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ACTION OF PROSTAGLANDIN DEHYDROGENASE INHIBITORS ON PROSTAGLANDIN UPTAKE IN RAT ISOLATED LUNG

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1 The effects of some inhibitors of 15-hydroxy-prostaglandin dehydrogenase (PGDH) have been studied on the inactivation of prostaglandin E_2 (PGE_2) and 16, 16-dimethyl PGE_2 in rat isolated lung.

2 Bioassay was used to show that the inactivation of PGE_2 and its methyl analogue, was inhibited by frusemide (10^{-3} M), N-ethyl maleimide (10^{-5} M) and 5, 5'-dithio-bis-2-nitro-benzoic acid (10^{-2} M), but not by caffeine (2.5×10^{-3} M).

3 The efflux of radioactivity from lung following injection of [^{14}C]-PGE_2 was faster in lungs treated with frusemide, N-ethyl maleimide or bromcresol green, than in untreated lungs.

4 Caffeine (10^{-3}, 10^{-2} M) did not change the rate of ^{14}C-efflux from lungs following injection of [^{14}C]-PGE_2.

5 From these results it is concluded that those PGDH inhibitors that prevented prostaglandin inactivation in isolated lungs did so by inhibiting uptake of prostaglandin rather than by inhibiting PGDH.

Introduction

Inactivation of prostaglandin E_2 (PGE_2) passing through the pulmonary circulation comprises two distinct steps, uptake of substrate from the extracellular space and subsequent metabolism by intracellular enzymes, most significantly 15-hydroxy-prostaglandin dehydrogenase (PGDH). Inhibitors of the uptake process, for instance, bromcresol green and other indicator dyes (Bito & Baroody, 1975; Bakhle, Jancar & Whittle, 1978) and di-phloretin phosphate (Crutchley & Piper, 1975; Bakhle *et al.*, 1978) have been described and so have inhibitors of the enzyme PGDH such as frusemide, caffeine and N-ethyl maleimide (Hansen, 1976). It seemed reasonable to assume that, for analogues of PGE_2 that are substrates for the uptake process but not for the enzyme PGDH, inactivation in the pulmonary circulation would not be affected by inhibitors of PGDH. As the experiments described below show, this and other assumptions were not justified.

Methods

Lungs were removed from male rats, anaesthetized with pentobarbitone (administered intraperitoneally), and perfused via the pulmonary artery with warmed (37°C), gassed (95% O_2 and 5% CO_2) Krebs solution at 8 ml/min as described previously (Alabaster & Bakhle, 1970). For bioassay the effluent superfused two strips of hamster stomach (Ubatuba, 1973) or rat stomach (Vane, 1957) attached to isotonic Harvard transducers and the contractions of these assay tissues were displayed on a Watanabe potentiometric pen recorder. Injections of prostaglandins (0.05 to 0.2 ml) were made into the pulmonary artery cannula ('al') or below the lung to pass directly over the assay tissues ('dir'). Inactivation was measured as described earlier (Bakhle *et al.*, 1978). In each experiment control inactivation was measured before the inhibitors were infused and their effects on inactivation determined.

In two experiments, effluent containing [^{14}C]-PGE_2 and metabolites was collected for 10 min and extracted, after acidification to pH 3, with diethyl ether. The organic phase was separated and then evaporated under reduced pressure. The residue was taken up in methanol and applied to silica gel thin layer chromatography plates (Merck). The plates were developed in ethyl acetate: formic acid (80:1 v/v); the F-IV system of Andersen (1969), for 15 cm. After drying, marker compounds PGE_2, 15-oxo-PGE_2 and 13, 14-dihydro-PGE_2 were identified by spraying the chromatogram with anisaldehyde (Kiefer, Johnson & Arora, 1975). The chromatogram was cut into 1 cm sections, each section soaked in 1 ml methanol for 4 h and then scintillant was added. Corrections were made for quenching.

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For the measurement of $T\frac{1}{2}$, radioactive prostaglandin E_2 ([1-^{14}C]-PGE_2, 55 mCi/mmol, Radiochemical Centre, Amersham) was injected (0.1 ml; 1.0 μg; 2×10^4 d/min) and the effluent collected in 2 drop fractions for up to 5 min. The drop rate (drops/min) was measured immediately before and after a collection. The value of $T\frac{1}{2}$ was the time taken (to the nearest fraction collected) for half the injected radioactivity to appear in the effluent. Inhibitors were infused (0.1 ml/min) into the pulmonary arterial cannula for 20 min before, during and after the injection of PGE_2. In two lungs, ^{125}I-human serum albumin (Radiochemical Centre, Amersham) was injected (0.1 ml; 10^4 d/min) and the effluent collected as before for estimation of $T\frac{1}{2}$.

I am grateful to Dr J. Pike (Upjohn) for a gift of 16, 16-dimethyl PGE_2, 15-oxo-PGE_2 and 13, 14-dihydro PGE_2, and to Hoechst for a gift of frusemide. Other compounds were obtained from the following sources: PGE_2, caffeine, 5,5′-dithio-bis-2-nitrobenzoic acid, N-ethyl maleimide (Sigma), bromcresol green (Hopkins & Williams).

Results

Inhibition of prostaglandin E_2 and 16, 16-dimethyl PGE_2 inactivation

Four compounds, described as inhibitors of PGDH *in vitro*, were tested for inhibition of the inactivation of PGE_2 in rat isolated lung. As shown in Table 1, all but caffeine were inhibitors in the perfused lung. In two experiments, the effect of one inhibitor of prostaglandin inactivation, frusemide, was confirmed radiochemically by adding [^{14}C]-PGE_2 to the unlabelled PGE_2 used in bioassay. Lung effluent was collected for 10 min during an assay of PGE_2 inactivation. Effluent was extracted and the extract analysed for PGE_2 and metabolites by thin layer chromatography. The radioactivity associated with PGE_2 and its metabolites was measured. When the PGE_2 was given directly over the tissues ('dir'), 84% of the radioactivity co-chromatographed with the PGE_2 marker. Injected into the pulmonary circulation ('al'), only 12% of the radioactivity was PGE_2 and 84% chromatographed as the 15-oxo derivatives. Frusemide (10^{-3} M), infused through the lung, reduced the proportion of 15-oxo-PGE_2 to 29% and the proportion of unchanged PGE_2 was increased to 62%.

The inactivation of 16, 16-dimethyl PGE_2 was also inhibited by the three compounds affecting PGE_2 inactivation, whereas caffeine was again without effect (Table 1). These experiments were carried out by bioassay and the effect of frusemide on the inactivation of 16, 16-dimethyl PGE_2 is illustrated in Figure 1. The slow onset of the contraction of the hamster stomach strip to the methyl analogue injected 'al' (third contraction) is typical of this analogue (Bakhle *et al.*, 1978). However, after frusemide, the contraction to the 'al' injection was rapid in onset and of greater magnitude, showing inhibition of inactivation.

Table 1 Effects of inhibitors of prostaglandin dehydrogenase (PGDH) on prostaglandin inactivation in rat isolated lung

Inhibitor (M)	*% inhibition of inactivation (mean ± s.e. mean)* PGE_2	*16,16-dimethyl PGE_2*
Frusemide		
(10^{-3})	60 ± 6 (6)[1]	33 ± 7 (6)
N-ethylmaleimide		
(10^{-5})	67 ± 3 (7)	37 ± 8 (3)
5:5′-Dithio bis-2-nitrobenzoic acid		
(10^{-3})	16 ± 2 (8)	—
(10^{-2})	44 ± 6 (7)	56 ± 13 (6)
Caffeine		
(10^{-3})	6 (2)	
(2.5×10^{-3})	6 ± 5 (6)	11 ± 8 (6)

[1] Number of experiments

All the values shown with the exception of those for caffeine represent a significant effect ($P < 0.05$; t test). The effects of caffeine are not significantly different from zero (t test or Wilcoxon). These values are derived from bioassay experiments. The control value for inactivation of PGE_2 was 93 ± 1% ($n = 36$) and that of 16,16-dimethyl PGE_2 54 ± 3% ($n = 28$).

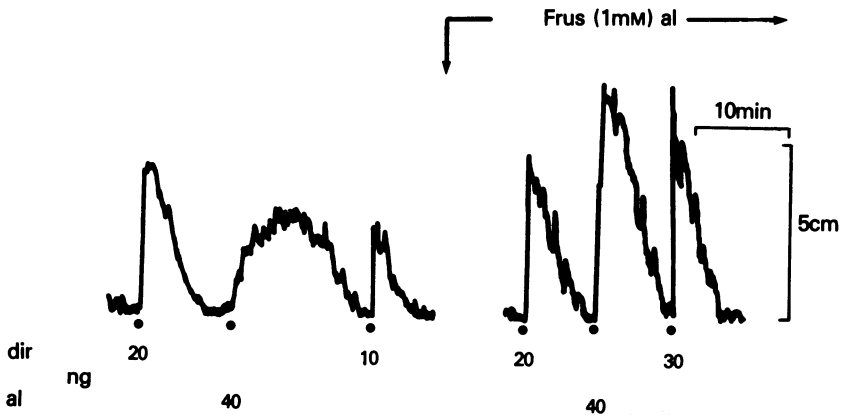

Figure 1 Effect of frusemide on inactivation of 16, 16-dimethyl prostaglandin E_2 (16 Me_2 PGE_2) in rat isolated lung. The two parts of the trace are from the same experiment and the gap between them represents about 20 min. The trace shows the responses of a hamster stomach strip superfused with effluent from a rat lung perfused with Krebs solution. In the first, left-hand section, the injection of 40 ng 16Me_2 PGE_2 through the pulmonary circulation ('al') caused a slowly developing response of the strip, the peak of the contraction being between that caused by 20 and 10 ng injected directly to the tissue ('dir'). After 20 min of infusing frusemide (1 mM) through the pulmonary circulation, the injection of 40 ng 16Me_2 PGE_2 caused a rapid response of the assay tissue almost equivalent to 30 ng 16Me_2 PGE_2 injected directly. Thus in the presence of frusemide, more 16Me_2 PGE_2 survives passage through the pulmonary circulation, i.e. inactivation was inhibited.

Effect of inhibitors on washout of radioactive prostaglandin E_2

Washout of PGE_2 radioactivity from untreated lungs had a $T\frac{1}{2}$ value of about 25 s, higher than that for ^{125}I human serum albumin (Table 2). Frusemide and N-ethyl maleimide, both inhibitors of inactivation, as shown with bioassay, reduced $T\frac{1}{2}$ to almost half the control value whereas caffeine, ineffective as an inhibitor of inactivation, was also without effect here. Bromcresol green, an inhibitor of prostaglandin uptake, and an inhibitor of prostaglandin inactivation, also reduced the $T\frac{1}{2}$ to approximately half its normal value. Where the $T\frac{1}{2}$ value had been changed by an inhibitor, 30 min after stopping the infusion of the inhibitor, $T\frac{1}{2}$ values returned to control levels.

Discussion

In this series of experiments it had been hoped to assess the effect of PGDH inhibition on prostaglandin inactivation in isolated perfused lung by the use of compounds known to inhibit PGDH in cell-free preparations. The experimental design was based on two assumptions both of which proved to be unjustified. First it was assumed that the inactivation of the PGE_2 analogue, 16, 16-dimethyl PGE_2, which is not a substrate for PGDH, would therefore be unaffected by the presence of PGDH inhibitors whereas inactivation of PGE_2 itself would be inhibited. However, the bioassay experiments showed that where PGE_2 inactivation was reduced, for instance by frusemide and N-ethyl maleimide, so also was the inactivation of the methyl analogue. There are differences in the

Table 2 Efflux of ^{14}C from isolated lung after injection of [^{14}C]-prostaglandin E_2; effect of inhibitors of prostaglandin E_2 inactivation

Treatment of lung	$T\frac{1}{2}$ (*s*)
Control	25.2 ± 0.8 (27)[1]
Bromcresol green	
10^{-5} M	12.5 ± 1.1* (6)
Frusemide	
10^{-3} M	12.5 ± 1.8* (5)
N-ethylmaleimide	
10^{-5} M	17, 14 (2)
10^{-2} M	11.0 ± 1.0* (3)
Caffeine	
10^{-3} M	26.2 ± 3.1 (5)
10^{-2} M	27.0 ± 3.3 (5)
^{125}I-human serum albumin	9, 6 (2)

[1]Number of lungs. The value $T\frac{1}{2}$ is the time taken for half the injected radioactivity to emerge from the lung and is expressed as mean ± s.e. mean.
*Significantly different ($P < 0.05$) from control, untreated lungs.

degree of inhibition between these two substrates, which probably reflect the relative affinities of the uptake system for each substrate and for the inhibitors. Such differences have been observed before (Bakhle *et al.*, 1978). These results suggested that PGDH inhibitors were acting on the uptake process for prostaglandins.

The second assumption was that, by analogy with 5-hydroxytryptamine, inhibition of the intracellular enzyme would enable substrate to accumulate unchanged in the lung and appear slowly in the effluent after the bolus had passed (Alabaster & Bakhle, 1970). This would, for instance, lead to a slower washout of radioactivity from the lung following injection of [^{14}C]-PGE_2 in the presence of PGDH inhibitors. The converse of this effect had already been reported, i.e. the more rapid washout of radioactivity derived from ^{14}C-$PGF_{2\alpha}$ in rat lung during infusion of the uptake inhibitor, bromcresol green (Bito & Baroody, 1975). $T\frac{1}{2}$ was also reduced in the experiment described here i.e. the rate of radioactivity washout was increased in the experiments with bromcresol green. Although frusemide and N-ethyl maleimide were expected to produce a slower efflux of radioactivity and thus to increase $T\frac{1}{2}$, they in fact reduced $T\frac{1}{2}$ to almost the same value as observed after bromcresol green. Thus, both sets of experiments, bioassay and radiochemical, led to the same unexpected conclusion that PGDH inhibitors prevented prostaglandin inactivation in perfused lung by preventing uptake rather than metabolism.

One explanation of these results would be to postulate that the uptake process for PGE_2 is not capable of functioning against the increased concentration gradient caused by the increased intracellular level of prostaglandin consequent on PGDH inhibition. This explanation is unlikely because, as Anderson & Eling (1976) have shown, PGE inactivation in rat lung is controlled by PGDH activity rather than by the uptake of substrate. Indeed, they suggest that prostaglandin can be taken up and, if PGDH is saturated, be returned unchanged to the perfusate. Further evidence against this explanation is the effect on the methyl analogue which, as it is not a substrate for PGDH, cannot be affected by PGDH inhibitors alone.

An alternative explanation is to accept that some inhibitors of PGDH are also inhibitors and, perhaps more potent inhibitors, of the uptake process for prostaglandins in lung. This dual action is not without precedent as di-phloretin phosphate (DPP) prevented metabolism of PGE_2 by homogenates of guinea-pig lung (Crutchley & Piper, 1974) and also inhibited inactivation of 16, 16-dimethyl PGE_2 (Bakhle *et al.*, 1978), thus showing an action on both PGDH and on uptake.

However, it could be argued that the highly ionized DPP molecule could not enter the cell to inhibit PGDH and thus its effects must be exerted at the cell surface. This argument cannot apply to frusemide nor to the recent experiments with probenecid (Bito, Baroody & Reitz, 1977). In the latter work (published while this paper was in preparation) probenecid, an inhibitor of $PGF_{2\alpha}$ metabolism in homogenates and perfused lung, reduced the $T\frac{1}{2}$ for [^{3}H]-$PGF_{2\alpha}$ in rat lung to the same value as the $T\frac{1}{2}$ for [^{14}C]-sucrose. Similar effects were produced by DPP and BCG. Thus, for $PGF_{2\alpha}$ also it seems that inhibition of metabolism in perfused lung may be due more to inhibition of uptake than to inhibition of PGDH.

Although these experiments were designed to study the effects of PGDH inhibition on prostaglandin inactivation, they have, in fact, provided more information about the uptake process. For instance, this uptake process seems to have two chemically distinct groups of inhibitors, the highly ionised organic molecules like bromcresol green and other indicator dyes and di-phloretin phosphate, and those, less polar, compounds capable of reacting with sulphydryl groups like frusemide and N-ethyl maleimide. Other sulphydryl-reactive compounds which are inhibitors of prostaglandin inactivation, like ethacrynic acid and *p*-chloro-mercuribenzoate (Crutchley & Piper, 1974) may also act by blocking uptake in the perfused lung although they are known to inhibit PGDH *in vitro*. The two classes of inhibitor should help to define further the chemical features of the uptake system for prostaglandins in lung.

The effect of PGDH inhibition on inactivation in whole lung still remains to be determined and it may be that with a different type of PGDH inhibitor, lacking an effect on uptake, the two original assumptions would be justified. It is nevertheless interesting that, in the presence of inhibitors, the uptake step seems to be more readily affected and becomes rate-limiting whereas under normal conditions, the rate of PGE inactivation is limited by PGDH action and not by uptake (Anderson & Eling, 1976). This and previous work (Bakhle *et al.*, 1978) suggest also that 16,16-dimethyl PGE_2 could be used as a means of investigating the prostaglandin uptake processes as distinct from prostaglandin metabolism by PGDH in lung or in other tissues. Finally, the present experiments re-emphasize the importance of uptake processes, under normal conditions or after drug treatment, as determinants of the fate of vasoactive hormones in the lung.

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