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08:45 h

INVOLVEMENT OF CALMODULIN IN PLATELET REACTION.
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Calmodulin is a ubiquitous Ca^{2+}-binding protein, modulating a number of Ca^{2+}-dependent cellular reactions. In blood platelets, involvement of calmodulin has been demonstrated in the activation of myosin light chain kinase and of phospholipase A_2. In the present study, involvement of this protein in platelet reaction was thoroughly investigated, utilizing phenothiazines(chlorpromazine and trifluoperazine) known as selective inhibitors of calmodulin.

Phenothiazines in micromolar concentration completely inhibited platelet aggregation and secretion induced by ADP epinephrine, collagen, thrombin or Ca^{2+}-ionophore. They also completely inhibited the aggregation triggered by arachidonate or by the mixture of thromboxane A_2 and prostaglandin endoperoxides, which is probably due to the inhibition of activation of myosin light chain kinase. As another possible involvemnt of calmodulin in the reaction, the effect of phenothiazines on the release of arachidonate from phospholipids was studied in stimulated platelets to which radioactive arachidonate had been incorporated. The release was dose-dependently inhibited by either of phenothiazines. Then, their effect on the hydrolysis of phosphatidylinositol by platelet sonicates was investigated, using ^{3}H-myoinositol phosphatidylinositol as substrate. Either of phenothiazines suppressed the hydrolysis in the micromolar concentration, indicating their inhibition on phosphatidylinositol specific phospholipase C.

These observations suggest involvement of calmodulin in at least two steps of platelt reaction: one is the step of the release of arachidonate from phospholipids (the activation of phospholipase C in addition to phospholipase A_2) and the other is the step of contraction of platelet actomyosin after the formation of thromboxane A_2 (the activation of myosin light chain kinase).

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09:00 h

CALMODULIN BINDING PROTEINS IN PLATELET ACTOMYOSIN.
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Platelet actomyosin (thrombosthenin) possesses a myosin-linked Ca^{2+} regulation and Ca^{2+} sensitivity is conferred to it by calmodulin through myosin light chain kinase. Calmodulin binding proteins if they are present in the actomyosin complex may have an important regulatory role in the contractile mechanism of platelet activation. To test this possibility an aceton powder was made from platelet actomyosin and extracted with an 8 M urea containing buffer. The extract was examined for the presence of calmodulin binding proteins by alkaline urea polyacrylamide gel electrophoresis. It was shown by this technique that some proteins in the actomyosin complex can form a Ca^{2+} dependent complex with both calmodulin and skeletal muscle troponin C (TNC is closely related to calmodulin) even in the presence of 8 M urea. Calmodulin binding proteins could be isolated from the extract by affinity chromatography in 8 M urea on TNC-Agarose column. 3 major proteins of 270 K, 61 K and 23 K molecular weight were eluted by EGTA and each of them was able to bind to calmodulin or TNC if Ca^{2+} was present. At least one of these calmodulin binding proteins exerted a troponin I like effect when tested on reconstituted skeletal muscle actomyosin and the 23 K protein showed a close similarity to troponin I, the inhibitory protein of the actin linked Ca^{2+} regulatory system in skeletal muscle. It is presumed that calmodulin binding proteins may have a dual role in the regulation of platelet actomyosin. They can inhibit the Ca^{2+} dependent phosphorylation of myosin light chain and one or more of them may also exert an actin linked inhibitory effect.

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09:15 h

PLATELET CALMODULIN MEDIATES THE CALCIUM DEPENDENT ACTIVATION OF FACTOR XIII.
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Transglutaminases are ubiquitous in cells and require calcium for their activation. The factor XIII zymogen, present in plasma and in the platelet cytosol, requires for its activation both a limited proteolytic activity on the catalytic subunit, "a", and, in the use of the plasma enzyme, calcium for dissociating subunit "a" from the carrier subunit "b". Calcium is also necessary for exposing the reactive sulfhydryl group. We have recently suggested a role for the platelet factor XIII in the generation of calcium-dependent cross-linking processes in platelets. Since calmodulin is present in considerable amounts in the platelet cytosol and is known to regulate the activity of various calcium-dependent enzymes and cellular reactions, we have investigated its possible role in factor XIII activation. Since the "a" subunit of platelet factor XIII is identical to its plasma counterpart, the more easily purified plasma zymogen was used. The effect of calmodulin on the two calcium-dependent steps of factor XIII activation was investigated following thrombin-stimulated hydrolysis of the "a" subunit. Platelet calmodulin was found to enhance by at least 3 fold the calcium-dependent unmasking of the reactive sulfhydryl groups which were titrated with ^{14}C-iodoacetamide. Calmodulin also enhanced by at least 4-fold the calcium-dependent dissociation of the b subunit from its complex with the thrombin-hydrolyzed "a" subunit. The calmodulin mediation of the calcium-dependent steps of factor XIII activation may be important for regulating the factor XIII-dependent cross-linking reactions in platelets and is reminiscent of the calcium-related regulatory role of fibrinogen on factor XIII activation which could prevail in plasma. An investigation of the possible role of calmodulin on other tissue transglutaminases is warranted.

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EXPRESSION OF THE Fc RECEPTOR ON HUMAN PLATELETS IS REGULATED BY CYCLIC AMP CHANGES.
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The Fc receptor of human platelets is involved in their injury by immune complexes composed of drugs, viruses, or bacteria and corresponding IgG antibody. Using Protein A-bearing staphylococci coated with isolated human IgG Fc fragment as a functional probe for the Fc receptor, we measured factors regulating its expression on human platelets. Isolated Fc fragment in the fluid phase and not bound to Protein A-bearing staphylococci remained without a measurable effect on human platelets isolated from plasma proteins. Fc fragment bound to Protein A-bearing staphylococci caused their aggregation and release of ^{3}H-serotonin. Prostaglandin I_2 (PGI_2, prostacyclin) known as the most potent activator of platelet adenylate cyclase blocked the interaction between Fc fragment-coated staphylococci and human platelets. This effect was dose- and time-dependent. A corresponding rise in platelet cAMP levels was observed in parallel experiments. Refractoriness of PGI_2-treated platelets was persistent up to 2 hr and was paralleled by a sustained increase in platelet cAMP level. Thus, a rise in platelet cAMP level induced by PGI_2 is associated with a reduced expression of the Fc receptor on human platelets.