SEPARATE MECHANISMS FOR THE INHIBITION OF PLATELET AGGREGATION BY ADENOSINE AND 2-CLOROADENOSINE. R. Apitz-Castro and C.R. Torres. IVIC, Depto. Bioquímica, Aptdo. 1827, Caracas 101, Venezuela.

The mechanism by which adenosine (Ado) and 2-cloroadenosine (Cl-Ado) inhibit platelet aggregation is not clear. In order to get some insight into the mode of action of these compounds, we studied the effect of Cl-Ado on the uptake of Ado by intact platelets, the effect of these compounds on the endogenous phosphorylation of specific plasma membrane proteins, and its effect on the carboxymethylation pattern of plasma membrane proteins in intact platelets. Cl-Ado does not modify the uptake of Ado by intact platelets, nor is itself incorporated into the platelet's pool of nucleotides. Phosphorylation of plasma membrane proteins is not affected by Cl-Ado; however, Ado produces a selective increase in the phosphorylation of one plasma membrane component of glycoproteic nature. As has been reported, phosphorylation of this glycoprotein is also modulated by cAMP (BBA, 455:371, 1976). Although the electrophoretic pattern of carboxymethylated plasma membranes is unaffected by Ado or Cl-Ado, it was found that the former markedly increases the label of all the susceptible proteins, while Cl-Ado selectively protects a single membrane component. Electrophoretically, this component seems to be related to the above mentioned glycoprotein. The results reported suggest that Ado and Cl-Ado interact with different components of the plasma membrane, impairing platelet aggregation through different mechanisms. In the case of Ado, two ways seem operative: a) A cAMP-like stimulation of a specific membrane glycoprotein and b) A more general perturbation of the membrane structure, perhaps through an Ado-carrier complex (Acta Med. Scand. 525:169, 1971). Cl-Ado seems to interact solely on the external surface of the plasma membrane, suggesting that the transmembrane phospho-glycoprotein previously described is in some way closely related to the ADP-receptor of the platelet plasma membrane.

ENERGY REQUIREMENT FOR MAINTENANCE OF ADENYLATE ENERGY CHARGE AND METABOLIC ATP IN PLATELETS DURING STARVATION. J.W.N.Akkerman,G.Gorter and J.J.Sixma. Dept.of Haematology,University Hospital Utrecht,The Netherlands.

Energy requirements for maintenance of stable adenylate energy charge (AEC)and metabolic ATP(ATP-m)level were studied in gel filtered platelets at various degrees of starvation. Platelets gel filtered and subsequently incubated during 40 min.at 37 °C with 1mM CN^- and without glucose consumed their glycogen at a rate of 0.79 ± 0.23(± SD,n=6)μmol glycosyl residues $.min^{-1}.10^{-11}$ cells.During this period AEC and ATP-m decreased linearly with time at rates of $5\text{-}6.10^{-3}$ and 0.75-1.05% of total radioactive adenine nucleotides $.min^{-1}.10^{-11}$ cells respectively. Addition of 25-1000μM glucose increased lactate production and decreased the fall of AEC and ATP-m proportional to the amounts of glucose added.Glycogenolysis remained active below 100μM glucose but ceased at higher glucose concentrations.From these data ATP-m production from glycogenolysis and glycolysis was calculated and compared with the decrease of steady state levels of AEC and ATP-m.A production of 3μmol ATP-m$.min^{-1}.10^{-11}$ cells was required to maintain initial AEC and ATP-m level.At lower rates of ATP-m production these values fell without reaching stable steady state levels in a lower range.After 40-50 min variations in AEC and ATP-m ceased and lactate formation stopped,leaving the cells in a state of hybernation. Subsequent addition of glucoserestored lactate accumulation,AEC and ATP-m.On the basis of formation and steady state levels of ATP-m its consumption was calculated.A lowering production was not completely met by a lowering consumption.Energy consumption in resting platelets is therefore partly independent from energy production.

INTERACTIONS OF THE ADP ANALOGS, 2'-O-METHYL ADP AND ADENINE 9-β-D-ARABINOFURANOSIDE 5'-DIPHOSPHATE AND CATECHOLAMINES WITH HUMAN PLATELETS. B.H. Ragatz, P.G. Iatridis, S.G. Iatridis, S.G. Markidou, M.F. Asterita and J. Gadarowski, Northwest Center for Medical Education, I.U. School of Medicine, Gary, Indiana, U.S.A. and School of Medicine, University of Athens, Greece.

The two analogs 2'O-methyl ADP (Me'ADP) and adenine 9-β-D-arabinofuranoside 5'-diphosphate (A-Ara-DP) are compounds with stereochemical modification in the pentose moiety of ADP. These compounds have been added individually to citrated platelet rich plasma (PRP) and the concentration dependent interaction of each with platelets has been studied turbidimetrically using a dual channel Payton Aggregometer under the following experimental conditions: variable amounts of compound only; variable amounts of compound challenged at 30" or 5' by an aggregation-stimulating ADP concentration; or variable amounts of compound plus aggregation-stimulating concentrations of epinephrine (EPI) or norepinephrine (NOR) added after 30".

Data obtained indicate that both compounds induce reversible aggregation at high concentrations, and that neither compound competes effectively with ADP added after 30". Both compounds delay the secondary wave of aggregation when ADP is added after 5'. Both compounds also exhibit mild dose dependent cooperative effects when epinephrine or norepinephrine are added 30" later.

The ADP analogs, Me'ADP and A-Ara-DP, exhibit weak interactions with the platelet ADP receptor, indicating the importance of the ribose conformation in this case. The mild cooperative effects for either of these analogs plus EPI or NOR also suggest that the platelet ADP and catecholamine receptors are independent but interacting. Supported by USPHS HL-15425.