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RELATIONSHIP BETWEEN ELEVATION OF CYCLIC-3',5'-GMP (cGMP) AND AGGREGATE FORMATION IN HUMAN PLATELETS

M. Edgecombe, M.C. Scrutton and R. Kerry
Department of Biochemistry, King's College, London WC2R 2LS, U.K.
and Ciba-Geigy Pharmaceuticals, Horsham, U.K.

Maximal 3- to 5-fold increases in platelet cGMP levels as measured by specific radioimmunoassay are observed on stimulation of aspirin-treated platelet-rich plasma with saturating doses of ADP, adrenaline, 5HT, PAF, thrombin, 9,11-epoxymethano-PGH_2 (U44069), collagen, ristocetin and the Ca^{2+}-ionophore ionomycin. The dose/response curves for the elevation in cGMP induced by these agents are either superimposable on, or lie to the right of, those describing the rate or extent of the aggregatory response as measured by an increase in light transmittance. The increase in cGMP induced by ADP is totally inhibited by addition of PGI_2 or forskolin with dose/response relationships superimposable on those observed for inhibition of the aggregatory response. No increase in cGMP is observed if platelets are stimulated by PAF or ionomycin in an unstirred system or when aggregation induced by ADP is prevented by addition of a monoclonal antibody which recognises the glycoprotein IIb/IIIa complex.

Addition of the fibrinogen γ-chain C-terminal decapeptide ($\gamma_{402-411}$) or α-chain tetrapeptide ARG-GLY-ASP-SER prevents aggregation and the increase in cGMP induced by PAF. The γ-chain decapeptide also completely prevents the increase in cGMP induced by ristocetin, but the α-chain tetrapeptide is ineffective in this respect. Both peptides inhibit to some extent aggregate formation induced by ristocetin.

The data demonstrates a strong correlation between aggregate formation and the increase in the platelet cGMP levels and support the previous postulate that platelet-platelet contact causes activation of guanylate cyclase. No relationship is apparent between the effects of the various agents tested on cGMP levels and their known ability to increase cytosolic Ca^{2+} concentration. (Supported by SERC and Ciba-Geigy.)

PLATELET AGGREGATION

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COMPARISON OF THE PLATELET AGGREGATION INDUCED BY THREE THROMBIN-LIKE ENZYMES OF SNAKE VENOMS AND THROMBIN. C.M. Teng and F.N. Ko. Pharmacological Institute, College of Medicine, National Taiwan University, Taipei, Taiwan, R.O.C.

Acutin was isolated from Agkistrodon acutus venom and batroxobin and thrombocytin were isolated from Bothrops atrox venom. These three thrombin-like enzymes had different specificity for platelet activation and fibrinogen clotting. The clotting activities were 700, 170 and 7 μ/mg for batroxobin, acutin and thrombocytin, respectively. They induced aggregation and ATP release of washed rabbit platelets. The aggregating activities were 10^2, 10^4 and 10^5 times less than that of thrombin for thrombocytin, acutin and batroxobin, respectively basing on the clotting unit. The platelet - activating potency was correlated with their effectiveness on the retractility and elasticity of the clots. Platelet aggregation induced by thrombin or thrombocytin could be inhibited by heparin with antithrombin III while that by acutin or batroxobin could not. The thrombin-like enzymes did not induce aggregation of thrombin-degranulated platelets even fibrinogen was added. Indomethacin showed weak inhibition on the aggregation while the ADP - scavenging system, creatine phosphate/creatine phosphokinase, or apyrase inhibited the aggregation induced by the three thrombin-like enzymes but not that by thrombin. In the presence of EGTA, only thrombin could induce ATP release from platelets. It is concluded that the aggregation induced by thrombin-like enzymes is different from that of thrombin and mainly due to ADP released from platelets.

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AUSTRALIAN SNAKE VENOMS AND THEIR EFFECT UPON HUMAN PLATELETS. L.R. Marshall and R.P. Herrmann. Department of Haematology, Royal Perth Hospital, Perth, Western Australia.

The in vitro effect of Australian snake venoms on human citrated plasma has been documented and the majority induce coagulation, in keeping with the common clinical presentation of D.I.C. following envenomation. The effect of these venoms upon platelets in vitro has hitherto not been studied extensively and clinical evidence is conflicting, some cases with thrombocytopaenia have been reported. Twenty Australian venoms were tested, 19 elapids and one hydrophiid (Enhydrina schistosa). Four crotalid snake venoms from the Americas and S.E. Asia were also tested. All of the venoms (1 mg/ml) were investigated for their ability to aggregate both fresh washed platelets (200 x 10^9/l) resuspended in modified Ardlie's buffer pH 7.35 and formaldehyde fixed platelets (200 x 10^9/l) in phosphate buffered saline pH 7.35 using a dual channel Chronolog aggregometer. Samples were taken for electron microscopy (EM).

All elapid venoms induced aggregation in fresh platelets, some only minimally and often after a long lag phase. EM studies revealed only clumping without degranulation of the platelets. This was in marked contrast to the crotalid venoms where rapid aggregation and gross degranulation occurred. The hydrophiid venom failed to induce aggregation of the fresh platelets, however upon addition of normal plasma gross aggregation and degranulation was demonstrated. Aggregation of fixed platelets was negligible in the presence of the majority of elapid and the hydrophiid venoms. The crotalid venoms however did induce aggregation, although to a lesser extent than with the fresh platelets.

The elapid venoms, along with the others studied, required metabolically active platelets to exert their maximal effect. Crotalid and hydrophiid venoms were more active against platelets than the elapid venoms. The hydrophiid venom's action on platelets was unique in that a plasma co-factor appeared to be required and this is the subject of further investigations.