by thrombin and ADP (Nature 1980, 283,195), we investigated the relationship between cAMP levels and fibrinogen receptor availability. Platelets separated from plasma proteins were briefly exposed to a low thrombin concentration (0.05 U/ml) followed by hirudin to inactivate free thrombin. Binding of ¹²⁵I-fibrinogen and cAMP levels were determined in parallel samples. A dose-dependent rise in platelet cAMP levels from 3.3 pM to 10.3 pM/10⁸ platelets in response to PGI₂ (3×10^{-5} M - 3×10^{-8} M) was accompanied by a corresponding inhibition of ¹²⁵I-fibrinogen binding. The degree of the cAMP increment correlated with binding inhibition ($r=0.96$). The inhibition of ¹²⁵I-fibrinogen binding by PGI₂ was sustained up to 120 min and was paralleled by a persistent rise in cAMP level. Stimulation of platelet cAMP synthesis "from within" by a ribosylation of the nucleotide regulatory component with subunit A₁ of cholera toxin also increased cAMP levels and inhibited fibrinogen receptor mobilization.

These results provide evidence that "up and down" regulation of fibrinogen receptor in platelets is linked to changes in cAMP levels induced by different types of adenylyl cyclase antagonists and agonists.

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ON THE SEPARATION OF THROMBOPLASTIC AND PLATELET STIMULATION ACTIVITY AND ON THE PHOSPHOLIPID CONTENT OF VARIOUS TISSUE EXTRACTS. C.M. Kirchmaier, N. Bender and K. Breddin. Division of Angiology, Department of Internal Medicine, J.W. Goethe University, Frankfurt/ M., F.R.G.

Homogenisates of different human and animal tissue induce rapid and reversible platelet changes, enhance platelet aggregation and induce platelet retention. These findings led to the hypothesis, that paravascular and other tissues contain a "Hemostasis Activating Factor" HAF, which is locally liberated at the site of a tissue injury. Enzymatic digestion of crude tissue extracts by phospholipase D and Neuraminidase destroyed the platelet stimulating activity. Separation by thin layer chromatography using chloroform-methanol (70:30) as eluant resulted in five zones. Staining by different media showed phospholipid, protein and glycoprotein compounds. Testing different lipids and phospholipids we found that Lyso-dimethylcephaline and Sphingosine induced morphologic platelet changes in high concentrations (10^{-3} g%) while other compounds were almost inactive. The tissue extracts contained a very weak thromboplastic, but some partial thromboplastic activity. Using Amicon CF25 cones the platelet stimulating activity was found in the ultrafiltrate which contained no thromboplastic or partial thromboplastic activity. The ultrafiltrate higher molecular weight supernatant showed a strong partial thromboplastic activity, but only a slight platelet stimulating activity. The partial thromboplastic activity in tissue extracts from different organs (subcutaneous tissue, lung, muscle, vessels, brain) showed a large variation and did not correlate with platelet stimulating effects. So for instance brain extracts showed the well known partial thromboplastic activity while they contained only a weak platelet stimulating activity.