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10:00 h

MODULATION OF ADENYLATE CYCLASE ACTIVITY IN PLATELETS BY β_2-GLYCOPROTEIN I. Inger Schousboe, Department of Biochemistry C, University of Copenhagen, DK-2200 Copenhagen, Denmark.

β_2-Glycoprotein I, purified from human serum, has been shown to bind to negatively charged phospholipids. Such phospholipids are essential reactants in several surface mediated reactions. In blood coagulation, platelets, to which β_2-glycoprotein I recently has been shown to bind, furnish a major portion of these phospholipids. Concomitant with this binding, a change in platelet adenylate cyclase activity has been observed. The purpose of this study is to further investigate the effect of β_2-glycoprotein I on the adenylate cyclase activity in platelets.

Human platelets isolated immediately after bleeding were washed in buffered isotonic solutions at low ionic strength. At 0 °C intact or sonicated platelets were incubated with various concentrations of β_2-glycoprotein I and prostaglandin E_1. Intact platelets were then sonicated, platelet membranes isolated and frozen at -180 °C, and adenylate cyclase activity of the membranes determined.

The results showed that adenylate cyclase activity in platelets increases about 4 times when intact platelets are preincubated with β_2-glycoprotein I at saturation. This increase is also seen when low concentrations of PGE_1 ($\leq$ 1µM) are present in the preincubation mixture. There was no increase in adenylate cyclase activity when sonicated platelets were incubated with β_2-glycoprotein I.

On the basis of these results it is suggested that in vivo β_2-glycoprotein I may have one or both of the following functions. 1) It may prevent circulating platelets from aggregation by protecting the negatively charged phospholipids and/or by increasing the adenylate cyclase activity and 2) it may catalyze the deaggregation of aggregated platelets.

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10:15 h

INACTIVATION OF PURIFIED HUMAN PLATELET CYCLIC AMP PHOSPHODIESTERASE BY THE AFFINITY LABEL 2'-O-IODOHYDRIN-P-CYCLIC AMP. P.G. Grant, R.F. Colman, A.K. Sinha and R.W. Colman. Thrombosis Research Center, Temple University School of Medicine, Philadelphia, PA and Department of Chemistry, University of Delaware, Newark, DE, USA.

Cyclic AMP phosphodiesterase (PDE) is a regulatory enzyme in human platelets. Inhibitors of this enzyme raise intracellular cAMP which prevents platelet activation. Little is known about the biochemistry of this enzyme. PDE was isolated from human platelet concentrates by nitrogen bomb cavitation. The specific activity of PDE in cell lysate was 0.064 nmoles cAMP hydrolysed/min/mg protein at 22°, 1 µM cAMP. Eighty percent of the activity appeared in the 100,000 x g supernatant fraction. Chromatography was performed on DEAE cellulose equibrated with 50 mM Tris-acetate pH 6.0, 3.75 mM 2-mercaptoethanol. A linear gradient with a limiting salt concentration of 1.0 M Na acetate separated two peaks of PDE activity. The first had a K_m for cAMP of >100 µM; the second had a K_m for cAMP of 5 µM. The lower K_m enzyme was further purified by adsorption on blue dextran Sepharose in 50 mM Tris pH 7.5, 2 mM $MgCl_2$ followed by affinity elution with 1 mM cAMP in the same buffer. These steps resulted in a 1700 purification of the enzyme (113 nmoles/min/mg). The compound 2'-O-iodohydrin-p-cAMP (IH-cAMP) is a cAMP derivative with an alkylating side chain. Incubation of PDE with 5 mM IH-cAMP at 37° resulted in 88% inactivation of the enzyme at 15 hours, compared to a control, with a corrected pseudo-first-order rate constant of 0.144 h^{-1}. When cAMP (100 µM) was included in the inactivation mixture the corrected pseudo-first-order rate constant decreased to 0.064 h^{-1}. Thus, a 20-fold excess of cAMP protected 56% against inactivation by IH-cAMP. The inhibition was not reversed by gel filtration of the inactivated enzyme which removed IH-cAMP. These results suggest that IH-cAMP reacts with the active site of PDE to irreversibly inactivate the enzyme. IH-cAMP should prove to be a useful tool in understanding the chemistry of the active site of this important enzyme.

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10:30 h

INHIBITION OF PLATELET ADHESION TO COLLAGEN BY PROSTAGLANDINS (PGI_2, PGE_1, PGD_2) ITS POSSIBLE CORRELATION WITH cAMP/ADENYLATE CYCLASE SYSTEM. A. Karniguian, Y.J. Legrand, J.P. Caen. Unité de Recherche sur la Thrombose et l'Hémostase (U 150 INSERM) Hôpital Saint Louis, 75475 PARIS Cedex 10 France.

The purpose of this work was to investigate if the inhibitory effect of Prostacyclin (PGI_2) on the ex vivo platelet adhesion to rabbit subendothelium (under defined conditions at a shear rate of 860 sec^{-1}) is explained by an inhibition of platelet adhesion to collagen. We therefore applied a sensitive method to measure the adhesion of ^{111}In labelled platelets to collagen ; this method has permitted to check the effect of very low concentrations of PGI_2 and also of two other prostaglandins (PGs) which have been shown to inhibit platelet aggregation : PGE_1 and PGD_2. An inhibition of adhesion has been observed which was short lasting with PGE_1, and of longer duration (more than 5 minutes) with PGI_2 and PGD_2 : the maximum inhibition using 1 uM of each compound was 92 % for PGI_2, 69 % for PGD_2 and 59 % for PGE_1. This inhibition was going in parallel with platelet cAMP increase and ceased when the cAMP decreased. Theophylline (10^{-3}M), a phosphodiesterase inhibitor, potentiated the inhibitory effect of PGE_1 on platelet adhesion. The addition of exogenous N^6 - $O^{2'}$ dibutyryl cAMP (10^{-5} - 10^{-3}M) reproduces the inhibitory effect of PGs on platelet adhesion, suggesting that PGs act on platelet adhesion to collagen via adenylate cyclase/cAMP system. Prostaglandins caused an inhibition of collagen induced platelet release, this inhibition was the same, wether it was measured on the α granules (βthromboglobulin) or dense bodies (ADP and 5HT) content. The dose of prostaglandins which provokes a 50 % inhibition (ID_{50}) is higher for the release : (PGI_2 ID_{50} : βTG = 7.2 10^{-11}M, 5HT = 7 10^{-11}M, PGE_1 ID_{50} βTG = 10^{-9}M, 5HT = 0.6 10^{-9}M, PGD_2 ID_{50} : βTG = 1.2 10^{-9}M, 5HT = 1.6 10^{-9}M) than for platelet adhesion (PGI_2 ID_{50} = 7 10^{-10}M, PGE_1 ID_{50} = 10^{-8}M, PGD_2 ID_{50} = 7.5 10^{-7}M.

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10:45 h

ROLE OF CYCLIC AMP IN THE INHIBITION OF HUMAN PLATELET FUNCTIONS BY QUERCETIN,A FLAVONOID THAT POTENTIATES PGI_2 EFFECT. J.-P. Cazenave, A. Beretz, A. Stierlé and R. Anton. Laboratoire de Biologie et de Pharmacologie des Plaquettes. Centre de Transfusion Sanguine et Laboratoire de Pharmacognosie, Faculté de Pharmacie, Strasbourg, France.

Injury to the endothelium (END) and subsequent platelet (PLAT)interactions with the subEND are important steps in thrombosis and atherosclerosis.Thus,drugs that protect the END from injury and also inhibit PLAT function are of interest.It has been shown that some flavonoids(FLA),a group of compounds found in plants,prevent END desquamation in vivo, inhibit cyclic nucleotide phosphodiesterases(PDE)and inhibit PLAT function.We have studied the structure-activity relationships of 13 purified FLA on aggregation and secretion of ^{14}C-5HT of prelabeled washed human PLAT induced by ADP,collagen(COLL) and thrombin(THR).All the FLA were inhibitors of the 3 agents tested.Quercetin(Q),was the second best after fisetin.It inhibited secretion and aggregation with I_{50} of 330µM against 0.1 U/ML.THR, 102µM against 5µM ADP and 40 µM against COLL.This inhibitory effect is in the range of that of other PDE inhibitors like dipyridamole or 3-isobutyl-1-methylxanthine.The aggregation induced by ADP, COLL and THR is at least mediated by 3 mechanisms that can be inhibited by increasing cAMP levels.We next investigated if Q,which is a PDE inhibitor of bovine aortic microsomes,raises PLAT cAMP levels. cAMP was measured by a protein-binding method. ADP-induced aggregation(5µM) was inhibited by PGI_2 (0.1 and 0.5 nM).Inhibition was further potentiated(1.7 and 3.3 times) by 10µM Q, which alone has no effect on aggregation.The basal level of cAMP(2.2 pmol/10^8PLAT) was not modified by Q (50 to 500µM).Using these concentrations of Q,the rise in cAMP caused by PGI_2(0.1 and 0.5nM) was potentiated in a dose dependent manner.Q potentiated the effect of PGI_2 on the maximum level of cAMP and retarded its breakdown.Thus Q and possibly other FLA could inhibit the interaction of PLAT with the components of the vessel wall by preventing END damage and by inhibiting PLAT function through a rise in cAMP secondary to PDE inhibition and potentiation of the effect of vascular PGI2 on PLAT adenylate cyclase.