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A dual action of histamine on guinea-pig lung vessels

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Summary

1. In isolated guinea-pig lungs perfused through the pulmonary artery, histamine caused a rise in perfusion pressure which was converted to a fall by a prior injection of mepyramine.

2. Evidence is provided to show that at least part of the histamine effect was due to its direct action on pulmonary vascular tissue, and was largely independent of bronchomotor tone.

3. Neither the pressor nor the depressor effects were modified by adrenoceptor blocking agents, phentolamine and propranolol, in doses which reversed or blocked the effects of noradrenaline, adrenaline or isoprenaline. The actions of histamine could therefore not be attributed to catecholamine release. The involvement of cholinergic mechanisms was also excluded since atropine failed to influence the histamine effects.

4. It is suggested that the mepyramine-sensitive pressor, and the mepyramine-resistant depressor effects of histamine, were mediated by different receptors.

5. It is proposed that a vasodilator action of histamine leading to a partial obstruction of the pulmonary airways could be part of the explanation for the relative ineffectiveness of mepyramine in blocking anaphylactic bronchoconstriction in the guinea-pig.

Introduction

Ash & Schild (1966) classified the effects of histamine in terms of actions mediated by at least two types of receptors. H_1 receptors, present in, for example, guinea-pig ileum and bronchi (Arunlakshana & Schild, 1959) mediate actions which are specifically antagonized by low concentrations of antihistamine drugs. Other actions which are not so antagonized, such as stimulation of gastric secretion (Ashford, Heller & Smart, 1949), and stimulation of isolated atria (Tredelenburg, 1960) would appear to be mediated by a different receptor or receptors. Several inhibitory actions of histamine on smooth muscle not blocked by antihistamine drugs have also been described: inhibition of rat uterus (Ash & Schild, 1966), inhibition of cat tracheal muscle (Maengwyn-Davies, 1968), and relaxation of sheep bronchial muscle (Eyre, 1969). In a previous study (Okpako, 1972), it was observed that after mepyramine, the pressor action of histamine in perfused guinea-pig

lung, was converted to a depressor effect. The present experiments examine this dual action of histamine in more detail.

Methods

Perfused guinea-pig lung

Guinea-pigs of either sex weighing approximately 450 g were killed by neck fracture and after tying the trachea, lungs and heart were isolated. An incision was made in the right ventricle and the auricles were split open. A flexible cannula was tied into the pulmonary artery and the vascular bed perfused with Krebs solution pre-warmed to 37° C and aerated with 5% CO_2 in oxygen. The composition of the Krebs solution was (mM): NaCl 119·0, KCl 4·7, $CaCl_2$ 2·5, $MgCl_2$ 1·2, $NaHCO_3$ 25·0, NaH_2PO_4 1·2, and glucose 11·5. Perfusion was by means of a Watson–Marlow constant flow inducer (MRHE 7 and MRHE 88). The whole preparation was enclosed in a constant temperature perfusion chamber (Easterbrook Glass Co.). Drugs were injected into the pulmonary artery as previously described (Okpako, 1971). Changes in perfusion pressure were measured by means of a water manometer.

In five experiments, the lungs were perfused through the trachea as well as through the pulmonary artery. The tracheal perfusion was as described by Luduena & Fisher (1967), the effluent emerging from scarifications on the pleural surface. According to these authors, a contraction of the bronchioles (bronchoconstriction) causes a rise in tracheal perfusion pressure. For the simultaneous perfusion, the flow inducer was fitted with a 'split clamp track' (Watson–Marlow Ltd.) each track taking 3·2 mm bore silicone tubing. Changes in pulmonary arterial and tracheal perfusion pressures were separately measured manometrically.

Pulmonary arterial strip preparation

Details of this preparation have been described elsewhere (Okpako, 1972). Guinea-pigs weighing at least 500 g were killed and the pulmonary artery cut close to the right ventricle. The cut end was held by fine forceps, the artery separated from adjacent tissue and cleared up to the bifurcation. A short length of artery was obtained by cutting just above the bifurcation. The tissue obtained was split open and cut spirally giving a strip of arterial circular muscle 3·0–4·5 cm long. The tissue was suspended in Krebs solution at 36° C in a 6 ml bath. The contractions of the tissue were recorded isotonically on a smoked drum (magnification $\times 13$). The tension on the tissue was 200 mg and the recording lever was gently vibrated by a Saxby Vibrator (Palmer Ltd.). The Krebs solution was aerated with 5% CO_2 in oxygen.

Drugs used were: (−)-adrenaline hydrochloride, atropine sulphate (BDH), histamine acid phosphate, (−)-isoprenaline sulphate, (±)-propranolol hydrochloride (Inderal, ICI), phentolamine mesylate (Rogitine, Ciba), acetylcholine hydrochloride, mepyramine maleate (Anthisan, May & Baker), cyproheptadine hydrochloride (MSD). All doses are expressed in terms of the salt. All drugs were freshly diluted in Krebs solution and injected in a volume of 0·1 ml.

In all experiments on perfused lung except that shown in Fig. 5, antagonists were injected as single doses into the pulmonary artery.

Results

Base-line perfusion pressure

At a flow rate of 5 ml/min the base-line pressure stabilized after about 20 min at 95–120 mm H_2O. Thereafter, there was a steady spontaneous increase in the base-line pressure in the course of the experiment. Table 1 shows the extent of increase in base-line pressure in four lungs perfused at 5 ml/min for 4 hours.

The increase in resistance after prolonged perfusion was associated with visible changes in the appearance of the lungs. Lungs perfused for longer than 3 h gradually changed from white to grey and looked oedematous. Sensitivity to pressor agents also diminished.

Effect of histamine and its modification by mepyramine

Histamine always caused an increase in perfusion pressure. The effect was immediate and lasted 10–20 min depending on the dose. In the dose range used, the responses increased with increasing dose up to a maximum and then declined. Frequently, but not always, the largest dose of histamine (20 μg) gave a biphasic response, an initial rise followed by a fall below the base-line and then a return to the original base-line (Fig. 1). The decline in response with large doses of histamine was not necessarily due to loss of sensitivity, since after the response to a large dose, the bigger response to a smaller dose was usually unaltered.

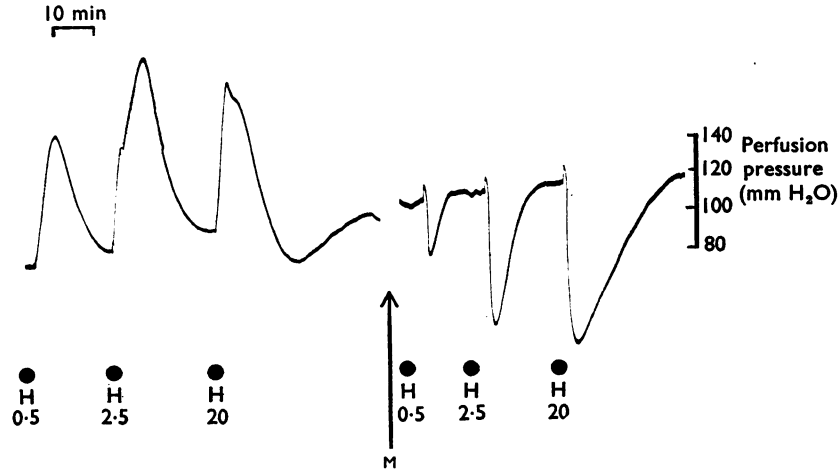

FIG. 1. Guinea-pig lungs perfused through pulmonary artery. Changes in perfusion pressure caused by three doses of histamine (μg) before and after the injection of mepyramine (250 μg) at M. Mepyramine itself caused a rise in perfusion pressure. Time interval between the two sets of responses was 30 minutes.

TABLE 1. *Increase in base-line pressure during perfusion of guinea-pig lungs* (4 *experiments*)

Perfusion time (min)	Base-line pressure mm H_2O	±SD
Initial	105	10·8
30	117·5	21·5
60	113	39·8
90	142	43·9
120	137·5	41·1
150	136·2	42·1
180	141·5	34·3
210	148	29·5
240	170	42·4

After the injection of mepyramine, the pressor response to histamine was converted to depressor. The depressor response was dose-related (Fig. 1b). The response was immediate and lasted 5–20 min, depending on the dose. The inhibitory response after mepyramine was not a reflection of increased tone after prolonged perfusion because when mepyramine was present either in the perfusion fluid or injected at the start of the experiment, the response to histamine was also depressor.

Dose-response curves were constructed by serial injection of 0·05, 0·5, 2·5 and 20 μg of histamine before and after the injection of mepyramine. The doses were given once in each of twelve lungs and repeated in six after 25 μg of mepyramine and in the other six after 250 μg mepyramine. The results are plotted in Fig. 2. The depressor responses to histamine were significantly greater at all dose levels after 250 μg of mepyramine than they were after the 25 μg dose ($P<0{\cdot}001$).

Repeated injections of histamine after mepyramine

This experiment was designed to test the persistence of the mepyramine effect after a single injection, and to determine whether there was tachyphylaxis of the depressor response to histamine. Tachyphylaxis of the response might be expected if histamine was acting indirectly.

The persistence of the mepyramine effect was tested by first giving 0·5, 2·5 and 20 μg histamine serially and then repeating these doses at intervals of 30 min, 2 h

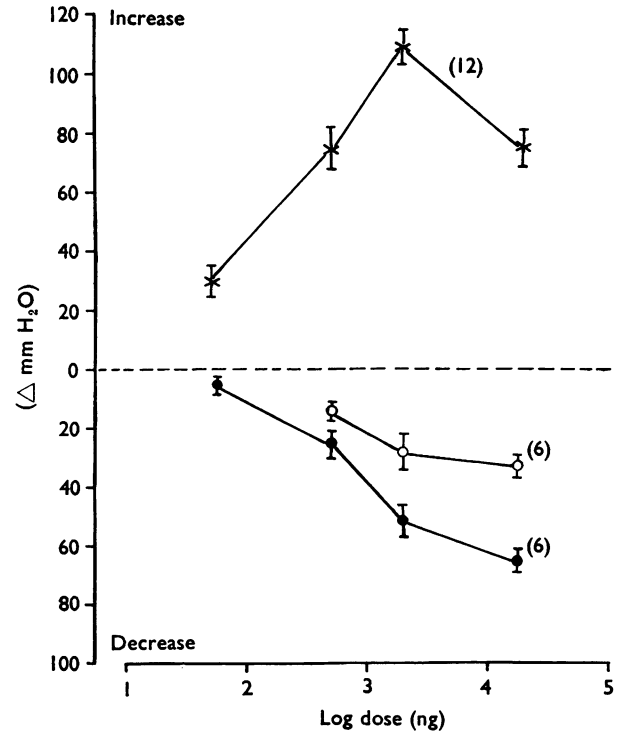

FIG. 2. Guinea-pig lungs perfused through pulmonary artery. Log dose-response curves to histamine alone (×——×), histamine after the injection of mepyramine (25 μg) (○——○), and histamine after mepyramine (250 μg) (●——●). The figures in parentheses refer to the number of lungs from which the means were obtained. The vertical bars indicate standard errors of the mean.

and 4 h after a single injection of 250 μg mepyramine. The results were as shown in Table 2. It is apparent that after a single dose of 250 μg, the effect of mepyramine in converting the histamine pressor response to depressor persisted for longer than 4 hours.

In each of three other lungs, histamine (2·5 μg) was given followed by mepyramine (250 μg). Thirty minutes later, histamine was repeated and again every 10 min until 8 doses had been given. The results were as shown in Fig. 3. In each lung, the response to histamine was pressor before the injection of mepyramine. In each case, the response was depressor after mepyramine. There was no apparent tachyphylaxis in the depressor response in any of the three lungs.

TABLE 2. *Persistence of the effect of mepyramine* (250 μg) *in perfused guinea-pig lungs*

Dose of histamine (μg)	Responses before mepyramine (mm $H_2O \pm$ standard error) All responses were pressor	
0·5	52±2·5	
2·5	100±3·3	
20·0	83±3·3	
	Responses after mepyramine (250 μg) All responses were depressor	
	Time after mepyramine injection (min)	Response mm H_2O±S.E.
0·5		19±0·4
2·5	30	40±1·7
20·0		56±5·4
0·5		25±1·7
2·5	120	58±4·8
20·0		70±3·6
0·5		13±0·9
2·5	240	35±4·4
20·0		56±4·8

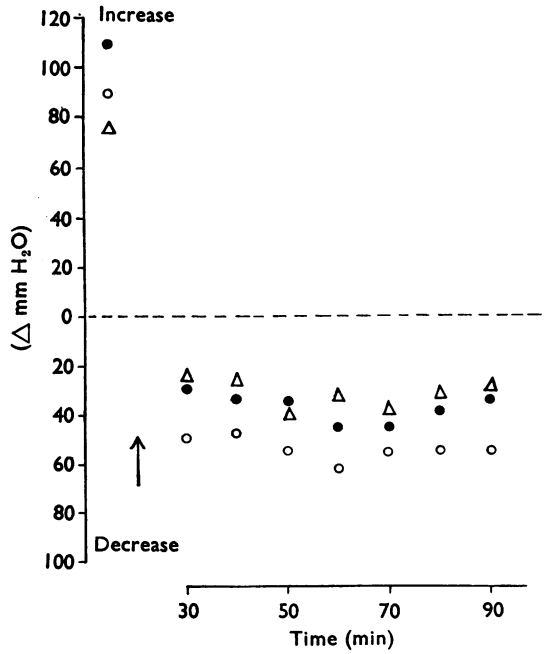

FIG. 3. Guinea-pig lungs perfused through pulmonary artery. Responses to histamine (2·5 μg) in three separate experiments before and after the injection of mepyramine (250 μg) at the arrow. In each lung the response was pressor before mepyramine. After mepyramine, the responses elicited every 10 min were depressor and remained unchanged for 90 minutes.

Adrenoceptor blocking agents

Since histamine is known to release catecholamines from a variety of sources, it was thought relevant to study the effect of some adrenoceptor blocking agents on the depressor response to histamine after mepyramine. In this preparation noradrenaline and adrenaline usually caused a biphasic, and isoprenaline always a depressor, response. These responses were not modified by mepyramine (250 μg). After phentolamine (1 mg) all responses became depressor, the responses to adrenaline and noradrenaline being greatly enhanced. After propranolol (250 μg) responses to adrenaline and noradrenaline became pressor while the responses to isoprenaline were abolished. Neither phentolamine nor propranolol had any effect on the depressor response to histamine. A typical experiment is shown in Fig. 4. It can be seen that the biphasic response to adrenaline was converted to a marked pressor response after propranolol. The depressor response to histamine was unchanged. In other experiments where noradrenaline and isoprenaline were used, the results were similar. Propranolol always enhanced the pressor response to noradrenaline and abolished the depressor effect of isoprenaline but if anything, usually enhanced the depressor response to histamine. Similarly, phentolamine always blocked the pressor, and enhanced the depressor, component of nor-

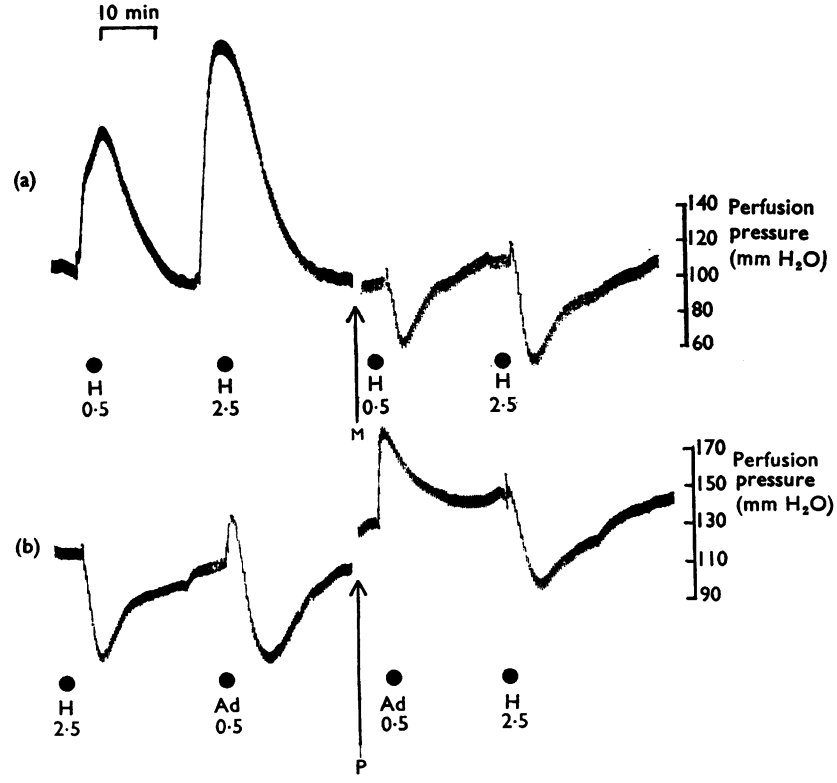

FIG. 4. Guinea-pig lungs perfused through pulmonary artery. (a), Inversion of the pressor response to histamine (H) after the injection of mepyramine (250 μg) at M. (b), The effect of propranolol (250 μg) injected at P. The biphasic response to adrenaline (Ad) was converted to a pressor response. The depressor response to histamine was unchanged; (a) and (b) are from the same experiment. Histamine and adrenaline doses in μg.

adrenaline and adrenaline responses, but had no effect on the depressor response to histamine. It seemed that the histamine response in this preparation was not mediated through the release of catecholamines.

Atropine and cyproheptadine

Atropine (0·5 μg/ml) present in the perfusion fluid or cyproheptadine (250 μg) injected in a single dose failed to modify the depressor response to histamine after mepyramine. The possibility of the involvement of cholinergic mechanisms or of 5-hydroxytryptamine release was thus excluded.

Lungs simultaneously perfused through trachea and pulmonary artery

Histamine caused a rise in both pulmonary arterial and tracheal perfusion pressures. The rise in the arterial perfusion pressure always occurred sooner than the rise in the tracheal pressure. The mean difference in the latent periods was 23 s (n=5) after injecting 0·5 μg histamine. The rise in tracheal perfusion pressure, however, persisted long after the arterial pressure had returned to base-line. In the experiment illustrated in Fig. 5, isoprenaline 0·1 μg was injected into the pul-

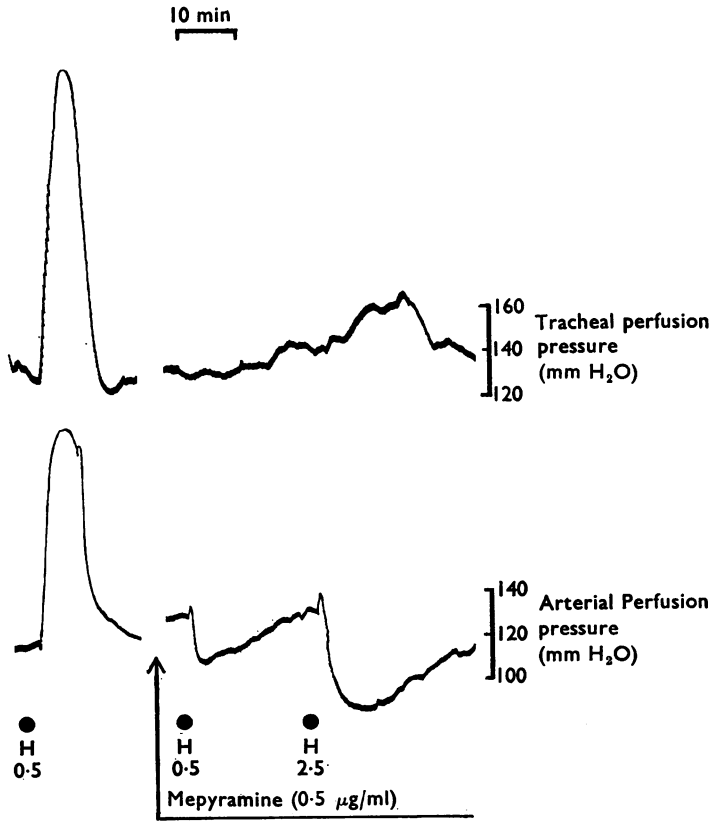

FIG. 5. Guinea-pig lungs perfused simultaneously through trachea and pulmonary artery. The rise in tracheal perfusion pressure caused by histamine (H) was antagonized by mepyramine 0·5 μg/ml present in the perfusion fluid. The rise in pulmonary artery perfusion pressure caused by histamine was converted to a fall. At the peak of the rise in tracheal pressure, 0·1 μg isoprenaline was injected into the pulmonary artery.

monary artery at the peak of the tracheal pressure rise in order to facilitate relaxation. In the presence of mepyramine, the histamine-induced rise in tracheal pressure was reduced or abolished. Under the same conditions, histamine always caused a fall in arterial perfusion pressure (see Fig. 5).

Isolated pulmonary arterial strip preparation

Specificity of the actions of histamine and catecholamines. This preparation responded by contraction to histamine as it did to adrenaline and noradrenaline. The potency of the amines decreased in the order of adrenaline>noradrenaline> histamine. In five separate preparations, isoprenaline, in doses comparable to those effective for adrenaline and noradrenaline, had no effect. It caused neither contraction nor relaxation, and it failed to inhibit histamine-induced contractions.

The contractions caused by histamine were specifically antagonized by mepyramine (see Fig. 6). In the presence of mepyramine 2 ng/ml the doses of histamine had to be increased approximately 50-fold in order to produce the same contractions as before mepyramine. The maximum response was not changed. The contractions to noradrenaline were slightly increased in the presence of this concentration of mepyramine.

The contractions caused by the catecholamines were specifically antagonized by phentolamine. In the presence of phentolamine (50 ng/ml) the dose-ratio for noradrenaline was approximately 5 while responses to histamine were unchanged. It was concluded that histamine contracted the circular muscle of the pulmonary artery by stimulating histamine receptors on the smooth muscle wall and not indirectly through catecholamine release.

Lack of an inhibitory action of histamine after mepyramine. An inhibitory action of histamine could not be demonstrated in the isolated artery preparation. In the presence of three different concentrations of mepyramine, histamine caused neither relaxation of the tissue nor did it inhibit catecholamine-induced contractions.

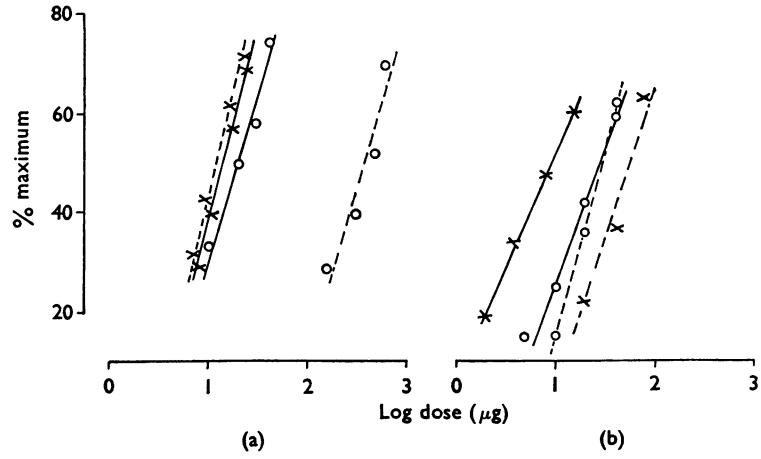

FIG. 6. Pulmonary arterial strip preparation. Log dose response lines to histamine ○——○ and noradrenaline ×——× alone, and in the presence of (a) mepyramine 2 ng/ml and (b) phentolamine 50 ng/ml. Histamine in the presence of antagonist ○- - -○; noradrenaline in the presence of antagonist ×- - - -×. (a) and (b) are from different experiments. Each point was a mean of at least 5 results.

Discussion

In lungs perfused simultaneously through the trachea and pulmonary artery (Fig. 5), the rise in arterial perfusion pressure caused by histamine always preceded the rise in tracheal perfusion pressure. Mepyramine reduced or blocked the histamine-induced bronchoconstriction, while converting the rise in arterial perfusion pressure into a fall. These results suggested that the histamine-induced changes in pulmonary vascular resistance were at least in part due to a direct action on pulmonary vascular smooth muscle. This conclusion is consistent with the finding that in the dog, the increase in pulmonary vascular resistance in response to stimulation of carotid sinus baroreceptors (Daly & Daly, 1957) and the decrease caused by stimulation of carotid body chemoreceptors (Daly, 1957), were independent of bronchomotor tone.

The results demonstrated two actions of histamine on the pulmonary vascular bed of the guinea-pig; a mepyramine-sensitive pressor action and a depressor action which was unmasked after mepyramine. The bell-shaped nature of the histamine dose-response curve would suggest that the pressor component of the histamine response was predominant at low doses, the depressor component becoming marked at high dose levels.

Although histamine can release catecholamines from, for example, adrenal medulla (Burn & Dale, 1926), stomach (Paton & Vane, 1963) chick intestine (Everett & Mann, 1967), and myocardial circulation (Parratt, 1969), the evidence from the present experiments pointed to a direct action of histamine on pulmonary vascular tissue. Adrenoceptor blocking agents (phentolamine and propranolol) in doses which blocked or reversed catecholamine responses had no effect on either the pressor or the depressor effect of histamine. During repeated injection of histamine, there was no tachyphylaxis which might be expected if histamine was acting indirectly. In the perfused whole lung there was sometimes a reduction in the pressor response during repeated application of histamine. This was accounted for in terms of increased base-line perfusion pressure. Since vagal reflex mechanisms would not operate in the isolated lung, and because atropine failed to modify the histamine responses, the possibility that histamine stimulated 'lung irritant receptors' (Mills & Widdicombe, 1970) was excluded.

Although the possibility that histamine caused a release of a depressor substance cannot be excluded on the basis of the present experiments, it seemed more likely that the actions of histamine on the pulmonary vascular bed were due to the stimulation of two different types of receptors. The pressor effect was mediated by H_1 receptors of Ash & Schild (1966) since this effect was specifically antagonized by mepyramine. The depressor effect would appear to be due to the stimulation of a different histamine receptor, probably the same type of receptor mediating other mepyramine-resistant actions of histamine (see **Introduction**). The failure to demonstrate a depressor action of histamine in the pulmonary arterial strip preparation suggested a difference in the regional distribution of histamine receptors in the pulmonary vascular bed. The receptors mediating the depressor effect might be present in a higher proportion in arterioles and capillaries. Burn & Dale (1926) showed that in dogs, cats and monkeys, histamine caused vasoconstriction of large arteries and vasodilatation of arterioles and capillaries.

It is of interest to note that the present experiments and others (Nagasaka, Boukaert, Shaepdryver & Heymans, 1964; Okpako, 1972) have also shown

apparent regional differences in the distribution of adrenoceptors. Both α- and β-adrenoceptors were shown to be present in the whole lung. The presence of β-adrenoceptors was not, however, demonstrable in the isolated pulmonary arterial strip preparation. β-Adrenoceptors were also apparently present in a higher proportion in the arterioles and capillaries (see also Day & Dixon, 1971).

Recent findings indicate that changes in pulmonary vascular tone may influence airways resistance (Oskoui & Aviado, 1969; Boissier, Advenier, Guidicelli & Viars, 1971). The results presented here (Fig. 5) show that while mepyramine antagonized histamine-induced bronchoconstriction, at the same time it revealed a pulmonary vasodilator effect of histamine. In anaphylaxis, during which the concentration of histamine in the vicinity of lung vessels is high, such an effect may well be marked. If, as proposed by Boissier *et al.* (1971), pulmonary vasodilatation increases airways resistance, this effect of histamine could contribute to the relative ineffectiveness of mepyramine in blocking anaphylactic bronchoconstriction. The importance of such an effect would depend on the contribution of other vasoactive mediators of anaphylaxis, e.g., prostaglandins E_2 and $F_{2\alpha}$ (Piper & Vane, 1969; Okpako, 1972).

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