0605
08:45 h

ARACHIDONATE-INDUCED FIBRINOGEN BINDING TO THROMBIN-DEGRANULATED RABBIT PLATELETS IS INDEPENDENT OF RELEASED ADP.
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When human or rabbit platelets are stimulated with ADP, fibrinogen (Fbg) binding sites are revealed, the platelets bind Fbg and aggregate. Since stimulation with other aggregating agents (arachidonate, collagen or ionophores) releases platelet granule contents, including ADP and Fbg, it is difficult to determine whether these agents cause aggregation or Fbg binding that is independent of ADP. Therefore we treated rabbit platelets with thrombin (0.73 U/ml) to release at least 90% of their dense granule contents, as measured by ^{14}C-serotonin, washed and resuspended them, and studied aggregation and Fbg binding upon stimulation with ADP or arachidonate. In the presence of Fbg, thrombin-degranulated platelets (TDP) aggregate in response to ADP or arachidonate at concentrations that aggregate untreated platelets, although TDP aggregate somewhat less extensively. When TDP are aggregated with 50 μM arachidonate, they lose up to 9% of their remaining serotonin, corresponding to a concentration of ADP in the suspending medium of not more than 0.06 μM, which does not aggregate TDP or cause detectable Fbg binding. When creatine phosphate/creatine phosphokinase (CP/CPK) is added at a concentration that abolishes aggregation in response to 1 μM ADP, it reduces aggregation caused by arachidonate by only 18%. Binding studies with ^{125}I-Fbg show that stimulation of TDP with either ADP or arachidonate results in specific Fbg-binding similar to the binding to ADP-stimulated normal platelets. CP/CPK almost completely inhibits binding induced by ADP but reduces binding induced by arachidonate by only 30%. Aggregation and binding studies with TDP using a combination of arachidonate with low concentrations of ADP failed to show synergistic effects. Thus arachidonate causes aggregation and Fbg binding to TDP that are independent of ADP, although the magnitude of these effects may be increased by released ADP.

0606
09:00 h

ROLE OF PROSTAGLANDINS IN PLATELET FIBRINOGEN RECEPTOR EXPOSURE.
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Fibrinogen binding to membrane receptors exposed by agonists such as ADP and thrombin is a prerequisite for platelet aggregation. However, a role for platelet prostaglandins in this process remains to be clarified. To assess whether platelet prostaglandins can expose fibrinogen receptors, we examined the effects of aspirin (ASA) and indomethacin on fibrinogen binding to ADP and epinephrine-stimulated platelets. Fibrinogen binding was measured by incubating gel-filtered human platelets (GFP) with ^{125}I-labeled fibrinogen, $CaCl_2$ and ADP or epinephrine at 37°C for 3 min without stirring. Free and platelet-bound ^{125}I-fibrinogen were separated by rapid sedimentation of the platelets through silicone oil. The platelet release reaction, measured as ^{14}C-serotonin secretion, did not occur under these conditions. Fibrinogen binding in response to 1-2μM ADP or 10μM epinephrine was inhibited 40-60% by preincubation of platelet-rich plasma with 1 mM ASA for 20 min at 37°C. Similar results were produced by adding 25μM indomethacin to GFP. The inhibitory effect of ASA on ADP-stimulated fibrinogen binding could be overcome by increasing the ADP concentration while the inhibition of epinephrine-stimulated fibrinogen binding could not. However, stimulation of aspirin-treated platelets with both 10μM epinephrine and the prostaglandin endoperoxide PGH_2 (1μM) restored the extent of fibrinogen binding to that of control platelets stimulated by 10μM epinephrine alone. Scatchard analysis demonstrated that ASA decreased the number but not the affinity of the exposed fibrinogen binding sites. Identical results were obtained if ASA was ingested before blood donation. These studies demonstrate that prostaglandins synthesized in response to platelet stimulation can expose fibrinogen receptors on the platelet surface. Furthermore, these studies support the concept that platelet aggregation can occur through mechanisms independent of platelet ADP secretion.

0607
09:15 h

FIBRINOGEN (FIBR) BINDING IS NECESSARY FOR INCORPORATION OF ^{125}I-LABELED MEMBRANE PROTEIN INTO THE PLATELET CYTOSKELETON.
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Platelets stimulated with ADP bind fibr specifically, and this ability correlates well with their ability to aggregate when they are shaken with ADP and fibr. Others found that platelets incubated with chymotrypsin (CT) bind and aggregate with fibr without stimulation by ADP; as with ADP-induced stimulation, these responses are inhibited by excess EDTA. Phillips et al. showed that thrombin activation produced a large Triton-insoluble cytoskeleton in the presence of either EDTA or $CaCl_2$, but that ^{125}I-labeled glycoprotein(s) IIb-III were incorporated into the cytoskeleton only when $CaCl_2$ was present and aggregation occurred. This system cannot be used to assess the role of fibr in membrane glycoprotein incorporation, as exogenous fibr is not required for thrombin-induced aggregation. We therefore used CT-treated platelets labeled with ^{125}I. They were suspended in calcium-poor Hepes-buffered Tyrode's solution at 22°C for 30 min with 2.5 mM EDTA or 1 mM $CaCl_2$ with or without 100 ug/ml fibr. Only samples with $CaCl_2$ + fibr aggregated, and similar samples prepared with CT-treated platelets which had first been incubated with EDTA at 37°C for 15 min failed to aggregate despite recalcification. Cytoskeletons were prepared by adding an equal vol of 2% Triton X-100-5 mM EGTA-0.1 M Tris pH 7.4 and centrifuging 1 hr later. Cytoskeletal protein was 10-25% of total and did not vary with aggregability. CT-treated platelets showed more ^{125}I bands than untreated labeled platelets. The two bands with highest M_r on SDS-PAGE were present only when the platelets aggregated during incubation, i.e., under conditions in which fibr is bound. Possibly the dimeric fibr molecule must link two surface molecules before they can attach to the cytoskeleton.

0608
09:45 h

PLATELET AGGREGATION INDUCED IN VIVO BY HEPARIN AND HEPARIN FRACTIONS.
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As we have reported, heparin-induced platelet aggregation in vitro varies among heparin subfractions, being generally less with lower molecular weights and having a reciprocal relationship with antithrombin affinity.

We now have studied heparin-induced platelet aggregates in vivo by the technique of Wu and Hoak using arterial blood from unanesthetized rabbits. Porcine mucosal heparin was fractionated by gel filtration into high molecular weight (ave. 15,000 Daltons) or low molecular weight (ave. 6,000 Daltons) preparations. IV administration of commercial porcine mucosal heparin (spec. act. 150 u/mg) or high (spec. act. 183 u/mg) or low (spec. act. 208 u/mg) molecular weight fractions was followed by an increase in the platelet aggregate ratio compared with preinjection control values. The rise in platelet aggregate ratio with heparin was significantly different from the effect of a saline placebo (n=8) but was not significantly different among rabbits receiving the commercial heparin (n=9) or the high (n=8) or low (n=8) molecular weight preparations. Peak rise in circulating aggregate ratio occurred 2 minutes after the injection, and values returned to control levels within 15 to 30 minutes. There was no change in platelet count in blood collected in EDTA, suggesting that the aggregates were not removed from the circulation in vivo.

Heparin fractions of low molecular weight were further separated according to antithrombin affinity by an antithrombin binding technique. In 8 rabbits low molecular weight/high antithrombin affinity heparin (spec. act. 480 u/mg) did not cause formation of platelet aggregates. The results were significantly different from those with commercial heparin ($p=0.05$) or with the other heparin fractions ($p=0.06$).

Clinical use of low molecular weight heparin of high antithrombin affinity may lead to fewer heparin-induced platelet effects and to an improvement in anticoagulant therapy.