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The actions of histamine and prostaglandins $F_2\alpha$ and E_2 on pulmonary vascular resistance of the lung of the guinea-pig

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In the guinea-pig isolated perfused lung, prostaglandin $F_2\alpha$ ($PGF_2\alpha$) and histamine increased, and prostaglandin E_2 (PGE_2) decreased, resistance to fluid flow. $PGF_2\alpha$ and histamine caused contractions of the pulmonary artery circular muscle preparation while PGE_2 inhibited contractions caused by other agonists. It was concluded that the changes in resistance to fluid flow caused by the three agents was partly or wholly due to their direct action on pulmonary vascular tissue. Mepyramine abolished the pressor response to histamine but had no effect on the pressor response to $PGF_2\alpha$. Furthermore, there was no evidence of the involvement of adrenergic mechanisms in the action of the prostaglandins. It is suggested that $PGF_2\alpha$ probably plays a role in the pulmonary vasoconstriction caused by anaphylaxis in guinea-pigs pretreated with mepyramine.

After death from anaphylactic shock, the guinea-pig is often found to have the right heart distended with blood (Auer & Lewis, 1910). This effect has been associated with increased pulmonary vascular resistance (Hahn, Giertz & Schmutzler, 1961). It is also a common observation that anaphylactic shock in the guinea-pig isolated perfused lung is accompanied by a marked rise in the perfusion pressure (e.g. Brocklehurst, 1960). Thus at least part of the increased resistance to blood flow seen *in vivo* must be a consequence of antigen-antibody reaction occurring locally in the lungs.

The apparent pulmonary vasoconstrictor effect of anaphylaxis seems worthy of study in view of its probable contribution to the circulatory collapse characteristic of this reaction in the guinea-pig. Furthermore, it is important to ascertain whether the increased resistance to blood flow arises from the direct action of anaphylactic mediators on lung vessels or from a mechanical occlusion of these vessels because of intense bronchoconstriction. This paper describes the effects on pulmonary vascular resistance *in vitro*, of three substances known to be released during anaphylaxis in guinea-pig lung: histamine (Bartosch, Feldberg & Nagel, 1932), prostaglandins $F_2\alpha$ and E_2 (Piper & Vane, 1969). The actions of these substances on the circular muscle of the pulmonary artery are also described.

METHODS

Whole lung preparation

Guinea-pigs of either sex weighing 350–400 g obtained locally were killed by neck fracture and the trachea immediately exposed and clamped. The animal was bled, the chest opened and an incision made in the right ventricle and another in the left atrium. Warm Krebs solution was flushed into the pulmonary artery through the incision in the right ventricle; this removed most of the blood from the pulmonary vascular bed. Heart and lungs were then removed to a dish and a flexible polythene

cannula inserted through the right ventricular incision and tied into the pulmonary artery. Most of the heart was then cut away. The lungs were perfused with Krebs solution containing atropine 10^{-7} g/ml, prewarmed to 37° and aerated with 5% carbon dioxide in oxygen. Perfusion was by means of a Watson-Marlow constant flow inducer adjusted to deliver 5 ml/min.

Changes in perfusion pressure induced by drugs were measured by means of a water manometer connected to a side arm of the pulmonary arterial cannula. The whole preparation was enclosed in a glass perfusion chamber (Easterbrooke Glass Co. Ltd.) and was undisturbed throughout the rest of the experiment. Drugs were administered through a 0·5 mm bore polythene tubing inserted in the pulmonary artery cannula in a manner previously described (Okpako, 1971).

Pulmonary arterial strip preparation

Guinea-pigs, 500 g or more, were killed by neck fracture and bled. The heart was exposed and the pulmonary artery excised by cutting close to the right ventricle, separated from adjacent tissue and cleared up to the bifurcation. The two branches were cut and the artery removed to a dish containing Krebs solution, and the tube obtained was cut to give a sheet of tissue 1·5–2 cm^2 which was then partially cut through on alternate sides to give a strip of circular muscle 4–5 cm long. This was suspended in aerated (5% CO_2 in O_2) Krebs solution at 37° in a 10 ml bath and tied to frontal lever (magnification $\times 13$). The tension on the tissue was 200 mg; the preparation was gently vibrated by a Saxby Vibrator (C. F. Palmer Ltd.).

Drugs

Drugs used were: histamine acid phosphate, atropine sulphate, isoprenaline sulphate, noradrenaline hydrochloride, propranolol hydrochloride (Inderal, ICI), phentolamine mesylate (Rogitine, Ciba). All doses were expressed in terms of the salt. Pure prostaglandins E_2 and $F_2\alpha$ (PGE_2 and $PGF_2\alpha$) were gifts from Dr. J. E. Pike (Upjohn Company, Kalamazoo). Prostaglandin stock solutions (10 mg/ml) were made in 96% ethanol and diluted in Krebs solution just before use.

RESULTS

Whole lung preparation

At a perfusion rate of 5 ml/min the perfusion pressure stabilized after about 15 min, at 100–120 mm H_2O for up to 2 h. Thereafter it increased steadily spontaneously; it was therefore necessary to reduce the flow rate to maintain a steady perfusion pressure. This spontaneous increase in pressure probably corresponded with the development of oedema in the lungs (see Broder & Schild, 1965).

Histamine and $PGF_2\alpha$ caused an increase in pulmonary vascular resistance (PVR) in all preparations at threshold doses from 0·05 to 0·2 μg in 15 preparations. In most lungs the two drugs were equipotent both in magnitude and duration of action. Dose-response relations could not be compared because high doses of histamine and $PGF_2\alpha$ caused a long-lasting increase in PVR which failed to return to base-line even after 45–60 min perfusion.

PGE_2 caused a decrease in PVR in all preparations at threshold doses of 0·02–0·1 μg in 13 preparations. Isoprenaline caused a similar effect. Dose-response curves were compared by random application of different doses of the two drugs in each of 3

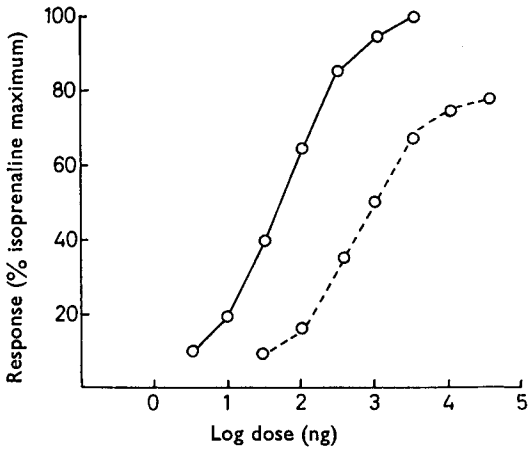

FIG. 1. Resistance to fluid flow in perfused guinea-pig lung. Dose-response curves to isoprenaline (○——○) and prostaglandin E_2, (○---○) random doses in three lungs. Each response decreased the resistance and was expressed as a percentage of the maximum response to isoprenaline.

lungs. Each response was then expressed as a percentage of the maximum response to isoprenaline. The results in Fig. 1 show that PGE_2 was about ten times less potent than isoprenaline on a weight basis. The maximum of the PGE_2 dose-response curve was less than that of isoprenaline.

The effect of mepyramine

After a single injection of mepyramine (10 μg) the pressor response to histamine was either abolished or converted to a small depressor response while the response to PGF_2 was unaffected (Fig. 2). This suggested that the action of PGF_2 was not mediated through activation of histamine receptors or the release of histamine.

Adrenoceptor antagonists

In view of some reports implicating adrenergic mechanisms in the action of some prostaglandins, the effects of phentolamine and propranolol on the responses to PGE_2 and $PGF_2\alpha$ were investigated. In these experiments the responses to the drugs were first elicited, then a single dose of either phentolamine or propranolol was slowly infused into the pulmonary artery cannula. Propranolol usually produced variable pressor effects that returned to base-line in 5–10 min. The same doses of drugs were

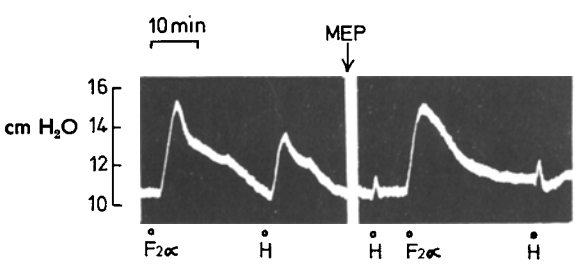

FIG. 2. Resistance to fluid flow in perfused guinea-pig lung. Responses to $PGF_2\alpha$ (4 μg) and histamine (H) (2 μg). After injecting mepyramine (MEP) (10 μg) the pressor response to histamine was either abolished or converted to a small depressor response. The pressor response to $PGF_2\alpha$ was unaffected.

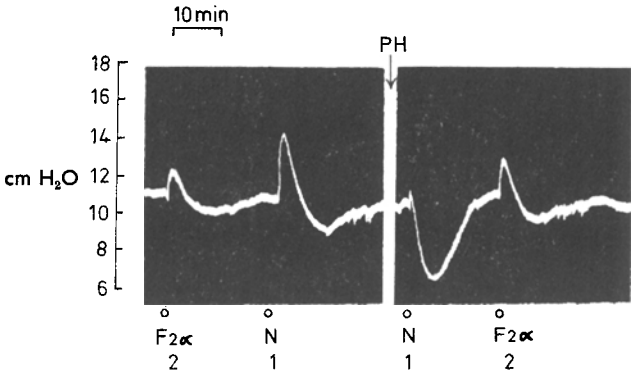

FIG. 3. Resistance to fluid flow in guinea-pig perfused lung. Responses to $PGF_2\alpha$ (2 μg), and noradrenaline (N) (1 μg). After the injection of 50 μg of phentolamine (PH) the biphasic response to noradrenaline was converted to a depressor response. The pressor response to $F_2\alpha$ was unaffected.

repeated. Fig. 3 shows that phentolamine (50 μg) converted the pressor response to noradrenaline into a depressor response, without affecting the response to $PGF_2\alpha$. The depressor response was always blocked by propranolol. This dual action of noradrenaline, consistently observed in this preparation, demonstrated the presence of both α- and β-adrenoceptors in the pulmonary vascular bed of the guinea-pig lung and their stimulation by noradrenaline. The result confirmed those of Nagasaka, Bouckaert & others (1964). It is further seen from Fig. 4 that propranolol (25 μg), which completely abolished the depressor response to isoprenaline, had no effect on the depressor response to PGE_2. It was concluded that the actions of prostaglandins E_2 and $F_2\alpha$ in this preparation were not mediated through activation of adrenoceptors nor through the release of catecholamines.

Pulmonary arterial strip preparation

$PGF_2\alpha$ causes bronchoconstriction in the guinea-pig, as does histamine. PGE_2 relaxes the bronchiolar smooth muscle in this species (Main 1964). Thus it can be argued that the changes in resistance to fluid flow caused by these substances were secondary mechanical effects arising from changes in bronchiolar smooth muscle tone. The actions of these substances were therefore assessed on the circular muscle of the pulmonary artery.

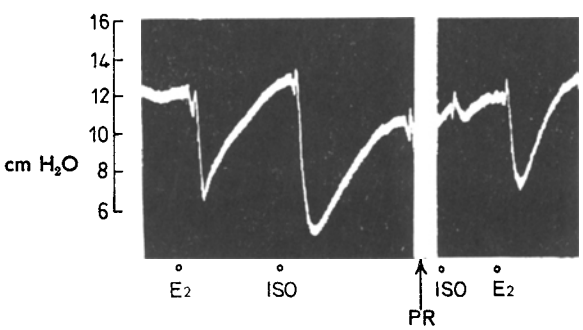

FIG. 4. Responses to PGE_2 (2 μg) and isoprenaline (ISO) (0·2 μg). After injecting propranolol (PR) (25 μg) the depressor response to isoprenaline was abolished. The depressor response to E_2 was unaffected.

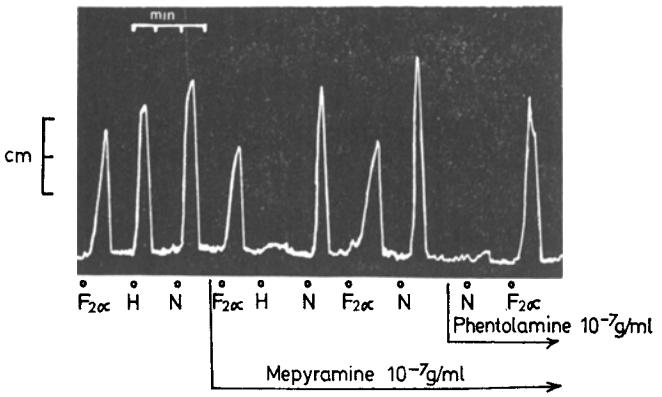

FIG. 5. Guinea-pig pulmonary arterial strip preparation. Contraction to $PGF_2\alpha$ (6 μg), histamine (H) (2 μg) and noradrenaline (N) (1 μg). Contractions to histamine and noradrenaline were specifically blocked by mepyramine and phentolamine respectively. Phentolamine tended to enhance contractions to $PGF_2\alpha$. Bath volume 10 ml. Drug contact time 1 min. Time interval between doses: 5 min.

This preparation responded by contraction to noradrenaline in doses of 10–50 ng/ml. When the preparation was first set up, contractions to the same dose of drug would continue to increase for up to 1 h. Thereafter the contractions remained stable for several hours.

Effects of histamine and $PGF_2\alpha$. Both histamine and $PGF_2\alpha$ caused dose-related contractions of the pulmonary arterial strip preparation at threshold doses between 50–200 and 200–500 ng/ml respectively. There was no evidence of tachyphylaxis to either drug in this preparation. As shown in Fig. 5 the contractions produced by $PGF_2\alpha$ were resistant to the action of phentolamine and mepyramine. As in the whole lung, the effects of $PGF_2\alpha$ were usually slightly enhanced in the presence of phentolamine.

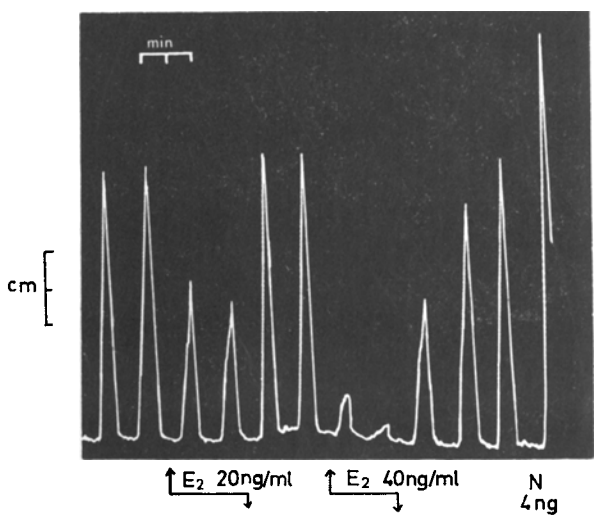

FIG. 6. Guinea-pig pulmonary arterial strip preparation. Effect of PGE_2 on responses to noradrenaline. All unlabelled contractions were elicited by noradrenaline (N) (2 μg). One minute before the 3rd and 4th contractions and before the 7th and 8th, PGE_2 was added to the bath to produce the concentrations shown. Bath volume 10 ml. Drug contact time: 1 min. Time interval between doses: 5 min.

Effects of PGE_2. PGE_2 in doses of up to 1 μg/ml produced no observable effects in this preparation, but regularly antagonized contractions to histamine and noradrenaline. Doses of 5 ng/ml were sufficient to cause a marked inhibition of the contractions to these agonists. The effect was dose-dependent (Fig. 6). With high doses PGE_2 (100 ng/ml or more) the inhibition persisted even after the prostaglandin had been washed out. In one experiment in which 400 ng/ml of E_2 was used, it took 55 min for the response to noradrenaline to return to normal after washing out PGE_2. The shorter duration of action in the whole lung is probably a reflection of rapid metabolism (Piper, Vane & Wyllie, 1970). Relaxation of the arterial strip could only be demonstrated at high doses (1 μg/ml) if the tone of the muscle was raised by prior addition of histamine to the bath.

Isoprenaline in doses of up to 10 μg/ml caused neither contraction nor relaxation of this tissue even after α-adrenoceptor blockade in histamine-treated preparations. Neither did it inhibit the contractions caused by histamine or noradrenaline. It was concluded that the actions of PGE_2 in this preparation were unrelated to β-adrenoceptor stimulation.

DISCUSSION

$PGF_2\alpha$ increases pulmonary arterial pressure in cats (Anggard & Bergstrom, 1963) and in dogs (Durcharme, Weeks & Montgomery 1968). The present results of the effects of $PGF_2\alpha$ in guinea-pig lung are consistent with previous findings. The effects of PGE_2 on pulmonary circulation appear not to have been studied previously, although PGE_1, whose actions are qualitatively similar, decreases pulmonary vascular resistance in the dog (Maxwell, 1967) and in the isolated blood-perfused lungs of cats and rabbits (Hauge, Lunde & Waaler 1967). The present results show that PGE_2 causes a decrease in pulmonary vascular resistance of isolated perfused lung of the guinea-pig.

There was no evidence of the involvement of adrenergic mechanisms in the action of the two prostaglandins. In this respect these results are consistent with those of Hauge & others (1967) who showed that the depressor effect of PGE_1 in the rabbit perfused lung was not affected by propranalol or phentolamine treatment. However, the present results differ from those of Davies & Withrington (1969) who showed that in the dog the splenic vasodilatation caused by $PGF_2\alpha$ was converted to vasoconstriction after treatment with phenoxybenzamine.

The actions of the three drugs on resistance to fluid flow appear to be by direct effect on pulmonary vascular tissue. Histamine and $PGF_2\alpha$, which caused increases in PVR in perfused whole lung, also caused contractions of the circular muscle of the pulmonary artery; PGE_2 which caused a decrease in PVR in whole lung also inhibited contractions produced by other agonists in the arterial strip. It is noteworthy that although histamine and $PGF_2\alpha$ were approximately equipotent in their pressor actions, the latter was up to five times less potent than histamine in the pulmonary arterial strip. Perhaps the smaller blood vessels are more sensitive to the action of $PGF_2\alpha$ than the pulmonary artery. Ducharme, Weeks & Montgomery (1968) concluded that the pressor action of $PGF_2\alpha$ in the perfused hind limb of the dog was primarily a result of veneconstriction.

It is not possible yet to determine the role of these substances in the production of pulmonary vasoconstriction during anaphylaxis since the overall effect would depend on how much of each was released, and other unidentified substances may also play

a part. $PGF_2\alpha$, which was found to be released in greater amounts than PGE_2 in guinea-pig lung anaphylaxis (Piper & Vane, 1969), was also less susceptible to metabolism in guinea-pig lung than PGE_2 (Piper & others, 1970). Furthermore, Hahn & others (1961) found that anaphylactic shock in guinea-pigs pretreated with mepyramine was characterized by intense pulmonary vasoconstriction without bronchoconstriction. $PGF_2\alpha$ may well play an important role in this effect.

Acknowledgements

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