1290

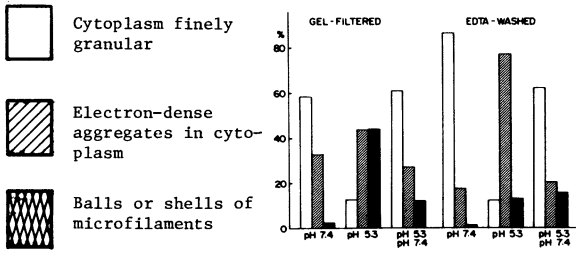
COMPARISON OF THE TIME COURSE FOR ADP-INDUCED SHAPE CHANGE, AGGREGATION AND ONSET OF REFRACTORINESS. JG Milton, C. Glushak, T. Wong and MM Frojmovic. Physiology, McGill University, Montreal, Canada.

Platelet morphology has been classified in our laboratory by phase-contrast microscopy into discocyte (D), disco-echinocyte (DE) and sphero-echinocyte (SE). Following the addition of 1-20 μM ADP to citrated platelet-rich plasma (PRP), platelet morphology changes as D→DE→SE with the maximum in DE occurring at 6-10 sec and that for SE at 1-3 min, and then slowly returns to D over the course of ~30 min. Here we compare the time courses for early platelet aggregation (PA) and the onset of refractoriness, to the time course for platelet shape change, the latter measured in initially mixed unstirred PRP diluted with plasma to N≤ 50,000 μl^{-1} to minimize aggregation. PA was measured microscopically as the % decrease in single platelets and refractoriness was measured by aggregometry from ADP dose-response curves determined as a function of time after initial stimulation. The time course for PA was similar to that for the appearance of DE. The DE at the time of maximal PA had pseudopods, but a main body geometry which differed only slightly from that of a D. The time course for onset of refractoriness at 2 μM ADP resembled that for the appearance of SE, indicating that the lifetime for single platelets able to participate in PA is < 60 sec. In contrast to ADP, PA induced by 10 μM adrenalin was maximal at ~30 sec and during this time there was little shape change (< 20% decrease in D). Thus although PA can occur without shape change, the rate is 3-4 fold faster if shape change occurs. The similarity of the time course and ADP concentration dependence for PA and DE formation suggest an intimate relationship between these processes. Moreover, the above results emphasize the transient nature of single DE participation in aggregation, and in particular point out that the relatively slowly formed 'sphered' platelets (SE) are not sticky as single platelets and do not participate in early aggregation.

1291

REVERSIBLE AND IRREVERSIBLE CHANGES IN PLATELET CYTOSKELETON, ENERGY CHARGE AND SECRETION BY EXPOSURE TO pH 5.3. **E.H. Mürer, G.J. Stewart and G. Tuszynski. Thrombosis Research Center, Temple University Medical School, Philadelphia, PA 19140, U.S.A.**

The effects of exposure to low pH on platelets isolated from plasma by saline-EDTA washing or by gel filtration were compared. Platelets isolated by the two methods responded similarly with respect to nucleotide metabolism, secretion and leakage. Both showed decrease in adenylate energy charge and metabolic ATP level after exposure to pH 5.3 and reversal to almost normal levels after return to pH 7.6, while the pH 5.3-induced secretion (about 50% of preabsorbed serotonin) was continued after the return to higher pH and reached about 80%. The response of the cytoskeleton of EDTA-washed and gel-filtered platelets to low pH differed quantitatively. Analysis of transmission electron micrographs (TEM):



TEM of Triton-insoluble residue showed similar structures and SDS-PAGE showed primarily actin (42,000d) and likely myosin (200,000d). Conclusion: The method of isolation did not affect the response of platelets to low pH when studied by platelet nucleotide metabolism, secretion or leakage but did affect the response when studied by the organization of the cytoskeleton (actin filaments).

1292

SOME OBSERVATIONS ON PLATELET AMINO ACID TRANSPORT SYSTEMS. T.U. Yardimci, A. Özbilen and O.N. Ulutin. Hemostasis Res. Center, Cerrahpasa Med. Fac. Istanbul Univ., and Pharmaceutical Sciences Fac. of Instanbul Academy, Istanbul, Turkey.

We have studied the transport systems for amino acids in platelets. Na+/K+ dependent active transport systems were found to be responsible for the transport of amino acids through the platelet membrane (Km's being at uM ranges). We have also isolated the binding proteins for amino acids from platelet membranes as the carriers involved in these active transport systems by cold osmotic shock procedure. Each amino acid besides being transported by a specific active transport system may be subject to transport by group amino acid transport systems.

Group amino acid transport systems are classified by countertransport experiments as follows:

Neutral amino acid group transport systems:
IA: glycine, alanine, serine, threonine
IB: valine, leucine, isoleucine, serine, threonine
IC: cysteine, methionine, proline
Basic amino acid group transport systems:
IIc: lysine
IIB: histidine, arginine
Acidic amino acid group transport systems:
III A: Aspartic acid, glutamic acid
Aromatic amino acid group transport systems:
IV_c: Phenylalanine, tyrosine, histidine, proline.

1293

THE ROLE OF SUPEROXIDE DISMUTASE(SOD) IN THE PLATELET FUNCTIONS. A. Kimura, K. Fujimura and A. Kuramoto. Department of Medicine, Research Institute for Nuclear Medicine and Biology, Hiroshima University, Hiroshima, JAPAN

We previously reported that SOD and catalase(CAT) as well as glutathione peroxidase(GPx) exist in human platelets, which are known to protect membrane or enzyme from oxidative damage and might regulate the prostaglandin synthesis by scavenging active oxygen(O_2^-, H_2O_2, $^1O_2$, HO·), and that the compensatory high SOD activity might be important for the maintenance of normal platelet function in the cases of glutathione reductase deficiency and the cases of myeloproliferative disorders with decreased platelet CAT and/or GPx. In order to make clear the roles of SOD, further study was done by utilizing diethyldithiocarbamate(DDC); specific inhibitor of SOD.

DDC inhibited the activity of platelet SOD after two hours incubation in a dose dependent manner. Platelet treated with 10^{-4} M of DDC inhibited the SOD activity to 86 % of control and showed the increase of platelet aggregability induced by ADP, epinephrine and collagen. The higher concentration of DDC (10^{-3} M) treated platelet returned to show nearly the same maximal aggregability as that of control, and platelets with 16 % residual activity (10^{-2} M DDC) rather showed the inhibition of aggregation. The basal malondialdehyde (MDA) was not changed at any concentration of DDC treated. However, N-ethylmaleimide(NEM) or thrombin-induced MDA production of DDC treated platelet showed an almost similar manner to platelet aggregability; increased at low concentration of DDC and decreased at higher concentration.

These results suggested that SOD plays a regulatory role on platelet function and metabolism, possibly by affecting the pathway of prostaglandin biosynthesis.