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INHIBITORY EFFECT OF EXOGENOUS ARACHIDONIC ACID OR LINOLEIC ACID ON RABBIT PLATELET AGGREGATION AND RELEASE REACTION. M. Cattaneo, R.L. Kinlough-Rathbone and J.F. Mustard, McMaster University, Hamilton, Ontario, Canada.

In contrast to other release-inducing agents (e.g. thrombin) arachidonic acid (AA) releases only 40-50% of amine storage granule contents and although low concentrations induce aggregation, high concentrations do not. Several theories have been proposed to explain these observations: 1) AA or its products inactivates the cyclo-oxygenase; 2) the products of AA increase platelet cAMP; 3) lipoxygenase products are inhibitory; 4) unsaturated fatty acids (UFA) perturb the cell membrane. Using washed rabbit platelets we examined the effect of AA on platelet function. In these experiments aspirin-treated platelets (ASA 5.5 mM) were exposed to AA (230 μM) for 15 min. and then to PGE_1 (10 μM) for 30 min. The platelets were then resuspended. These platelets did not aggregate to ADP (9 μM) and their response to thrombin (0.02-0.05 U/ml) was impaired in contrast to control, ASA-treated platelets not exposed to AA. Non-ASA-treated platelets exposed to AA (230 μM), deaggregated with PGE_1, and then resuspended also did not aggregate in response to ADP (9 μM) collagen, AA (230 μM) or thrombin (0.02-0.05 U/ml). When platelets pretreated with ASA and AA were mixed 1:1 with normal platelets and the mixture stimulated with AA (230 μM), the AA-treated platelets did not release their granule contents whereas the normal platelets did. These results do not support the hypothesis that the inhibitory effect of AA on platelet aggregation and release is primarily due to inhibition of cyclo-oxygenase or an increase in cAMP caused by AA products. It seems unlikely that inhibition by AA can be due to products of the lipoxygenase pathway, because the effect persists when the platelets are washed and resuspended. Similar results were obtained by incubating platelets with linoleic acid (230 μM). This evidence is compatible with the hypothesis that UFA can inhibit platelet function by perturbing the cell membrane. This effect may be related to changes in receptor availability.

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THROMBOXANE SYNTHESIS IN FATTY ACID-MODIFIED HUMAN PLATELETS. S.W. Needleman, A.A. Spector, J.C. Hoak, Departments of Medicine and Biochemistry and the Cardiovascular Center, Univ. of Iowa College of Medicine, Iowa City, IA. 52242 USA

Epidemiologic evidence suggests that ingestion of polyunsaturated fatty acids (PUFA) retards arterial thrombo-occlusive diseases. Consequently, we investigated whether PUFA might influence production of platelet thromboxane A_2, a potent agonist of platelet aggregation. A culture system containing 2.7μM PGE_1 was devised in order to maintain viability of washed human platelets for as long as 24 hr at 30°C during supplementation with linoleic (18:2) or oleic (18:1) acid. Platelets were then washed, aggregated with 0.12 U/ml thrombin and the media was assayed for thromboxane B_2 (TX) by radioimmunoassay. 24 hr control platelets released comparable TX to freshly washed platelets (283 pmol/ml ± 44[SEM] vs 205 ± 40). At 200μM fatty acid concentrations, there was a 30-55% decrease in TX for 18:2 and a 20-30% decrease for 18:1 enriched platelets compared to control. Inhibition was not observed earlier than 4 hr after exposure to fatty acid. Inhibition was more pronounced when the fatty acids were incubated at 37°C than at lower temperatures. At lower concentrations of fatty acid, less inhibition was observed, and in some instances, enhancement of TX release was actually noted. Aggregation was recorded on a Payton aggregometer and analyzed for the slope of the initial linear phase (M_{ag}). M_{ag} for 24 hr control and freshly washed platelets was comparable. At 200μM fatty acid, for 18:2 compared to control, M_{ag} was decreased 23-56%, whereas for 18:1 M_{ag} was decreased 22-34%. These findings are in agreement with our previous report of diminished prostacyclin synthesis in similarly modified endothelial cells, and suggest that changes in PUFA availability may influence TX synthesis in the intact human platelet.

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PHOSPHOLIPID COMPOSITION AND PHOSPHOLIPID-MODIFYING AND PROSTAGLANDIN-SYNTHESISING ENZYMES IN HUMAN PLATELET SURFACE AND INTRACELLULAR MEMBRANES ISOLATED BY FREE FLOW ELECTROPHORESIS. N. Crawford*, M. Lagarde≠, S. Menashi* and F. Carey†. Department of Biochemistry, Royal College of Surgeons of England, London, U.K.*, INSERM-U63, Laboratoire d'Hemobiologie, Lyon, France≠, and Biochemistry Department, I.C.I. Pharmaceuticals Division, Alderley Park, Cheshire, UK†

A mixed membrane fraction prepared from human platelets which have been exposed to neuraminidase and surface labelled with ^{125}I-Lens culinaris separates into three vesicle subpopulations in a high voltage free flow electrophoresis chamber [Bender Hobein/MSE]. One fraction [N_I] which is devoid of the lectin label has exclusive localisation of NADH cytochrome-c-reductase activity with high enrichment [12-14 fold]. The two less electronegative fractions [N_{II} and N_{III}] account for the whole of the lectin label and have adenylate cyclase activity. Fraction N_I which is believed to be of intracellular origin has a low cholesterol/phospholipid ratio [0.291 ± 0.014], negligible sphingomyelin and has a high fluidity as measured by fluorescence polarisation using diphenyl hexatriene. Fractions N_{II} and N_{III}, believed to represent two different surface membrane domains, have high cholesterol/phospholipid ratios [0.606 ± 0.027 and 0.739 ± 0.054] are rich in sphingomyelin and have significantly higher microviscosities than N_I. The polypeptide profile of N_I is also particularly distinctive. Using 2[1-^{14}C] arachidonyl phosphatidyl choline and 2[1-^{14}C] arachidonyl diacyl-glycerol as substrates, phospholipase-A_2 and diglyceride lipase activities have been measured in the three membrane fractions. Both enzymes are almost exclusively located in the intracellular membrane fraction N_I with high enrichment ratios with respect to homogenate activities. Similarly cyclooxygenase and thromboxane synthetase activities are also largely confined to the intra-cellular membranes. Full analytical data for the phospholipid and fatty acid compositions of the surface and intracellular membrane fractions will also be presented.

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CIS-UNSATURATED FATTY ACIDS INHIBIT PLATELET FUNCTION BY PERTURBATION OF THE PLATELET MEMBRANE. D.E. MacIntyre , R.L. Hoover, M.J. Karnovsky, E.W. Salzman. Dept. Surg., Beth Israel Hosp., Dept. Path., Harvard Med. School, Boston, Dept. Pharm., U. Glasgow, Scotland.

The uptake of free fatty acids into surface membranes has been shown to affect lymphocyte capping and fibroblast adhesion and growth. Using human gel filtered platelets (GFP), we examined the effects of saturated (S), cis-unsaturated (CU) and trans-unsaturated (TU) fatty acids (FA) on platelet morphology, shape change, aggregation, 5HT release, TxB_2 production and diphenyl hexatriene (DPH) polarization, an index of membrane lipid organization. FA (1-30 μM) did not alter platelet morphology. CUFA (16:1, 18:1, 18:2, 18:3, 20:1, 20:2) but not TUFA (18:1, 18:2) or SFA (14:0, 16:0, 18:0, 20:0) inhibited thrombin (T)-induced platelet aggregation and 5HT release. Up to 15 minutes after addition of ^{14}C-FA to GFP, >90% of platelet ^{14}C was present as free FA. Under these conditions only CUFA inhibited T-induced platelet shape change and primary or secondary aggregation induced by ADP, U46619, collagen or arachidonate. Responses to A23187 were unaffected. T-induced TxB_2 production was reduced by CUFA. The antiplatelet effects were evident despite the presence of inhibitors of adenylate cyclase (2',5'-dideoxy adenosine, 100 μM) or cyclo-oxygenase (indomethacin, 30 μM), but were reversed by resuspension of FA-GFP in FA-free buffer. Analysis of fluorescence decay of DPH in platelet membranes revealed lifetime heterogeneity, indicative of distinct lipid domains. Only CUFA caused a decrease in DPH polarization of intact platelets and platelet membranes. These findings suggest that inhibition of receptor mediated platelet responses by CUFA is produced by perturbation of the platelet membranes in specific lipid domains.

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