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Characterisation of the phospholipase A_2 isoforms supporting prostacyclin production synthesis by endothelial cells

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Endothelial cells line the luminal surface of blood vessels where they release hormones including prostacyclin (PGI_2) that dilates blood vessels, inhibits platelet aggregation and protects against atherogenesis. PGI_2 is produced by the sequential actions of three enzymes: phospholipase A_2 (PLA_2) cleaves membrane phospholipids to liberate arachidonic acid (AA), which is converted by cyclooxygenase (COX) into PGH_2, which is then converted to PGI_2 by PGI_2 synthase. Although numerous PLA_2 isoforms exist, it is thought to be the Ca^{2+}-dependent cytosolic PLA_2 ($cPLA_2$) isoform that supports prostaglandin synthesis (1,2). However, it has been proposed that PGI_2 release from endothelial cells induced by thrombin or tryptase is dependent upon Ca^{2+}-independent $iPLA_2$ (3). To test this proposal, we examined the contribution of $cPLA_2$ and $iPLA_2$ to PGI_2 synthesis using blood-outgrowth endothelial cells (BOECs) from healthy volunteers and from a $cPLA_2$-deficient patient (2), as well as intact mouse arteries.

BOECs were grown from blood of healthy donors (n=4) and a patient lacking $cPLA_2$ (2). These cells are an established model of endothelium from vessels (4). BOECs were incubated with the $cPLA_2$ inhibitor pyrrophenone (0.1-10µM) or vehicle (0.1% DMSO; 30min). They were then incubated with thrombin (1U/ml; PBS) or Ca^{2+} ionophore (A23187; 30µM; DMSO 0.1% final) to activate endogenous $iPLA_2$ and $cPLA_2$ enzymes, respectively, or with AA (50µM; ethanol 0.05% final), which stimulates PGI_2 formation without the requirement of PLA_2. In parallel, aortic rings prepared from mice (male, BALB/c, 10 weeks; n=6) were incubated with pyrrophenone, the $iPLA_2$ inhibitor bromoenol lactone (BEL; 0.1-10µM) or vehicle (0.1% DMSO), as above. Mouse aortic rings do not respond robustly to thrombin, so for these experiments, PGI_2 release was stimulated with acetylcholine (ACh; 1µM; saline). In this tissue Ach, as for thrombin, acts via a G_q-protein coupled receptor signalling pathway. In both assays, PGI_2 release was determined after 30min incubation by measurement of its breakdown product, 6-keto$PGF_{1\alpha}$ using ELISA.

BOECs released PGI_2 in response to A23187, thrombin and AA (vehicle, 160±19pg/ml; thrombin, 1853±1089; A23187, 608±180pg/ml; AA, 2035±836pg/ml; all $p<0.001$ by Wilcoxon signed rank test). PGI_2 release induced by thrombin was inhibited in a concentration-dependent manner by the $cPLA_2$ inhibitor pyrrophenone ($-\log IC_{50}$; 6.8±0.3, $p=0.02$ by Kruskal-Wallis test), as was release of PGI_2 induced by A23187 ($-\log IC_{50}$; 7.1±0.7, $p=0.05$ by Kruskil-Wallis test). Release of PGI_2 from BOECs incubated with the COX substrate, AA, which bypasses the need for PLA_2 activity, was not affected by pyrrophenone. In agreement, BOECs from a $cPLA_2$-deficient individual did not produce PGI_2 in response to thrombin or A23187 but

responded normally to exogenous AA. In mouse aortic rings, ACh stimulated robust production of PGI_2 which was inhibited in a concentration-dependent manner by pyrrophenone ($-\log IC_{50}$; 6.2±0.5; p=0.04 by Kruskil-Wallis test) but unaffected by the $iPLA_2$ inhibitor, BEL (ACh; 1374±252pg/ml, ACh+10μM BEL; 1377±201pg/ml, p=0.94 by Mann-Whitney test).

These data demonstrate that $cPLA_2$ and not $iPLA_2$ is the dominant isoform driving PGI_2 production by endothelial cells and intact arteries.

(1) Adler DH et al, JCI 118:2121, 2008. (2) Brooke MA et al, Gut *In press*, 2013 (3) Sharma J et al, Biochem 49:5273, 2010. (4) Starke RD et al, Blood, 121:2773, 2013.