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PLATELET ACTIVATION AND AGGREGATION BY GAS BUBBLES IN VITRO.
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After diving, in decompression sickness and after extra-corporeal circulation in membrane oxygenators, there is often a reduction in the number of circulating platelets. Gas bubbles seem to be the common denominator for these quite different conditions, and it is assumed that gas bubbles activate the platelets although by unknown mechanisms. We have used gas bubbles as a platelet agonist in an aggregometer-like apparatus. The bubbles produced platelet aggregation similar to that caused by "classical" agonists, e.g., ADP, epinephrine etc. The gas-liquid interface was essential for this aggregation, and the bubble diameter, rather than the type of gas or the total number of bubbles, determined the potency of this agonist. Electron microscopical studies revealed that single platelets and platelet aggregates with numerous, short pseudopods adhered to the bubble wall. The platelet aggregation caused by gas bubbles was abolished by metabolic blockers (2-deoxyglucose and antimycin A), EGTA, phosphodiesterase inhibitors (theophylline, caffeine, papaverine, dipyridamole), adenylate cyclase activators (PGE_1, adenosine, AMP), or a combination of low concentrations of phosphodiesterase inhibitors and adenylate activators. Theophylline which is regarded as a weak inhibitor of platelet activation in general, was an especially potent inhibitor of gas bubble-induced platelet aggregation whereas cyclooxygenase inhibitors (acetyl salicylic acid, indomethacin), adrenoreceptor blocker (yohimbine), guanylate cyclase activators (azide, nitroprusside) and trifluoperazine had little or no effect. Bubble-induced aggregation is thus similar to the primary, and not the secondary, aggregation caused by classical agonists. However, the ineffectiveness of guanylate cyclase activators distinguishes bubble-induced aggegation from "classical" primary aggregation.

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INCREASED AGGREGATION AND SECRETION RESPONSES OF HUMAN PLATELETS WHEN LOADED WITH THE CALCIUM FLUORESCENT PROBES QUIN2 AND FURA-2.
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Incubation of human platelets with the fluorescent dye esters quin2-AM (10 μM) or fura-2-AM (1 μM) makes possible the direct measurement of intracellular free calcium ($[Ca^{2+}]_i$). Under these conditions, basal levels of $[Ca^{2+}]_i$ of 120 ± 16 nM (n=23) using quin2 and 137 ± 15 nM (n=5) using fura-2 can be measured. Both probes record comparable increases of $[Ca^{2+}]_i$ after stimulation with ADP, thrombin, PAF, or U-46619. Incorporation into human platelets of quin2 or fura-2 at the concentrations used to monitor $[Ca^{2+}]_i$ leads to the activation of platelets. This was shown by increased aggregation and secretion responses of quin2 or fura-2 loaded platelets after stimulation with ADP (5 μM), PAF (1 μM) and with low concentrations of thrombin (0.015 U/ml), collagen (0.5 μg/ml), the endoperoxide analog U-46619 (0.5 μM) or the calcium ionophore A 23187 (1 μM). Quin2 and fura-2 mediated platelet activation could be due to altered arachidonic acid metabolism, since it was partly inhibited by prior treatment with the cyclooxygenase inhibitor acetylsalicylate (1 mM). In contrast, platelets loaded with higher concentrations of calcium chelators (20 to 100 μM quin2-AM) exhibited diminished aggregation responses to all aggregating agents. This latter effect was accompanied by increased fluidity of the platelet plasma membrane bilayer and by the exposure of a new pool of membranes at the outer surface of platelets, as monitored with trimethylammonium-diphenylhexatriene (TMA-DPH) in platelets loaded with the non-fluorescent calcium probe analog MAPT. Platelet shape change, as measured in the aggregometer, was dose-dependently inhibited after loading of quin2 (10-50 μM quin2-AM), even at concentrations which potentiated aggregation. We conclude that incorporation of intracellular calcium chelators alters platelet responses, including at concentrations used to monitor intracellular calcium changes.

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EPINEPHRINE INDUCED POTENTIATION OF ARACHIDONATE AGGREGATION IN QUIN 2 LOADED PLATELETS IS NOT MEDIATED BY ELEVATION OF CYTOSOLIC CALCIUM.
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Previous studies have demonstrated that chelation of ionized cytosolic calcium by Quin 2 leads to a refractory state in platelets. However, epinephrine (Epi) induced membrane modulation restored the sensitivity of Quin 2 loaded platelets to the action of agonists. Further studies with Quin 2 and Fura 2 suggested that Epi induced recovery of sensitivity by refractory platelets to aggregation by arachidonate does not require elevation of cytosolic calcium. To further delineate the role of calcium in membrane modulation, we followed phosphoinositol metabolism and myosin light chain phosphorylation using radio-labeled platelets. The total amount of PI metabolites generated after exposure to Epi, AA or Epi + AA were significantly less than that formed after 0.1 u thrombin stimulation. Quin 2 at 40 μM concentration had no inhibitory effect on PI hydrolysis. However, at this concentration it effectively blocked AA induced aggregation. Although Epi treatment restored the sensitivity of Quin 2 loaded platelets to the action of AA, it did not enhance the formation of increased quantitites of PI metabolites. Similar to earlier studies, Quin 2 loading effectively blocked phosphorylation of myosin light chain (20 K). Although a combination of Epi + AA restored some phosphorylation of 20 K protein in Quin 2 loaded platelets, the degree of phosphorylation was significantly less than that achieved in control stimulated platelets. Results of these studies suggest that Epi induced restoration of sensitivity to refractory, Quin 2 loaded platelets is not mediated by 1) significant elevation of cytosolic calcium, 2) enhanced production of PI metabolites, 3) increased phosphorylation of 20 K protein. Epi induced membrane modulation is a novel, independent mechanism capable of restoring sensitivity to agonists in platelets with compromised function.

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ADRENALINE ACTIVATES HUMAN PLATELETS BUT IS NOT PER SE AN AGGREGATING AGENT. EFFECTS ON PLATELET MORPHOLOGY, MEMBRANE FLUIDITY, FIBRINOGEN BINDING, CYTOPLASMIC FREE CALCIUM AND PROTEIN PHOSPHORYLATION.
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Adrenaline (Adr) is generally considered as a full agonist able to induce *in vitro* the aggregation of human platelets and could play an important role *in vivo* in the appearance of thrombotic disorders when catecholamine levels are increased. Adr (2.5 M) induces the aggregation and secretion of 41 % of preloaded ^3H-serotonin in human platelets in citrated plasma. This effect is not seen in plasma collected on 50 ATU/ml hirudin, and is due to the generation of traces of thrombin during blood collection and not to a direct effect of citrate itself, such as the lowering of plasma free calcium. With washed human platelets suspended in Tyrode's buffer containing 2 mM Ca^{2+}, 0.35 % albumin and apyrase, Adr (0.1 - 100 M) does not cause shape change, aggregation or secretion of serotonin and does not modify platelet ultrastructure as judged by electron microscopy. Adr (1-100 M) does not change platelet membrane fluidity, as studied with the lipophilic fluorescent probe TMA-DPH. Adr has no direct effect on fibrinogen binding to intact platelets, intracellular Ca^{2+} levels measured with quin2, or phosphorylation of 20 KDa or 47 KDa polypeptides, whereas all these parameters are modified after stimulation with ADP or thrombin. Adr potentiates the action of all types of aggregating agents on aggregation, secretion, intracellular Ca^{2+} levels, membrane fluidity, fibrinogen binding or protein phosphorylation. This effect is also seen with alpha2-adrenergic agonists (noradrenaline, alpha-methyl noradrenaline, clonidine) and is inhibited by alpha2-adrenergic antagonists such as yohimbine. The potentiation of platelet aggregation by Adr is not modified by prior incubation of the platelets with 1mM aspirin for 15 min. This study shows that Adr alone does not induce modifications of morphology, metabolism or function of intact and functional washed human platelets and that Adr cannot be considered *per se* as an aggregating agent. However, Adr interacts with alpha2-adrenergic receptors on human platelets and potentiates biochemical and aggregatory responses induced by other platelet agonists.

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