PGI_2 release from guinea-pig lungs: detection and bioassay

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Prostacyclin (PGI_2) is an unstable product of arachidonic acid (AA) metabolism which has potent vasodilator and anti-aggregatory activity. Generation of PGI_2 from prostaglandin (PG) endoperoxides by aortic microsomes was first demonstrated by Moncada, Gryglewski, Bunting & Vane (1976). 6-oxo-$PGF_{1\alpha}$, one of the stable breakdown products of PGI_2, has been found in the perfusate of guinea-pig lungs after perfusion with AA and also in perfusate from sensitized guinea-pig lungs after antigen challenge (Dawson, Boot, Cockerill, Mallen & Osborne, 1976; Boot, Cockerill, Dawson, Mallen & Osborne, 1977).

The experiments described here show the release of PGI_2 from perfused lungs challenged with AA, using a differential bioassay technique which utilized the different half-lives of the unstable products, and platelet aggregation studies.

Guinea-pig lungs were removed, inflated with air, and perfused through the pulmonary artery with oxygenated Krebs bicarbonate solution (7 ml/min; 37°C). The outflow from the pulmonary circulation superfused a cascade of 5 isolated assay tissues: (1) Rabbit aortic spiral (1); (2) Rabbit mesenteric (or coeliac) artery spiral; (3) Rabbit aortic spiral (2); (4) Rat fundic strip and (5) Rat colon. A mixture of antagonists (Gilmore, Vane & Wyllie, 1968) plus indomethacin (1 μg/ml final concentration) was infused into the Krebs' solution superfusing the assay tissues. A delay coil was inserted into the cascade such that the lung perfusate was delayed 2 min between passing over the second bioassay tissue and reaching the third tissue. Since thromboxane (TxA_2) has a short half life (30 s at 37°C), this effectively removed TxA_2 from the perfusate, so that assay tissues below the delay coil could be used to quantitate more accurately the PGE_2 and $F_{2\alpha}$, PGH_2 and PGI_2 released by a lung AA challenge. The assay tissues were calibrated to injections of PGH_2, PGE_2, $PGF_{2\alpha}$ and in some experiments to TxA_2 generated from human platelet microsomes (Needleman, Moncada, Bunting, Vane, Hamberg & Samuelsson, 1976).

AA (2–10 μg) injected into the pulmonary circulation released substances which contracted Rabbit aortic spiral (1) and rabbit mesenteric spiral and rat fundic strip and relaxed the rat colon (inhibition of spontaneous contractions). The colon was contracted by PGH_2, PGE_2 and $PGF_{2\alpha}$ and therefore this effect on the colon was possibly due to the presence of PGI_2. When 15-hydroperoxy arachidonic acid (15-HPAA), a specific inhibitor of PGI_2 synthetase (Gryglewski, Bunting, Moncada, Flower & Vane, 1976) was infused through the pulmonary circulation at 4 μg/ml for 20 min and the lung challenged with AA 20 min later, responses of the rabbit aortic spiral (1) and rabbit mesenteric spiral were potentiated by 16–50% and 90–200% respectively, while responses of the rat fundic strip were reduced by 40–80% and the inhibitory response of the colon reversed to a slight contraction. This effect is consistent with a reduction of a PGI_2 component of AA-induced release since PGI_2 is known to relax arteries and contract the rat fundic strip (Gryglewski *et al*, 1976). The results could not be attributed to PGI_2 generation by the bioassay tissues.

Further evidence for PGI_2 release from guinea-pig lungs was obtained from platelet aggregation studies. Aliquots of lung perfusate collected after an AA challenge inhibited aggregation of rabbit platelet rich plasma induced by either AA or ADP. This anti-aggregatory activity declined on standing at room temperature and was absent in perfusate from 15-HPAA pretreated lungs.

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