0283

08:45 h

CORRELATION OF TENSION GENERATION AND MYOSIN LIGHT CHAIN PHOSPHORYLATION IN A THROMBIN ACTIVATED PLATELET CONTRACTILE MODEL. J. Daniel, R. Sevy, and L. Salganicoff. Thrombosis Research Center and Dept. of Pharmacology, Temple Univ. School of Med., Phila., Pa. 19140.

Previous experiments have established that a 20,000 daltons protein which becomes phosphorylated when platelets are stimulated by thrombin is a myosin light chain. A model of thrombin activated platelets, in which the tension generated by contraction of the platelet aggregate is measured under isometric conditions, was used to study the relationships between steady-state myosin light chain phosphorylation and tension generation. After thrombin activation the platelet aggregate contracted, and in the contracted state myosin light chain was found to be phosphorylated. Relaxation induced by either PGI_2 (which activates adenyl cyclase) or 120 mM K_2SO_4 containing 2 mM EGTA (which is presumed to deplete cytoplasmic Ca^{++}) was accompanied by dephosphorylation of myosin light chain. Recontraction of the relaxed preparation with either ADP (10^{-4}M) or epinephrine (10^{-6}M) in the presence of Ca^{++} was accompanied by rephosphorylation of myosin light chain. The relationships between isometric tension, cAMP production and myosin light chain phosphorylation and dephosphorylation were studied in the contracted thrombin activated preparation subsequently relaxed with graded doses of PGI_2 ($3x10^{-9}$ to $3x10^{-6}$M), such that complete dose-response curves were obtained. There was a striking positive correlation between the level of isometric tension and the degree of phosphorylation of myosin light chain. Over the same dose range of PGI_2 there was no increase in cAMP levels, within the error of the detection procedure, until the ED-50 (approx. $3x10^{-8}$M) for relaxation with PGI_2 was exceeded. At the same time that relaxation and myosin light chain dephosphorylation occurred in response to PGI_2, there was phosphorylation of a 22,000 daltons band; relaxation with K_2SO_4-EGTA did not affect this band. The above experiments are consistent with the hypothesis that myosin light chain phosphorylation is directly involved in platelet contraction

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09:00 h

ASSOCIATION OF α-ACTININ WITH THE PLASMA MEMBRANE OF HUMAN BLOOD PLATELETS. Jan J. Sixma, Marion E. Schiphorst and Brigitte H. Jockusch. Department of Hematology, State University Utrecht, The Netherlands and EMBO-Laboratory, Heidelberg, West-Germany.

The association of α-actinin with the plasma membrane of human blood platelets was investigated using immunoadsorbed antibodies against α-actinin from porcine skeletal muscle and against actin from chicken gizzard. Platelet membranes enriched 10 times in radiolabelled wheat-germ-agglutinin were prepared utilizing the polylysine-bead technique of Cohen et al (1977). Immunofluorescent staining showed the presence of actin and α-actinin associated with these membranes.

Platelets were ectolabelled with lactoperoxydase-iodination. A crude membrane preparation was solubilized with Triton X 100, and incubated with anti α-actinin or appropriate control antibodies. The formed immune complexes were bound to Protein A-Sepharose beads, and subjected to 2-D-electrophoresis with i.e.f. in the first dimension. α-actinin from these membranes was not radiolabelled, had an apparent mol wt of 100 kdaltons and a pI of 6.7, similar to an α-actinin preparation from porcine skeletal muscle that was investigated in parallel. The location of α-actinin in the 2-D pattern was different from that of glycoprotein III. No specific glycoprotein was found bound to α-actinin under the experimental conditions. Treatment of membranes with non-ionic detergents and 'protein perturbants' indicated that α-actinin is an 'extrinsic protein'. These data indicate that α-actinin as well as actin are closely associated with the plasma membrane, linked by electrostatic interaction. Both actin and α-actinin were not exposed on the outside in a resting platelet.

0285

09:15 h

THROMBIN-INDUCED RESPONSES IN HUMAN PLATELETS DIFFER IN THEIR REQUIREMENT FOR RECEPTOR OCCUPANCY: EVIDENCE FOR TIGHT COUPLING BETWEEN OCCUPANCY AND FORMATION OF PHOSPHATIDIC ACID. Holm Holmsen, Carol A. Dangelmaier and Holm-Kjetil Holmsen. Thrombosis Research Center, Temple University, Philadelphia, PA 19140 U.S.A.

Thrombin causes secretion of dense granule constituents from platelets. The time course and extent of this response is unaltered if thrombin is rapidly neutralized after secretion has started, indicating that a sustained occupancy of the thrombin receptors is not required. Using a 50-fold excess of hirudin to neutralize thrombin, we show here that the shape change and aggregation responses also are independent on sustained receptor occupancy. In contrast, the secretion of acid hydrolases, liberation of [^{3}H-]arachidonate (from phospholipids in platelets prelabeled with [^{3}H]arachidonate) and formation of [^{32}P]phosphatidic acid (in platelets prelabeled with ^{32}P-orthophosphate) induced by thrombin were immedately stopped by hirudin. However, the incorporation of ^{32}P into phosphatidylinositol and breakdown of [^{3}H]phosphatidyl inositol continued unaffected after thrombin removal. Thrombin-induced platelet responses can thus be subdivided into two classes according to their requirement for receptor occupancy: The first class (shape change, aggregation, dense granule secretion phosphatidylinositol breakdown and phosphatidylinositol synthesis) requires a short, initial occupancy, while the second class (acid hydrolase secretion, arachidonate liberation and phosphatidic acid synthesis) requires an occupancy that is sustained as long as the response is executed. The increased turnover of phosphoinositides is a cyclic process, and our results show that only one step - the formation of phosphatidic acid from diacylglyceride and ATP - is tightly coupled to receptor occupancy by the agonist.

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09:45 h

ADRENALINE ACTIVATES HUMAN PLATELETS BUT DOES NOT CAUSE PRIMARY AGGREGATION IF THROMBIN GENERATION IS INHIBITED BY HIRUDIN. J.-P. Cazenave, A. Sutter, S. Hemmendinger, M.-L.Wiesel, F. Lanza and J. Daver. Laboratoire de Biologie et de Pharmacologie des Plaquettes,Centre de Transfusion Sanguine, Strasbourg et Centre d'Immunologie P.Fabre,Castres, France.

Adrenaline(ADR) affects human platelets(PLAT) by binding to α receptors,inhibiting adenylate cyclase and translocating Ca^{2+} across the plasma membrane.In citrated PLAT-rich plasma(CIT-PRP), ADR induces primary aggregation(1^{st}AGG), which may be followed by 2^{nd}AGG due to the activation of the arachidonate(AA) pathway and the release(REL) of ADP. ADR acts synergistically with other aggregating agents.In contrast,ADR does not aggregate suspensions of washed human PLAT(SWHP) and hirudin(HIR)-PRP.To determine the role of traces of thrombin(THR) on AGG and REL of ^{14}C-5HT of prelabeled PLAT induced by ADR,we have used SWHP and PRP anticoagulated with CIT(13mM), HIR(30 U./ml) or both and examined the effects of addition of HIR(30 U./ml),a specific inhibitor of THR. ADR(1-10μM) does not cause AGG or REL of SWHP,even in the presence of added HIR or CIT. However,ADR(1-10μM) potentiates the effects of ADP and AA on AGG and REL.This is not inhibited by HIR. THR alone (0.02 U./ml) causes shape change of SWHP,addition of ADR(4.5μM) causes extensive AGG and REL, which are inhibited by HIR.In CIT-PRP, ADR(1-10μM)causes 1^{st} AGG followed by 2^{nd}AGG and REL, addition of HIR to CIT-PRP has no effect on AGG and REL. Aspirin(ASA) and/or CP/CPK inhibit 2^{nd}AGG-induced by ADR in CIT-PRP. Addition of ASA+CP/CPK+HIR does not inhibit ADR-induced 1^{st}AGG in CIT-PRP.After addition of CIT to HIR-PRP, ADR does not AGG PLAT.In contrast,ADP causes 1^{st}AGG without REL in HIR-PRP and addition of CIT to HIR-PRP induces 2^{nd}AGG and REL.However,ADR causes 1^{st}AGG and REL, if PRP was prepared from blood to which HIR has been added at the same time of CIT or later.In conclusion:1) ADR does not cause 1^{st}AGG in SWHP or HIR-PRP if the PLAT have not been exposed to THR during their isolation; 2) traces of THR change the response of PLAT to ADR independently of the effect of CIT on Ca^{2+} concentrations; 3) the use of CIT-PRP does not prevent completely THR generation.