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UPTAKE AND INACTIVATION OF PROSTAGLANDIN E_2 METHYL ANALOGUES IN THE RAT PULMONARY CIRCULATION

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1 The fate of (15S)-15-methyl prostaglandin E_2 methyl ester and 16,16-dimethyl prostaglandin E_2 in the pulmonary circulation of rat isolated lungs was compared with that of prostaglandin E_2 by means of bioassay.

2 Calculated on the basis of height of response of the assay tissues, the inactivation of prostaglandin E_2 was 96 ± 1%, of 15-methyl prostaglandin E_2 methyl ester, 53 ± 6% and of 16,16-dimethyl prostaglandin E_2, 50 ± 4%.

3 Responses of the hamster stomach strip to the prostaglandin E_2 analogues passing through the pulmonary circulation were prolonged and slower in onset than those to the analogue given directly to the tissue. No such difference was observed with prostaglandin E_2.

4 Bromocresol green, bromothymol blue, bromocresol purple and thymol blue (10^{-5} M) all inhibited the inactivation of the three prostaglandins studied, as did diphloretin phosphate (1.5×10^{-6} M). All five inhibitors also reversed the shape change in response seen after transpulmonary injection of 16-16-dimethyl prostaglandin E_2.

5 We conclude that the inactivation of the methyl analogues is due to uptake, as they are not substrates for prostaglandin dehydrogenase.

6 The lung may act as a depot for some compounds taking them up from the pulmonary vessels and later releasing them slowly into the systemic circulation.

Introduction

The greater potency and longer duration of action of two prostaglandin analogues (15S)-15-methyl prostaglandin E_2 methyl ester and 16,16-dimethyl prostaglandin E_2, when compared with prostaglandin E_2 *in vivo* (see Main & Whittle, 1975) has been attributed to the resistance of the two methyl analogues to metabolism by 15-hydroxy-prostaglandin dehydrogenase (PGDH) (Weeks, DuCharme, Magee & Miller, 1973). Since this enzyme catalyses the rate-limiting step in the pulmonary inactivation of prostaglandin E_2 (Nakano, Änggård & Samuelsson, 1969) we investigated the fate of these two methyl analogues in rat isolated lungs in comparison with that of prostaglandin E_2 itself.

A preliminary account of this work has been presented to the Physiological Society (Bakhle, Jancar & Whittle, 1977).

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Methods

Isolated lungs of male rats (150–250 g body wt.) were perfused via the pulmonary artery with oxygenated Krebs solution at 37°C as described earlier (Alabaster & Bakhle, 1970). The composition of the Krebs solution was (mM): $NaHCO_3$ 25, NaCl 120, KCl 417, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2 and glucose 5.6. The lung perfusate was directed over isolated smooth muscle preparations for bioassay and the contractions of the assay tissues recorded via isotonic transducers coupled to a multichannel Watanabe recorder. The assay tissues were strips cut from the forestomach pouch of the rat stomach (Vane, 1957) and the hamster stomach (Ubatuba, 1973).

The assay tissues were also constantly superfused (0.1 ml/min) with a mixture of antagonists (methysergide, 0.56 μM; mepyramine, 0.35 μM; hyoscine, 0.33 μM; phenoxybenzamine, 0.33 μM; propranolol, 7.5 μM and indomethacin, 10 μM; the final concentrations are given). Prostaglandins were injected (0.1–0.3 ml) either directly over the assay tissues, or

into a cannula lying in the pulmonary artery of the isolated lung.

Materials

Prostaglandins, stored in methanol (−5°C) were made up freshly in 0.9% w/v NaCl solution (saline) or Krebs solution following evaporation of the methanol, when required. The free acid of prostaglandin E_2 and 16,16-dimethyl prostaglandin E_2 and the methyl ester of (15S)-15 methyl prostaglandin E_2 were used. The indicator dyes: bromocresol green, bromocresol purple, bromothymol blue and thymol blue (Hopkins & Williams), and diphloretin phosphate (Leo AB, Hesingborg, Sweden) were dissolved in saline immediately before use.

Results

Bioassay

In this study both the rat stomach strip and the hamster stomach strip have been used for bracketing bioassy of the prostaglandins. The response of the superfused rat stomach strip to prostaglandin E_2 was slow, with only 6–10 responses per hour being feasible. Furthermore, the methyl prostaglandin analogues produced responses of even longer duration. Thus the number of observations which could be obtained during the limited lifetime of an isolated perfused lung (approximately 1.5 h) was severely limited. In contrast, the responses of the superfused hamster stomach strip to prostaglandin E_2 were very rapid, as found with this tissue in an organ bath (Ubatuba, 1973), and thus 20 to 30 responses per hour could be achieved. The responses of this tissue to the methyl prostaglandin E_2 analogues tended to be of longer duration than those obtained with prostaglandin E_2.

The superfused hamster stomach strip was less sensitive to prostaglandin E_2 than the rat stomach strip, threshold values for contractions on the two tissues being 10 to 20 ng and 1 to 2 ng respectively. Likewise, the 16,16-dimethyl analogue of prostaglandin E_2 was less active on the hamster stomach strip than on the rat stomach strip, the threshold values being 10 to 20 ng and 1 to 2 ng respectively. The hamster stomach strip showed a steeper dose-response relationship than the rat stomach strip to both prostaglandins, confirming the original findings with this preparation (Ubatuba, 1973).

The sensitivity of the hamster stomach strip to (15S)-15 methyl prostaglandin E_2 methyl ester was found to vary with the age of the preparation. Although the sensitivity of the tissue to prostaglandin E_2 and its 16,16-dimethyl analogue was retained for 3 to 5 days, providing the tissue was stored in Krebs solution (4°C), the sensitivity to the 15-methyl analogue, which was equiactive with the 16,16-dimethyl analogue on fresh tissue, declined rapidly on storage. After 3 days' storage in Krebs solution (4°C) the tissue failed to respond to even high doses of the 15-methyl analogue (100–400 ng), although infusion of this analogue (6 ng/ml) did potentiate the actions of prostaglandin E_2 twofold. This change in sensitivity to the methyl ester of (15S)-15 methyl prostaglandin E_2 may reflect changes in the tissue esterase activity during storage.

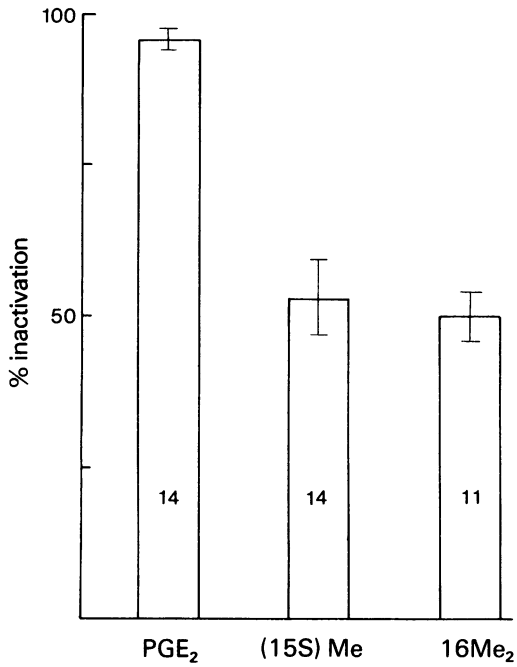

Figure 1 Inactivation of prostaglandin E_2 (PGE_2), (15S)-15 methyl prostaglandin E_2 methyl ester ((15S)Me) and 16,16-dimethyl prostaglandin E_2 ($16Me_2$) after a single passage through the pulmonary circulation of rat isolated lung. The results, shown as % inactivation, are expressed as the mean; vertical lines show s.e. mean. The number of experiments is shown in each column.

Inactivation of prostaglandins in the pulmonary circulation

In our bracketing bioassay, the inactivation of prostaglandins was measured by a comparison of the heights of responses to agonists injected through the pulmonary circulation with the responses to the agonists injected directly over the tissues. On this basis, all three prostaglandins were inactivated on a single passage through the rat isolated lung, with prostaglandin E_2 being extensively inactivated (Figure 1). However, with the two methyl prostaglandin ana-

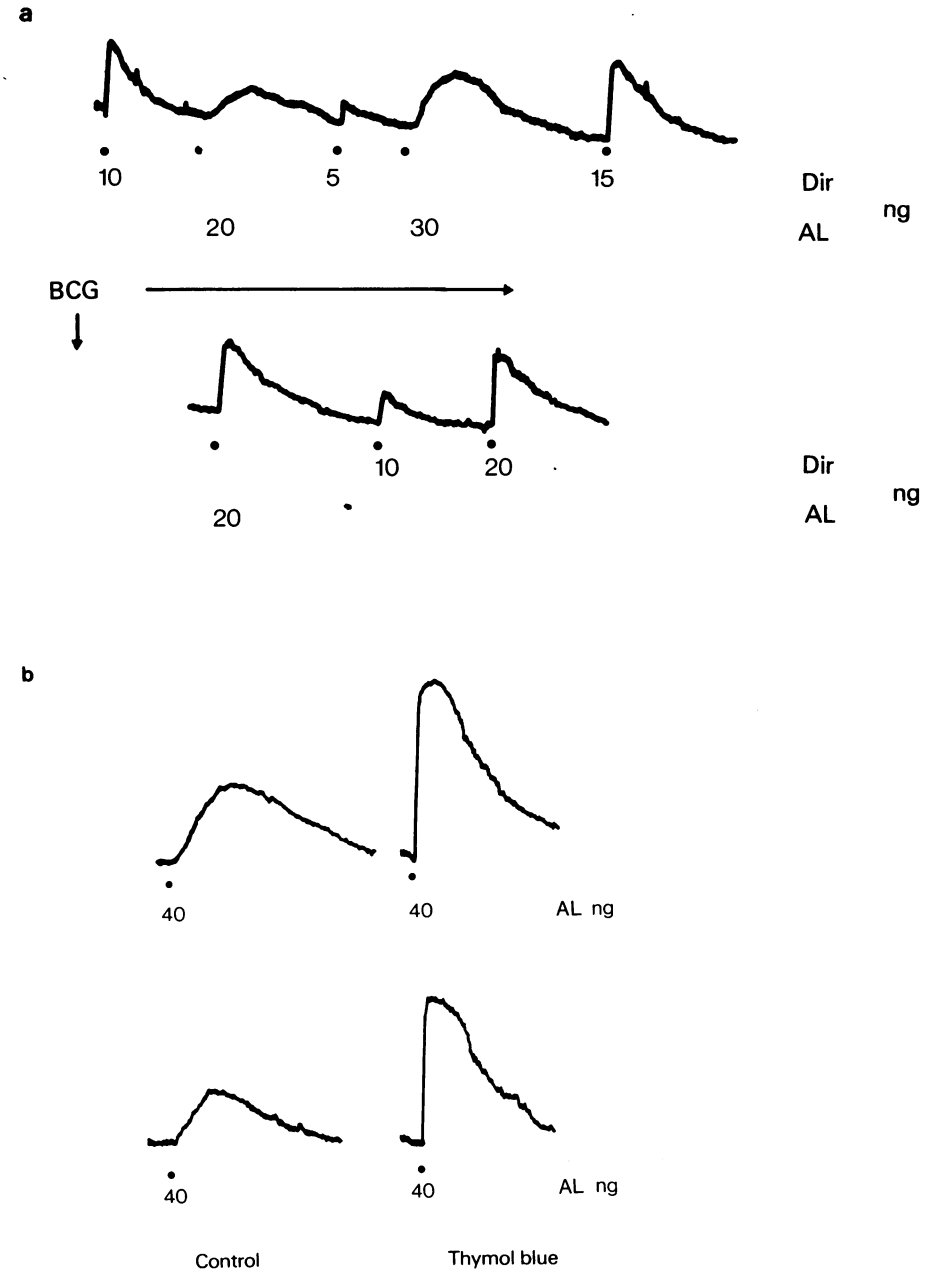

Figure 2 Inactivation of 16,16-dimethyl prostaglandin E_2 in rat isolated perfused lung and the effects of indicator dyes using the hamster stomach strip as assay tissue. (a) The trace shows that the slower onset and relaxations of the assay tissue to the prostaglandin analogue when injected across the lung (AL) compared to direct administration (Dir) is abolished by the concurrent infusion of bromocresol green (BCG, 10^{-5} M) through the lung. The height of the responses are also increased by BCG, indicating reduced inactivation. (b) These traces, taken from two separate experiments show the shape and height of the response of the assay tissue to the prostaglandin analogue injected through the lung and the effects of thymol blue (10^{-5} M).

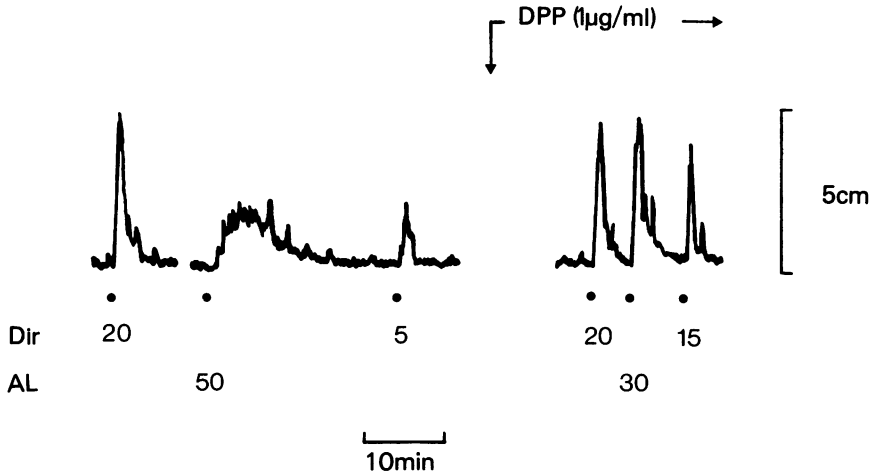

Figure 3 Effects of diphloretin phosphate (DPP; 1 μg/ml, 1.5 × 10^{-6} M) infused through the lung on inactivation of 16,16-dimethyl prostaglandin E_2. The trace shows that DPP not only increased the height of the assay tissue response injected across the lung (AL) (showing decreased inactivation of the analogue), but the shape of the response became comparable to that observed when injected directly to the tissues (Dir).

logues, both the height and the shape of the response to the hamster stomach strip to injections via the pulmonary circulation were altered. Comparison with the direct responses (Figure 2) show that the effects of 16,16-dimethyl prostaglandin E_2 on the tissues, when injected via the lung, were slower to reach their maximum and were more prolonged than when injected directly to the tissues. Similar changes in response shape following transpulmonary administration were observed with the 15-methyl analogue, but not with prostaglandin E_2, which gave responses of rapid onset with both routes of administration.

Inhibition of prostaglandin inactivation

The effect of the indicator dye, bromocresol green (BCG), which has been shown to inhibit the uptake of prostaglandin $F_{2\alpha}$ by rat lung (Bito & Baroody, 1975), was investigated in our system. A continuous infusion of BCG (10^{-5} M) through the lung, (a concentration shown to inhibit prostaglandin $F_{2\alpha}$ uptake; Bito & Baroody, 1975) reduced the inactivation of prostaglandin E_2 and its two methyl analogues, estimated in terms of contraction heights of the assay tissue (Table 1). At this concentration, BCG had little effect on the shape of the responses of the assay tissues to the prostaglandins injected directly. However, as shown in Figure 2, BCG not only increased the height of the contraction of the tissue to 16,16-dimethyl prostaglandin E_2 when injected through the lung (indicating reduced inactivation) but increased the rate of onset of contraction and subsequent relaxation of the assay tissue, the response thus resembling that observed with direct administration to the tissue. Similar results were obtained with another indicator dye, thymol blue (10^{-5} M) which, when perfused through the lung, likewise increased the rate of onset of the response of the hamster strip

Table 1 Inhibition of prostaglandin inactivation in rat isolated perfused lung

Inhibitor	*Concentration* (M)	% *inhibition of inactivation* *prostaglandin* E_2	*15-methyl* PGE_2 *methyl ester*	*16,16-dimethyl* PGE_2
Bromocresol green	10^{-5}	75 ± 13 (8)	48 ± 14 (5)	75 ± 11 (11)
Thymol blue	10^{-5}	42 ± 5 (5)	27 ± 9 (5)	64 ± 11 (6)
Bromothymol blue	10^{-5}	43 ± 6 (6)	44 ± 6 (7)	—
Bromocresol purple	10^{-5}	26 ± 8 (10)	31 ± 7 (5)	—
Diphloretin phosphate	1.5 × 10^{-6}	46 ± 1 (4)	—	57 ± 3 (6)

Results shown as % inhibition of control inactivation of the prostaglandins as estimated by bioassay, are expressed as mean ± s.e. mean, where (*n*) is the number of observations.

to the methyl prostaglandin E_2 analogues injected through the lung. In contrast, although both these indicator dyes increased the height of contraction of the assay strip to transpulmonary-administered prostaglandin E_2 (indicating decreased inactivation) they did not alter the shape of the response.

Two other indicator dyes, bromothymol blue and bromocresol purple had comparable effects. The inactivation of the prostaglandins returned to the control values within 10 to 15 min of terminating the infusion of these dyes through the lung.

Another highly charged molecule, diphloretin phosphate (DPP) inhibited the inactivation of prostaglandin E_2 in rat lungs and also inhibited the inactivation of the 16,16-dimethyl analogue (Table 1). This inhibitor of inactivation also altered the shape of responses to 16,16-dimethyl prostaglandin E_2 injected through the pulmonary circulation (Figure 3).

Discussion

The hamster stomach strip has in our experiments shown two major advantages over the rat stomach strip as an assay tissue for E-type prostaglandins. First, the faster response and relaxation enabled us to carry out more determinations within the limited life of each isolated lung preparation. Second, the rapid response of this tissue enabled us to follow more closely rapid changes in biological activity appearing in the effluent from the lung. Thus, when 16,16-dimethyl prostaglandin E_2 was injected through the pulmonary circulation, the slower onset and subsequent relaxation easily observed on the hamster stomach strip could not be detected on the rat stomach strip because the latter tissue has normally a slow rate of response and relaxation.

Our experiments indicate that the two methyl prostaglandin E_2 analogues, in spite of resistance to 15-hydroxy-prostaglandin dehydrogenase (PGDH) the major prostaglandin metabolising enzyme in lung, appear to be inactivated on a single passage through the pulmonary circulation. One explanation of this could be that the methyl analogues are substrates for the prostaglandin-Δ-13 reductase enzyme and that the inactivation reflects production of the 13,14-dihydromethyl analogues which could be less active than the parent compounds on the bioassay tissues. However, it is thought that this Δ-13 reductase enzyme acts only on the 15-oxo products following initial metabolism by PGDH (Änggård, 1971).

We therefore suggest that the methyl analogues are taken up from the vascular space in the lung during passage through the pulmonary circulation but, as they are resistant to enzymatic degradation by PGDH, they reappear in the vascular space unchanged. This sequence of events leads to a reduction in the peak concentration of the bolus, which in our system is measured as inactivation, together with a slow onset and a prolongation of the response when the methyl analogues are injected through the lung. Thus, the inactivation of the analogues that we measure is not due to chemical transformation into metabolites, but due to a temporal biophysical redistribution of substrate. A similar prolongation of response has been associated with uptake of 5-hydroxytryptamine into pulmonary endothelial cells in the presence of monoamine oxidase inhibitors followed by re-entry of the 5-hydroxytryptamine into the perfusate (Alabaster & Bakhle, 1970). With prostaglandin E_2 the duration of the responses was the same whether the injections were made directly, or through the lung. This is because all the prostaglandin E_2 taken up by the lung is metabolized (except at very high substrate supply rates, Anderson & Eling, 1976) and only that part of the prostaglandin E_2 bolus that is not taken up and remains in the vascular space, survives unchanged in the effluent to contract the assay tissue.

Our hypothesis is supported by the experiments with the prostaglandin uptake inhibitors. It has previously been shown that the indicator dye BCG inhibited prostaglandin $F_{2\alpha}$ uptake without affecting PGDH in rat lung (Bito & Baroody, 1975). In our experiments BCG inhibited the apparent inactivation of the methyl analogues whilst altering the shape of the bioassay tissue response to trans-pulmonary injections to one with a more rapid onset and faster relaxation. The other indicator dyes we have used, thymol blue, bromothymol blue, bromocresol purple, also produced qualitatively similar changes in prostaglandin inactivation, suggesting that they, too, are uptake inhibitors and that the property of prostaglandin uptake inhibition may be distributed widely among this type of large organic molecule. Uptake inhibition seems also to be the mechanism by which DPP prevents inactivation of 16,16-dimethyl prostaglandin E_2, the shape changes being the same as those produced by BCG. Furthermore, with these prostaglandin analogues which are not susceptible to PGDH, inhibition of inactivation by DPP is more likely to be due to inhibition of uptake than to the potential inhibition of PGDH (Crutchley & Piper, 1975). The most obvious chemical similarity between the dyes and DPP is that they are large organic anions of strong acids. This may be the sole chemical requirement for prostaglandin uptake inhibition. Their highly ionised character and large size would also tend to exclude them from cells and thus to confine their site of action to the external surface of cell membranes where uptake systems are located.

The inactivation process for prostaglandins in lung has therefore two limiting factors of uptake and susceptibility to PGDH and both factors appear to have

different specificities. Thus, prostaglandins may be substrates for both systems (prostaglandins of the E and F series; Ferreira & Vane, 1967) for neither system (prostaglandin B_2) or substrates for either the PGDH (A-type prostaglandins; Nakano *et al.*, 1969; Anderson & Eling, 1976) or as in the present work with methyl analogues of prostaglandin E_2, substrates for just the uptake system.

Our findings also suggest that, if a substrate is taken up by lung but not metabolized, then the lung can act as a buffer, smoothing out large changes in venous blood content and act as a depot, slowly releasing active substrates into arterial blood. In our context, therefore, the increased potency and longer duration of action of 16,16-dimethyl and the (15S)-methyl analogues of prostaglandin E_2 *in vivo* in the rat (Main & Whittle, 1975) may be due respectively to a reduced pulmonary metabolism of the analogues and the continued release of analogue taken up in the lung. This work thus emphasizes that, even in the absence of enzymatic transformation, passage through the pulmonary circulation can affect the pharmacokinetics of blood-borne substances.

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