

Differential role of phospholipase A₂ isoforms in release of prostacyclin and prostaglandin E₂ from human endothelial cells

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Prostaglandins (PGs), which include prostacyclin and PGE₂, are powerful hormones formed from arachidonic acid (AA) by the enzyme cyclo-oxygenase (COX) coupled with downstream synthase enzymes. AA is normally contained in membrane phospholipids but released when cells are activated by the actions of phospholipase A₂ (PLA₂) enzymes. PLA₂ is expressed in a range of isoforms, which fall into three key families; iPLA₂, cPLA₂ and sPLA₂. iPLA₂s are calcium independent whilst cPLA₂s and sPLA₂s are activated by calcium. Endothelial cells are particularly enriched with COX and their release of PGs contributes to cardiovascular homeostasis. We have previously shown that prostacyclin release from endothelial cells lacking cPLA₂ is greatly reduced (1), suggesting that this isoform is important in these cells. However others suggest that iPLA₂ drives AA release in endothelial cells (2). In the current study, we have compared to effect of cPLA₂, iPLA₂ and sPLA₂ on PG release by cultured endothelial cells.

Methods: Blood outgrowth endothelial cells (BOEC) from healthy volunteers were isolated and cultured as described previously (3). Cells were plated on 96-well plates and stimulated with IL-1 β (1ng/ml) for 24 hours before the addition of selective inhibitors of cPLA₂ (pyrrophenone; PYR; 0.01-1 μ M), iPLA₂ (bromo-enol lactone; BEL; 0.1-3 μ M) or sPLA₂ (YM26734; 1-30 μ M) or vehicle (0.1% DMSO) for 30mins. Cells were then stimulated for a further 30 minutes with thrombin (1U/ml; PBS) to activate PLA₂ or with AA (50 μ M; ethanol 0.05% final), which provides substrate for PG formation directly. Prostacyclin (measured as its stable breakdown product 6-keto PGF_{1 α}) and PGE₂ were measured by ELISA.

Results: Basal release (pg/ml) of PGE₂ (203.4 \pm 60) and prostacyclin (365.2 \pm 61.9) were increased respectively by thrombin (815 \pm 95, 2296.0 \pm 362.3) and AA (1412.7 \pm 283.9, 3761.1 \pm 584.1), (p<0.05 one-way ANOVA followed by Dunnett's post-test). In thrombin stimulated cells, pyrrophenone (PYR) inhibited both prostacyclin and PGE₂ release whilst bromo-enol lactone (BEL) inhibited prostacyclin release only (Figure 1). YM26734 (30 μ M) had no effect on either PGE₂ or prostacyclin release (p>0.05; t-test). None of the PLA₂ inhibitors tested affected PG release from cells stimulated with AA (p>0.05; one-way ANOVA followed by Dunnett's post-test).

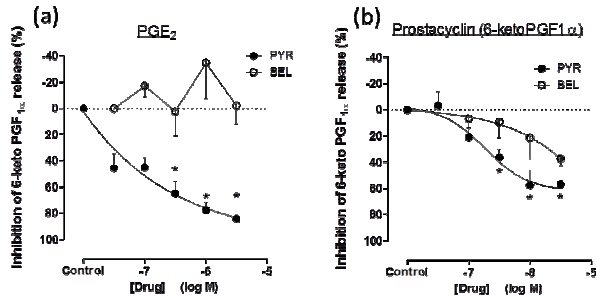


Figure 1: Effect of pyrophenone (PYR) or bromoenol lactone (BEL) on thrombin induced PGE₂ (a) or prostacyclin (b) release from BOECs. Data is mean ± S.E.M. for n=4 experiments. Data was analysed by one-way ANOVA followed by Dunnett's post-test. *p<0.05.

Conclusions: These results suggest that AA release in endothelial cells is regulated by iPLA₂ and cPLA₂, but not sPLA₂ and that intracellular compartmentalisation may link iPLA₂ activity to prostacyclin release selectively versus PGE₂ release.

References:

- (1) Reed DM *et al.* 2014. pA2 online 111:186P;
- (2) Sharma J *et al.* (2010). *Biochemistry* **49**: 5473-5481;
- (3) Starke RD *et al.* 2013. *Blood* **121**: 2773-2784.