Wednesday, July 15, 1981

Oral Presentations

Platelets – XIV

Calcium, Calmodulin

08:00–09:30 h

Platelets – XV

Cyclic AMP

09:45–11:00 h

Grand Ballroom West

0525

08:00 h

EFFECTS OF EXOGENOUS CALCIUM ON IONOPHORE A23187-INDUCED PLATELET STIMULATION. B. Lages, C. Kruger and H. J. Weiss. Department of Medicine, Columbia University and St. Luke's-Roosevelt Hospital Center, New York, N.Y. U.S.A.

The ionophore A23187 is known to induce platelet(P) secretion in the presence or absence of extracellular calcium(Ca), thus providing evidence that endogenous Ca participates in the secretory mechanism. To explore further the interaction of A23187 with P Ca, we have compared the P responses to A23187 in the presence and absence of extracellular Ca using stirred gel filtered P in Ca-, Mg-free Tyrode's containing EGTA or EGTA plus Ca. With Egta alone, A23187 induced an immediate increase in light transmission(LT) which was not associated with aggregation, and a rapid secretion of 14C-5HT, ATP+ADP, and acid hydrolases which was complete within 30 sec. Increasing Ca, however, caused a progressively greater lag in secretion response(up to 60 sec.) but did not decrease the extent of secretion. With Ca/EGTA, LT changes were associated with visible P aggregation. In vivo ASA treatment reduced the extent of both secretion and aggregation but did not alter the effect of Ca on the secretion time course. Similar patterns of LT changes with EGTA vs aggregation with Ca/EGTA occurred in response to thrombin, but Ca did not cause comparable effects on the time course of thrombin-induced secretion. These results suggest that the P response to A23187 when intracellular Ca is utilized differs from that when Ca is supplied externally and that this is not due solely to the presence of aggregation. We hypothesize that these different responses may reflect changes in an endogenous P Ca pool which participates in P secretion.

0526

08:15 h

HUMAN PLATELET ACTIVATION IN THE ABSENCE OF AGGREGATION: A CALCIUM-DEPENDENT PHENOMENON INDEPENDENT OF THROMBOXANE FORMATION. S. Levy-Toledano, J. Maclouf, P.A. Bryon, H. de la Baume, E. Savariau, R. Hardisty and J.P. Caen. INSERM U 150, Hôpital Lariboisière, 6 rue Guy Patin, Paris, France.

In response to the ionophore A 23187, thrombasthenic platelets undergo a change in light transmission (LT) using either PRP or washed platelets. This change was accompanied by a normal release of ^{14}C serotonin (5HT) and thromboxane (TX) synthesis in absence of aggregation; lactic dehydrogenase determinations showed no evidence of platelet lysis. Ultrastructural qualitative electron microscopy revealed the formation of the central gel mass and the central apposition of organelles but the platelets were loosely packed. This ultrastructural change which was accompanied by biochemical phenomena was quantified; this includes an increase of the elongation coefficient, a decrease of the circularity coefficient as well as a decrease of the percentage of granules and surface connecting system in comparison with the total volume of the cell.

The change in light transmission was not inhibited by non-steroidal anti-inflammatory drugs (aspirin, indomethacin, flurbiprofen) suggesting a dissociation between a normal release of ^{14}C-5HT and an absence of TX formation. Moreover it was not inhibited by creatine phosphate/creatine phosphokinase, prostaglandin E_1 or cytochalasine and/or colchicine; it was then not dependent on ADP, cAMP or the integrity of microfilaments and microtubules. However chlorpromazine, TMB8 and dibucaine, drugs which interfere with intracellular membrane transport of calcium ions inhibited this platelet activation (change in LT, ^{14}C-5HT release or TX synthesis).

The stimulation of internal calcium fluxes by the ionophore was sufficient to induce platelet activation in the absence of aggregation; it was found to be independent of TX formation.

0527

08:30 h

THE ROLE OF CALCIUM IN PLATELET MICROTUBULE ASSEMBLY-DISASSEMBLY. M.Kikuchi, Y.Ikeda, M.Handa, S.Matsuda, H.Muraki, K.Toyama, M.Yamamoto, K.Watanabe, Y.Ando Department of Hematology and Department of Clinical Pathology, Keio University, Tokyo, Japan.

Microtubules exist in a dynamic equilibrium between polymerized and depolymerized forms in human platelets, playing a major role to maintain the discoid shape of platelets. It has been previously shown that the interaction of aggregating agents with platelets leads to a rapid but transient disassembly of microtubules. (Steiner and Ikeda, J.Clin. Invest. 63:443,1979) In this paper, the role of calcium in the equilibrium between assembled and disassembled microtubules was investigated. The respective pools of soluble and polymerized tubulin were "frozen" by addition of a glycerol-dimethyl sulfoxide-containing medium to platelet rich plasma, preincubated with 2 µM A23187 for various time intervals. The two pools of tubulin were estimated by measuring the colchicine binding activities of total and polymerized tubulin according to the method of Wilson.

Resting platelets were found to contain 56.2 ± 2.7 µg tubulin per 10^9 platelets, of which 56.7 % was in polymerized form. Addition of A23187 to platelet rich plasma produced a transient decrease in the pool of polymerized tubulin within 30 sec., followed by a return to base-line values within 2 min.. TMB-8, a known intracellular calcium antagonist, abolished this transient decrease in polymerized tubulin induced by A23187 in a concentration dependent manner, while indomethacin or acetylsalycylic acid did not.

These findings may indicate the important role of intracellular calcium in microtubule assembly-disassembly.