Role of COX-1, COX-2 and phospholipase A₂ in prostacyclin release from aortic tissue of mature mice in vitro

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Prostaglandin I₂ (PGI₂), formed by the concerted actions of cyclo-oxygenase (COX-1 or COX-2) and prostacyclin synthase, is a potent inhibitor of platelet activation and an important cardioprotective hormone. COX-1 is present constitutively in aortic endothelium of young adult mice and is responsible for the production of PGI₂ in the aortic arch (Lundberg et al. 2009, PA2 online 035P). PGI₂ releasing capacity is greatly reduced in vessels from mature (12 months old) COX-1⁻/⁻ mice (Lundberg et al. 2009, PA2 online 002P). However, COX-2 may be expressed in vessels with age and could therefore be associated with the cardiovascular side effects of non-steroidal anti-inflammatory drugs (NSAIDs).

Here, we have extended our previous observations and compared PGI₂ release from aortic tissue of mature (10-16 month old) wild type, COX-1⁻/⁻ and COX-2⁻/⁻ mice. We have also investigated the role of phospholipase A₂ (PLA₂) activation in vitro by assessing the effects of calcium ionophore, A23187, on PGI₂ release from vessels of each type of mouse. Incubation of tissues with diclofenac was used to validate COX isoforms as the source of PGI₂.

Mice were killed by CO₂ and the thoracic aorta excised, cleared of connective tissue and cut into 5 equally sized segments. Tissues were equilibrated for 60 minutes in individual wells of 96-well plates containing DMEM either with (triplicate incubations) or without diclofenac (100μM; duplicate incubations) in a humidified incubator (37°C at 5% CO₂). Media was then replaced and tissues incubated for 30 minutes; samples were taken for baseline measurements, before addition of A23187 (50μM) for a further 30 minutes. Media was stored at -80°C until PGI₂ was measured as its breakdown product 6-ketoPGF₁α by ELISA.

Aortic tissue from wild type or COX-2⁻/⁻ mice released low levels of PGI₂ unless stimulated with calcium ionophore A23187 (Figure 1). A23187-induced PGI₂ release was strongly inhibited by diclofenac (Figure 1). Aortae from COX-1⁻/⁻ mice released negligible levels of PGI₂, even in the presence of...
A23187. These observations support the idea that in both young and mature mice, COX-1 is responsible for the majority of vascular PGI$_2$ production.