

Friday, July 17, 1981

Oral Presentations

Platelets – XXIII

Function, Metabolism

08:00–09:30 h

Thrombosis, Clinical – XIII

Platelet Reactions

09:45–11:00 h

Grand Ballroom Centre

0835

08:00 h

EXTRACELLULAR CALCIUM AND PLATELETS. P.M. Taylor and S. Heptinstall. Department of Medicine, University Hospital Nottingham, England, UK.

To gain more information on the role of extracellular Ca in platelet behaviour, the movement of ^{45}Ca between plasma and platelets has been studied. Two experimental procedures have been used: platelets were either studied in plasma that contained near-physiological levels of divalent cations or were studied in divalent cation-depleted plasma.

There was a continuous movement of Ca from plasma into platelets when the latter were suspended in plasma that contained near-physiological levels of divalent cations. The uptake was linear with time (2.0 to 2.5 ng ion Ca/ 10^9 platelets/60 mins) and was faster at 37°C than at 25°C. The amount of Ca taken up by the platelets increased as the extracellular Ca level was increased and was markedly inhibited by Mg. Sr did not affect the uptake. EGTA displaced only a small amount of the Ca that associated with the platelets which indicated that Ca was taken up into an intracellular pool rather than simply bound to the platelet surface. The relevance of this movement of Ca into the cells to platelet behaviour has not been established.

Studies using platelets suspended in divalent cation-depleted plasma showed that extracellular Ca was in equilibrium with Ca bound at or near the platelet surface. The binding of Ca was time-dependent but saturable (0.30 to 0.50 ng ion Ca/ 10^9 platelets/30 mins), and the majority was readily displaced by EGTA. The amount of Ca bound to the cells increased as the extracellular Ca level was increased but was little affected by an excess of either Mg or Sr. More Ca bound to platelets when they were incubated at 25°C than at 37°C. This was because platelets lost their ability to bind Ca when they were incubated at 37°C in divalent cation-depleted plasma. This phenomenon was time-dependent and irreversible and was paralleled by a loss in the ability of the platelets to aggregate. These Ca binding sites would seem to be relevant to the aggregation process.

0836

08:15 h

THE WISKOTT-ALDRICH SYNDROME (WAS): STUDIES ON A POSSIBLE DEFECT IN MITOCHONDRIAL ATP REGENERATION IN PLATELETS. W.van Brederode, G. Gorter and J.W.N. Akkerman. Department of Haematology, University Hospital Utrecht, The Netherlands.

WAS is a severe, X-linked disorder, characterized by eczema, immunodeficiency and an increased bleeding tendency caused by thrombocytopenia and platelet malfunction. There is a diminished epinephrine-induced aggregation response and an abnormal mitochondrial CO_2 production during platelet activation. From this, Shapiro et al (The Lancet 1978) concluded that WAS-platelets have a defect in mitochondrial ATP regeneration, which could be employed for detection of WAS-carriers, who are clinically normal and have only minor platelet defects. The test consists of an epinephrine-induced aggregation in the presence of an inhibitor of glycolytic ATP production (deoxyglucose, 2 DG), and showed impaired second wave aggregation in obligate carriers but not in normal controls. We tested 4 unrelated obligate WAS-carriers and found impaired aggregations in all. Five out of 7 female relatives also showed aggregation abnormalities, suggestive for WAS-carriership. However, in 8 out of 15 normal controls (males and females) the test was also positive. The nature of a possible defect in mitochondrial ATP supply was further studied in gel-filtered platelets by analyzing the metabolic ATP level before and during epinephrine-induced aggregation in the presence of inhibitors of glycolysis and glycogenolysis and during incubation in substrate-depleted medium. These studies showed that mitochondrial energy generation depended on sugar supply either from glycolysis or glycogenolysis and was unable to maintain a normal metabolic ATP level when these pathways were inhibited. Incubation with 2DG led to a fall in metabolic ATP and - consequently - to an impaired epinephrine-induced aggregation. The fall of metabolic ATP (2DG present) was much steeper in platelets from 2 unrelated WAS-patients than in cells from normal controls; most (but not all) obligate carriers showed intermediate values. It is concluded that the impaired epinephrine-induced aggregation in the presence of 2DG in WAS reflects disturbances in ATP homeostasis, which are consistent with a mitochondrial defect.

0837

08:30 h

ASSESSMENT OF ENERGY COSTS OF SECRETORY RESPONSES IN PLATELETS BY ABRUPT ARREST OF ATP REGENERATION. J.W.N. Akkerman, G. Gorter and H. Holmsen. Department of Haematology, University Hospital Utrecht, The Netherlands, and Specialized Center for Thrombosis Research, Philadelphia, PA, U.S.A.

A new method has been developed for the quantitative assessment of energy consuming processes in platelets. Under carefully controlled metabolic conditions ATP resynthesis is abruptly blocked by a cocktail of metabolic inhibitors. This leads to a fall in metabolic ATP, which is linear with time between 0 and 30 sec after addition of the inhibitors. Evidence is presented that this fall reflects the velocity by which the platelets consume metabolic energy prior to addition of the inhibitors. Resting platelets consume 4 μmol ATP equivalents/min/ 10^{11} cells at 37°C and 0.5 μmol (same units) at 15°C. When thrombin (5 U/ml) is included in the inhibitor-mixture, aggregation and secretion of dense granules (^3H -serotonin), α -granules (β -thromboglobulin) and lysosomal granules (N acetyl β glucosaminidase) follow despite the arrest in ATP resynthesis. The fall in metabolic ATP is now much steeper, reflecting an increase in energy consumption during these functions. Using changes in temperature as a means to affect secretion and energy metabolism, secretion velocity (measured between 0 and 10 sec after thrombin addition) can be compared with simultaneous energy consumption (measured between 0 and 30 sec after thrombin addition). At a consumption of 12 μmol ATP/min/ 10^{11} cells secretion velocity of dense-, α - and lysosomal granules is 100, 95 and 50% of uninhibited suspensions, respectively. At 6 μmol (same units) these percentages are 70, 35 and 25%. If thrombin is added after addition of the inhibitors thereby initiating secretion at lowered metabolic ATP levels, secretion is slower as metabolic ATP is lower. Again lysosomal granule secretion is more inhibited than α -granule secretion, which is slower than dense granule secretion. These data reflect an increasing need for metabolic energy in the order dense-, α - and lysosomal granule secretion.