Time
09.15 **0854** HUMAN PLATELETS POSSESS AN INDUCIBLE AND SATURABLE RECEPTOR SPECIFIC FOR FIBRINOGEN.

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Fibrinogen is required for ADP-dependent aggregation of human platelets; but the molecular basis for the requirement is not known. Fibrinogen, rendered free of fibronectin, plasminogen, VIII, II and XIII, was radiolabelled (^{125}I-fg) and used as the probe for a platelet receptor. Platelets were isolated by differential centrifugation, washed in calcium-free Tyrode's solution containing 0.2% albumin and isolated by exclusion from Sepharose CL 2B. ^{125}I-fg bound to platelets only after ADP stimulation, and binding was characteristic of a saturable receptor. Specificity was established by demonstration that fibrinogen completely inhibited binding of ^{125}I-fg, competing with the same affinity, whereas other proteins were without effect. SDS polyacrylamide gel electrophoresis of the platelets after reduction demonstrated ^{125}I polypeptide chains with migration identical to Aα, Bβ and γ chains; and fibrinopeptide A was on the bound ^{125}I-fg indicating that fibrinogen per se is bound. Scatchard analysis of binding at optimal concentration of ADP ($\geq$ 5 μM) indicated 4,644 $\pm$ 186 receptors with a dissociation constant Kd = 1.3 x 10^{-7} M, comparable to that estimated directly from the aggregation reactions. Receptors induced at different ADP concentrations appeared to have the same affinity for fibrinogen, indicating the probable existence of only a single class of receptors. We conclude that a specific saturable receptor is rapidly induced on human platelets by ADP, and postulate that this event may play a significant role in the observed in vitro aggregation phenomenon and perhaps the primary hemostatic function of platelets. Supported by NIH grant HL-16411.

09.30 **0855** EXPOSURE OF A HUMAN PLATELET FIBRINOGEN RECEPTOR BY ADP.

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Fibrinogen is a cofactor for the aggregation of human platelets by ADP but its precise role is not known. In order to clarify the function of fibrinogen in platelet aggregation, we measured the binding of ^{125}I-labeled human fibrinogen to gel-filtered human platelets before and after platelet stimulation by ADP. Incubations were performed without stirring to prevent platelet aggregation and secretion. Platelet-bound and free ^{125}I-fibrinogen were separated by centrifugation of the platelets through silicone oil. Specific fibrinogen binding was that ^{125}I-fibrinogen which could be displaced from the platelets by a 10-fold excess unlabeled fibrinogen. Specific fibrinogen binding required platelet stimulation by ADP and either Ca^{+2} or Mg^{+2}. Specific binding reached equilibrium within 60 sec., demonstrated saturation kinetics, and did not occur with thrombasthenic platelets. Scatchard analysis demonstrated a single class of binding sites with a K_d of 25 $\pm$ 3.9 µg/ml and 39,000 $\pm$ 5,000 binding sites per platelet. When the extent of ADP-induced fibrinogen binding to unstirred platelets was compared to the extent of aggregation of stirred platelets induced by the same concentrations of ADP, a correlation of 0.96 was seen. This study demonstrates that a uniform population of fibrinogen receptors is exposed on the platelet surface by ADP. Furthermore, we suggest that the fibrinogen molecules bound to the platelet as a result of ADP stimulation are directly involved in the platelet aggregation response.

09.45 **0856** MOBILIZATION OF FIBRINOGEN RECEPTOR SITES ON HUMAN PLATELETS STIMULATED WITH ADP IS PREVENTED BY PROSTACYCLIN

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Fibrinogen is a plasma factor required for aggregation of human platelets by ADP. The mechanism of platelet-ADP-fibrinogen interaction was studied by measuring the equilibrium binding of ^{125}I-fibrinogen to human platelets separated from plasma proteins. Binding of ^{125}I-fibrinogen to platelets not stimulated with ADP was low and unaffected by an excess of unlabelled fibrinogen. However, when platelets were stimulated with 4µM of ADP, there was an eightfold increase in the number of available binding sites for human fibrinogen with affinity constant of $1.9 \times 10^{9} M^{-1}$. This striking increase in fibrinogen receptor sites on human platelets was specific for ADP as contrasted to ATP, AMP, and adenosine. Prostacyclin (Prostaglandin I_2, PGI_2), a novel prostaglandin produced by the blood vessel wall, completely blocked this ADP-induced increase in fibrinogen receptor sites on human platelets. The effect of PGI_2 was prompt and concentration dependent, reaching maximum at 10^{-9}M. 6-keto $PGF_{1\alpha}$, a stable derivative of PGI_2, did not have such an effect. Thus movement of fibrinogen receptor sites on human platelet membrane stimulated with ADP is prevented by PGI_2. This represents a new biologic property of this vascular hormone and contributes to better understanding of its potent inhibitory effect in vitro and in vivo on ADP-induced platelet aggregation requiring mobilization of fibrinogen receptor.