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Effects of pulmonary metabolites of prostaglandins E_2 and $F_{2\alpha}$ on guinea-pig respiratory tract

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The effects of three pulmonary metabolites of prostaglandins E_2 and $F_{2\alpha}$ on the guinea-pig tracheal muscle *in vitro* and on lung resistance *in vivo* were investigated. Prostaglandin $F_{2\alpha}$, 13, 14-dihydro $PGF_{2\alpha}$ and 13,14-dihydro 15-oxo $PGF_{2\alpha}$ produced contractions on the tracheal muscle *in vitro*. 15-oxo $PGF_{2\alpha}$ relaxed some tracheal preparations but stimulated others, the stimulant action having a threshold dose range some 10–25 times lower than the relaxant doses. Prostaglandin $F_{2\alpha}$ and 13,14-dihydro $PGF_{2\alpha}$ increased lung resistance in anaesthetized guinea-pigs. Prostaglandin E_2 and its three pulmonary metabolites produced relaxation of guinea pig trachea *in vitro* and decreased lung resistance *in vivo*. All three metabolites were less active than PGE_2 in both the systems. The *in vitro* effects of $PGF_{2\alpha}$ and its metabolites were selectively blocked by polyphloretin phosphate.

Postaglandins E_2 and $F_{2\alpha}$ are present in pulmonary tissues (Änggård, 1965; Karim, Sandler & Williams, 1967) and the lungs are also involved in the metabolism of these prostaglandins. The pulmonary metabolites as well as the parent prostaglandins have pharmacological effects on airway smooth muscle. Generally, though not invariably, prostaglandins of the E series relax and those of the F series contract smooth muscle of the respiratory tract (see Smith, 1976 for references).

Dawson, Lewis & others (1974) reported that 15-oxo $PGF_{2\alpha}$ is more potent than $PGF_{2\alpha}$ in contracting guinea-pig tracheal and human bronchial muscles *in vitro*. However, on various airway and lung dynamics parameters in intact anaesthetized dogs, 15-oxo $PGF_{2\alpha}$ was relatively inactive compared to $PGF_{2\alpha}$ (Wasserman, 1975).

There is also a controversy regarding the potencies of the pulmonary metabolites of PGE_2 on bronchial smooth muscle. Boot, Dawson & Harvey (1976) found 15-oxo PGE_2, 13,14-dihydro PGE_2 and 13,14-dihydro 15-oxo PGE_2 to be less active than PGE_2 in relaxing respiratory tract smooth muscle *in vitro*. In contrast Crutchley & Piper (1975) found 15-oxo PGE_2 to be 1–1·8 times more potent than PGE_2 and the other two metabolites as active as PGE_2 in relaxing guinea-pig trachea *in vitro*. The effect of the pulmonary metabolites of PGE_2 and $PGF_{2\alpha}$ on lung resistance in guinea-pigs has not been investigated before.

In view of limited studies on the effects of prostaglandin metabolites on respiratory tract smooth muscles and the reported differences in the results, I have investigated the effects of three pulmonary metabolites of PGE_2 and $PGF_{2\alpha}$ on the guinea-pig tracheal chain preparation *in vitro* and on guinea-pig lung resistance *in vivo*.

MATERIALS AND METHODS

In vitro *preparation*

Guinea-pig tracheal chains were prepared according to the method of Akcasu (1952). Each chain, containing 4 to 5 rings of trachea was suspended in a 25 ml organ bath containing Krebs-Ringer solution of the following composition (g litre^{-1}): sodium chloride 6·9; potassium chloride 0·35; calcium chloride ($2H_2O$) 0·37; potassium dihydrogen orthophosphate 0·14; magnesium sulphate ($7H_2O$) 0·11; glucose 1·98; sodium bicarbonate 2·1. The bathing fluid was maintained at 37° and aerated with 5% carbon dioxide in oxygen. The pH of the bath fluid was 7·4.

A load of 0·3 g was applied to the tissue and allowed to equilibrate for an hour before testing the effects of prostaglandins. Isometric contractions or relaxations were measured using a force tension transducer connected to a recording device. The dose cycle ranged from 25–40 min and contact time from 7 to 12 min. The log dose response was exercised for all compounds. Suitable submaximal doses of the metabolites of PGE_2 and $PGF_{2\alpha}$ were compared with similar doses of respective parent compounds using a four point bioassay method.

Effect of polyphloretin phosphate

Polyphloretin phosphate (PPP), a selective antagonist of prostaglandins (Eakins, 1972) was tested for its action on the effects of prostaglandin metabolites on guinea-pig tracheal chain preparation *in vitro*. PPP was added to the bath 1 min before the addition

of the agonist. Carbachol or histamine served as controls.

In vivo preparation

A modification of James' (1969) method for measuring lung resistance in anaesthetized animals was used. Guinea-pigs, 300–400 g, were anaesthetized with intraperitoneal injections of 30 mg kg^{-1} pentobarbitone sodium. A ventilation cannula was inserted into the trachea and connected to a Palmer miniature ideal pump, set at 74 strokes min^{-1} and a stroke volume of 3·5 ml. A side-arm of the outflow was connected to a non-return water valve, set at a pressure of 10 cm water. Intrapulmonary pressure was measured by a pressure transducer connected to the outflow. The jugular vein was cannulated for intravenous administration of drugs. The carotid artery was cannulated and connected to another pressure transducer for simultaneous measurement of blood pressure for monitoring the state of the animal. An interval of 10–15 min was allowed between doses. All drugs were made up to the required final dilution in 0·9% sodium chloride chloride solution. The relaxant effects of PGE_2 and metabolites were monitored indirectly by measuring the differences in increase of airway resistance to standard doses of histamine before and 1 min after intravenous injection of prostaglandins.

RESULTS

Guinea-pig tracheal chain preparations in vitro

The potencies of six pulmonary metabolites were compared with their respective parent compounds, PGE_2 or $F_{2\alpha}$. $PGF_{2\alpha}$, 13,14-dihydro $PGF_{2\alpha}$ and 13,14-dihydro 15-oxo $PGF_{2\alpha}$ produced dose-related contractions of guinea-pig trachea. 15-oxo $PGF_{2\alpha}$ in three out of six experiments relaxed the smooth muscles and in the remainder three contracted. Threshold doses for the relaxant effect ranged between 0·2 and 1·32 μg ml^{-1} whilst that for the stimulant action varied between 0·008 and 0·032 μg ml^{-1}. 13,14-Dihydro $PGF_{2\alpha}$ was about as potent as $PGF_{2\alpha}$ and 13,14-dihydro 15-oxo $PGF_{2\alpha}$ was the least active metabolite (Table 1).

PGE_2, 15-oxo PGE_2, 13,14-dihydro PGE_2 and 13,14-dihydro 15-keto PGE_2 relaxed the guinea-pig tracheal muscles. All three metabolites were much less active than PGE_2 (Table 1). Sometimes PGE_2 and its metabolites caused an initial transient contraction (cf. Sweatman & Collier, 1968).

Effect of polyphloretin phosphate (PPP)

Over a wide range of concentrations (0·8 to 400 μg ml^{-1}), PPP inhibited the stimulant effect of $PGF_{2\alpha}$, 15-oxo $PGF_{2\alpha}$, 13,14-dihydro $PGF_{2\alpha}$ and 13,14-dihydro 15-oxo $PGF_{2\alpha}$ on trachea. The contractile effects of histamine and carbacohol were not altered by PPP which itself reduced the resting tone of the tracheal muscle at all concentrations, 0·8 to 400 μg ml^{-1}. It was therefore not possible to test its effect on the relaxant effects of PGE_2 and its three metabolites.

Table 1. *Relative potencies of $PGF_{2\alpha}$ and PGE_2 metabolites on guinea-pig tracheal chain preparation* in vitro, *relative to respective parent $PGF_{2\alpha}$ or PGE_2 taken as 1 (mean ± s.e.).*

Metabolites	Effect	Potency Mean n = 6	Potency Range	Threshold dose range μg ml^{-1}
15-oxo $PGF_{2\alpha}$	Cons.	1·097	0·36 –1·54	0·008–0·032
	Rel.	0·013	0·0025–0·02	0·2 –1·32
13,14-Dihydro $PGF_{2\alpha}$	Cons.	1·23 (±0·19)	0·47 –1·62	0·02 –0·20
13,14-Dihydro 15-oxo $PGF_{2\alpha}$	Cons.	0·081 (±0·0034)	0·0049–0·15	0·02 –0·80
15-oxo PGE_2	Rel.	0·16 (±0·0074)	0·04 –0·25	0·10 –0·40
13,14-Dihydro PGE_2	Rel.	0·12 (±0·007)	0·09 –0·16	0·01 –0·20
13,14-Dihydro 15-oxo PGE_2	Rel.	0·057 (±0·013)	0·04 –0·11	0·10 –0·80

Guinea-pig lung resistance in vivo

$PGF_{2\alpha}$ and 13,14-dihydro $PGF_{2\alpha}$ increased guinea-pig lung resistance in a dose-dependent manner the latter showing a potency nearly equalling $PGF_{2\alpha}$ (Table 2). The other two metabolites, 15-oxo $PGF_{2\alpha}$ and 13,14-dihydro 15-oxo $PGF_{2\alpha}$ up to 160 μg kg^{-1} (higher doses were not used because of the insolubility of the compounds in physiological saline) did not increase lung resistance.

PGE_2 and its three pulmonary metabolites reduced lung resistance as measured by a reduction in the bronchoconstrictor effects of standard doses of histamine. Responses were dose related. All three metabolites however were weaker bronchodilators when compared with PGE_2 (Table 2).

DISCUSSION

The three metabolites of PGE_2 relaxed guinea-pig tracheal muscle *in vitro* and produced a decrease in lung resistance *in vivo*. However, in both *in vitro* and *in vivo* situations, the metabolites were less active than PGE_2. The *in vitro* results with the three PGE_2 metabolites are in agreement with those reported by Boot & others (1976) and for 13,14-dihydro PGE_2 and 13,14-dihydro 15-oxo PGE_2 the

Table 2. *Relative potencies of $PGF_{2\alpha}$ and PGE_2 metabolites on the guinea-pig lung resistance preparation* in vivo, *relative to respective parent compounds taken as 1 (mean ± s.e.).*

Metabolites	Effect	Potency Mean n = 3	Range	Threshold dose range μg kg⁻¹
15-oxo $PGF_{1\alpha}$		No response up to 160 μg kg⁻¹		
13,14-Dihydro $PGF_{1\alpha}$	Cons.	0·89	0·59 –1·22	16–62
13,14-Dihydro 15-oxo $PGF_{1\alpha}$		(±0·13)* No response up to 160 μg kg⁻¹		
15-oxo PGE_1	Dil.	0·043	0·017–0·084	0·35–4·0
13,14-Dihydro PGE_2	Dil.	0·67	0·30 –1·17	0·02–0·2
13,14-Dihydro 15-oxo PGE_2	Dil.	0·028	0·023–0·039	2·0 –4·0

* n = 4.

results are similar to those reported by Crutchley & Piper (1975). However, for 15-keto PGE_2, Crutchley & Piper (1975) have reported a potency of 0·8–1·8 (mean 1·3) compared with PGE_2 whereas in the present study and that of Boot & others (1975) this metabolite is much less active than PGE_2. The effects of the three metabolites of PGE_2 on guinea-pig lung resistance *in vivo* have not been previously reported. The most potent of the metabolites was 13,14-dihydro PGE_2 which was about as active as PGE_2. Boot, Dawson & Osborne (1976) reported that PGE_2 and 15-oxo PGE_2 relaxed guinea-pig bronchial smooth muscle, the metabolite having similar activity to the parent compound.

Amongst the metabolites of $PGF_{2\alpha}$, 13,14-dihydro $PGF_{2\alpha}$ is a slightly more potent stimulant of guinea-pig tracheal muscle *in vitro* than $PGF_{2\alpha}$. 13,14-Dihydro 15-oxo $PGF_{2\alpha}$ is approximately 10 times less active than $PGF_{2\alpha}$. Dawson, Lewis & others (1974) have previously shown that on the guinea-pig trachea, 15-oxo $PGF_{2\alpha}$ was 2–3 times more active than $PGF_{2\alpha}$. I have found 15-oxo $PGF_{2\alpha}$ to be as potent as $PGF_{2\alpha}$ in contracting guinea-pig trachea and also to possess a relaxant component which is seen at a higher concentration. This phenomenon is not unlike that observed for PGE_1, E_2, A_1 and $F_{1\alpha}$ on isolated small resistance arteries of the rabbit (Strong & Bhor, 1967). In guinea-pig lung experiments *in vivo*, 13,14-dihydro $PGF_{2\alpha}$ was about as active as $PGF_{2\alpha}$ in increasing lung resistance. The results with 15-oxo $PGF_{2\alpha}$ and 13,14-dihydro $PGF_{2\alpha}$ are in agreement with those reported in the dog by Wasserman (1975).

In the physiological situation, $PGF_{2\alpha}$ is converted to 15-oxo $PGF_{2\alpha}$ by the enzyme 15-hydroxy dehydrogenase and the metabolite is readily reduced to 13,14-dihydro 15-oxo $PGF_{2\alpha}$ by the enzyme 13,14-prostaglandin reductase. Cornette, Harrison & Kirton (1974) reported that the blood concentration of $PGF_{2\alpha}$ in the rhesus monkey is 10–20 times lower than that of 13,14-dihydro 15-oxo $PGF_{2\alpha}$ while that of 15-oxo $PGF_{2\alpha}$ is slightly higher than that of $PGF_{2\alpha}$. It is therefore likely that in the normal situation, during metabolic degradation, $PGF_{2\alpha}$ is readily transformed into 13,14-dihydro 15-oxo $PGF_{2\alpha}$ which possesses little biological activity.

There is evidence to suggest that prostaglandins are involved in bronchospasm (Smith, 1976). The relevant effect of 15-oxo $PGF_{2\alpha}$ may relate to the observations of Gardiner & Collier (1974) and Dawson & Boot (1977) who suggest that the response of respiratory muscle to PGs may relate to the initial tone of the tissue although this has not been my experience. Imbalance of PG metabolizing enzymes has been suggested as playing a role in the aetiology of asthma by Dawson & Sweatman (1975), whilst commenting on the biological activity of parent PGs and their metabolites and has also been developed by Mathé (1976), and their present findings add support to these views.

PPP has previously been shown to antagonize some of the effects of PGE_2 and $PGF_{2\alpha}$ (Eakins, 1972). Mathé, Strandberg & Aström (1971) have demonstrated that PPP is capable of blocking the constrictor effect of $PGF_{2\alpha}$ but not the relaxant action of PGE_1 or PGE_2 on isolated human bronchi. On the contrary, Puglisi (1973) reported that PPP antagonized PGE_1-induced relaxation but not $PGF_{2\alpha}$-induced contraction on isolated guinea-pig trachea. PPP, however, has not previously been tested for antagonistic action against prostaglandin metabolites on bronchial smooth muscle of any species. In my preparations of guinea-pig trachea *in vitro*, PPP was shown to block the action of all three $PGF_{2\alpha}$ metabolites. Control doses of carbachol or histamine producing similar responses were not affected by PPP. At the doses tested, PPP consistently produced relaxation of guinea-pig tracheal muscle tone. In this relaxed state it was difficult to observe the antagonistic action of PPP on the relaxant effect of PGE_2 or its three metabolites.

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