Br. J. Pharmac. (1972), **45**, 284–298.

Effects of histamine, 5-hydroxytryptamine and bradykinin on the vascular system of isolated lungs of the guinea-pig and the influence of phenylbutazone on these effects

P. N. AARSEN AND A. ZEEGERS

Pharmacological Laboratory, University of Amsterdam, Polderweg 104, Amsterdam (0), The Netherlands

Summary

1. In isolated lungs of the guinea-pig perfused through the pulmonary artery repeated doses of histamine progressively increased pulmonary arterial pressure and reduced fluctuations in tracheal pressure. Phenylbutazone almost completely abolished the effect of histamine on the arterial pressure and suppressed the progression of the effect on tracheal pressure.

2. The effects of 5-hydroxytryptamine and bradykinin did not show such progression.

3. The fractions of radioactivity remaining in the lungs after doses of ^{14}C-histamine did not differ significantly after successive doses. The outflow of radioactivity from previously loaded lungs was greatly increased by histamine, bradykinin and 5-hydroxytryptamine. This increase was not affected by papaverine.

4. Successive doses of histamine, but not 5-hydroxytryptamine, produced a progressive increase in weight of the lungs and this effect was accompanied by a progressive increase in arterial pressure. Both effects were strongly suppressed by phenylbutazone.

5. It is concluded that repeated administration of histamine causes an accumulation of fluid in the lungs probably mainly in the interstitial spaces, which results in an inhibition of the tracheal pressure fluctuations. Phenylbutazone prevents this effect by suppressing the vasoconstrictor action of histamine without affecting the increased vascular permeability.

Introduction

Alternate addition of histamine and bradykinin to isolated lungs of the guinea-pig perfused through the pulmonary artery causes a blockage of the airways by oedema formation (Aarsen, 1966). On repeated administration of histamine alone, but not of 5-hydroxytryptamine, the inhibitory effect on the propagation of the external pressure fluctuations through the lungs as measured in the trachea, increases and becomes partly irreversible. This phenomenon as well as the histamine-induced rise of pulmonary arterial pressure, were found to be increased by a low dose of bradykinin and suppressed by non-steroid anti-inflammatory agents (Aarsen, 1969).

Since Wilhelm (1962) showed that synthetic bradykinin markedly increased the permeability of the skin vessels in guinea-pigs, whereas 5-hydroxytryptamine caused only a small increase, it was supposed that these differences in sensitivity might explain the observed differences between the effects on the guinea-pig lungs of histamine and bradykinin on the one hand and 5-hydroxytryptamine on the other hand. In the present investigation the experiments were repeated with radioactively labelled histamine and histidine; the efflux of the radioactive isomers as well as their retention in the lung tissues has been studied.

Methods

Isolated guinea-pig lungs with vascular perfusion

The method was essentially the same as that used by Bhattacharya & Delaunoix (1955) and has been previously described (Aarsen, 1969). Female albino guinea-pigs, weighing 200–300 g, were injected intraperitoneally with urethane, 1,250 mg/kg, and 5 mg of pentobarbitone sodium per animal. Fifteen minutes later the trachea was cannulated and heparin, 500 I.U., was given intravenously. The animal was bled and the pulmonary artery cannulated for perfusion. After the heart was cut off through the atria, the lungs with the trachea were removed and suspended in the perspex cylinder by means of the cannulae (Fig. 1).

The lung vessels were perfused with Krebs solution containing (mM): NaCl 118·4; KCl 4·7; $CaCl_2$ 2·5; $MgSO_4$ 1·2; KH_2PO_4 1·2; $NaHCO_3$ 24·9; glucose 11·1. The solution was equilibrated with 5% CO_2 in O_2 at 37° C. The finger pump was adjusted to give a constant flow of about 22 ml/minute. Since this flow appeared to be independent of changes in pulmonary vascular resistance within the range occurring in the present experiments, the pulmonary arterial perfusion pressure was assumed to be proportional to vascular resistance. This pressure as well as the tracheal pressure fluctuations were recorded by means of Elema–Schönander pressure transducers EMT 490B.

In the experiments in which the left atrial pressure was artificially increased, an outflow cannula was fixed in the left auricle and the heart was not cut off. A loop of thread 0·6 mm in thickness was placed around this cannula at a distance of about 1 cm from the atrium. Both ends of the loop were pulled through a fixed metal tube, bore 1·4 mm, to the outside of the perspex cylinder, and connected to a vertical sliding adjustment attachment. By screwing up this attachment the loop around the cannula could be tightened and the cannula constricted against the front-side of the metal tube.

Changes in weight of lungs were determined by placing the lungs inside a glass cylindrical vessel surrounded by a water jacket, where they hung freely on a string. This string was fastened at its lower end to the vertically bent parts of the cannulae in the pulmonary artery and the trachea, and its upper end to a Pixie 8101 transducer (Endevco Laboratories). In these experiments the atmospheric pressure outside the lungs was not changed so as to avoid interference with the weighing. Furthermore, the outflow of the finger pump had to be decreased to 13 ml per min in order to perfuse the lung vessels at a pressure corresponding to that of the previous experiments.

The solutions under test were added during 30 s to the perfusion fluid by a continuous infusion apparatus at a speed of 0·5 ml/min at 10 min intervals. The

increases in pulmonary arterial perfusion pressure and in weight, mentioned in Table 5, are the maximal values obtained during the indicated period.

Tracer preparation, dosing, sampling and counting

[Ring-2-^{14}C]-histamine dihydrochloride and [ring-2-^{14}C]-L-histidine were used in a solution of 10 μg/ml distilled water, containing 2·9 and 2·8 μCi/ml respectively. According to the dose either 0·05, 0·1 or 0·2 ml of these solutions was added by means of a standardized syringe and a Hamilton dispenser TB600 over a period of 20 seconds. The cannula was then flushed by administration of 0·1 ml Krebs solution in 10 s. When the addition of radioactive histamine was preceded by 0·1 ml of a bradykinin solution (250 ng/ml), this volume was injected over a period of 30 seconds. When the effects of repeated doses of radioactive histamine were studied, the doses were always administered at 10 min intervals. The radioactive

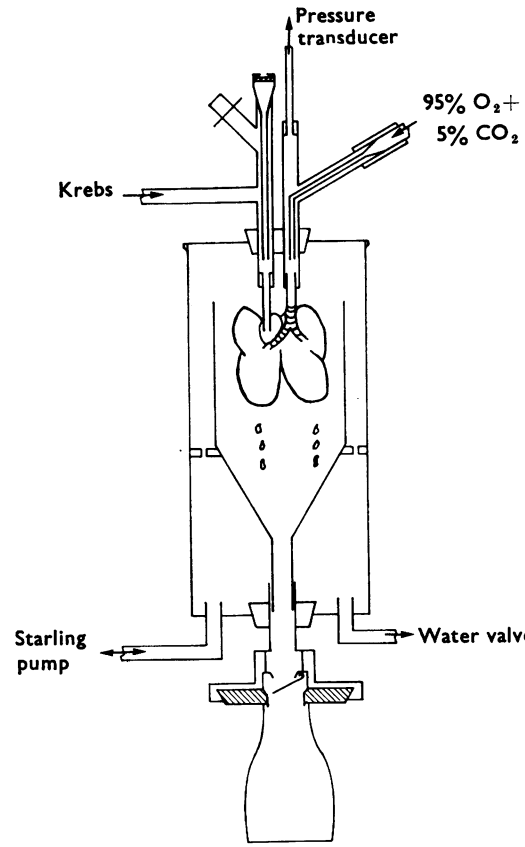

FIG. 1. Schematic drawing of perspex cylinder used to suspend isolated guinea-pig lungs by means of the cannula in the trachea and the pulmonary artery. The tracheal cannula is attached to a tube with a side-arm through which a polythene cannula with a diameter of 1·0 mm is pushed to fill the bronchial tree with 5% CO_2 in O_2. The main tube is connected to a pressure transducer. The cannula in the pulmonary artery is attached to a tube with three connexions: one to the perfusion system and a pressure transducer; the second serving as an outlet for air and the third with a thin polythene injection cannula. The pressure in the cylinder was alternately made positive and negative by means of a Starling respiration pump. The effluent from the lungs was collected once a minute in polythene flasks screwed on a slide.

lung effluent was collected in polythene flasks screwed on a slide carrying 8 flasks, connected to the perspex cylinder as shown in Fig. 1. This slide was moved to replace the flask at 1 min intervals. Three ml of each fraction was added to 17 ml of a liquid scintillation fluid consisting of: 10 g 2,5-diphenyloxazole ; 500 mg *p*-bis-[2-(5-phenyloxazolyl] benzene ; 80 g naphthalene ; 143 ml toluene ; 429 ml dioxane ; and 429 ml ethylene glycol monomethyl ether (Cellosolve). The radioactivity of each sample was measured in a liquid scintillation counter (Nuclear Chicago). The mean efficiency (±S.E.M.) of this liquid scintillation fluid estimated by means of *n*-^{14}C-hexadecane as a reference, was found to be 70·9 ± 0·25% (n=6). The radioactivity remaining in the lungs was estimated by the combustion technique described by Buyske, Kelly, Florini, Gordon & Peets (1966).

Drugs

The radioactive compounds [ring-2-^{14}C]-histamine dihydrochloride and [ring-2-^{14}C]-L-histidine were obtained from Philips Duphar, and *n*-[1-^{14}C]-hexadecane from the Radiochemical Centre, Amersham. The other substances used were: histamine acid phosphate (BDH), 5-hydroxytryptamine creatinine sulphate (BDH), synthetic bradykinin (BRS 640, Sandoz), sodium phenylbutazone (Butazolidin, Geigy), and papaverine hydrochloride. Unless indicated otherwise the doses are expressed in terms of their salts.

Statistical analysis

The two-tailed t test for paired differences was used to assess the significance of the progressive increase in effects after successive doses of a drug. The tests used for comparing the difference of two means are based on unknown variances which cannot be assumed equal (Natrella, 1963). The level of significance chosen was α=0·05.

Results

Tracheal pressure fluctuations and pulmonary arterial pressure

Four successive additions of 1 μg of ^{14}C-histamine dihydrochloride caused markedly increasing effects both on the amplitude of the tracheal pressure fluctuations and on the pulmonary arterial perfusion pressure (Fig. 2). Moreover, the tracheal pressure fluctuations did not completely return to their original amplitude. Figure 2 shows that this partial irreversibility was even more marked when a dose of 25 ng of bradykinin was given before the histamine. It was suppressed to a large extent by the addition of phenylbutazone (10 μg/ml) to the perfusion fluid. Table 1 shows the mean increase in the effects after four successive doses of histamine, with and without a preceding low dose of bradykinin (25 ng); the effects of repeated doses of 5-hydroxytryptamine are also shown. Under these conditions the increase in the histamine effects was not changed by the low dose of bradykinin. There is a great difference in the duration of the inhibition of tracheal pressure fluctuations induced by histamine and 5-hydroxytryptamine. This duration was measured from the injection of the substance under test until the pressure fluctuations had returned to a steady level. After the fourth addition of histamine, with or without bradykinin, the duration of the inhibition of tracheal pressure fluctuations lasted respectively on an average 191 and 171 s longer than after the first

TABLE 1. *Increase in effects of repeated doses (4x) of histamine (H), with and without bradykinin (B), and 5-hydroxytryptamine (5-HT) on tracheal pressure fluctuations (TP) and pulmonary arterial pressure (PAP), and the influence of phenylbutazone (10 μg/ml perfusion fluid)*

Treatment	*n*	Phenylbutazone	Inhibition of TP				Increase in PAP	
			IV		IV-I		IV	IV-I
			decrease (%)	duration (sec)	decrease (%)	duration (sec)	(cm H_2O)	(cm H_2O)
1 μg H	3	—	73± 23	267±57·3	54±19·6	171±57·3	3.5±2·0	2.5±1·8
1 μg H+25 ng B	6	—	82± 7·5	272±22·9	54±11·2	191±24·9	4.5±0·6	2.3±0·8
1 μg H+25 ng B	3	+	55±12·9	90±22·7	43±12·2	39±21·0	0.9±0·3	—0.4±0·3
1 μg 5-HT	6	—	84± 5·5	141±19·0	46± 5·2	29±20·7	2.6±0·6	0.9±0·6
1 μg 5-HT	6	+	64± 6·9	131±16·8	46± 9·4	19±27·7	1.9±0·2	0.9±0·2

The mean effects (±S.E.M.) after the fourth dose in the course of each treatment and their increase over the first dose are indicated respectively by IV and IV-I.

addition, whereas this difference averaged 29 s with 5-hydroxytryptamine (Table 1). This increase in duration of the histamine effect, in particular, was significantly suppressed by phenylbutazone (10 μg/ml).

A significantly greater difference in increase of arterial pressure between the fourth and first dose was found for histamine than for 5-hydroxytryptamine (Table 1). The pressor effect of histamine as well as its progressive increase after successive doses, was suppressed by phenylbutazone, whereas that of 5-hydroxytryptamine remained essentially unaltered.

Bradykinin causes a bronchoconstrictor effect in guinea-pig lungs *in vivo* (Collier, Holgate, Schachter & Shorley, 1959; 1960), while responses to repeated doses of bradykinin decline (Collier & Shorley, 1960). In the present investigation on

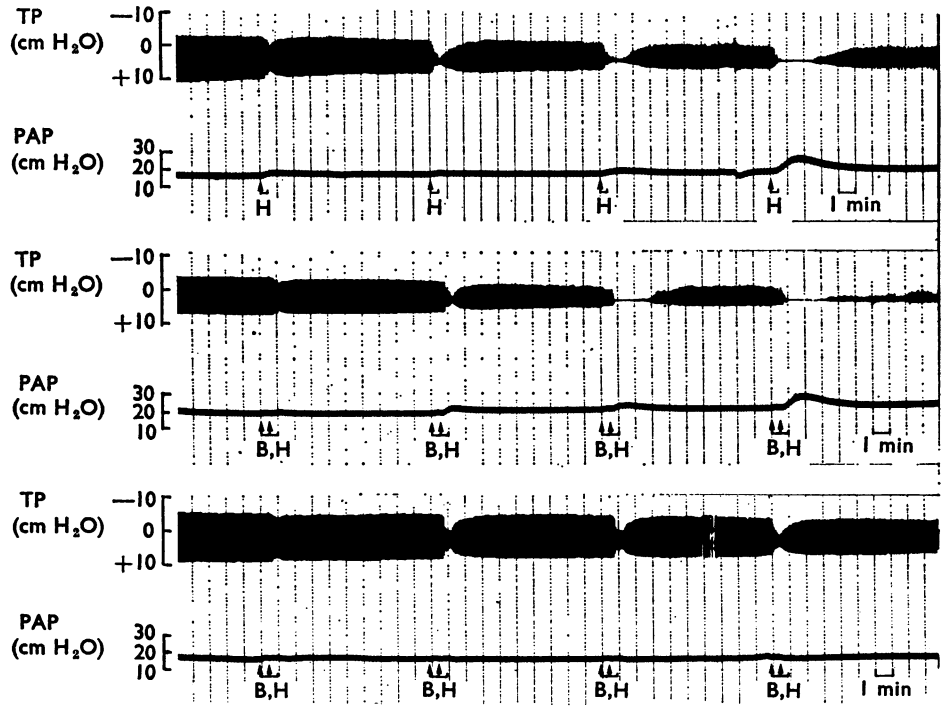

FIG. 2. Tracheal pressure fluctuations (TP) and the pulmonary arterial perfusion pressure (PAP) of isolated lungs of the guinea-pig, to which four successive doses of 1 μg of histamine dihydrochloride (H) alone (upper curves), and in combination with a preceding dose of 25 ng bradykinin (B) (middle and lower curves) were given at 10 min intervals. In the third experiment (lower curves) phenylbutazone (10 μg/ml) had been added to the perfusion fluid. Time scale, 1 min.

TABLE 2. *Mean effects* (±S.E.M.) *of repeated administration* (6*x*) *of bradykinin on tracheal pressure fluctuations* (*TP*) *and the pulmonary arterial pressure* (*PAP*)

	n	I	II	III	IV	V	VI
Decrease in TP (%)	4	49 ±9·2 (ir)	44 ±13·7	23 ±10·2	35 ±16·2	42 ±17·6	53 ±16·9
Increase in PAP (cm H_2O)	4	1·7±0·85	0·5± 0·04	0·5± 0·17	0·4± 0·09	0·8± 0·32	1·7± 0·80

The successive doses are indicated by roman numerals. The percentage decrease in TP is based on the amplitude measured in the last min before each addition. The case where this inhibition was irreversible is indicated by (ir).

isolated lungs with vascular perfusion, the first addition of 25 ng bradykinin caused a small vasoconstrictor effect without any change in the amplitude of the tracheal pressure fluctuations. Therefore, the effect of repeated doses of 75 ng bradykinin was studied. The mean results of 4 experiments, in which 6 doses of bradykinin were administered at 10 min intervals, are summarized in Table 2. The standard errors indicate that there was a great variation in the sensitivity to bradykinin. The mean vasoconstrictor effect fell after the first dose and rose after the fourth dose but none of these differences were statistically significant. The changes in tracheal pressure fluctuations were also not statistically significant.

Uptake of ^{14}C-histamine in the tissues

The level of radioactivity found in the effluent of the experiments referred to in the three upper rows of Table 1 is shown in Figs. 3a, b and c. The radioactivity in cpm/ml effluent of each fraction collected after the 1st, 2nd, 3rd and 4th doses of ^{14}C-histamine is plotted against time. Most of the added ^{14}C-histamine appeared in the lung effluent within the first min after commencing injection. The mean fractions of added radioactivity remaining in the lung after this first min following the first and fourth additions are summarized in Table 3. None of these values differ significantly from each other. Therefore the progressive increase in

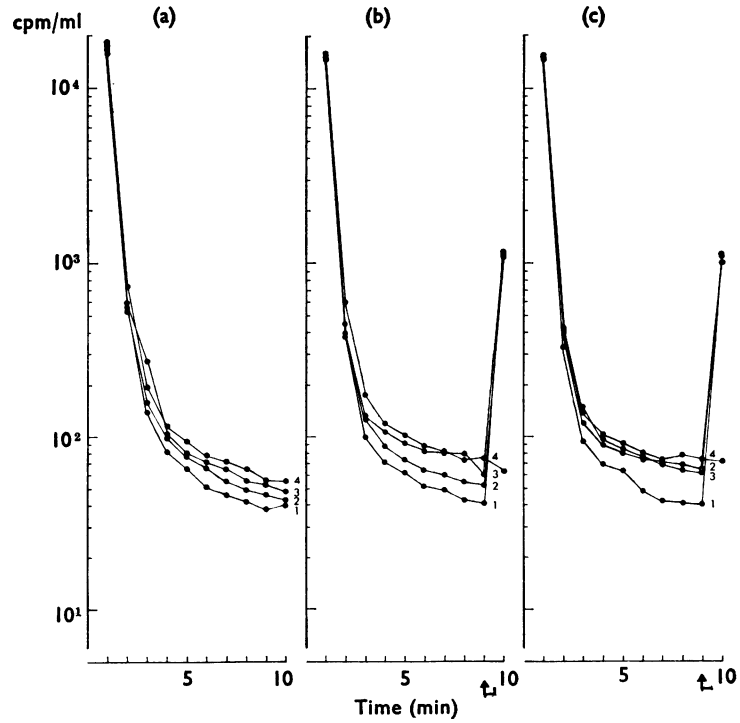

FIG. 3. Radioactivity per ml effluent from isolated vascular perfused lungs of the guinea-pig after four successive doses of 1 μg ^{14}C-histamine (a) without, and (b) and (c) with a preceding addition (indicated by arrow) of 25 ng of bradykinin. In (c) 10 μg of phenylbutazone per ml was added to the perfusion fluid. The curves after the 1st, 2nd, 3rd and 4th doses of ^{14}C histamine are numbered 1, 2, 3 and 4 respectively. Each point in (a) and (c) is the mean of 3 experiments, in (b) of 6 experiments.

effect after repeated doses of histamine on the one hand and the suppressing influence of phenylbutazone on this increase on the other hand cannot be ascribed to changes of the amounts of histamine 'extracted' by the tissues within the first minute of the determination. In agreement with this is the finding that the mean amount of ^{14}C recovered from the lungs by combustion at the end of the experiment, was 1·0 and 1·1% of the total load, respectively, in experiments with and without phenylbutazone in the perfusion fluid. A further finding was that the outflow of radioactivity per min increased considerably after addition of 25 ng of bradykinin. This increase was neither significantly influenced by successive administrations nor by phenylbutazone (Figs. 3b and c). Amidopyrine (10 μg/ml) was also found ineffective in 3 experiments.

Outflow of ^{14}C from the lungs

Since in the preceding experiments the dose of bradykinin was followed at once by radioactive histamine, the duration and course of the effect of bradykinin on the outflow of radioactivity could not be studied. Therefore, in a further 6 experiments, the lungs were loaded with 2 μg of ^{14}C-histamine in both the 1st and 21st min; while 25 ng of non-labelled bradykinin was added in the 11th and 31st minute. Table 4 shows that the mean ($\pm$S.E.M.) radioactivity present in the effluent collected during the min before the administration of bradykinin (control period) was $83 \pm 6{\cdot}2$ cpm/ml, i.e. $0{\cdot}245 \pm 0{\cdot}017$% of the amount of radioactivity added 9 min before. During the min when bradykinin was injected the outflow increased up to $2{,}168 \pm 81{\cdot}6$ cpm/ml effluent, that is about 26 times more than in the control period. This increase was short-lasting; within 3 min the outflow of radioactivity had returned to its original value. To exclude a possible vasomotor influence of bradykinin on this increase in outflow of radioactivity, the experiments were repeated in the presence of papaverine (25 μg/ml). This had no significant influence on the outflow of radioactivity induced by bradykinin (Table 4). Moreover, the influence of bradykinin was studied in the same way on the outflow of the acid dye phloxin (sodium salt of tetrabromotetrachlorofluorescein, mol wt 829·7). After addition of 0·1 ml of a 1% solution of this dye the effluent was colourless to the eye within 1·5 s; after 9 min, less than one-thousandth of the dose could be detected spectrophotometrically. This trace was not increased by addition of bradykinin. Therefore it was concluded that the most likely explanation for the increase in outflow of radioactivity caused by bradykinin was an increased leakage of radioactivity from the interstitial spaces, and not a liberation of radioactivity remaining in recesses of the vascular system. Moreover, histamine and 5-hydroxytryptamine, two other substances known to increase vascular permeability, also increased the outflow of radioactive material (Table 4). On a molar basis brady-

TABLE 3. *Mean fractions* ($\pm$S.E.M.) *of added amounts of radioactivity remaining in the lungs one min after starting the injection of ^{14}C-histamine; the injection lasted* 30 *sec. The roman numerals indicate the number of the addition*

Treatment	*n*	Fraction remaining in lungs in % of added dose I	IV
Histamine (1 μg)	3	$18{\cdot}1 \pm 11{\cdot}0$	$13{\cdot}7 \pm 9{\cdot}8$
Histamine (1 μg)+bradykinin (25 ng)	6	$19{\cdot}7 \pm 10{\cdot}7$	$17{\cdot}4 \pm 5{\cdot}3$
Histamine (1 μg)+bradykinin (25 ng) in the presence of phenylbutazone	3	$22{\cdot}0 \pm 4{\cdot}1$	$18{\cdot}0 \pm 5{\cdot}0$

TABLE 4. *Mean increase (±S.E.M.) in outflow of radioactivity from isolated lungs of the guinea-pig induced by bradykinin, histamine and 5-hydroxytryptamine (5-HT). The lungs were loaded 10 min before each administration of drug. In all experiments both loading and administration of drug were done twice in succession, except in those marked with an asterisk. In some groups of experiments papaverine (25 μg/ml) was added to the perfusion fluid to suppress vasoconstrictor effects of the drugs*

Loading	*n*	Papaverine added	Drug	Cpm/ml effluent			
				Last min before	During and after addition		
					1st min	2nd min	3rd min
^{14}C-Histamine (2 μg)	6	—	25 ng Bradykinin	83±6·2	2,168± 81·6	369±60·6	108±11·9
,,	3	+	25 ng Bradykinin	40±3·8	2,175±252·8	237±20·5	51± 2·3
(*) ,,	5	—	250 ng Histamine	68±4·9	2,266± 56·0	111± 5·0	98±11·5
^{14}C-Histidine (2 μg)	3	—	25 ng Bradykinin	51±1·8	1,199± 86·2	53± 3·4	31± 0·8
,,	3	—	500 ng 5-HT	38±1·2	1,843± 36·8	76± 2·0	39±2·8
^{14}C-Histidine (1 μg)	3	+	250 ng Histamine	36±4·1	1,413± 81·1	82±12·1	47± 5·8

TABLE 5. *Effects of 4 successive doses of 1 μg histamine and 1 μg 5-hydroxytryptamine base, on the weight of guinea-pig lungs compared with effects on pulmonary arterial perfusion pressure (PAP); the influence of phenylbutazone (10 μg/ml perfusion fluid) is also shown. The mean effects (±S.E.M.) of the fourth dose of each drug and the increase in effect relative to that of the first dose are indicated respectively by IV and IV-I*

Treatment	Phenylbutazone	*n*	Increase in PAP (cm H_2O)		*P*-value	Increase in weight (g)		*P*-value
			IV	IV-I		IV	IV-I	
Histamine	—	6	10·6±1·16	9·0±1·08	<0·05	0·65±0·048	0·55±0·053	<0·05
Histamine	+	6	3·4±0·67	2·2±0·35		0·15±0·056	0·11±0·037	
5-Hydroxytryptamine	—	6	1·6±0·41	0·5±0·23	>0·05	0·09±0·093	0·06±0·075	>0·05
5-Hydroxytryptamine	+	6	1·7±0·27	0·9±0·23		0·05±0·026	0·05±0·022	

kinin was about 33 times more active than histamine. In our experiments the sensitivity of the guinea-pig lungs to histamine and 5-hydroxytryptamine was of the same order in contrast to the findings of Wilhelm (1962). In three series of experiments the lungs were loaded with ^{14}C-histidine instead of ^{14}C-histamine. No explanation can be given for the smaller amount of radioactivity in the effluent in these experiments (Table 4), even though 2 μg of the ^{14}C-histidine solution used contained 0·02 μCi less than the same dose of ^{14}C-histamine solution.

Effects on the weight of the lungs

A marked increase in effect is seen after repeated doses of histamine, but such an increase does not occur after repeated doses of 5-hydroxytryptamine. If the increasing effect of histamine is caused by accumulation of fluid in the lungs, then a gain in weight should be found. The effect of repeated doses of these amines on the weight of the lungs and on the perfusion pressure was therefore investigated. In 6 experiments histamine and 5-hydroxytryptamine, both in a dose of 1 μg base, were compared, as shown in Figure 4. After the successive doses of histamine the increase in perfusion pressure rose progressively, as did the duration of the effect. The progressive effect on the weight was even more pronounced; after the 3rd and 4th dose the weight did not return to its original level within the interval of 10 min between the doses. In contrast, the effect of 5-hydroxytryptamine was not increased. Although the rise in perfusion pressure after the first dose was not

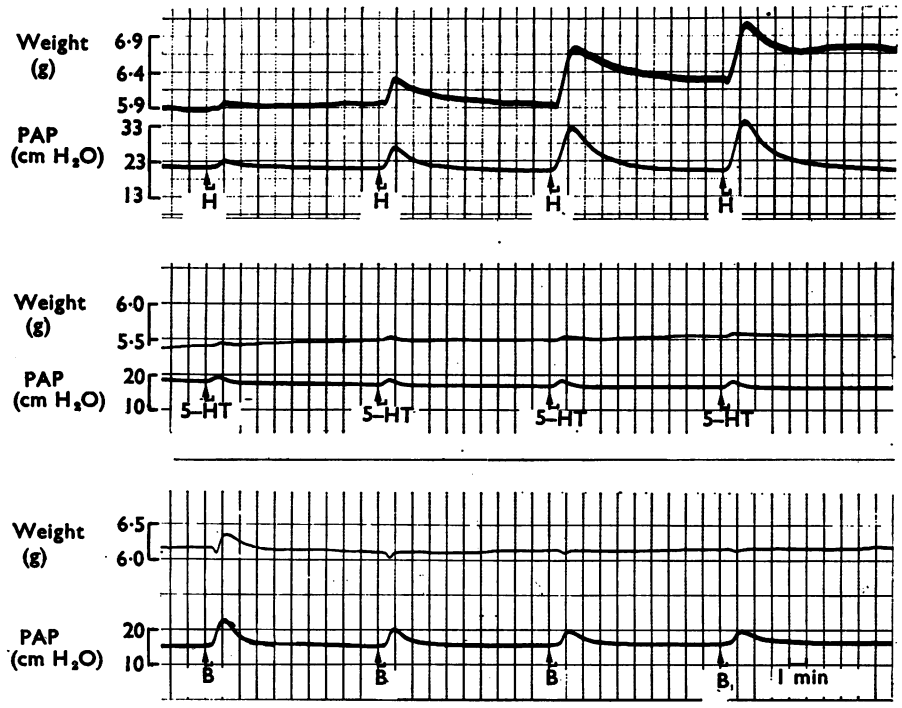

FIG. 4. Changes in weight and pulmonary arterial perfusion pressure (PAP) of isolated lungs of the guinea-pig, to which four successive doses of 1 μg of histamine base (H) (upper tracings); 1 μg of 5-hydroxytryptamine base (5-HT) (middle tracings), and 75 ng of bradykinin (B) (lower tracings) were added. Time scale, 1 min.

much different from that after the first dose of histamine, this rise remained practically unaltered after subsequent additions of 5-hydroxytryptamine. The results of the experiments are summarized in Table 5. In agreement with the previous experiments, phenylbutazone (10 μg/ml perfusion fluid) strongly suppressed the progression of the effects of histamine, whereas the small progression found after repeated doses of 5-hydroxytryptamine was not significantly influenced by phenylbutazone (Table 5). Bradykinin in a dose of 25 ng caused a small vasoconstrictor effect only after the first addition and had hardly any effect on the weight of the lungs. A dose of 75 ng of this polypeptide produced a considerable vasoconstriction of the lung vessels, which decreased upon successive additions as shown in Fig. 4. In three experiments the mean rise in perfusion pressure was successively: 7·3; 4·0; 2·7 and 2·2 cm water. The influence of the first dose of 75 ng bradykinin on the weight was biphasic; in all 3 experiments a slight decrease in weight (0·05 g on average) was followed by an increase (0·27 g on average). Subsequent additions

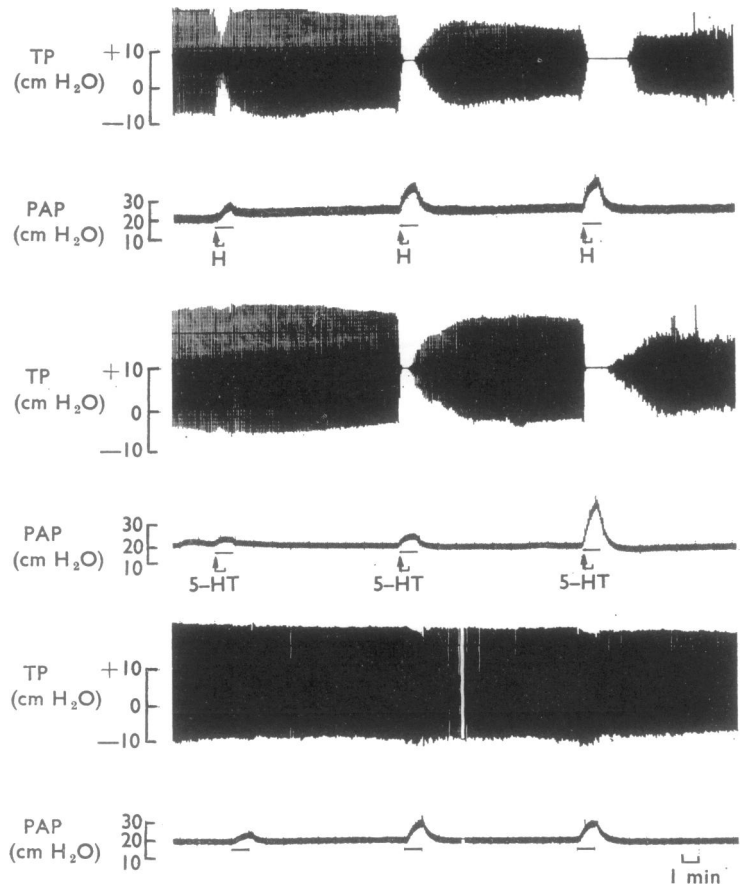

FIG. 5. Tracheal pressure fluctuations (TP) and pulmonary arterial perfusion pressure (PAP) of isolated lungs of the guinea-pig perfused with Krebs solution, to which phenylbutazone (10 μg/ml) was added. The left atrial outflow was restricted to approximately the same extent during 3 periods of 1 min at 10 min intervals, as indicated by the horizontal bars. This restriction of outflow was combined with the administration of 1 μg of histamine base (H) (upper tracings); or with 1 μg of 5-hydroxytryptamine base (5-HT) (middle tracings). No drug was added in the control experiments (lower tracings). Time scale 1 min.

usually caused only a small decrease in weight. In the presence of phenylbutazone the effects of bradykinin on both the perfusion pressure and the weight were completely abolished.

Effect of increased atrial pressure

Since the progressive, partly irreversible increase in effects caused by repeated doses of histamine were related to an increase in weight and were suppressed by phenylbutazone, it was assumed that an accumulation of fluid in the lungs took place caused by a rise in hydrostatic pressure induced by histamine. To obtain evidence for this assumption the influence of an artificially increased pressure on the responses to repeated additions of histamine and of 5-hydroxytryptamine in the presence of phenylbutazone (10 μg/ml perfusate) was investigated. Phenylbutazone was added to suppress the vasomotor effects of the amines. The atrial pressure was increased four times for one minute, starting at the moment the drug under test was added. In three control experiments, in which neither histamine nor 5-hydroxytryptamine was added, the increased atrial pressure in itself did not affect the tracheal pressure fluctuations (Fig. 5, lower curves). Combination of elevation of atrial pressure with the administration of either 1 μg of histamine base or 1 μg of 5-hydroxytryptamine base in the presence of phenylbutazone caused a progressive increase in the height and duration of the effect on the tracheal pressure fluctuations. Furthermore, this inhibition was partly irreversible. In four experiments the mean increase in the duration of the inhibitory effect on the tracheal pressure fluctuations was 207 s after 3 administrations of histamine and 228 s after 3 administrations of 5-hydroxytryptamine. In spite of the fact that the impairment of the left atrial flow was approximately the same in all experiments, the rise of the pulmonary arterial pressure was increasing during each successive treatment, especially when either histamine or 5-hydroxytryptamine was added (Fig. 5). This progression after successive treatments is probably a secondary effect due to a decrease in compliance caused by fluid accumulation.

Discussion

Histamine, 5-hydroxytryptamine and bradykinin cause broncho- and vasoconstriction in isolated lungs, and furthermore, increase the vascular permeability. However, upon repeated administration of these substances to isolated guinea-pig lungs performed through the pulmonary artery, a difference between these compounds was observed. The effect of histamine on both the tracheal pressure fluctuations and the pulmonary arterial pressure increased progressively and became partly irreversible ; this progression of the histamine effects as well as the vasoconstrictor action of histamine, was suppressed by phenylbutazone. The effect of 5-hydroxytryptamine, on the other hand, was not progressive and the vasoconstriction was not affected by phenylbutazone.

After successive administrations of bradykinin the lung vessels seemed to be most sensitive to the first dose and less sensitive to subsequent doses. The tracheal pressure fluctuations decreased slowly and gradually and did not return to their original height after the first dose. From the second (sometimes the third dose) up to and including the sixth dose, the inhibitory effect on the tracheal pressure set in more rapidly and became completely reversible. This pattern of responses is more or less the opposite to that found after repeated doses of histamine, and

thus it is concluded that the reversible decrease in amplitude is probably due to a bronchoconstrictor effect. This conclusion assumes that another causal factor must be involved in the slow irreversible decrease in amplitude, found after the first addition of bradykinin.

Experiments with ^{14}C-histamine demonstrated that the fractions of added radioactivity remaining in the lungs after 1 min were similar for the successive doses of histamine alone or combined with a preceding small dose of bradykinin. However, bradykinin itself caused a large increase in the outflow of radioactivity which was not affected by either phenylbutazone or papaverine. Non-labelled histamine and 5-hydroxytryptamine also had a similar effect on lungs loaded 10 min earlier with either ^{14}C-histamine or ^{14}C-histidine. This effect seems to be more or less similar in magnitude for the 3 compounds in the concentrations used and was not affected by papaverine. Since the lung vessels were perfused with about 200 ml of salt solution during this period of 10 min and since most of an intra-arterial addition of 1 mg of phloxin passed the vascular bed within 1·5 s, the increased outflow of radioactivity is probably due to an induced increase in leakage of radioactivity from the interstitial spaces.

Histamine, in contrast to both 5-hydroxytryptamine and bradykinin, caused a progressive increase in both the weight and pulmonary arterial pressure. Thus, repeated doses of histamine probably cause an accumulation of fluid in the lungs, an effect that is suppressed by phenylbutazone. If the accumulation of fluid is caused essentially by an increased pressure in the blood vessels the permeability of which has been increased, these results can only be explained if histamine acts on different sites in the pulmonary vascular bed from 5-hydroxytryptamine and bradykinin. Gilbert, Hinshaw, Kuida & Visscher (1958) demonstrated, using isolated perfused dog lungs, that histamine usually caused a greater increase in the venous resistance than in the arterial resistance, whereas 5-hydroxytryptamine usually increased the arterial resistance more than the venous resistance. They also found a good correlation between the change in lung weight and the change in venous resistance.

With bradykinin the reaction of the lung vessels to the first dose was found to be quite different from that to succeeding doses. The first dose caused a marked vasoconstriction and had a biphasic effect on the weight ; a short decrease followed by a longer lasting increase. This indicates that the first dose of bradykinin may cause vasoconstriction at two sites in the vascular bed. The succeeding doses, only causing a decrease in weight, may then act on a precapillary site in the vascular bed as Hauge, Lunde & Waaler (1966) concluded from their experiments on the rabbit.

The determination of weight does not differentiate between different causes of the weight changes, e.g. it is impossible to obtain an estimate of the part of the increase in weight due to increased filling of the vessels. The magnitude of this filling capacity depends on the distension of the vessels, which in its turn may be counteracted by the hydrostatic pressure in the interstitial spaces due to an influx of fluid. Lunde & Waaler (1969) demonstrated, using isolated perfused lung preparations, that an increase in venous pressure in the left atrium up to about 15 mmHg caused a rapid increase in weight up to a stable level, indicating a transvascular flux of fluid until a new transvascular fluid equilibrium was reached. When the pressure in the left atrium, and hence the capillary hydrostatic pressure, was elevated above a certain level and maintained long enough then the weight

gradually increased, leading to visible oedema formation. When the elevated pressure was decreased again Lunde & Waaler (1969) saw first a rapid fall in weight followed by a slowly diminishing decrease. However, in this case the weight did not return to its original level. This first phase was thought to reflect a reduction in vascular capacity and the second a gradual, incomplete reabsorption of transvascular fluid.

In the experiments on weight presented in this paper the increase induced by histamine in both weight and vascular pressure fell quite slowly and in most cases returned to the original levels after the first and second dose (Fig. 4). To what extent this gradual decrease in weight is due to a reduction in vascular capacity is unknown. However, after the third and fourth doses the changes in weight did not correspond to the changes in vascular pressure. The pulmonary arterial pressure now rose to an average of 24·7 and 28·6 cm H_2O respectively. By this increase the critical level for irreversible fluid accumulation in interstitial spaces may have been passed since the weight did not return to its original level. It should be noted that this did not lead to visible fluid accumulation in the trachea.

From these findings the following conclusions can be drawn; first, the steady progress and irreversibility of the effect of histamine is caused by an accumulation of fluid in the interstitial spaces caused by a combination of increased permeability and increased intravascular pressure. Second, the effect of phenylbutazone on the accumulation of fluid in the lungs occurring after repeated administration of histamine is due to its inhibitory activity on the vasoconstrictive action of histamine. These conclusions are supported by the finding that, in lungs continuously treated with phenylbutazone, short-lasting artificial venous pressure elevations concomitant with repeated administrations of histamine could reproduce the progressive increase in inhibitory effect on the tracheal pressure fluctuations. Although 5-hydroxytryptamine and bradykinin cause changes in vascular permeability similar to those produced by histamine, they do not cause an accumulation of fluid in the interstitial spaces, possibly because their vasoconstrictor effects, reflected in an increase in pulmonary arterial pressure, are located more in the arterial part of the vascular system, than are the vasoconstrictor effects of histamine. The fact that the response to 5-hydroxytryptamine also increased progressively, when the atrial outflow was restricted is another indication that the vasoconstriction of these substances normally did not result in a predominant increase in venous pressure.

We should like to thank Prof. Dr. C. van der Meer for his advice and helpful criticism during the investigation and the preparation of this manuscript.

REFERENCES

AARSEN, P. N. (1966). The influence of analgesic antipyretic drugs on the responses of guinea-pig lungs to bradykinin. *Br. J. Pharmac. Chemother.*, **27**, 196–204.

AARSEN, P. N. (1969). Effects of bradykinin and anti-inflammatory agents on the response of isolated guinea-pig lungs to histamine. *Acta physiol. pharmac. neerl.*, **15**, 223–242.

BHATTACHARYA, B. K. & DELAUNOIX, A. L. (1955). An improved method for the perfusion of isolated lung of guinea-pig. *Arch. int. Pharmacodyn.*, **101**, 495–501.

BUYSKE, D. A., KELLY, R., FLORINI, J., GORDON, S. & PEETS, E. (1966). Determination of tritium and C^{14} in biological samples by rapid combustion techniques. In: *Advances in Tracer Methodology*, Vol. 1, ed. Rothschild, S., pp. 185–191. New York: Plenum Press.

COLLIER, H. O. J., HOLGATE, J. A., SCHACHTER, M. & SHORLEY, P. G. (1959). An apparent bronchoconstrictor action of bradykinin and its suppression by some anti-inflammatory agents. *J. Physiol., Lond.*, **149**, 54P.

COLLIER, H. O. J., HOLGATE, J. A., SCHACHTER, M. & SHORLEY, P. G. (1960). The bronchoconstrictor action of bradykinin in the guinea-pig. *Br. J. Pharmac. Chemother.*, **15**, 290–297.

COLLIER, H. O. J. & SHORLEY, P. G. (1960). Analgesic antipyretic drugs as antagonists of bradykinin in the guinea-pig. *Br. J. Pharmac. Chemother.*, **15**, 601–610.

GILBERT, P. G., HINSHAW, L. B., KUIDA, H. & VISSCHER, M. B. (1958). Effects of histamine, 5-hydroxytryptamine and epinephrine on pulmonary hemodynamics with particular reference to arterial and venous segment resistances. *Am. J. Physiol.*, **194**, 165–170.

HAUGE, A., LUNDE, P. K. M. & WAALER, B. A. (1966). The effect of bradykinin, kallidin and eledoisin upon the pulmonary vascular bed of an isolated blood-perfused rabbit lung preparation. *Acta physiol. scand.*, **66**, 269–277.

LUNDE, P. K. M. & WAALER, B. A. (1969). Transvascular fluid balance in the lung. *J. Physiol., Lond.*, **205**, 1–18.

NATRELLA, M. G. (1963). *Experimental Statistics, National Bureau of Standards Handbook* 91, United States Department of Commerce, National Bureau of Standards, pp. 3–3.1.2. Washington D.C.: U.S. Government Printing Office.

WILHELM, D. C. (1962). The mediation of increased vascular permeability in inflammation. *Pharmac. Rev.*, **14**, 251–280.

(*Received January* 20, 1972)