## Inhibition of the pulmonary inactivation of prostaglandins in rabbit *in vivo*

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Prostaglandins (PGs) of the E and F series are efficiently inactivated in the pulmonary circulation of several species *in vivo* (Ferreira & Vane, 1967) and of guinea-pig *in vitro* (Piper, Vane & Wyllie, 1970). This inactivation can be strongly inhibited *in vitro* by di-4-phloretin phosphate (DPP) at concentrations well below those causing antagonism of PG actions (Crutchley & Piper, 1973, 1974). The present study investigates whether DPP inhibits pulmonary inactivation of $PGE_2$ by rabbits *in vivo*.

Male Dutch rabbits 1.8 to 2.5 kg were anaesthetized by pentobarbitone sodium 40 mg/kg i.v. A polyethylene cannula was introduced retrogradely into the aortic arch via the right carotid artery for i.a. infusions. A similar cannula for i.v. infusion was introduced into the superior vena cava via the right jugular vein. Blood pressure was recorded from the left carotid artery or a femoral artery. Saline, or DPP (in solution, in saline) was infused continuously into the left jugular vein. $PGE_2$ infusions of 1.5 min duration were given i.a. or i.v. and the resulting falls in blood pressure measured. Control and test measurements were taken in the same animals.

$PGE_2$ was much more potent via the i.a. route, the difference between i.a. and i.v. responses being taken as a measure of pulmonary inactivation. DPP 25 to 100 $\mu g\ kg^{-1}\ min^{-1}$ potentiated the i.v. responses and shifted the dose responses curve for i.v. $PGE_2$ to the left, to approximate to that for i.a. doses (n = 18). DPP had no effect on the i.a. responses, suggesting that potentiation of i.v. effects was due to decreased inactivation of $PGE_2$ in the lung. Potentiation of i.a. responses by DPP was seen, however, with longer infusions of $PGE_2$ (6 to 8 min). This was presumably due to the cumulative effects of circulating i.a. doses. No direct antagonism of $PGE_2$ was seen.

DPP 25 to 100 $\mu g\ kg^{-1}\ min^{-1}$ also potentiated the depressor actions of i.v. infusions of $PGF_{2\alpha}$ and the increase in gastrointestinal motility due to i.v. infusions and injections of PGs $E_2$ and $F_{2\alpha}$ (n = 5). This was presumably due to impaired pulmonary PG inactivation.

The percentage inhibition of pulmonary inactivation by these doses of DPP was 75% at maximum but this caused marked potentiation of PG effects, emphasizing the importance of pulmonary inactivation of PGs.

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## Factors influencing the turnover of prostaglandin synthetase

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Evidence is accumulating that prostaglandin generation is of physiological and pathological importance, so the mechanism(s) by which intracellular levels of prostaglandin synthetase are regulated are clearly of interest. Continuous *de novo* synthesis of enzymes is a property of all viable cells and one method by which the cellular concentration of certain types of enzymes can be rapidly regulated (in response to hormone stimulation for example) is by changes in their rate of synthesis or destruction. Lands, LeTellier, Rome & Vanderhoek (1973) have shown that *in vitro* prostaglandin synthetase catalyses its own destruction during substrate oxygenation. If such 'self-destruction' occurs *in vivo*, a tissue such as the kidney which has a high biosynthetic capacity (Somova, 1973) should demonstrate continuous synthesis of the enzyme also.

To test this possibility we injected (i.p.) four male rats (200-250 g) with 4 mg cycloheximide

**Table 1** Effect of cycloheximide on PG biosynthesis and *de novo* protein synthesis*

| *Time (hr)* | *p moles $^{14}$C-lysine incorporated into protein* | *p moles $^{14}$C-PGs synthesized* | *p moles 15-keto $PGE_2$ synthesized* |
|---|---|---|---|
| 0 | 65.32 | 7.8 | 58.9 |
| 1 | 50.34 | 8.9 | 33.0 |
| 2 | 23.19 | 10.7 | 14.1 |
| 3 | 21.64 | 12.2 | 16.4 |
| 4 | 15.34 | 10.9 | 14.4 |
| 5 | 12.93 | 11.0 | 13.9 |
| 6 | 8.08 | 10.4 | 14.1 |
| 7 | 6.96 | 10.7 | 11.3 |

* Each figure is the mean of two results

(an inhibitor of protein synthesis). Three hours later the rats were killed and the kidneys were removed. Prostaglandin biosynthesis in kidney homogenates was measured by bioassay (see Gilmore, Vane & Wyllie, 1968) after 10 min incubation at 37° C with 20 μg added arachidonic acid. Protein synthesis was quantitated in slices of kidney by measuring the incorporation of (0.1 μc) $^{14}$C-lysine into protein during 1 h in a shaking water bath at 37° C. The mean prostaglandin biosynthesis in the two control animals was 82.2 p moles/kidney. Surprisingly in rats treated with cycloheximide prostaglandin production was 428.4 p moles/kidney, even though the protein synthesis was inhibited by 63.7%. One reason for this apparent stimulation of synthesis could be that the prostaglandin metabolizing enzymes were being blocked. To check this possibility, 14 male rats were injected with 4 mg cycloheximide. Each hour, thereafter, two of the rats were killed and the kidneys removed. Prostaglandin biosynthesis here was measured by the conversion of $^{14}$C-arachidonic acid to labelled $PGE_2$ and $F_{2\alpha}$, which were extracted from the aqueous homogenates and isolated by t.l.c. after 10 min incubation in a shaking water-bath at 37° C. Unlabelled prostaglandin $E_2$ (10 μg) was added to homogenates to protect labelled prostaglandins from destruction by metabolizing enzymes. Metabolism of the prostaglandins was measured by the conversion of (0.01 μCi) $^3$H-$PGE_2$ to its 15-keto derivative, which was extracted from aqueous homogenates after 10 min incubation at 37° C and isolated by t.l.c. Protein synthesis was measured as before.

Table 1 clearly shows that although the capacity of the kidneys to synthetize prostaglandins is undiminished even when protein synthesis was reduced by 80-90%, the capacity of the kidney to metabolize prostaglandin was greatly reduced. A preliminary study excluded the possibility that this was a direct blocking action of the drug.

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