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Selective inhibition of thromboxane B_2 accumulation and metabolism in perfused guinea-pig lung

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1 U46619, a prostaglandin H_2 endoperoxide analogue and thromboxane A_2 agonist, dose-dependently inhibited accumulation and metabolism of thromboxane B_2 in the isolated perfused lung of the guinea-pig. At similar doses prostaglandins E_1, E_2, $F_{1\alpha}$, $F_{2\alpha}$, I_2, 5,6-*trans*-PGE_2 and 8-*iso*-PGE_1 were ineffective.

2 U46619 did not affect accumulation and metabolism of prostaglandin $F_{2\alpha}$ under similar conditions.

3 The pulmonary disposition of thromboxane B_2, which occurs by uptake into pulmonary cells or binding to a specific macromolecular component, is mediated by a mechanism distinct from that handling prostaglandin $F_{2\alpha}$. The possible relevance of these findings to the pulmonary disposition of thromboxane A_2 is discussed.

Introduction

Thromboxane B_2 is metabolized in the isolated perfused lung of the guinea-pig to a single product identified by gas chromatography-mass spectrometry as 13,14-dihydro-15-keto-thromboxane B_2 (Blair, Dollery, Hoult, Peers, Robinson & Waddell, 1982; Robinson, Peers, Waddell, Blair & Hoult, 1982). At a concentration of 10 ng/ml (27 nM) there was 30% conversion in a single pass, whereas conversion of prostaglandin $F_{2\alpha}$ to its 15-keto and 13,14-dihydro-15-keto metabolites was 87% under similar conditions (Robinson *et al.*, 1982). Both substances also accumulate within the lung according to measurements of tissue-to-medium ratios (Blair *et al.*, 1982; Robinson *et al.*, 1982), either as a result of carrier-mediated uptake into pulmonary cells or binding to a cellular component.

We show here that thromboxane B_2 accumulation and metabolism is selectively inhibited in a dose-dependent manner by a structurally related analogue which at similar doses spares prostaglandin $F_{2\alpha}$.

Methods

Isolated ventilated lungs of the guinea-pig (male Dunkin-Hartley, 450–550 g) were set up as previously described (Robinson *et al.*, 1982) and perfused via the pulmonary artery at 10 ml/min with warmed well-oxygenated Krebs solution. After 5 min equilibration, thromboxane B_2 (final concentration 10 ng/ml (27.0 nM), containing 0.02 μCi/ml [$^3H_8$]-thromboxane B_2, specific activity 100 Ci/mmol, New England Nuclear GmbH) and inulin [^{14}C]-carboxylic acid (final activity 5.0 nCi/ml, specific activity 13.5 mCi/mol, Amersham International, used as extracellular space marker) were infused into the perfusion fluid for a further 5 min. In competition experiments other prostaglandins were co-infused during this period of 5 min.

The extent of pulmonary metabolism of thromboxane B_2 was quantified radiochromatographically (Robinson *et al.*, 1982). After thin layer chromatography of resuspended perfusate extracts in a solvent containing ethyl acetate: acetone: acetic acid (90:10:1, v/v), radioactive peaks were located by scanning and by reference to authentic iodine-visualized standards (R_F 0.30 and 0.45 for thromboxane B_2 and its 13,14-dihydro-15-keto metabolite, respectively), and the extent of conversion measured by liquid scintillation spectrometry.

Accumulation of ^{3}H and ^{14}C in the lung was measured after Soluene-350 solubilization of 100 mg and 300 mg aliquots of homogenized lung (Robinson & Hoult, 1982), and the results are expressed as the tissue-to-medium ratio after correction for counting efficiency and background activity.

All prostaglandins and thromboxanes were from the Upjohn Company, Michigan, U.S.A., with the

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Table 1 The effect of co-perfused prostaglandins on the accumulation and metabolism of thromboxane B_2 (27 nM) in isolated perfused lung of guinea-pig

Prostaglandin co-perfused	n[a]	*Tissue/medium ratio*[b] *[^{3}H]-TXB_2*	*[^{14}C]-inulin*	*Metabolism (%)*[b,c]
None	10	3.39 ± 0.10	0.17 ± 0.01	29.1 ± 3.0
$PGF_{2\alpha}$, 0.9 μM	3	4.66 ± 0.18***	0.14 ± 0.01	28.4 ± 3.3
PGE_1, 1 μM	6	3.16 ± 0.18	0.15 ± 0.01	25.6 ± 5.9
PGE_2, 1 μM	2	3.39 ± 0.14	0.14 ± 0.01	27.9 ± 8.9
$PGF_{1\alpha}$, 1 μM	3	3.19 ± 0.22	0.13 ± 0.01***	39.1 ± 8.8
PGI_2, 1 μM	3	2.95 ± 0.13*	not tested	21.6 ± 2.8
5,6-*trans* PGE_2, 1 μM	3	3.70 ± 0.16	0.18 ± 0.01	20.8 ± 4.6
8-*iso*-PGE_1, 1 μM	3	3.72 ± 0.16	0.15 ± 0.01	23.6 ± 5.3
U46619, 0.3 μM	3	2.88 ± 0.20*	0.14 ± 0.01	23.3 ± 7.9
U46619, 0.6 μM	3	1.74 ± 0.08***	0.16 ± 0.01	13.2 ± 0.5*
U46619, 0.9 μM	3	1.10 ± 0.05***	0.13 ± 0.01**	4.5 ± 1.1**
U46619, 1.0 μM	3	0.53 ± 0.02***	0.16 ± 0.01	0 ***

[a] n = number of lungs perfused.
[b] The number of determinations for tissue/medium ratios is $6 \times n$, results are means ± s.e.mean. Estimates of metabolism are means ± s.e.mean calculated from the average of duplicate measurements for each lung.
[c] In all experiments a correction factor (ranging from 5–10%) was applied to the percentage of radioactivity appearing in the metabolite zone that was due to impurities in the radiolabel after storage (2–5%) or to non-specific transformation during extraction (3–5%). The correction factor was checked in replicate in each experiment.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ with respect to control. (Student's unpaired t test).

exception of prostacyclin sodium salt which was from the Wellcome Research Laboratories, Beckenham, Kent.

Results

Table 1 shows that under control conditions thromboxane B_2 was 29.1 ± 3.0% converted to 13,14-dihydro-15-keto-thromboxane B_2. There was substantial accumulation of thromboxane-associated tritium within the lung, giving a tissue-to-medium ratio of 3.39 ± 0.10 compared to the value of 0.17 ± 0.01 for the extracellular space marker inulin. Perfusion of each lung for only 10 min avoided oedema which may occur during prolonged perfusion of isolated lungs with physiological salt solutions lacking colloid oncotic agents, but allowed the achievement of apparent steady state conditions (see Robinson & Hoult, 1982).

Co-perfusion with prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$, or the synthetic isomers 8-*iso*-PGE_1 and 5,6-*trans*-PGE_2, all at 37 times higher concentration than that of thromboxane B_2 ($F_{2\alpha}$ at 33 times higher), did not significantly alter the pulmonary disposition of thromboxane B_2 according to either the tissue-to-medium ratio or the extent of metabolism (Table 1). Prostacyclin, also at 1 μM, produced a 13% reduction in tritium retention, significant at the 2% level, and a small but not statistically significant reduction in thromboxane B_2 metabolism. In additional experiments (data not shown) co-perfusion with 10 μM prostaglandin E_2 inhibited thromboxane-associated tritium retention (0.61 ± 0.02, $P < 0.001$), abolished thromboxane B_2 metabolism ($P < 0.002$) and reduced the retention of inulin (0.13 ± 0.01, $P < 0.02$).

The prostaglandin endoperoxide H_2 analogue U46619 (15**S**-hydroxy-11α, 9α-epoxymethano-prosta-5Z, 13E-dienoic acid), which possesses thromboxane A_2-like activity (Coleman, Humphrey, Kennedy, Levy & Lumley, 1981), proved to be a potent concentration-dependent inhibitor of thromboxane B_2 metabolism and accumulation in the perfused guinea-pig lung (Table 1). Both parameters were reduced at 0.3 μM U46619 and complete prevention of thromboxane B_2 metabolism was obtained at 1.0 μM. In some experiments there was a small but statistically significant reduction of inulin retention, possibly as a result of the vasoconstrictor effect of U46619 on the pulmonary vascular bed, but there was no simple dose-relationship.

In further experiments we tested the effect of U46619 on the accumulation and metabolism of prostaglandin $F_{2\alpha}$ infused at 10 ng/ml (28.2 nM). At a concentration of 0.6 μM U46619 (sufficient to produce statistically significant reductions in accumulation and metabolism of thromboxane B_2, see Table 1) there was no effect in four lungs on the disposition of prostaglandin $F_{2\alpha}$. Inactivation was 85.8 ± 0.5% in the presence of U46619 compared to a control value of 87.2 ± 3.9%, and the tissue-to-medium ratios were 1.64 ± 0.03 and 1.59 ± 0.06 respectively. Even at 1.2 μM U46619 inhibition was slight: metabolism of prostaglandin $F_{2\alpha}$ was reduced by 18.7% (not

significant) and prostaglandin $F_{2\alpha}$-associated tritium was reduced by 15.0% ($P<0.002$, tests on three lungs).

These results demonstrate the relative selectivity of U46619 as inhibitor of the pulmonary accumulation and metabolism of thromboxane B_2.

Discussion

The principal conclusion from these experiments is that the thromboxane A_2 mimic,U46619, is a potent and selective inhibitor of the pulmonary accumulation and metabolism of thromboxane B_2. At 1.0 μM there was complete suppression of thromboxane B_2 metabolism and 84% reduction in the thromboxane B_2-associated accumulation of tritium, whereas at this concentration seven other prostaglandins were essentially inactive. However, U46619 did not materially affect the accumulation or metabolism of prostaglandin $F_{2\alpha}$ within the lung.

Several lines of evidence suggest that prostaglandins must be taken up into lung cells by a saturable transmembrane carrier mechanism prior to enzymatic inactivation and release back into the pulmonary circulation (see Robinson & Hoult, 1982, for discussion and references). Eling and colleagues have shown that the 'unidirectional flux' (i.e. uptake) and consequent metabolism of one prostaglandin may be inhibited in a concentration-dependent manner by a second structurally-related prostaglandin added simultaneously (Anderson & Eling, 1976; Eling, Hawkins & Anderson, 1977).

Our experiments provide analogous evidence concerning the accumulation and metabolism of thromboxane B_2, except that the inhibitor (U46619) does not affect the inactivation of prostaglandin $F_{2\alpha}$ at any of the doses tested or accumulation except at the highest dose tested. The implication is that the accumulation and metabolism of thromboxane B_2 is dependent on a mechanism distinct from the one which handles prostaglandin $F_{2\alpha}$. This interpretation is supported by the fact that prostaglandin $F_{2\alpha}$ itself does not affect thromboxane B_2 metabolism (although it alone of the prostaglandins tested caused a significant increase in the thromboxane B_2 tissue-to-medium ratio, but this remains unexplained).

Moreover, by further analogy with prostaglandins it seems reasonable to suppose that in this context 'accumulation' must be equivalent to uptake into pulmonary cells rather than to extracellular binding to a structurally specific macromolecule (similar to an external 'receptor'), but to date there are no definitive methods for distinguishing these two possibilities. However, the close coupling implicit in our data between specific accumulation and metabolism, along with our previous findings of similarities between the disposition of thromboxane B_2 (Robinson *et al.*, 1982) and carrier-mediated transport of prostaglandin $F_{2\alpha}$ (Robinson & Hoult, 1982) in terms of temperature sensitivity and susceptibility to protein synthesis inhibition, together with the identification here of an apparently specific inhibitor of accumulation, incline us to the view that there may also be a pulmonary uptake mechanism for thromboxane B_2.

Direct validation of this hypothesis awaits the demonstration of prostaglandin and thromboxane metabolism in intact cultured pulmonary cells. It has previously been assumed that pulmonary vascular endothelial cells are responsible for prostaglandin metabolism in the intact lung on account of their large surface area and capacity to metabolize other vasoactive substances, but pulmonary artery endothelial cells in culture do not inactivate prostaglandins to any significant extent (Ody, Dieterle, Wand, Stalder & Junod, 1979). However, pulmonary capillary cells have not yet been studied and may be active in this respect.

Our results may also have a bearing on thromboxane A_2 metabolism in lung. Thromboxane A_2 is generated in the lung under various circumstances, and the 13,14-dihydro-15-keto metabolite of thromboxane B_2 has been detected in anaphylactic lung perfusates (Dawson, Boot, Cockerill, Mallen & Osborne, 1976; Anhut, Peskar & Bernauer, 1978), and may arise by metabolism of thromboxane B_2 in the manner we have demonstrated. However, the half-life of thromboxane A_2, although short (ca. 30 s in aqueous solution at 37°C, Hamberg, Svensson & Samuelsson, 1975), is probably too long to account for this, considering the timescale of events within the perfused lung.

There are two alternative options. The first is that released thromboxane A_2 is metabolised to its 13,14-dihydro-15-keto metabolite following uptake of thromboxane A_2 into lung cells and enzymatic transformation, followed by release and hydrolysis to its thromboxane B_2 counterpart. The second possibility is that thromboxane A_2 is synthesized and released within the cells containing the metabolizing enzymes and inactivated before release. This seems less likely because the great majority of studies on prostaglandin and thromboxane synthesis and release suggest that these substances are released from cells to function as extracellular chemical messengers; nevertheless, Vargaftig, Chignard & Benveniste (1981) have indirect evidence that in platelets, functionally active thromboxane A_2 may be generated within the cells without apparent release. It is not known whether this concept has any validity for other tissues.

It is therefore possible that, like thromboxane B_2, thromboxane A_2 can itself be taken up and enzymatically transformed in the guinea-pig lung. To date this has not been tested directly because of the technical

difficulties associated with the instability of aqueous solutions of thromboxane A_2. Our findings that U46619 (which is an agonist at thromboxane A_2 receptors, and therefore must bear a close conformational resemblance to it) as well as other thromboxane A_2-like agonists or antagonists (e.g. U44069, the 9α,11α- isomer of U46619, and pinane thromboxane A_2; Robinson, C. & Hoult, J.R.S., unpublished experiments), interfere with pulmonary thromboxane B_2 accumulation and metabolism suggest that thromboxane A_2 could possibly itself be subject to the same processes. If this were so, it would necessitate revision of present concepts concerning the mechanisms which restrict the actions of thromboxane A_2 *in vivo*. Clearly, this requires further testing.

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