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Inhibitors of prostaglandin dehydrogenase (Ph CL 28A and Ph CK 61A) increase output of prostaglandins from rat isolated lung

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1 Two potent inhibitors of prostaglandin dehydrogenase (PGDH), Ph CL 28A and Ph CK 61A, have been investigated for their effects on prostaglandin catabolism and synthesis in rat isolated lung.

2 Both CL 28A (0.3 μM) and CK 61A (0.5 and 5 μM) markedly increased the survival of prostaglandin E_2 (PGE_2) and $PGF_{2\alpha}$ on a single passage through the pulmonary circulation.

3 Both inhibitors delayed the efflux of ^{14}C following injection of [^{14}C]-PGE_2 through the pulmonary circulation.

4 Output of PGE_2 and $PGF_{2\alpha}$ but not that of 6-oxo-$PGF_{1\alpha}$ from exogenous arachidonic acid (AA) was increased by CL 28A.

5 Output of all three prostaglandins from endogenous AA stimulated by calcium ionophore A23187 was increased by CL 28A.

6 With CK 61A, output of 6-oxo-$PGF_{1\alpha}$ from exogenous AA was not increased but that from endogenous AA was increased by either concentration of this inhibitor.

7 We conclude that it is possible to increase output of biologically active prostaglandins from lung by preventing their inactivation *in situ*.

8 The apparent selectivity of PGI_2 synthesis from endogenous AA to potentiation by the inhibitors may have therapeutic possibilities.

Introduction

The lungs synthesize and inactivate several prostaglandins (Bakhle & Ferreira, 1985). Thus the prostaglandins that emerge from the lung in the pulmonary vascular compartment are the net result of these two opposing properties. Output of both active prostaglandins and inactive prostaglandin metabolites has been demonstrated from lung stimulated *in vivo* (Forstermann *et al.*, 1981) or from perfused isolated lungs (Dawson *et al.*, 1976; Robinson *et al.*, 1984). We have therefore argued that it should be possible to increase the output of prostaglandins from lung by inhibiting the inactivation process for prostaglandins during the stimultion of their synthesis. Previous attempts to demonstrate this theoretically possible effect have been inconclusive, probably because the inhibitors of prostaglandin inactivation used also had other properties (Bakhle & Pankhania, 1985). Recently, highly potent and selective inhibitors of 15-hydroxy prostaglandin dehydrogenase (PGDH) have been described, with minimal inhibitory potency towards cyclo-oxygenase (Berry *et al.*, 1983; 1985). This gave us the opportunity to test our hypothesis without interference from other pharmacological properties. The most potent compound, Ph CL 28A and a less potent congener Ph CK 61A were studied in rat isolated lungs, perfused with Krebs solution, for their effects on prostaglandin inactivation and output from exogenous and endogenous arachidonic acid (AA). Some of these results have been communicated to the British Pharmacological Society (Bakhle & Pankhania, 1987).

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Methods

Male Wistar rats (200–250 g) were used. They were anaesthetized with pentobarbitone (60 mg kg^{-1}; i.p.) and the lungs were removed and perfused via the

pulmonary artery with Krebs solution (gassed with 95% O_2; 5% CO_2 and warmed to 37°C) at 8 ml min^{-1} (Bakhle 1979). When prostaglandin inactivation was studied, the Krebs solution contained indomethacin (3 $\mu g\,ml^{-1}$). After a 10 min period of perfusion, the assays were started.

Bioassay

Inactivation of prostaglandin E_2 (PGE_2) or $PGF_{2\alpha}$ was measured with rat or hamster stomach strips superfused with lung effluent (Bakhle, 1979). The responses to bolus injections of prostaglandin (300–1000 ng) into the pulmonary circulation were compared, in a bracket assay, with those to injections of prostaglandin (10–100 ng) given directly to the assay tissues.

Each lung served as its own control, assay in the absence of drug preceding an assay during the perfusion of drug in the Krebs solution through the pulmonary circulation. Drug perfusions were started 15 min before, and continued throughout, the assay. In our assays, the effects of drugs on the assay tissues are allowed for because responses to prostaglandin standards are also obtained in the presence of drug. Since the reponse to prostaglandin in lung effluent is measured in terms of the response to the standards, this procedure corrects for any change in sensitivity of the tissue due to the effects of the infused drug.

Efflux of radioactivity: $t_{\frac{1}{2}}$ assay

Here the effluent from rat lungs was collected in 4-drop fractions (approximately 3 s) immediately before and after an injection of ^{14}C-labelled prostaglandin (500 ng; 0.01 μCi; 0.1 ml) into the Krebs perfusate flow entering the lung. The total radioactivity in each fraction was measured by liquid scintillation methods (Bakhle, 1982). The $t_{\frac{1}{2}}$ values were stable over the experimental period, e.g., successive assays for PGE_2 in untreated lungs, 30 min apart were 34 ± 3 s and 38 ± 5 s respectively ($n = 5$).

Metabolism and output of prostaglandins

The survival of exogenous prostaglandins was measured by giving a single bolus injection of 500 ng of PGE_2 or $PGF_{2\alpha}$ into the perfusate entering the lung. The effluent perfusate was collected in a single fraction for 5 min subsequently and a sample of this effluent taken for radioimmunoassay (RIA) of unchanged prostaglandin.

Output of prostaglandins from exogenous AA was measured following an injection of 10 μg AA and collecting lung effluent for 5 min. To study prostaglandin synthesis from AA in lung lipids (endogenous AA), the calcium ionophore A23187 (1–3 μg) was injected into the perfusate entering the lung. The lung effluent was collected for 5 min immediately following the ionophore. Only one injection was given to each lung and thus control and drug treated values were obtained from different lungs.

Radioimmunoassay

A sample of lung effluent (100 μl) from either procedure was assayed directly or after five-fold dilution with Tris-buffered saline, for PGE_2, $PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$. The assay procedures have been described previously (Watts *et al.*, 1982). Cross-reactivities of the anti-sera were as follows: anti-6-oxo-$PGF_{1\alpha}$, 6-oxo-$PGF_{1\alpha}$ = 100%, PGE_2 = 4.8%, $PGF_{2\alpha}$ = 5.4%, 15-oxo derivatives of these prostaglandins < 0.5%: anti-PGE_2, 6-oxo-$PGF_{1\alpha}$ n = 0.04%, PGE_2 = 100%, $PGF_{2\alpha}$ = 1.4%, 15-oxo-PGE_2 = 1.2%, 13,14-dihydro-15-oxo PGE_2 = 0.6%: anti-$PGF_{2\alpha}$, 6-oxo-$PGF_{1\alpha}$ = 0.08%, PGE_2 = 0.2%, $PGF_{2\alpha}$ = 100%, 15-oxo-$PGF_{2\alpha}$ = 0.07%, 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ = 0.8%. The coefficients of variation for each RIA were; 6-oxo-$PGF_{1\alpha}$, 6.6% and 6.8%; PGE_2, 5.4% and 7.9%, $PGF_{2\alpha}$, 7.4% and 5.8% for inter- and intra-assay variation respectively ($n > 4$). The lower limits of detection of the RIA were:-6-oxo-$PGF_{1\alpha}$, 0.1 ng ml^{-1}; for $PGF_{2\alpha}$ and for PGE_2, 0.2 ng ml^{-1}. The drugs in the concentrations used had little effect on the RIA of the prostaglandins. The mean difference in displacement over the range of standards used (0.16–20 ng ml^{-1}) was: 6-oxo-$PGF_{1\alpha}$ = 0.07%, PGE_2 = 1.7%, and $PGF_{2\alpha}$ = 1.6% for CL28A at 0.3 μM. For CK 61A at 5 μM, these values were: 6-oxo-$PGF_{1\alpha}$ = 1.6%, PGE_2 = 2.2%, and $PGF_{2\alpha}$ = 0.1%.

Materials

Arachidonic acid (Sigma, Poole) was dissolved in ethanol and stored under nitrogen at − 20°C. After evaporating the ethanol with a stream of nitrogen, the residue was taken up in 0.9% w/v NaCl solution and converted to the sodium salt of AA with Na_2CO_3. This aqueous solution was freshly made for each day's experiments. Prostaglandin E_2 or $PGF_{2\alpha}$ (Sigma, Poole) was stored in methanol solution at − 20°C. Methanol was evaporated and the prostaglandin redissolved in 0.9% w/v NaCl solution and diluted further to the desired concentration.

5, 8, 9, 11, 12, 14, 15 (n) [^3H]-6-oxo-$PGF_{1\alpha}$, (100 Ci $mmol^{-1}$) was obtained from New England Nuclear, Boston, Mass. U.S.A.; 5, 6, 8, 11, 12, 14, 15, (n)-[^3H]-PGE_2 (120 Ci $mmol^{-1}$), 5, 6, 8, 9, 11, 14, 15, (n)-[^3H]-$PGF_{2\alpha}$ (120 Ci $mmol^{-1}$) and [1-^{14}C] PGE_2 (58 mCi $mmol^{-1}$) for the $t_{\frac{1}{2}}$ assays were obtained from Amersham International (Amersham, Bucks). The calcium ionophore was generously provided by Dr W. Dawson (Lilly Research Centre, Windlesham, Surrey) and indomethacin by Merck Sharp and Dohme (Hod-

desdon, Herts). We are also grateful for a gift of nafazatrom (Bayer, U.K.). The antiserum to 6-oxo-$PGF_{1\alpha}$ was kindly donated by Dr J.A. Salmon (Wellcome Foundation, Beckenham, Kent) and the antisera to PGE_2 and $PGF_{2\alpha}$ have been prepared in this laboratory. Ph CL 28A (2-hydroxy-5-[[3,5-bis (methoxycarbonyl) phenyl]azo] - benzeneacetic acid) and Ph CK 61A (5-[4-carboxyphenyl)azo]-2-hydroxybenzeneacetic acid) were obtained from Mr H. Agback (Pharmacia).

Statistical methods

Results are given as mean values (± s.e.mean) from *n* lungs unless otherwise stated. In all experiments, except those using the calcium ionophore, each lung provided its own control value before drug infusion was started and those results have been analysed with a paired *t* test. With ionophore, only one injection was given to each lung and thus control and drug treated values were obtained from different lungs. Here the unpaired *t* test was used to assess significance of the difference between means. A value of $P < 0.05$ was taken to denote a significant difference.

Results

The first experiments were to establish the efficacy of the inhibitors in our experimental system towards prostaglandin inactivation. As shown in Table 1, CL 28A at 0.3 μM caused about a 2.5 fold increase in PGE_2 surviving a single passage through the pulmonary circulation as measured by bioassay. Comparable increases were seen for $PGF_{2\alpha}$, the substrate used by Berry *et al.* (1985). The other inhibitor, CK 61A, was studied only with PGE_2 as substrate and at either concentration caused marked increases in survival. In the bioassays, the survival of prostaglandin was assessed only from the peak height of the response, but a qualitative change was also seen in the response of the assay tissue to PGE_2 surviving pulmonary transit in the presence of either inhibitor. These responses were more prolonged than those obtained in the absence of inhibitor and were not accompanied by changes in response to standard doses of PGE_2 given directly to the assay tissues. We therefore also measured survival by RIA, using lung effluent collec-

Table 1 Survival of prostaglandin E_2 (PGE_2) and $PGF_{2\alpha}$ in rat isolated lung following treatment with prostaglandin dehydrogenase inhibitors

	Survival			
Inhibitor	*PGE_2*		*$PGF_{2\alpha}$*	
(concentration)	*Control*	*Treated*	*Control*	*Treated*
Bioassay				
CL 28A (0.3 μM)	8 ± 1 (6)	19 ± 3* (6)	3 ± 2 (5)	33 ± 6* (5)
CK 61A (0.5 μM)	6 ± 1 (5)	27 ± 5* (5)		
(5 μM)	6 ± 1 (5)	39 ± 9* (6)		
RIA				
CL 28A (0.3 μM)	3.5 ± 0.5 (5)	9.9 ± 0.6* (5)	0.5 ± 0.01 (4)	6.8 ± 1.1* (5)
CK 61A (0.5 μM)	2.0 ± 0.6 (4)	7.6 ± 0.3* (4)		
(0.5 μM)	2.0 ± 0.6 (4)	10.0 ± 0.5* (4)		

*Significantly different from control value; $P < 0.05$

Bioassay results are expressed as % survival of dose passing through the pulmonary circulation. RIA results are expressed as ng prostaglandin ml^{-1} of the 5 min effluent fraction. The values shown are the means (± s.e.mean) from the number of experiments shown in parentheses below each condition. In the bioassays, results are calculated from the peak height of the contraction of the assay tissue superfused with lung effluent. No allowance was made for the duration of the response (see text). For the RIA, lung effluent was collected for 5 min following the test dose of prostaglandin and the value shown represents the concentration in this fraction. The survival of either prostaglandin was markedly increased by any of the treatments, measured either by bioassay or RIA.

ted for 5 min and thus approximating more closely to the area under the curve of the bioassay response. By RIA, there were 3 to 5 fold increases in the concentration of PGE_2 surviving passage through the lung and a more than 10 fold increase in $PGF_{2\alpha}$ caused by either CL 28A or CK 61A.

In previous experiments (Bakhle, 1982), similar changes in the duration of the bioassay responses and increased survival of PGE_2 were associated with a prolonged efflux of radiolabel following injection of [^{14}C]-PGE_2 into the pulmonary circulation of rat lungs.

We therefore measured the effect of the two PGDH inhibitors on the efflux of ^{14}C from the isolated lung, following a bolus injection of [^{14}C]-PGE_2. This efflux was quantitated by the $t_{\frac{1}{2}}$ value, the time for 50% of injected ^{14}C to appear in the effluent. As shown in Figure 1, in isolated lungs without treatment, the efflux was maximal at about fraction 4, equivalent to almost 13 s after the injection and had a $t_{\frac{1}{2}}$ value of 29 s.

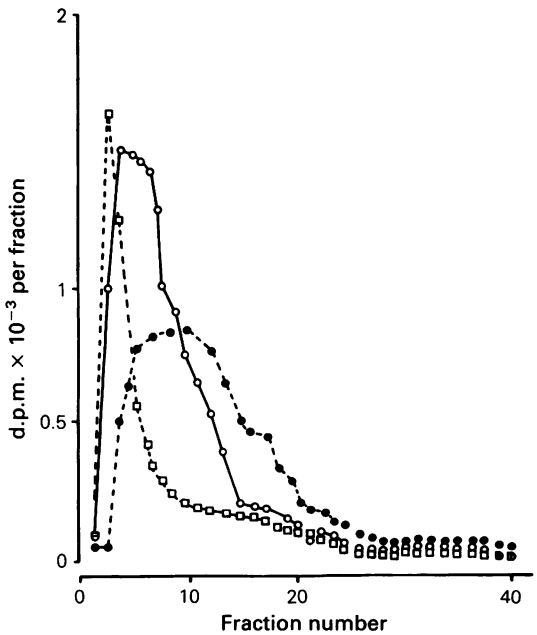

Figure 1 Effect of prostaglandin dehydrogenase inhibitors on ^{14}C-efflux from rat lung after [^{14}C]-prostaglandin E_2 ([^{14}C]-PGE_2). Effluent perfusate from rat isolated lungs was collected in 4 drop (ca. 3 s) fractions following a bolus injection of [^{14}C]-PGE_2. The total radioactivity in each fraction was measured and is shown in the figure for each fraction. Relative to the efflux in untreated lungs (○), CL 28A infusion (●) (and that of CK 61A, not shown) delayed the peak and prolonged the efflux of ^{14}C. Infusion of nafazatrom (□) increased the rate of efflux with an earlier peak and a shorter duration. $t_{\frac{1}{2}}$ of [^{14}C]-PGE_2: control 28s, CL 28A 41s, hafazatrom 22s.

In the presence of CL 28A, the peak was delayed to about fraction 11 and the $t_{\frac{1}{2}}$ increased by 40% to 41 s. Essentially similar changes were seen with CK 61A at 5 μM and the results of several such experiments are summarized in Table 2. Additional experiments using $PGF_{2\alpha}$, the substrate originally used by Berry *et al.* (1985), and CL 28A showed a comparable increase of $t_{\frac{1}{2}}$ in the presence of the inhibitor (Table 2). For comparison, we also used in these experiments another PGDH inhibitor, nafazatrom (Wong *et al.*, 1982). From Figure 1, it is clear that this inhibitor caused the maximal efflux to occur earlier than either under control conditions or with CL 28A infusion. The $t_{\frac{1}{2}}$ value in the presence of nafazatrom was reduced to almost 70% of the control value (Figure 1 and Table 2). This concentration of nafazatrom (37 μM) has been shown also to increase PGE_2 survival (by bioassay) to 33 ± 3% in rat isolated lungs (Bakhle & Pankhania, 1985).

Output of prostaglandins

The effect of infusions of CL 28A (0.3 μM) on the output of PGE_2, $PGF_{2\alpha}$ and PGI_2 (measured as 6-oxo-$PGF_{1\alpha}$) following metabolism of exogenous or endogenous AA are summarized in Table 3. Output of prostaglandin into lung effluent was measured under three conditions: basal, i.e. before stimulation; after exogenous AA and after stimulation with calcium ionophore to release endogenous AA. There was no effect of CL 28A on output under basal conditions on any of the prostaglandin assayed. Following exogenous AA, PGE_2 and $PGF_{2\alpha}$ output but not that of 6-oxo-$PGF_{1\alpha}$ was increased by CL 28A. When endogenous AA was liberated after stimulation with the calcium ionophore A23187 (3 μg), again PGE_2 and $PGF_{2\alpha}$ output was increased by CL 28A. However, under these conditions, i.e. metabolism of endogenous AA, CL 28A also increased the output of 6-oxo-$PGF_{1\alpha}$ more than 3 fold.

The results for CK 61A, tested at the two concentrations used earlier (0.5 and 5 μM), are summarized in Figure 2. At the lower concentration, the inhibitor decreased slightly the basal output of 6-oxo-$PGF_{1\alpha}$ but there was no effect on output from exogenous AA. However, output from endogenous AA was increased by about 50%. For PGE_2 and $PGF_{2\alpha}$, no effects were seen at this lower concentration. The 10 fold higher concentration of CK 61A (5 μM) produced a marked increase in basal output of 6-oxo-$PGF_{1\alpha}$, no change in output from exogenous AA and a 7 fold increase in output following ionophore stimulation of endogenous AA. After correction for basal output, net output from exogenous AA actually fell from 5.5 ± 0.6 ng ml^{-1} to 1.6 ± 0.9 ng ml^{-1} whereas net output from endogenous AA increased from 0.9 ± 0.2 ng ml^{-1} to 4.4 ± 0.7 ng ml^{-1} during infusion of CK 61A. The

Table 2 Effects of prostaglandin dehydrogenase (PGDH) inhibitors on efflux of ^{14}C from rat isolated lung following [^{14}C]-prostaglandin E_2 ([^{14}C]-PGE_2) or [^{14}C]-$PGF_{2\alpha}$

	$t_{\frac{1}{2}}$ (s)			
Inhibitor (concentration)	*PGE_2*		*$PGF_{2\alpha}$*	
	Control	*Treated*	*Control*	*Treated*
CL 28A (0.3 μM)	37 ± 2 (6)	63 ± 6* (6)	40 ± 3 (6)	68 ± 10* (8)
CK 61A (5 μM)	37 ± 2 (4)	71 ± 6* (6)		
Nafazatrom (37 μM)	32 ± 2 (6)	23 ± 2* (5)		

*Significantly different from control values; $P < 0.05$
The values in the table are the means (± s.e.mean) from the number of experiments shown. Note that nafazatrom shortens $t_{\frac{1}{2}}$ values whereas the other two PGDH inhibitors both prolong the $t_{\frac{1}{2}}$.

basal output of neither PGE_2 nor $PGF_{2\alpha}$ was raised above detectable levels by treatment with the higher concentration of CK 61A.

We also investigated the effects of this higher concentration of CK 61A on the output from endogenous AA using less ionophore, 1 μg instead of 3 μg as in the work summarized in Figure 2. Stimulation of endogenous AA turnover with 1 μg ionophore gave no detectable output of PGE_2 nor $PGF_{2\alpha}$ and stimulated output of 6-oxo-$PGF_{1\alpha}$ was the same as the basal level (0.4 ± 0.1 ng ml^{-1}) in 4 lungs. In the presence of CK 61A (5 μM) the lower dose of ionophore now caused net output of all three prostaglandins: 6-oxo-$PGF_{1\alpha}$, 3.8 ± 0.3 ng ml^{-1}; PGE_2, 2.1 ± 0.1 ng ml^{-1} and $PGF_{2\alpha}$, 0.5 ± 0.1 ng ml^{-1} ($n = 4$).

Discussion

Our experiments have shown these two inhibitors of PGDH to alter the pulmonary pharmacokinetics of exogenous prostaglandins and to increase the output of prostaglandin synthesized *in situ*. These effects have largely supported our original hypothesis but have also raised further questions.

Increased prostaglandin survival in isolated lungs after treatment with these two PGDH inhibitors was first shown by Berry *et al.* (1985) who used [^3H]-$PGF_{2\alpha}$ as substrate and radio-t.l.c. assay. Our results confirm and extend their results by using a different substrate and two different assays. Because we have used bolus injections of substrate, there were quantitative differences in the survival values between bioassay, which essentially depends on the peak concentration of prostaglandin and RIA, which reflects the total area under the peak of effluent prostaglandin. Nevertheless, all three assays show that the two drugs, CL 28A and CK 61A, are potent inhibitors of the inactivation of PGE_2 and $PGF_{2\alpha}$ in isolated lung. However, inactivation of prostaglandins in lung is a two-step process of uptake into cells followed by oxidation catalysed by intracellular PGDH. Thus increased survival can

Table 3 Effect of CL 28A on the output of prostaglandins from rat isolated lungs

	Output of prostaglandin (ng ml^{-1} effluent)					
	6-oxo-$PGF_{1\alpha}$		*PGE_2*		*$PGF_{2\alpha}$*	
	Control	*Treated*	*Control*	*Treated*	*Control*	*Treated*
Basal	1.8 ± 0.3	1.5 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	ND	ND
Exogenous AA (10 μg)	18.6 ± 4.2	20.4 ± 3.4	13.6 ± 2.5	23.6 ± 2.7*	1.6 ± 0.1	4.2 ± 0.3*
Endogenous AA (Calcium ionophore 3 μg)	3.4 ± 0.5	11.4 ± 1.7*	ND	1.2 ± 0.1*	ND	1.4 ± 0.2*

*Significantly different from control values; $P < 0.05$; ND = not detectable.
The values in the table are the means (± s.e.mean) from 4 experiments at each condition. No effects on basal output of all three prostaglandins were seen. Output of 6-oxo-$PGF_{1\alpha}$ was increased only following stimulation of endogenous arachidonic acid (AA).

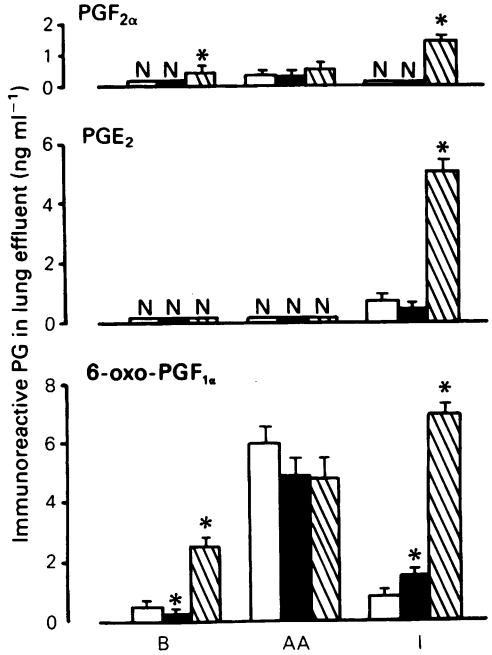

Figure 2 Effects of CK 61A on the output of prostaglandins (PGs) from rat isolated lungs. The heights of the columns represent the mean (+ one s.e.mean shown) values from 3–6 experiments in each condition. N = not detectable. For each prostaglandin (PG) results are presented under 3 conditions: basal (B) i.e. before stimulation; after exogenous arachidonic acid (AA) and after stimulation of endogenous AA by calcium ionophore (I). The three columns in each condition represent values in untreated lungs (open columns), during infusion of 0.5 μM CK 61A (solid columns) and infusion of 5.0 μM CK 61A (hatched columns). The major effects of the prostaglandin dehydrogenase inhibitor were to increase basal output and output stimulated by ionophore, both utilizing endogenous AA.

result from inhibition of either process and we have, in earlier work, sought to differentiate between the two options by bioassay and $t_{\frac{1}{2}}$ measurements (Bakhle *et al.*, 1978; Bakhle, 1979; 1980). In this study we have presented the effects of the two inhibitors on the $t_{\frac{1}{2}}$ value. This value is derived from measurements of total ^{14}C in lung effluent and as such does not distinguish unchanged PGE_2 from metabolite. It has, however, been established that inhibitors of uptake with no demonstrable effects on PGDH *in vitro*, like bromocresol green, dipyridamole and sulphinpyrazone decrease the $t_{\frac{1}{2}}$ values (Bito & Baroody, 1975; Uotila & Mannisto, 1981). Inhibitors of the enzyme PGDH should cause prolonged bioassay responses to prostaglandin passing through the pulmonary circulation and should increase $t_{\frac{1}{2}}$ values. These predictions were based on the results obtained with the PGDH resistant analogues of PGE_2, 15-methyl and 16-dimethyl PGE_2 (Bakhle *et al.*, 1978) and the argument has been further elaborated (Bakhle, 1979; 1980; 1982; Minty *et al.*, 1987). The two highly potent PGDH inhibitors, CL 28A and CK 61A, fulfilled our predictions satisfactorily, although the less potent inhibitor, nafazatrom, appeared to act more on the uptake step in isolated lung.

The effects of CL 28A and CK 61A on prostaglandin output from lung were also as expected from our original hypothesis, i.e. the output of prostaglandin was increased. The increased output of PGE_2 or $PGF_{2\alpha}$ consequent on PGDH inhibition is easily understood since they are readily inactivated in lung. However, both PGI_2 and its degradation product, 6-oxo-$PGF_{1\alpha}$, are not metabolized on passage through the pulmonary circulation (Dusting *et al.*, 1978; Hawkins *et al.*, 1978), presumably because they are not taken up by cells containing PGDH, for which enzyme *in vitro*, PGI_2 is a good substrate (McGuire & Sun, 1978). Nevertheless, the presence of 6,15-dioxo-$PGF_{1\alpha}$, presumably derived from the product of PGDH action on PGI_2 (15-oxo-PGI_2), has been demonstrated in lung effluent *in vivo* (Forstermann *et al.*, 1981) and in perfused lung (Dawson *et al.*, 1976; Robinson *et al.*, 1984). Thus PGI_2 synthesized *in situ* appears to be susceptible to PGDH although exogenous PGI_2 is not metabolized. Therefore, the predicted effect of selective PGDH inhibitors would be increased output of all three prostaglandins synthesized in the lung. However, the increase was not seen for all the prostaglandins measured. Whereas PGE_2 and $PGF_{2\alpha}$ output was increased with CL 28A using either exogenous or endogenous substrate, the output of 6-oxo-$PGF_{1\alpha}$ was not affected with exogenous AA but was clearly increased when the endogenous substrate turnover was stimulated by the ionophore.

The results with CK 61A are less clear-cut because of the greater effect on basal output, i.e. before stimulation at the higher concentration used. Nevertheless, at either concentration, output of 6-oxo-$PGF_{1\alpha}$ output from exogenous AA was not increased whereas that from endogenous AA was clearly greater in the presence of the inhibitor. Enhancement of PGE_2 and $PGF_{2\alpha}$ output by this inhibitor was most clear with the ionophore stimulation. Overall, the two inhibitors of PGDH were able to bring about the postulated increase in prostaglandin output during prostaglandin synthesis in the lung.

The major new question put by these results is why the increase in PGI_2 output appears to be dependent on the source of AA utilized for prostaglandin synthesis. This source-dependent effect was present at two concentrations of inhibitor and at two levels of

stimulation. It has been reported earlier for four other compounds, all inhibitors of prostaglandin inactivation in lung (Bakhle & Pankhania, 1985). These other compounds, bromcresol green, dipyridamole, sulphinpyrazone and nafazatrom, all have direct effects on cyclo-oxygenase (Al-Ubaidi & Bakhle, 1980; Blass *et al.*, 1980; Ali *et al.*, 1977; Marnett *et al.*, 1984) which made analysis of their effects on PGI_2 synthesis less clear. The two PGDH inhibitors used here have been shown specifically to lack activity towards cyclo-oxygenase (Berry *et al.*, 1983; 1985). Thus the present results strengthen the possibility that source-dependent increases in PGI_2 output are a real phenomenon.

The explanation for this phenomenon may be that the cells involved in PGI_2 synthesis from exogenous free AA are different from those stimulated by ionophore to liberate endogenous AA and that the activity or amount of PGDH in the two cellular environments is different. This explanation has some support. The profile of cyclo-oxygenase products formed in lung varies with the stimulus; exogenous AA or endogenous AA released by a variety of stimuli (Bakhle *et al.*, 1985a,b). There is also evidence for cell-selective synthesis of certain prostaglandins (Moncada & Vane, 1981; Lewis *et al.*, 1982). If there are differences in prostaglandin synthesizing enzymes in different cells, there could equally be differences in prostaglandin inactivating enzymes.

The differentiation between exogenous and endogenous substrate demonstrated here is probably too simple and different methods of liberating endogenous AA should be used in further studies with these inhibitors. Nevertheless, some interesting corollaries may be drawn. First, since the majority of prostaglandin synthesis *in vivo* is from endogenous substrate, i.e. from lipid esters and not from exogenous substrate, i.e. free blood-borne AA, a more relevant estimate of effects of inhibitors of prostaglandin inactivation on prostaglandin output would be gained from testing them with endogenous substrate rather than the more commonly used exogenous AA. Furthermore, some of the anti-platelet and anti-thrombotic activity of dipyridamole, sulphinpyrazone and nafazatrom could be due to a potentiation of local PGI_2 output at the vascular wall through an inhibition of inactivation processes. As mentioned earlier, such activity might not be expressed *in vitro* with exogenous AA. Lastly, since the output of PGI_2 from the endothelium is stimulated by thrombin, ATP, ADP, bradykinin etc. (Weksler *et al.*, 1978; Crutchley *et al.*, 1983; Hellewell & Pearson, 1984), the potentiation of PGI_2 output would be most marked at those sites in the vasculature where the endothelium is most under attack. This would ensure maximal local cytoprotection, without raising the circulating, bulk phase, concentration of PGI_2.

Overall, the effects of these two inhibitors of PGDH have been as predicted and they have given some support to our initial hypothesis. The substrate-dependent increase in 6-oxo-$PGF_{1\alpha}$ output, although compatible with previous findings, was unexpected. However, this phenomenon presents some intriguing possibilities for manipulating PGI_2 production *in vivo* which remain to be examined in more detail.

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