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**Cyclic AMP inhibits platelet aggregation by low concentrations of collagen but phosphorylation is unaffected**

J. Clark[1], S. Watson[1], S. Hill[2], S. P. Watson[1]. [1]Institute of Cardiovascular Sciences, University of Birmingham, Birmingham, United Kingdom, [2]School of Life Sciences, University of Nottingham, Nottingham, United Kingdom.

**Introduction** Platelets are exposed to prostacyclin and nitric oxide at the endothelium, which elevate cyclic AMP (cAMP) and cyclic GMP (cGMP) respectively. cAMP levels are also raised by activation of the G protein-coupled receptor, adenosine $A_{2A}$ which signals through the stimulatory G protein. Using collagen which signals through GPVI and integrin $\alpha_2\beta_1$ platelet receptors, the aim of this study was to investigate the cross-talk between adenosine $A_{2A}$ and GPVI receptors in platelet regulation via cAMP formation.

**Methods** Human venous blood was collected in anticoagulant citrate and washed platelets were prepared in modified Tyrodes buffer. For aggregation experiments, platelets were set to a final concentration of $2x10^8$/ml and for western blotting, $5x10^8$/ml. Aggregation was monitored by light transmission aggregometry with the addition of inhibitors, NECA (5'-N-ethylcarboxamidoadenosine) (100μM), Indomethacin (10μM) and Cangrelor (10μM). Secretion of ATP was measured using Chromo-lume reagent. Samples were lysed using 5x reducing sample buffer and tyrosine phosphorylation of PLCγ2 (Y1217, Y759), Syk (Y525/526, Y352, Y323) and LAT (Y200, Y171, Y132) were measured by western blotting. For spreading experiments washed platelets ($2x10^7$/ml) were incubated and allowed to spread on collagen-coated coverslips for 30 minutes. NECA and Forskolin (10μM) were added and incubated a further 30 minutes. Samples were stained with Alexa Fluor 488 phalloidin and imaged with confocal microscopy. Data analysis was performed using one-way ANOVA.

**Results** Addition of NECA to low dose collagen (1 & 2μg/ml) caused significant inhibition of platelet aggregation and secretion (n=6). Addition of NECA to high dose (10μg/ml) collagen stimulated platelets caused a small inhibitory effect (n=6). Forskolin (10μM) did not block collagen (10μg/ml)-induced phosphorylation of PLCγ2, Syk or LAT (n=5). Neither NECA (100μM) nor forskolin (10μM) altered platelet spreading on collagen (10μg/ml).

**Conclusions** Our data shows that cAMP inhibits low dose collagen-induced aggregation and secretion while only causing a small inhibitory effect on high dose collagen induced aggregation and secretion. This inhibition cannot be a consequence of PLCγ2, Syk and LAT tyrosine phosphorylation inhibition as the results from western blotting show that phosphorylation is not significantly affected. We hypothesise that cAMP is working further downstream and inhibiting $IP_3$-induced $Ca^{2+}$ release via the PKG-IRAG-$IP_3$ receptor complex.