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EFFECT OF SULPHASALAZINE ON PULMONARY INACTIVATION OF PROSTAGLANDIN $F_{2\alpha}$ IN THE PIG

P.G. HELLEWELL & J.D. PEARSON

A.R.C. Institute of Animal Physiology, Babraham, Cambridge CB2 4AT

1 The metabolism of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) 15 nM in 100,000 *g* supernatant fractions from piglet lung homogenates was inhibited by sulphasalazine with an IC_{50} value of 25 μM.

2 The piglet isolated lung perfused with Krebs solution, containing either albumin or Ficoll 70 to prevent oedema and vascular damage, efficiently metabolized $PGF_{2\alpha}$ given as a bolus injection (1 ng in 0.1 ml; 30 nM).

3 In Krebs solution containing Ficoll 70, sulphasalazine inhibited the pulmonary inactivation of $PGF_{2\alpha}$ in a dose-dependent manner with an IC_{50} value of 110 μM. No inhibition of inactivation by sulphasalazine was found when the perfusion fluid contained albumin, which is known to bind this drug effectively.

4 Analysis of the separated efflux profiles for $PGF_{2\alpha}$ and its metabolites with reference to the dilution curve for an extracellular marker provided evidence that sulphasalazine inhibited $PGF_{2\alpha}$ uptake into lung cells.

5 We conclude that the effect of sulphasalazine on pulmonary prostaglandin inactivation is primarily due to inhibition of prostaglandin transport, and not to inhibition of prostaglandin metabolism.

Introduction

Sulphasalazine (2-(3-carboxy-4-hydroxyazobenzene-4'-sulphonamido) pyridine) has been shown to inhibit the catabolism of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) 30 μM in homogenates from several tissues, in a variety of species, with an IC_{50} value of approximately 50 μM. This is due to inhibition of 15-hydroxyprostaglandin dehydrogenase (PGDH), the cytoplasmic enzyme responsible for primary degradation of prostaglandins; the drug has negligible effects on enzymes that may be involved in further catabolism, i.e. prostaglandin Δ-13 reductase, 9-hydroxyprostaglandin dehydrogenase and 15-ketoprostaglandin reductase (Hoult & Moore, 1978; 1980).

The inactivation of $PGF_{2\alpha}$ or PGE_2 by rat or guinea-pig lungs, isolated and perfused with Krebs solution, was inhibited by sulphasalazine in a dose-dependent manner (Hoult & Moore, 1980) and the authors suggested that this was probably due to inhibition of PGDH. Intravenous infusions of sulphasalazine into the anaesthetized rat also inhibited pulmonary inactivation of PGE_1 or PGE_2 (Hoult & Moore, 1980). However, Bakhle (1980) perfused lungs from three species with PGE_2 or a prostaglandin analogue known to be resistant to catabolism by PGDH and concluded that sulphasalazine exerted its action by preventing uptake of prostaglandin into intracellular compartments, rather than by direct inhibition of cytoplasmic PGDH.

In this study we have investigated in detail the mechanism by which sulphasalazine inhibits prostaglandin breakdown in the piglet isolated lung, perfused with Krebs solution supplemented with a colloid oncotic agent to prevent extravascular fluid leakage, and have compared its action with that in homogenates from the same tissue.

Methods

Catabolism of prostaglandin $F_{2\alpha}$ in cell-free homogenates

Newborn to 2 day old pigs (0.8–1.2 kg) were obtained from the Babraham herd. Each animal was given a subcutaneous injection of 500 units of heparin. Approximately 30 min later the animal was killed by stunning and exsanguination, the chest was opened, and the rib cage cut away. The ductus arteriosus was ligated and a cannula introduced into the pulmonary artery via the right ventricle. The pulmonary vascular bed was then flushed through for about 15 s with 50 ml of Krebs solution containing 4.5% (w/v) albumin and 50 u heparin. The composition of the Krebs solution was (mM): NaCl 118, KCl 4.7, KH_2PO_4 1.9, $MgSO_4$ 1.9, glucose 11.1, $NaHCO_3$ 25,

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$CaCl_2$ 1.3, and the pH was maintained at 7.4 (at 37°C) by gassing with O_2:CO_2 (95:5 by volume). The lungs were then excised, 6–8 g homogenized in 4 vol. 50 mM phosphate buffer (pH 7.5) and the 100,000 *g* cytosolic supernatant fraction prepared according to the method given by Hoult & Moore (1977).

Catabolism of $PGF_{2\alpha}$ was routinely determined by incubation, for 30 min at 37°C, of 80 µl subsamples of supernatant with substrate (final concentration 15 nM, containing 0.01 µCi [^{3}H]-$PGF_{2\alpha}$) and 5 mM NAD^+ in a total volume of 0.2 ml containing 0–500 µM sulphasalazine. $PGF_{2\alpha}$ and its metabolites were extracted (Hoult & Moore, 1977) and separated by chromatography on 0.25 mm silica gel layers containing fluorescent indicator (Polygram; Camlab, Cambridge) using ethyl acetate: acetone: glacial acetic acid (90:10:1 by volume) as the solvent system (Andersen, 1969). Chromatograms were cut in two fractions containing, respectively, $PGF_{2\alpha}$ and its metabolites, and radioactivity was determined in each fraction by liquid scintillation spectrometry after the addition of 3 ml Packard Picofluor Scintillation fluid. Values for percent metabolism were not corrected for any loss of ^{3}H from [^{3}H]-$PGF_{2\alpha}$ during metabolism.

Inactivation of prostaglandin $F_{2\alpha}$ in the perfused lung

Lungs were prepared as described in the previous section except that the left auricle was also cannulated via the left ventricle to collect effluent from the pulmonary veins. After flushing the pulmonary bed with Krebs solution containing albumin and checking for leaks, the trachea was cannulated and the lungs were excised and suspended in a water jacket at 37°C. The preparation was then inflated with approximately 50 ml air, and perfused at 37°C with Krebs solution containing a colloid oncotic agent at 4.5% w/v (one of: albumin, mol.wt. 69,000; dextran, mol.wt. 60,000–90,000; Ficoll 70, mol.wt. 70,000) with a flow rate of 10 ml/min using a Watson-Marlow peristaltic pump. None of the added colloid oncotic agents significantly altered the osmolarity of the Krebs solution. Arterial perfusion pressure and lung weight were continuously recorded via transducers on a dual channel recorder. Any lungs that developed gross oedema as judged by substantial (>20%) weight increase or visible swelling were discarded. The success of perfusion was assessed at the end of an experiment (usually after 60–90 min) by injection of Evans' blue dye bound to albumin into the pulmonary artery. Surface colouration of the lungs was noted and they were sliced to determine the extent of perfusion within each lobe (usually 70–90%).

Experiments were started after an equilibration period of 10–15 min perfusion. A 100 µl bolus of Krebs solution containing 0.5 µCi [^{3}H]-$PGF_{2\alpha}$ (30 nM) and 0.2 µCi [^{14}C]-sucrose was rapidly injected (1–2s) into the pulmonary artery and effluent was collected in 2 drop fractions (approx. 250 µl) for 60 s. Flow and drop rates were recorded before and after each injection. After one bolus injection into lungs perfused with drug-free Krebs solution the perfusate was changed for one containing a known concentration of sulphasalazine and after 10–15 min perfusion the injection was repeated. In most experiments the reversibility of the action of sulphasalazine was assessed by determining metabolism and recovery of radioactivity after the lungs were reperfused for 10–15 min with drug-free Krebs solution.

A 50µl subsample from each 2 drop fraction was taken for liquid scintillation counting. After corrections were made for background and channel overlap, results were expressed for each fraction as the percentage of total ^{14}C and ^{3}H injected. Further 50 µl subsamples from each 2 drop fraction were pooled, extracted, and the overall amount of $PGF_{2\alpha}$ metabolism determined by thin layer chromatography (t.l.c.). In certain experiments the amount of $PGF_{2\alpha}$

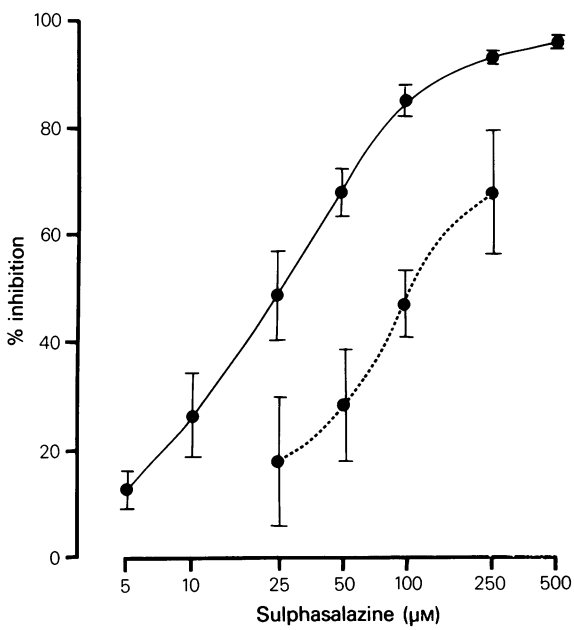

Figure 1 Inhibition of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) breakdown by sulphasalazine. Solid line shows the effect of sulphasalazine on metabolism of $PGF_{2\alpha}$ in 100,000 *g* supernatant fractions from pig lung. Each point represents the mean of 3–6 observations; vertical lines shows s.e.mean. Dashed line shows the effect of sulphasalazine on the inactivation of $PGF_{2\alpha}$ in the piglet isolated lung perfused with Krebs solution containing 4.5% (w/v) Ficoll 70. Each point represents the mean of 3–5 experiments; vertical lines show s.e.mean.

metabolism in each fraction was determined so that separated efflux profiles for $PGF_{2\alpha}$ and its metabolites could be constructed.

Chemicals

We are grateful to Dr H. Agback (Pharmacia AB, Sweden) and Dr J.R.S. Hoult (King's College, London) for generous gifts of sulphasalazine. [5,6,8,9,11,12,14,15(n)-^{3}H]-prostaglandin $F_{2\alpha}$ (sp. act. 180 Ci/mmol) and [U-^{14}C]-sucrose (sp. act. 382 mCi/mol) were purchased from Amersham International. Bovine albumin (Cohn fraction V) and dextran (mol. wt. 60,000–90,000) were purchased from Sigma, and Ficoll 70 from Pharmacia.

Results

Effect of sulphasalazine on prostaglandin $F_{2\alpha}$ catabolism in cell-free homogenates from lung

Under standard assay conditions approximately 70% of $PGF_{2\alpha}$ was metabolized in 30 min in the absence of sulphasalazine. Sulphasalazine inhibited breakdown of $PGF_{2\alpha}$ 15 nM in a dose-dependent manner (Figure 1; solid line) with an IC_{50} value (concentration required to inhibit control breakdown by 50%) of 25 μM.

Effect of sulphasalazine on prostaglandin $F_{2\alpha}$ inactivation in the perfused lung

In preliminary experiments lungs were perfused with Krebs solution without any added colloid oncotic agent, as used by Bakhle and co-workers (Alabaster & Bakhle, 1970 et seq.). Under these conditions, there was a rapid onset of oedema in the piglet lung. This was prevented by the addition of 4.5% (w/v) albumin to the perfusate, and the effects of sulphasalazine on $PGF_{2\alpha}$ inactivation were therefore studied using Krebs solution containing albumin.

Figure 2 is an example of the typical dilution curves obtained for ^{3}H and ^{14}C after a bolus injection of [^{3}H]-$PGF_{2\alpha}$ (30 nM) with [^{14}C]-sucrose into lungs perfused with drug-free Krebs solution. The ^{14}C curve represents the reference efflux pattern for a non-metabolized compound (of very similar mol. wt. to $PGF_{2\alpha}$) that only occupies extracellular space. Recovery of ^{14}C was almost complete (in this example 88% in 60 s), with more than 75% being recovered in under 25 s. Any significant broadening of the peak and diminution of recovery of ^{14}C was taken as evidence of damage to the lung vasculature; this conclusion was often substantiated by increasing lung weight. The efflux pattern for ^{3}H was different in several respects: the peak of ^{3}H efflux was much

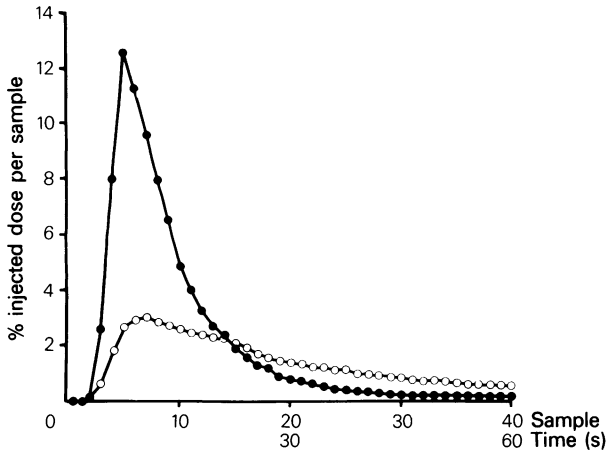

Figure 2 Efflux curves for ^{3}H and ^{14}C after bolus injection of [^{3}H]-prostaglandin $F_{2\alpha}$ ([^{3}H]-$PGF_{2\alpha}$) and [^{14}C]-sucrose into the piglet isolated lung. (○) ^{3}H; (●) ^{14}C.

lower than that of ^{14}C and occurred approximately 3 s after the ^{14}C peak; the efflux curve was much broader with a long tail above the ^{14}C curve; and the total recovery of ^{3}H in 60 s (in this example 56%) was significantly lower than that of ^{14}C. When the samples were pooled and extracted, most of the ^{3}H (in this example 60%) was found in metabolites of $PGF_{2\alpha}$.

Table 1 demonstrates that sulphasalazine at concentrations up to 500 μM was without effect on pulmonary metabolism of $PGF_{2\alpha}$ or on the recovery of ^{3}H or ^{14}C. These results contrast with those of Hoult & Moore (1980) and Bakhle (1980), where sulphasalazine at 50–100 μM significantly inhibited prostaglandin inactivation in isolated lungs perfused with albumin-free Krebs solution. Because sulphasalazine is known to be almost completely bound to albumin under the conditions of the experiments described above (Jansen, 1977), thus substantially reducing its concentration in free solution, the experiments were repeated with either dextran or Ficoll 70 as colloid oncotic agents in place of albumin.

Dextran has been used by Eling, Hawkins & Anderson (1977) and Linehan, Dawson & Wagner-Weber (1981) to supplement perfusion fluids in rat and cat isolated lungs. Table 2 shows that control levels of $PGF_{2\alpha}$ breakdown were similar when dextran (mol. wt. 60,000–90,000, 4.5% w/v) was used to replace albumin, and that there was a substantial and significant dose-dependent inhibition of metabolism in the presence of sulphasalazine. However, it was also clear that dextran had deleterious effects on the pulmonary vasculature. Oedema, monitored by rising lung weight, was evident after approximately 5 min of perfusion, and the increased leakiness of the

Table 1 Effect of sulphasalazine on the inactivation of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and recovery of injected radioactivity in piglet lungs perfused with Krebs solution containing 4.5% (w/v) albumin

	% metabolism	% recovery ^3H	% recovery ^{14}C
Initial control	65.5± 7.3 (8)	50.6±6.5 (7)	85.4± 8.8 (7)
Sulphasalazine 250 μM	68.6± 2.4 (5)	49.5±5.5 (4)	77.0±10.2 (4)
Sulphasalazine 500 μM	64.7±12.3 (4)	53.7±8.4 (3)	85.3± 9.2 (3)
Final control	74.8± 6.6 (7)	44.5±4.4 (6)	80.0± 4.1 (6)

Recoveries are expressed as percentage of total radioactivity injected ([^{3}H]-$PGF_{2\alpha}$ and [^{14}C]-sucrose) collected in 80 drops (approximately 60 s). Results are means ± s.e. (no. of experiments).

Table 2 Effect of sulphasalazine on the inactivation of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and recovery of injected radioactivity in piglet lungs perfused with Krebs solution containing 4.5% (w/v) dextran

	% metabolism	% inhibition of control metabolism	% recovery ^3H	% recovery ^{14}C
Initial control	75.4±9.1		28.7±19.1	66.7±22.0
Sulphasalazine 250 μM	25.0±4.3**	66.8± 5.7	53.0±19.5	60.3±20.5
Sulphasalazine 500 μM	16.9±3.3**	77.5± 4.4	57.7±23.7**	61.7±26.7
Final control	59.1±6.7**	21.7±10.8	28.3±20.2	59.0±24.6

Recoveries are expressed as percentage of total radioactivity injected ([^{3}H]-$PGF_{2\alpha}$ and [^{14}C]-sucrose) collected in 80 drops (approximately 60 s). Results are means ± s.e. of 3 experiments. Significant differences from control values at start of experiment revealed by paired t test; ** $P < .01$.

Table 3 Effect of sulphasalazine on the inactivation of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and recovery of injected radioactivity in piglet lungs perfused with Krebs solution supplemented with 4.5% (w/v) Ficoll 70

	% metabolism	% inhibition of control metabolism	% recovery ^3H	% recovery ^{14}C
Initial control ($n = 5$)	65.1±5.3		52.8± 2.3	87.4±7.3
Sulphasalazine 25 μM ($n = 3$)	54.2±8.0 *	16.7±12.4	56.7± 3.1	80.0± 4.4
Sulphasalazine 50 μM ($n = 3$)	47.0±6.8**	27.8±10.4	58.7± 1.2**	80.0± 6.3
Sulphasalazine 100 μM ($n = 4$)	34.4±4.2***	47.2± 6.5	64.5±11.7*	79.8±10.4
Sulphasalazine 250 μM ($n = 5$)	19.0±6.5***	68.8±11.9	71.4± 4.7***	84.4±10.8
Sulphasalazine 500 μM ($n = 2$)	21.8, 26.6	66.5, 59.1	78, 80	95, 100
Final control ($n = 5$)	64.7±5.8	0.6± 8.8	46.2± 3.0	81.0± 4.9

Recoveries are expressed as percentage of total radioactivity injected ([^{3}H]-$PGF_{2\alpha}$ and [^{14}C]-sucrose) collected in 80 drops (approximately 60 s). Means + s.e. are shown; n = no. of experiments. Significant differences from control values at start of experiment revealed by unpaired t test; * $P < 0.1$, ** $P < .01$, *** $P < .001$.

pulmonary bed was also apparent from the poorer recoveries within 60 s of ^3H and ^{14}C in the controls (compare Tables 2 and 1). In the presence of sulphasalazine the recovery of ^3H (but not of ^{14}C) increased with the inhibition of $PGF_{2\alpha}$ metabolism.

Because dextran induced oedema, a detailed investigation of the effects of sulphasalazine was not carried out with this colloid oncotic agent, and dextran was replaced with Ficoll 70 (mol. wt. 70,000; a copolymer of sucrose and epichlorhydrin). Lungs were successfully perfused for up to 90 min with Krebs solution containing 4.5% (w/v) Ficoll 70. There was no increase in lung weight, nor were there any visual changes indicative of oedema, and perfusion pressure remained constant (9.6 ± 3.6 mmHg, $n = 5$) throughout experiments. Under control conditions $PGF_{2\alpha}$ was inactivated to the same extent as in lungs perfused with albumin-supplemented Krebs solution and the recoveries of ^3H and ^{14}C were also the same. Sulphasalazine significantly inhibited $PGF_{2\alpha}$ breakdown in a dose-dependent manner, with corresponding increases in the recovery of ^3H (but not of ^{14}C) in 60 s, reaching a maximum inhibition by approx. 250 μM (Table 3). The IC_{50} value (see Figure 1; dashed line) was 110 μM, and the inhibition was fully reversible (bottom line of Table 3).

Subsamples from individual 2 drop fractions were extracted and chromatographed to determine the extent of $PGF_{2\alpha}$ metabolism. Figure 3a shows the separated dilution curves for $PGF_{2\alpha}$ and its metabolites in a control perfusion in which overall metabolism was 60%. The profile for the unmetabolized $PGF_{2\alpha}$ peaked at the same time as that of the extracellular marker, and there was no tail above the ^{14}C curve; the profile for $PGF_{2\alpha}$ metabolites, however, peaked approximately 7 s later, and had a per-

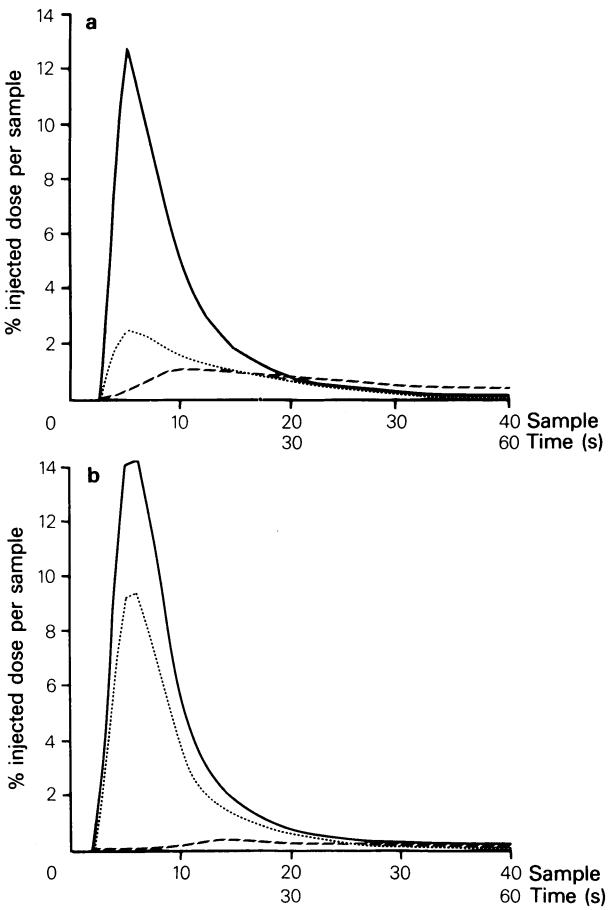

Figure 3 Efflux curves for ^3H and ^{14}C after bolus injection of [^3H]-prostaglandin $F_{2\alpha}$ ([^3H] $PGF_{2\alpha}$) and [^{14}C]-sucrose into the piglet isolated lung. Continuous line, [^{14}C]-sucrose; dotted line, [^3H]-$PGF_{2\alpha}$; dashed line, [^3H]-$PGF_{2\alpha}$ metabolites. (a) control; (b) in the presence of sulphasalazine 250 μM.

sistent tail above the ^{14}C curve. After the same lungs were perfused with Krebs solution containing sulphasalazine (250 μM; Figure 3b) there were substantial changes in the dilution curves. The peak of the unmetabolized $PGF_{2\alpha}$ still corresponded in time to the sucrose peak, although it was considerably higher than in the control; the $PGF_{2\alpha}$-metabolite dilution curve had a lower peak than in the control perfusion, but was still displaced by approximately 7 s and possessed a tail above the parent compound, but not above the extracellular marker. Overall metabolism was reduced to 25%.

Discussion

Sulphasalazine inhibited the metabolism of $PGF_{2\alpha}$ in 100,000 *g* supernatant fractions from piglet lung, as might be expected from its known inhibition of PGDH in several other species and tissues (Hoult & Moore, 1980). In these previous studies the metabolism of $PGF_{2\alpha}$ 30 μM was inhibited with an IC_{50} value for sulphasalazine of about 50 μM in all instances. In the present experiments using a much lower substrate concentration (15 nM) we found an IC_{50} value of 25 μM. Since the *K*m values of PGDH from a variety of tissues are about 20–50 μM (see Änggård & Oliw, 1976 and reference therein) we conclude that sulphasalazine cannot be a competitive inhibitor of PGDH. This is supported by data in a recent abstract (Berry, Hoult, Jeffery, Mak & Peers, 1982) but further work with purified PGDH is necessary to elucidate the mechanism of its inhibition by sulphasalazine.

Ody, Dieterle, Wand, Stalder & Junod (1979) showed that porcine lung slices metabolized $PGF_{2\alpha}$ and PGA_1 and there has been a brief report describing pulmonary metabolism of infused $PGF_{2\alpha}$ by the adult pig *in vivo* (Davis, Fleet, Harrison & Maule-Walker, 1980), but we are not aware of previous studies of prostaglandin inactivation in the porcine isolated perfused lung. The response to a bolus injection of a small mass (1 ng) of $PGF_{2\alpha}$ in the piglet lung preparation demonstrated that, in common with all other mammalian species so far studied, the pig possesses mechanisms for the pulmonary uptake and metabolism of prostaglandins and the release of prostaglandin metabolites to the perfusate. Data from this preparation can therefore be compared with those concerning the regulation of synthesis and metabolism of vasoactive agents, including prostaglandins, obtained in studies of large vessel porcine endothelial cells cultured *in vitro* (Trevethick, Olverman, Pearson, Gordon, Lyles & Callingham, 1981; Ager, Gordon, Moncada, Pearson, Salmon & Trevethick, 1982).

From our experiments we conclude that it is important to monitor lung weight and perfusion pressure and, in particular, to assess the recovery of prostaglandin and its metabolites in comparison with an extracellular marker (sucrose is convenient and has almost the same mol. wt. as $PGF_{2\alpha}$) to be confident that damage to the pulmonary bed is not occurring. In the piglet lung, albumin or Ficoll 70 provided satisfactory oncotic protection; in their absence or in the presence of dextran, oedema was rapid in onset. However, there may be species differences in the requirement for a colloid oncotic agent; for example, Bakhle and co-workers have routinely used albumin-free Krebs solution to perfuse rat lungs and have not commented on oedema formation.

The degree of inactivation of a small bolus injection of $PGF_{2\alpha}$ was independent of the oncotic agent used (albumin, dextran or Ficoll), in agreement with the findings of Robinson & Hoult (1982) that $PGF_{2\alpha}$ inactivation was not reduced in the presence of albumin; in contrast PGA_2 is very poorly inactivated when albumin is present in the perfusate (Hawkins, Wilson, Anderson & Eling, 1977; Robinson & Hoult, 1982) reflecting the greater affinity of PGA_2 than of $PGF_{2\alpha}$ for albumin (Raz, 1972).

The IC_{50} value for sulphasalazine against inactivation in the perfused lung was more than 4 fold higher than the value obtained for inhibition of PGDH. The concentration in the bolus injection was twice that in the cell-free system, but the peak $PGF_{2\alpha}$ concentration within the lung (except at very early times after injection) was calculated from the dilution curves to be at least 10 fold lower. Sulphasalazine therefore does not seem to act on PGDH in the perfused lung. There is, indeed, no evidence that sulphasalazine enters pulmonary cells; this could be tested directly by injecting radiolabelled sulphasalazine into the perfused lung. If the drug does not enter pulmonary cells its known effect on PGDH in cell-free systems cannot be relevant in the perfused lung, since PGDH is a cytoplasmic enzyme.

Prostaglandin inactivation in the pulmonary bed is thought to be due to uptake by a saturable carrier-mediated system (Anderson & Eling, 1976; Bito, Baroody & Reitz, 1977) followed by intracellular metabolism. Sulphasalazine could, therefore, inhibit pulmonary prostaglandin inactivation by blocking the carrier mediated uptake process. Inhibition of prostaglandin uptake has been directly shown for bromcresol green, by demonstrating that it inhibits inactivation in the perfused lung at concentrations that have no effect on PGDH (Bito & Baroody, 1975). Several other drugs including probenecid, frusemide, diphloretin phosphate and sulphasalazine reduce prostaglandin inactivation in the lung but also inhibit PGDH (Crutchley & Piper, 1974; Bito & Baroody, 1975; Eling *et al.*, 1977; Bakhle, 1979; Hoult & Moore, 1980). Therefore it is uncertain whether the action of these drugs in the perfused lung

is due primarily to effects on PGDH or whether they may inhibit prostaglandin transport.

The criterion that has been used most frequently to discriminate between inhibition of PGDH or inhibition of prostaglandin transport is that an inhibitor of uptake should decrease the time to recover a specific fraction (usually $T_{\frac{1}{2}}$) of the injected radioactivity (or should increase the recovery in a given time) from a bolus of prostaglandin. Uptake must be measured with reference to a marker of extracellular space to check that the drug does not have non-specific effects (such as restricting blood flow or causing leakage in the pulmonary bed) which would also decrease prostaglandin inactivation.

We have confirmed the observation of Bakhle (1980) that recovery of prostaglandin was more rapid in the presence of sulphasalazine. However, this finding alone is not conclusive evidence for uptake inhibition. Although inhibition of pulmonary 5-hydroxytryptamine (5-HT) metabolism by monoamine oxidase inhibitors increases $T_{\frac{1}{2}}$ values (Alabaster & Bakhle, 1970) it is not clear that this would be expected for inhibitors of PGDH, because unlike 5-HT and its metabolites which are predominantly retained within the lung for many minutes after a bolus injection (Alabaster & Bakhle, 1970; Rickaby, Linehan, Bronikowski & Dawson, 1981), prostaglandins and their metabolites are rapidly released.

Bakhle (1980) tested the effect of sulphasalazine on prostaglandin uptake by perfusing lungs with 16,16-dimethyl-PGE_2, which is not metabolized by PGDH, and found that in the presence of sulphasalazine responses of a bioassay tissue to the prostaglandin analogue occurred more rapidly and approached those of directly superfused 16,16-dimethyl-PGE_2. He thus concluded that sulphasalazine inhibited prostaglandin transport. Since there is no evidence that the analogue enters pulmonary vascular cells and in view of the reservations expressed above, it would be valuable to be able to confirm this result with radiolabelled 16,16-dimethyl-PGE_2 in the presence of a reference extracellular marker and of a colloid oncotic agent.

Analysis of the separated efflux curves for injected $PGF_{2\alpha}$ and its metabolites (Figure 3) demonstrated that unlike the $PGF_{2\alpha}$ metabolites, the bulk of non-metabolized $PGF_{2\alpha}$ in the presence or absence of sulphasalazine was recovered with an efflux profile similar to that of sucrose (i.e. with no delay relative to an extracellular marker). This observation, which agrees with other analyses of efflux profiles following bolus prostaglandin injection (Dawson, Cozzini & Lonigro, 1975; Anderson & Eling, 1976; Linehan & Dawson, 1979) implies that the bulk of the recovered non-metabolized prostaglandin did not leave the vascular space.

In contrast, after $PGF_{2\alpha}$ uptake, intracellular metabolism and release, the metabolite peak occurred approximately 7 s later than that of then non-metabolized $PGF_{2\alpha}$, suggesting that nearly all $PGF_{2\alpha}$ transported was metabolized. This was the case in both control and sulphasalazine-treated lungs. These findings provide the best evidence that sulphasalazine acts primarily as an inhibitor of prostaglandin transport in the perfused lung. The exact mechanism by which sulphasalazine interacts with the prostaglandin transport system and alters its kinetic parameters requires studies using tracer dilution techniques (Anderson & Eling, 1976; Linehan *et al.*, 1981; Syrota, Girault, Pocidalo & Yudilevich, 1982).

In conclusion, our experiments have demonstrated that sulphasalazine can efficiently inhibit prostaglandin inactivation in the porcine perfused lung, and that this action seems to be due to inhibition of prostaglandin transport rather than to inhibition of PGDH.

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