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Removal of 5-hydroxytryptamine in the pulmonary circulation of rat isolated lungs

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Summary

1. Rat isolated lungs perfused via the pulmonary artery with Krebs solution removed 92% of the 5-hydroxytryptamine (5-HT) infused through it. This degree of removal was independent of concentration in the range from 5 to 100 g/ml.

2. The removal of 5-HT by the lungs was inhibited by amitriptyline and desmethylimipramine (10^{-6}–10^{-5}M).

3. The monoamine oxidase inhibitors, mebanazine and iproniazid (10^{-6}–10^{-5}M), inhibited the initial removal slightly, but their main effect was to preserve the 5-HT taken up and this 5-HT slowly reappeared in the effluent from the lungs. Tranylcypromine (5×10^{-7}–10^{-6}M) showed a combination of amitriptyline-like and mebanazine-like effects on the 5-HT removal in rat lung.

4. Experiments with ^{3}H-5-HT showed that although under normal conditions only 10% of the radioactivity appeared in the lung effluent as 5-HT within the first 5 min, the rest of radioactivity administered could be recovered in the effluent over 50 min as a metabolite, probably 5-hydroxyindoleacetic acid.

5. The following amines were without effect on the removal of 5-HT by rat lungs: noradrenaline (6×10^{-7}M), normetanephrine (5×10^{-6}M), metaraminol (10^{-6}M), reserpine (10^{-6}–10^{-5}M) and phenoxybenzamine (10^{-5}M).

6. We conclude that the removal of 5-HT by rat lungs involves a process of uptake and metabolism rather than one of uptake and storage, but this process is not the catecholamine Uptake$_2$. The cells involved in this process might be either capillary endothelial cells or septal cells.

Introduction

The lung has been shown to play an important part in the metabolism of several vaso-active hormones (Vane, 1969). 5-Hydroxytryptamine (5-HT) is removed from the circulation by the lungs of the anaesthetized dog (Davies & Wang, 1965; Thomas & Vane, 1967), and isolated cat's lung perfused with blood also removed 5-HT (Gaddum, Hebb, Silver & Swan, 1953). Axelrod & Inscoe (1963) showed that after intravenous injections of radioactive 5-HT in the mouse, about half the circulating 5-HT was bound and retained by tissues in the first few minutes, and the rest was metabolized by enzymatic deamination. The lung and spleen seemed selectively to take up and bind the labelled 5-HT for long periods of time, since unchanged 5-HT could be detected in these tissues for up to a week. Thus it seems that 5-HT is removed from the circulation by the lung primarily by an

uptake and storage process and not by enzymatic inactivation. However, Eiseman, Bryant, Waltuch & Lexington (1964) found that in dog isolated lungs perfused with blood, 5-HT added to the recirculating blood was metabolized by the lungs to 5-hydroxyindoleacetic acid (5-HIAA) within 60 min. Since we found that isolated lungs of the rat perfused with Krebs solution also removed 5-HT we have studied this effect in rat lung in more detail, in an attempt to understand the mechanisms involved. A preliminary account of this work was given to the British Pharmacological Society (Alabaster & Bakhle, 1970).

Methods

Perfusion of lung and bioassay methods

Contractions of the rat stomach strip preparation (Vane, 1957) were used to assay 5-HT. Removal of noradrenaline by the lung was measured, either by relaxations of the rat stomach strips or contractions of spirally cut strips of rabbit aorta. One to three assay tissues were superfused in a cascade system with Krebs bicarbonate solution ($NaHCO_3$, 25 mM ; NaCl, 120 mM ; KCl, 4·7 mM ; $CaCl_2$, 2·5 mM ; KH_2PO_4, 1·2 mM ; $MgSO_4$, 1·2 mM ; glucose, 5·6 mM).

The assay tissues were allowed to equilibrate before the lungs were included in the circuit.

Wistar rats of either sex weighing between 150 and 200 g were anaesthetized with pentobarbitone (60 mg/kg intraperitoneally). The thorax was opened and heparin (500 i.u.) given intracardially. The animal was bled. For perfusion the pulmonary artery was cannulated with a flexible polythene cannula via a slit in the right ventricle. Most of the heart was cut away, including the left atrium, to allow the perfusion fluid to flow out freely. The trachea was cannulated, the lungs were dissected free. The lungs were flushed free of blood, inflated, and suspended by the trachea inside a funnel shaped chamber. The lungs were perfused at 8 ml/min via the pulmonary artery with oxygenated Krebs bicarbonate solution at 37° C. The lung perfusate was superfused over the assay tissues in the cascade below the lung chamber. The amount of 5-HT removed by the lungs was determined by comparing the contractions of the rat stomach strips to infusions of 5-HT made directly into the fluid superfusing the assay tissues with responses to infusions of 5-HT made into the pulmonary arterial cannula. Infusions were given for 3–5 min at 0·1–0·2 ml/min. The perfused lungs lasted for 1–3 h after which they became oedematous and were discarded.

Perfusion pressure was measured with a Statham pressure transducer attached to a side arm above the pulmonary arterial cannula. Contractions of the assay tissues were recorded with auxotonic levers fitted to Harvard transducers on a Watanabe type WTR 281 6-channel pen recorder.

Radioactivity experiments

Uniformly labelled tritiated 5-HT was used (Radiochemical Centre, Amersham: specific activity 6·2 Ci/mmol). The tritiated 5-HT was diluted with unlabelled 5-HT before use. During and after an infusion of tritiated 5-HT through the lungs, the lung perfusate was collected by an automatic fraction collector at 0·5 min intervals for the first 5 min after the start of the infusion, then at 1 min intervals for 5 min and then every 2 min. Aliquots (0·1 ml) of the fractions were added to

15 ml of Diotol liquid scintillator (Herberg, 1960) and the radioactivity counted in a Packard Tri-Carb spectrometer (model 315 EX).

In some experiments radioactive inulin (carboxyl ^{14}C-inulin, New England Nuclear, 1·54 mCi/g) was infused at a final concentration of 3 μCi/ml for 4 min. The perfusate was collected as before.

Chromatographic separation of 5-HT and metabolites

The samples of lung perfusate collected after an infusion of tritiated 5-HT through the lung were combined for the periods 0–2·5 min, 2·5–5 min, 5–10 min and 10–20 min, and then evaporated under reduced pressure to a fifth of their original volume. The concentrated perfusate was applied to Whatman cellulose phosphate P.81 ion exchange paper in volumes of 10–30 μl. The chromatogram was developed in a 0·2 M ammonium acetate/isopropanol (v/v 2:1) ascending solvent system at pH 6. In some experiments, Whatman diethyl amino-ethyl cellulose paper (DE 81) was used and developed in the same solvent system at pH 7·5. The chromatograms were developed for approximately 4 h (12 cm solvent front). The papers were dried and cut in 1 cm wide strips, and the strips added to vials containing 15 ml diotol and the radioactivity measured by liquid scintillation. In each experiment the results were corrected for the recovery of tritiated 5-HT added to control perfusate and carried through the evaporation and chromatography procedures. The average recovery was 72% $\pm$3·9 and no metabolite was detected. Unlabelled 5-HT and 5-HIAA, used as reference compounds, were detected on the chromatograms by spraying with Ehrlich's reagent (1% *p*-diethylaminobenzaldehyde in 96% ethanol) and exposure to hydrochloric acid vapour for 3–5 min.

Drugs

The following drugs were used: amitriptyline hydrochloride (Roche); desmethylimipramine hydrochloride (Geigy); iproniazid phosphate (Roche); 5-hydroxytryptamine creatinine sulphate (B.D.H.); (−)-noradrenaline (B.D.H.); mebanazine oxalate (I.C.I.); tranylcypromine sulphate (S.K.F.); reserpine (Ciba); (±)-normetanephrine hydrochloride (Calbiochem); metaraminol tartrate (Merck, Sharp & Dohme); phenoxybenzamine hydrochloride (S.K.F.); pentobarbitone sodium (Abbott); heparin (Boots); methysergide bimaleate (Sandoz); propranolol (I.C.); phentolamine mesolate (Ciba). All drugs were dissolved in saline (0·9% NaCl, w/v), except reserpine which was dissolved in 20% ascorbic acid solution (w/v) and then diluted with saline, and phenoxybenzamine which was dissolved in a small volume of ethanol and then diluted with saline.

Doses are given in molar concentrations or as the weight of the base per ml, and refer to the final concentration of the substance.

Results

The phrase " the removal of 5-HT " is used to describe the decrease in biological activity occurring when 5-HT passes through the pulmonary circulation of the rat isolated lung. It is used in preference to " uptake " or " inactivation " because both of these processes occur to varying degrees in the overall decrease according to the conditions of the experiment.

TABLE 1. *Removal of 5-HT by rat lungs and effect of drugs*

Drug	Concentration (M)	% 5-HT removed ±S.E.M.	% inhibition	No. of experiments	Significance *t* test value of *P*
Controls	—	92·3±0·3	—	57	
Amitriptyline	10^{-6}	82·5	14·1	2	
	5×10^{-6}	62·5	32·7	2	
	10^{-5}	52·4±2·7	44·3	5	<0·001
Desmethylimipramine	10^{-5}	55	40·5	2	
Mebanazine	10^{-5}	87·7±1·4	4·35	7	<0·05
Iproniazid	5×10^{-5}	95	—	1	
	10^{-4}	91	—	3	
Tranylcypromine	5×10^{-7}	92	—	2	
	10^{-6}	90·3	—	3	
	5×10^{-6}	57·3	37·5	3	
Reserpine	10^{-6}	93·5	—	2	
	10^{-5}	91	—	3	
	Pretreated (see text)	91·6	—	3	
Normetanephrine	5×10^{-6}	92·3±0·8	—	6	<0·8
Metaraminol	10^{-6}	91·3	—	3	
Phenoxybenzamine	10^{-5}	90	—	3	
Noradrenaline	6×10^{-7}	90	—	3	

The concentration of 5-HT in most infusions was 2·5 or 5×10^{-8}M (10 or 20 ng/ml) and they were given for 3–5 min. The degree of removal of 5-HT has been calculated from the peak height of the contractions of the assay tissues (rat stomach strips). Rats pretreated with reserpine received 2·0 mg/kg 48 and 24 h before the lungs were used.

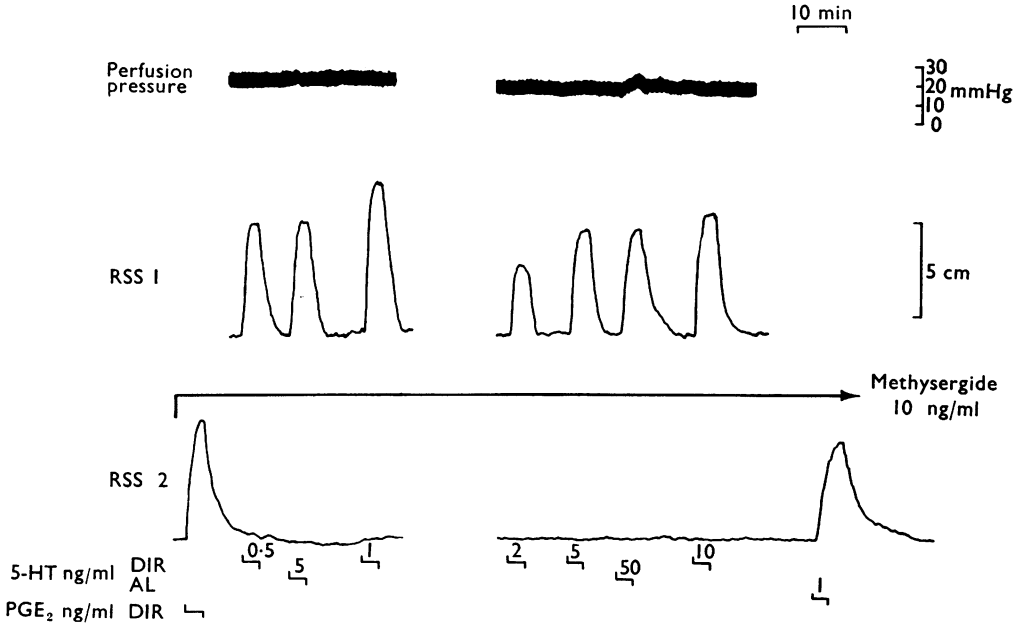

FIG. 1. Removal of 5-hydroxytryptamine by rat isolated lung. The top record shows lung perfusion pressure, and the middle and bottom records show contractions of rat stomach strips (RSS 1 and 2) superfused in series with effluent from lungs perfused with Krebs solution. Methysergide (10 ng/ml) was infused (as shown) continuously over the bottom preparation (RSS 2) to block responses to 5-HT. 5-HT was infused either directly to the assay tissues (DIR) or into the cannula in the pulmonary artery (AL). The same proportion of 5-HT was removed (90%) by the lung whether 5 ng/ml or 50 ng/ml 5-HT was infused. (Between the two sections, the load on the upper strip was increased and the recorder sensitivity reduced.) The lack of response of the lower strip showed that contractions of the upper strip were due to 5-HT and not to other substances released from or formed in the lungs. The lower strip was still sensitive to PGE_2 (1 ng/ml) during the methysergide infusion. Scales 10 min, 5 cm and 0–30 mmHg.

When first tested, 10–15 min after starting the perfusion, the lungs removed 92% (Table 1) of an infusion of 5-HT given into the cannula in the pulmonary circulation. The infusions were maintained until the assay tissues reached a steady contraction, which was usually 3–5 min. This high degree of removal of 5-HT was maintained for infusions repeated every 30 min for the duration of the experiment (1–3 h). The lungs also removed the same percentage of the 5-HT when the final concentration entering the lung was varied from 5 ng/ml to 100 ng/ml, although infusions of 10–40 ng/ml were used in most of the experiments. Concentrations up to 40 ng/ml did not increase the perfusion pressure which was usually 10–20 mmHg (1 mmHg≡1·333 mbar). Infusions of higher concentrations of 5-HT (50–100 ng/ml) increased the perfusion pressure by 5–20 mmHg. In Fig. 1, for instance, an infusion of 5-HT (5 ng/ml) through the lung (AL) is equipotent to 0·5 ng/ml given directly to the assay tissue (DIR); and no rise in perfusion pressure is seen. With 5-HT (50 ng/ml), the percentage removal is the same, although there is an increase in perfusion pressure of about 7 mmHg. Methysergide (10 ng/ml) allowed to flow over the assay tissues abolished the contractions of the rat stomach strips to infusions of 5-HT made directly to the assay tissues, and also to infusions of 5-HT through the lung, showing that the diminished contractor activity of such infusions was entirely due to residual 5-HT and not to other contractor substances released from the lung such as prostaglandins.

Effects of inhibitors of amine uptake

The tricyclic antidepressant drugs of the imipramine type inhibit catecholamine and 5-HT uptake in various systems (Todrick & Tait, 1969; Thoenen, Huerlimann & Haefely, 1964). Amitriptyline (10^{-6}–10^{-5}M) or desmethylimipramine (10^{-5}M) infused through the lung reduced the removal of 5-HT so that instead of 8%, between 20 and 50% of the infused 5-HT appeared in the perfusate (Table 1). These drugs were infused for 5–10 min before and during the 5-HT infusion through the lungs. An experiment with amitriptyline is illustrated in Fig. 2. An infusion of 5-HT (10 ng/ml) through the lungs was equipotent to about 5-HT (0·8 ng/ml) given directly to the assay tissues, representing a 92% removal. Amitriptyline

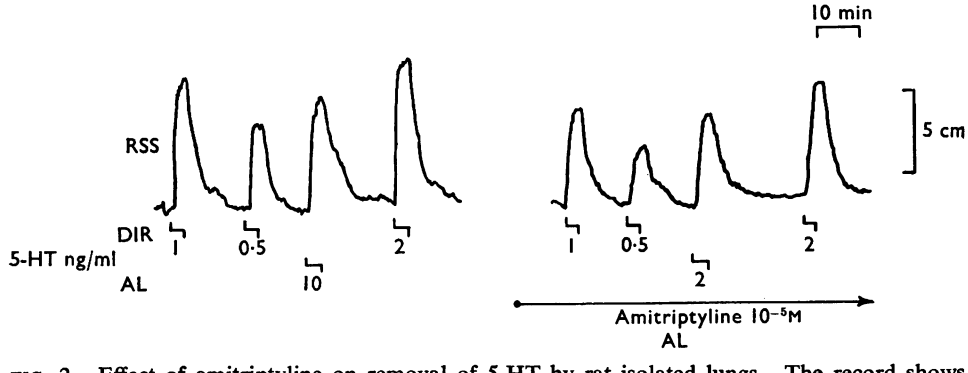

FIG. 2. Effect of amitriptyline on removal of 5-HT by rat isolated lungs. The record shows contractions of a rat stomach strip (RSS) superfused with effluent from lungs perfused with Krebs solution. 5-HT was infused directly to the assay tissue (DIR) or into the cannula in the pulmonary artery (AL). The first section shows about 92% removal of 5-HT in the untreated lung. Amitriptyline was then infused through the lungs and removal of 5-HT was reduced from >90% to about 50%. Scales, 10 min, 5 cm.

(10^{-5}M) infused through the lungs slightly reduced the sensitivity of the rat stomach strips to 5-HT. However, the infusion of 5-HT (2 ng/ml) through the lungs was now equivalent to about 1 ng/ml given directly, a removal of only 50%. Thus there was a six-fold increase in the proportion of 5-HT emerging from the lung. The effect of these drugs on 5-HT removal was essentially irreversible for the duration of the experiment.

Effect of monoamine oxidase inhibitors

The relevance of monoamine oxidase (MAO) to the pulmonary removal process for 5-HT is not established. Thus, although dog isolated lungs perfused with recirculated blood gradually oxidized exogenous 5-HT to 5-hydroxyindoleacetic acid (5-HIAA) (Eisemann *et al.*, 1964), Thomas & Vane (1967) found that the MAO inhibitor mebanazine was without effect on the proportion of 5-HT disappearing in the dog lung *in vivo*. However, MAO inhibition increased the 5-HT content of mouse lung after administration of the precursor 5-hydroxytryptophan (5-HTP) (Gershon & Ross, 1966). Several MAO inhibitors also block catecholamine uptake (Iversen, 1965a). We used mebanazine and iproniazid as examples of MAO inhibitors with little or no inhibition of catecholamine uptake, and tranylcypromine as a MAO inhibitor with uptake blocking properties (Iversen, 1965a). Mebanazine (10^{-5}M) infused before and during the passage of 5-HT through the lungs caused a small but reproducible inhibition of removal as shown by an increase in the height of contractions of the assay tissues, about twice as much 5-HT appearing in the perfusate as under normal conditions but iproniazid (10^{-4}M) did not change the initial removal of 5-HT. However, the most striking effect of both these MAO inhibitors was the prolongation of the contractions of the rat stomach strips after infusions of 5-HT through the lungs. The contractions persisted for 40–50 min as compared with a duration of 6–9 min in control lungs. There was no prolongation of the contractions induced by 5-HT infused directly to the stomach strips. Methysergide (10 ng/ml) abolished the prolonged contractions suggesting that they were due to 5-HT reappearing in the perfusate after an initial uptake into some structure in the lungs, and not due to the release from the lungs of other contractor substances, such as prostaglandins.

An experiment with mebanazine is shown in Fig 3. The two stomach strips showed that the removal of 5-HT by the lung was 92% before treatment of the lung with mebanazine. After the start of the mebanazine infusion, methysergide (10 ng/ml) was infused to reach the lower assay tissue only: this abolished the responses of the lower strip. The upper strip showed that the removal of 5-HT by the lung was now about 86% and the characteristic prolonged contraction was apparent. The response of the stomach strips to PGE_2 was not affected by this concentration of methysergide.

Tranylcypromine (5×10^{-7}—10^{-6}M) produced a small increase in the amount of 5-HT passing through the lung and a characteristic prolongation of the contractions of the stomach strips. At a higher concentration (5×10^{-6}M) an amitriptyline-like effect was seen ; there was a greater inhibition of removal and a less prolonged contraction of the assay tissues. This dual effect is illustrated in Fig. 4. The first panel shows that this lung removed 95% of 5-HT (10 ng/ml) infused through it. In the presence of tranylcypromine (10^{-6}M), the removal was reduced to 90% and there was a prolonged contraction of the assay tissue after the infusion of 5-HT

through the lung. In the last panel the tranylcypromine concentration was increased five-fold, and the removal of 5-HT was reduced to about 50%: the duration of contraction also reduced.

The results of these experiments suggested that 5-HT was removed by the rat lung by uptake into cells followed by either metabolism by monoamine oxidase,

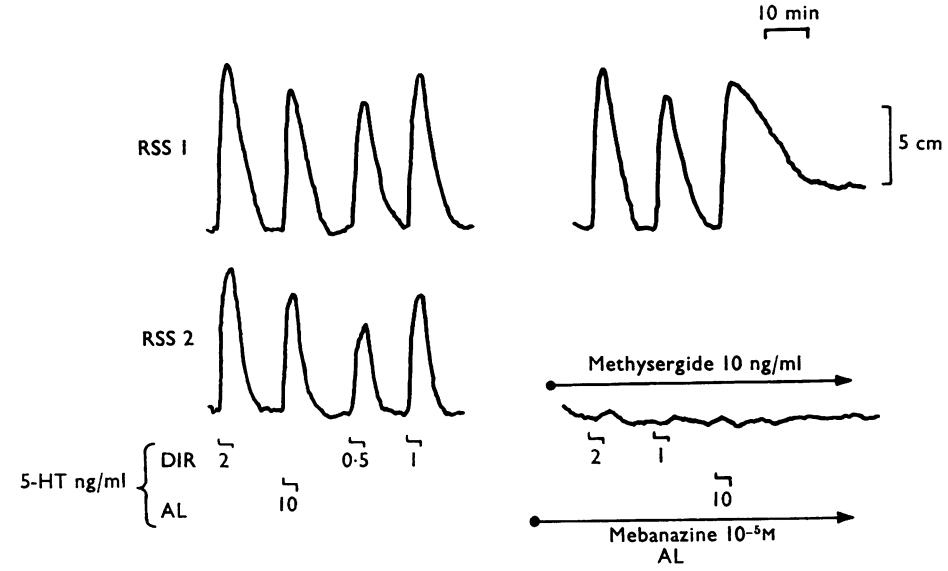

FIG. 3. Effect of mebanazine on removal of 5-HT by rat isolated lungs. The record shows contractions of two rat stomach strip preparations (RSS 1 and 2) superfused in series with the effluent from lungs perfused with Krebs solution. 5-HT infusions were given either directly to the assay tissues (DIR) or into the cannula in the pulmonary artery (AL). The first panel shows that the removal of 5-HT in the untreated lung was 90–95%. The second panel shows that when mebanazine was infused through the lungs as indicated, the lungs removed 80–90% of the 5-HT as judged by the peak of contraction of the upper stomach strip. However, the contraction of this tissue was prolonged. Methysergide infused over the lower assay tissue indicated that this prolonged response was due to 5-HT. Scales 10 min, 5 cm.

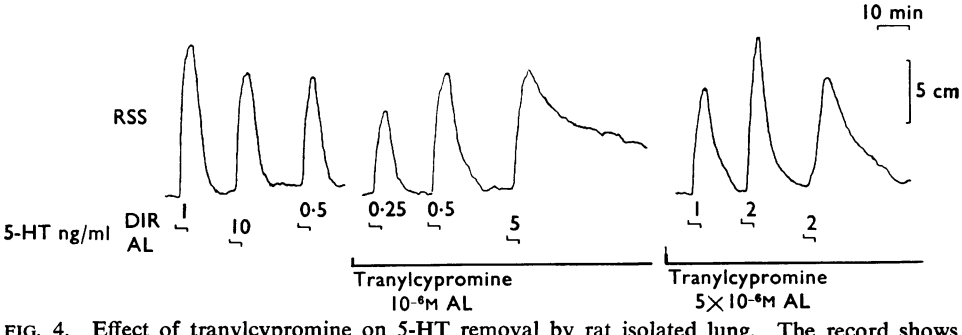

FIG. 4. Effect of tranylcypromine on 5-HT removal by rat isolated lung. The record shows contractions of a rat stomach strip (RSS) superfused with effluent from lungs perfused with Krebs solution. 5-Hydroxytryptamine (5-HT) infusions were given either directly to the assay tissues (DIR) or into the cannula in the pulmonary artery (AL). The first panel shows that the removal of 5-HT in untreated lungs was 94%. The second panel shows that tranylcypromine (10^{-6}M) infused through the lungs, slightly reduced the initial removal of 5-HT to 90% but prolonged the wash out of 5-HT from the lungs. The third panel shows the effect of a higher concentration of tranylcypromine, 5×10^{-6}M. Removal of 5-HT was reduced to about 50% and the prolonged contraction of the assay tissue was less marked. Scales, 10 min, 5 cm.

or storage, possibly in some type of granule. To decide between the relative importance of these alternatives, we repeated these experiments using radioactive 5-HT.

Experiments with radioactive 5-HT

When radioactive 5-HT was infused only to the assay tissues all the radioactivity was recovered and no metabolites could be detected. Radioactive 5-HT was infused through the lungs and after passing over the assay tissues the lung perfusate was collected in timed samples by an automatic fraction collector, as described in the methods. The radioactivity in the samples was measured by liquid scintillation spectrometry. The results of one such experiment are shown in Fig. 5 in which the radioactivity and the biological activity of the perfusate are plotted against time. The amount of radioactivity and biological activity entering the lung is indicated by the height of the bars. There was a substantial difference between the biological activity (about 10%) and the radioactivity (almost 50%), appearing in

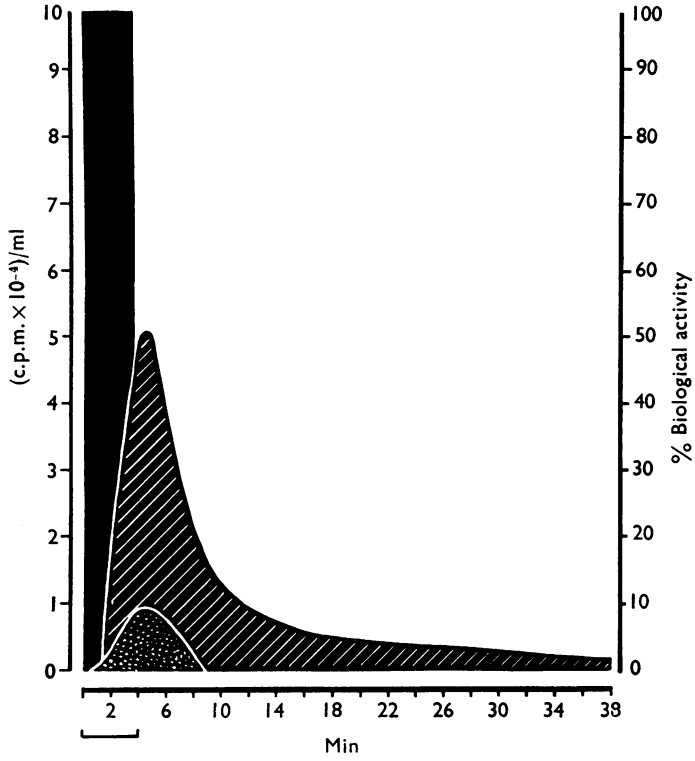

FIG. 5. Correlation between radioactivity and biological activity in the removal of ^{3}H-5-HT by rat isolated lungs. The solid black bar represents both the biological activity (as % of infused 5-HT) and the radioactivity (as (c.p.m. $\times 10^{-4}$)/ml) infused into the lungs. The horizontal line below the time axis represents the duration of the infusion (0–3 min). The hatched profile represents the radioactivity in the perfusate emerging from the lungs, sampled every 0·5 min from 0–5 min, every 1 min from 5–10 min, and every 2 min thereafter. The curve was drawn to join the experimental points so obtained. The stippled profile represents the contraction of the assay tissue (rat stomach strip) in response to the biological activity emerging from the lung. From the contraction of the stomach strip there is a peak concentration of 5-HT corresponding to about 10% of the infused dose (90% removal), and at the same time (approximately 5 min after the start of the infusion) 50% of the infused radioactivity appeared in the effluent from the lung.

the perfusate within the same period. Further, 90–95% of the radioactivity entering the lung was recovered in the perfusate within 40 min (see Table 2, control values).

The radioactivity in the perfusate was analysed by ion exchange chromatography on paper to separate labelled 5-HT from any other radioactive species. For this the fractions collected at 0·5 or 2 min intervals were combined over larger periods of time—that is 0–2·5 min—and so on as shown in Table 2. Aliquots of these samples were chromatographed with markers of 5-HT and 5-HIAA. Only two radioactive spots were obtained travelling with 5-HT and the oxidized metabolite 5-HIAA in two different solvent systems. R_f values were as follows: cellulose phosphate paper; 5-HT, 0·32; 5-HIAA, 0·84: diethylaminoethyl cellulose paper; 5-HT, 0·68; 5-HIAA, 0·17.

The amount of radioactivity associated with 5-HT in the combined samples is shown in Table 3 (see values for control). All the unchanged 5-HT had emerged from the lungs within 5 min.

The rate at which radioactivity washed out from the extracellular space in the lung was determined by infusing ^{14}C inulin for the same length of time as the 5-HT infusion. 92% of the inulin appeared in the perfusate within 5 min of the start of the infusion (Table 2).

Figure 6 illustrates the effect of amitriptyline and mebanazine on the radioactivity in the perfusate after an infusion of tritiated 5-HT through the lung, and Tables 2 and 3 summarize the results of the analysis of the perfusates. After amitriptyline

TABLE 2. *Percentage of administered radioactivity appearing in perfusate during the time intervals indicated, in minutes*

Treatment	0–2·5	2·5–5	5–10	10–20	20–30	30–40
Control (±S.E.M.) (n=8)	15·1 (±1·6)	32·4 (±0·7)	21·4 (±1·9)	13·7 (±0·8)	6·9 (±0·4)	4·0 (±0·3)
Amitriptyline 10^{-5}M (n=3)	20·7	50·0	19·3	5·9	1·8	0·6
Mebanazine 10^{-5}M (n=3)	13·1	26·3	18·6	15·2	8·2	4·3
Reserpine 10^{-5}M (acute) (n=2)	17·5	33·7	17·8	12·4	7·0	4·4
^{14}C-inulin (n=2)	39·5	52·8	7·5	0	0	0

Radioactivity in effluent from lung following infusions of ^{3}H-5-HT. The infusions were given for 3–5 min, and the total radioactivity administered was $2{\cdot}8 \times 10^6$ c.p.m. The effluent was collected from the beginning of the infusion (time=0), and then combined to give the larger time samples as described in **Methods**. The drugs were given by infusion for periods as described in the text. The infusion of ^{14}C-inulin was given in the same way as the 5-HT infusions and the total radioactivity given was $1{\cdot}5 \times 10^6$ c.p.m.

TABLE 3. *Percentage of administered radioactivity appearing as radioactive 5-HT in perfusate during the times indicated (min)*

Treatment	0–2·5	2·5–5	5–10	10–20
Control	1·6	3·3	0	0
Amitriptyline 10^{-5}M (n=3)	12·4	24·0	5·8	0
Control	5·1	6·9	0	0
Mebanazine 10^{-5}M (n=2)	10·4	16·8	7·6	4·2
Control	7·4		0	0
Reserpine 10^{-5}M (acute) (n=2)	8·2		0·1	0

Distribution of radioactivity in lung effluent between 5-HT and metabolite. Infusions of ^{3}H-5-HT ($2{\cdot}8 \times 10^6$ c.p.m.) were given for 3–5 min. Effluent was collected from the start of the infusion (time=0) and combined to give the larger samples as described in **Methods**. The proportion of ^{3}H-5-HT and ^{3}H-metabolite in each sample was determined, after chromatography of evaporated samples, by measuring the radioactivity associated with marker 5-HT and 5-HIAA.

the peak of radioactivity was higher, coinciding with the increase in biological activity, and the " tail " of the wash-out curve was much reduced ; 90% of the original radioactivity appeared within 10 min (Table 2). Chromatographic analysis of the perfusate showed a corresponding increase in the 5-HT content of the earlier fractions compared with the perfusate from untreated lungs (Table 3).

In the presence of mebanazine (10^{-5}M), the total radioactivity wash-out curve was not much different from the control curve, but there was a two-fold increase in unchanged 5-HT in the early fractions coinciding with the increase in biological activity. There was also persistence of 5-HT in the later fractions coinciding with the prolonged contractions of the assay tissues. In the first 20 min after the start of the infusion, about 40% of the administered 5-HT appeared unchanged in the perfusate, and the contractions of the assay tissues showed that 5-HT was still present at 40–60 min after the start of the infusion. Thus, although the initial uptake of 5-HT (calculated from the peak height of the contraction of the assay tissues) is about 88%, nearly half of the 5-HT taken up reappears in the perfusate giving an overall removal of between 50 and 60%.

Experiments with reserpine

Reserpine (10^{-6} and 10^{-5}M) was infused through the lungs for 15 min and 1–2 h later test infusions of 5-HT were made. The infusions of reserpine and 5-HT were not made concomitantly because the responses of the assay tissues to 5-HT were adversely affected by reserpine. The removal of 5-HT in these lungs was the same

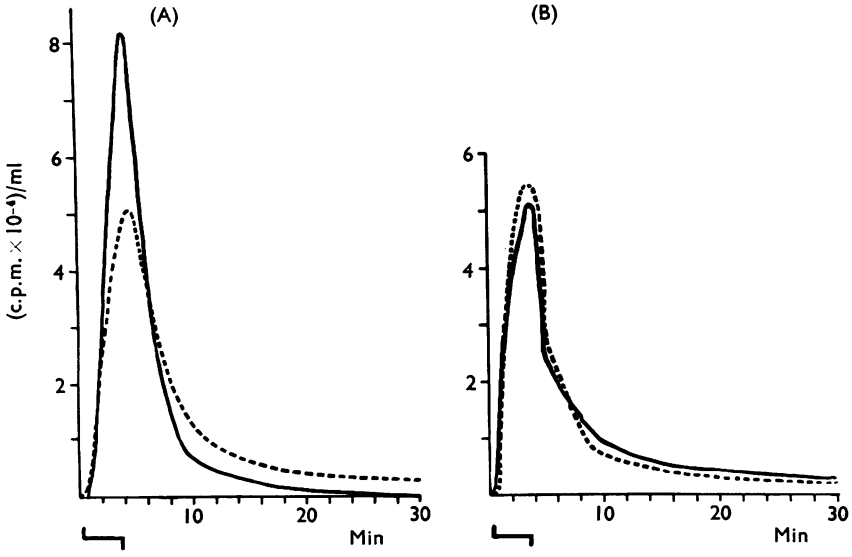

FIG. 6. Effect of (A) amitriptyline (10^{-5}M) and (B) mebanazine (10^{-5}M) on the removal of radioactive 5-HT by rat isolated lungs. The broken curve represents the radioactivity in the effluent from the lungs following an infusion of ^{3}H-5-HT (black bar below the time axis) through the lung under control conditions. About 50 min later, amitriptyline or mebanazine were infused through the lungs and an identical infusion of radioactive 5-HT made through the lungs. The radioactivity in the effluent from the treated lungs is shown by the solid curves. Amitriptyline increases the peak of radioactivity emerging and shortens the " tail " of the wash-out curve. Mebanazine has no effect on either the peak or the duration of the wash-out of radioactivity. All the curves were drawn to join the experimental values obtained, that is at 0·5 min intervals from 0–5 min, at 1 min intervals from 5 to 10 min and at 2 min intervals thereafter.

as in untreated ones (Table 2). In two experiments with radioactive 5-HT, reserpine infused at a concentration of 10^{-5}M produced no change in the time course of total radioactivity, or in the proportion of radioactive 5-HT and 5-HI AA separated by chromatography, in the perfusate (Table 3).

Effect of noradrenaline, normetanephrine, metaraminol and phenoxybenzamine

Hughes, Gillis & Bloom (1969) have shown that isolated lungs from the rat take up noradrenaline perfused through the pulmonary circulation, and the kinetic constants of this uptake are comparable with those of the uptake in rat heart obtained by Iversen (1963). To determine whether, in the rat lung, noradrenaline and 5-HT shared the same membrane transport system, we examined the removal of noradrenaline in our system and the effect which noradrenaline, and amines affecting noradrenaline uptake, might have on the removal of 5-HT.

When noradrenaline was infused through the rat lung, an average of 41% was removed. Thus, the noradrenaline removal mechanism was much less efficient than that for 5-HT. Furthermore, when noradrenaline (6×10^{-7}M, 100 ng/ml) was infused through the lung at the same time as 5-HT (5×10^{-8}M, 20 ng/ml) the removal of 5-HT was unaffected. In these experiments the assay tissues for 5-HT were made insensitive to noradrenaline by an infusion of propranolol (2 μg/ml) and phentolamine (100 ng/ml). Neither normetanephrine (5×10^{-6}M ; Fig. 7) nor metaraminol (10^{-6}M) infused through the lung affected the amount of 5-HT removed.

Phenoxybenzamine is a potent inhibitor of the extraneuronal uptake for catecholamines, Uptake$_2$ (Lightman & Iversen, 1969). We could not infuse phenoxybenzamine at the same time as 5-HT since phenoxybenzamine is an antagonist of

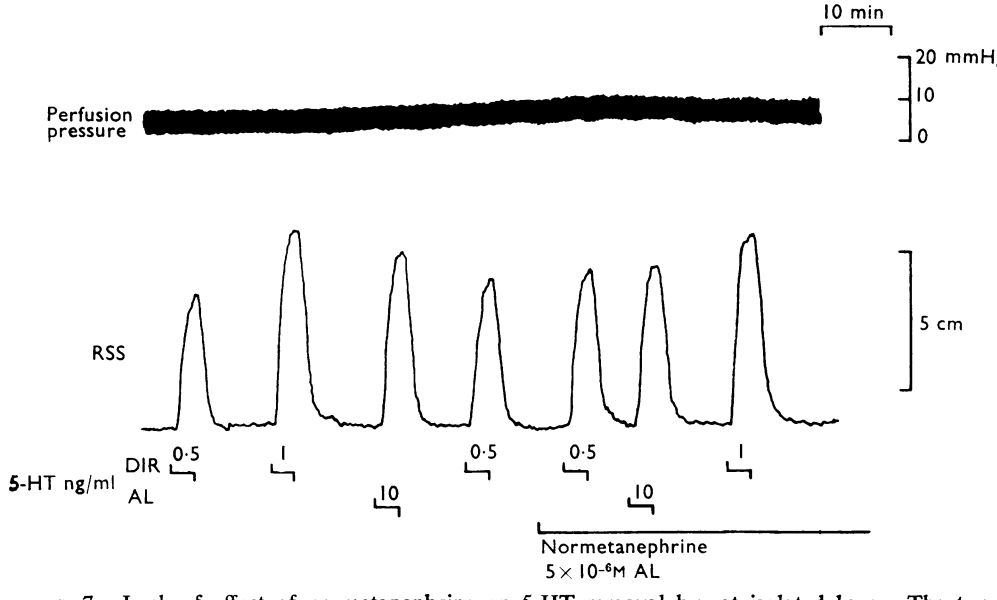

FIG. 7. Lack of effect of normetanephrine on 5-HT removal by rat isolated lung. The top record shows lung perfusion pressure and the lower record shows contractions of a rat stomach strip (RSS) induced by infusions of 5-HT given either directly to the assay tissue (DIR) or into the cannula in the pulmonary artery. Normetanephrine infused through the lungs, as shown by the solid line, had no effect on the removal of 5-HT. Scales, 10 min, 5 cm, 0–20 mmHg.

tryptamine receptors in the rat stomach strip (Vane, 1960). However, phenoxybenzamine inhibition of noradrenaline uptake is persistent and not quickly reversed (Eisenfeld, Landsberg & Axelrod, 1967). We therefore infused phenoxybenzamine (10^{-5}M) through the lungs for 30 min and allowed a further 5 min of normal Krebs perfusion before the lung perfusate was passed over the assay tissues. Under these conditions the removal of 5-HT by the lung was unchanged (Table 1).

Discussion

The isolated rat lung removes 5-HT from the perfusion fluid passing through the pulmonary circulation. The process is highly efficient, removing in a single passage 92% of the 5-HT. From the similarity in time course of radioactivity due to ^{14}C-inulin, and biological activity due to 5-HT in the perfusate following an infusion of these substances through the lung, it seems that the 5-HT that survives passage through the pulmonary circulation under normal conditions equilibrates only with the extracellular space. The high degree of removal is maintained when the concentration of 5-HT is raised from 5 ng/ml to 100 ng/ml. Our results suggest that the removal consists of an initial transfer of the amine from the perfusion fluid to a cell, a process which can be inhibited by amitriptyline and desmethylimipramine. This transfer may be by a membrane transport system analogous to monoaminergic transport in nerve endings. The next step is, however, not storage in granules or other sites resistant to enzymic degradation but enzymic attack by monoamine oxidase. The 5-HIAA thus formed is completely washed from the lung in 40–60 min. In the presence of MAO inhibitors, the initial uptake of amine is slightly reduced but the 5-HT which is taken up by the lung is spared from enzymic attack and reappears in the perfusate from the lung and may be detected biologically for up to 40 min after the infusion period. Thus, if after transport of 5-HT into the cell, binding occurs, it must be relatively loose so that under conditions of MAO inhibition, 5-HT slowly leaves the cell when the concentration of 5-HT in the perfusing fluid has fallen, after the end of the infusion.

The site of uptake and enzymic inactivation of 5-HT in rat lung is unknown, although some possibilities can be eliminated. For instance, 5-HT is thought to be taken up by the noradrenaline storage granules of sympathetic nerves in the guinea-pig vas deferens (Thoa, Eccleston & Axelrod, 1969) and about half the accumulated 5-HT was released in 2 h. In our system, it seems unlikely that 5-HT was accumulated by such nerve granules because of the rapid metabolism that we observed, and the lack of effect of reserpine on the removal of 5-HT by the lungs.

The mast cells of rat lung are known to contain 5-HT as well as histamine, and the 5-HT in mast cells is resistant to the releasing action of reserpine (Parrett & West, 1957). However, if mast cells are involved in the 5-HT removal in rat lung they must differ from other mast cells in that they allow fairly rapid metabolism of the 5-HT. Furano & Green (1964) found that mast cells from peritoneal fluid of rat could take up exogenous 5-HT but retained it unchanged for at least 24 h.

Another possible site of uptake is the blood platelet. These cells can take up 5-HT (Stacey, 1961), the uptake can be blocked by amitriptyline-like drugs (Todrick & Tait, 1969), and they are present in the lung capillaries in large numbers (Kaufman, Airo, Pollock & Crosby, 1965). However, 5-HT taken up by platelets is found in intracellular storage organelles (Born, 1963; Tranzer, Da Prada & Pletscher, 1966) and although platelets will oxidize exogenous 5-HT, the amount

of metabolism is small, for example, 3% in platelets incubated with 5-HT (10^{-6}M) for 2 h (Pletscher, Burkard, Tranzer & Gey, 1967). Since all the radioactivity was recovered after 40–60 min in our experiments and most of it was in the form of the metabolite 5-HIAA, it seems unlikely that platelets are involved. This does not exclude the possibility that *in vivo,* or in blood perfused lungs, part of the pulmonary removal of 5-HT may involve uptake into platelets in the lungs. Axelrod & Inscoe (1963), for example, found that after an intravenous injection of ^{14}C-5-HT in mice, a high concentration of radioactive 5-HT was found in the lung after 1 min and detectable quantities of unchanged 5-HT were present for at least a week. In mouse lung radioactivity derived from injected ^{14}C-5-HTP is retained for up to 72 h after injection (Gershon & Ross, 1966a, b). At 4 h after injection approximately 40% was due to ^{14}C-5-HT, and the amount of 5-HT was increased after MAO inhibition. However, the radioactivity was chiefly located in the interalveolar septa in cells tentatively identified as lung macrophages.

Hughes *et al.* (1969) have shown that the rat isolated lung concentrates infused noradrenaline, the uptake being inhibited by cocaine. The amine is metabolized by both monoamine oxidase and catechol-O-methyl transferase. They concluded from autoradiographic studies that the endothelial cells of the capillaries are important sites for this inactivation process. In our experiments noradrenaline was also removed by rat lung, but to a much lesser extent than 5-HT. Furthermore noradrenaline in concentrations of up to ten-fold excess in molarity—100 ng/ml—had no effect on 5-HT removal. Thus it seems that either noradrenaline and 5-HT are taken up by different sites or that 5-HT has a much greater affinity for a common uptake site than noradrenaline.

The uptake mechanisms for noradrenaline postulated by Iversen (1965b) have recently been further defined as an intraneuronal uptake, $Uptake_1$ and an extraneuronal uptake, $Uptake_2$ (Lightman & Iversen, 1969). $Uptake_2$ operates at all levels of noradrenaline concentration in the rat heart, but at low concentrations, that is, less than $1{\cdot}5 \times 10^{-5}$M (2·5 μg/ml), any noradrenaline taken up by this process is rapidly metabolized. The concentrations of 5-HT that we have studied are between $2{\cdot}5–5 \times 10^{-8}$M and we also observed rapid metabolism. Phenoxybenzamine has been shown to produce a marked reduction in the accumulation of extraneuronal noradrenaline and in the formation of metabolites in rat heart and vas deferens (Iversen & Langer, 1969; Eisenfeld *et al.*, 1967). Nevertheless neither this compound nor a selective $Uptake_2$ inhibitor, normetanephrine, nor metaraminol, a selective $Uptake_1$ inhibitor, has any effect on 5-HT removal in our preparation, at concentrations shown to produce marked or complete inhibition of the uptake of noradrenaline in the rat heart (Iversen, 1965b). Thus the uptake of 5-HT in rat lung is unlikely to be due to either of these processes.

A mode of amine binding to connective tissue which is unaffected by phenoxybenzamine has been described by Avakian & Gillespie (1968), and this binding is extracellular and specifically excludes a transport mechanism. This type of binding would therefore be incompatible with our findings of metabolism by MAO which is an intracellular enzyme, and of inhibition by the amitriptyline-like drugs.

It is clear therefore that the removal of 5-HT by rat lung does not involve any of the known uptake mechanisms for biogenic amines, and the characteristics of this process may define its own type of uptake mechanism. Furthermore oxidation of 5-HT by monoamine oxidase plays an essential role in the overall removal process,

in contrast to the disappearance of 5-HT in dog lung *in vivo* (Thomas & Vane, 1967), which is unaffected by MAO inhibitor.

However, this discrepancy may be due only to a difference between dog lung MAO and rat lung MAO. There is evidence that MAO in a single species exists as several iso-enzymes with variations in substrate and inhibitor specificity (Youdim, Collins & Sandler, 1969) and a similar variation between species is therefore not unlikely. It might be possible to demonstrate the involvement of MAO in the removal of 5-HT by dog lung by choosing another MAO inhibitor. This inhibitor specificity may explain the conflict between the results of Udenfriend, Weissbach & Bogdansky (1957), who could not affect 5-HT metabolism in the whole mouse with iproniazid, and those of Axelrod & Inscoe (1963) and of Gershon & Ross (1966a) who demonstrated an inhibition of 5-HT metabolism in the mouse using another MAO inhibitor, JB 516. These last authors have also shown that the radioactivity in mouse lung derived either from ^{14}C-5-HTP (Gershon & Ross, 1966b) or from ^{14}C-5-HT (personal communication to J. R. Vane quoted in Vane, 1969) is mainly in septal cells which could be either alveolar macrophages (as they suggest) or Type II alveolar cells (Heinemann & Fishman, 1969). Either these septal cells or the endothelial cells lining the lung capillaries (Hughes *et al.*, 1969) would be readily accessible to amines in the capillaries, but neither contain granules of the type generally associated with binding of biogenic amines. We would therefore postulate a removal process for 5-HT in the rat lung consisting of an initial transport into a cell, either endothelial or septal, and in normal circumstances, a subsequent oxidation by monoamine oxidase. Either or both of these steps may be inhibited with a consequent increase in the proportion of 5-HT appearing in the perfusate from the lung.

It would be interesting to see if the removal of noradrenaline by rat lung would fit one of the uptake mechanisms already established or if it would share the characteristics of 5-HT removal.

The inactivation of 5-HT in the pulmonary circulation has been demonstrated in cat lungs (Gaddum *et al.*, 1953), in guinea-pig and rat lungs perfused with Krebs solution (Alabaster & Bakhle, unpublished experiments and this paper) and in dog lungs *in vivo* (Thomas & Vane, 1967). If human lungs are capable of removing a similar proportion of 5-HT in the pulmonary circulation, they would provide a considerable defence against large amounts of 5-HT entering the arterial circulation in situations where the venous levels of 5-HT may be abnormally high as, for instance, in the carcinoid syndrome.

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