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THE RELATIONSHIP BETWEEN 5-HYDROXYTRYPTAMINE AND PARAQUAT ACCUMULATION INTO RAT LUNG

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Abstract—The uptake of 5-hydroxytryptamine (5HT) into rat lung slices has been shown to obey saturation kinetics and to be inhibited by imipramine, metabolic inhibitors and a sodium-free medium. The apparent K_m for the uptake process was found to be 3.3 μM with a V_{max} of 6 nmoles/g wet wt/min. Lung slices taken from rats given a dose of paraquat known to damage type I and type II lung epithelial cells showed inhibition of paraquat uptake but no inhibition of 5HT uptake. This together with the stimulation of paraquat accumulation into rat lung slices in a sodium-free medium leads to the conclusion that the uptake of paraquat and 5HT into the lung does not occur in the same cell type.

It has recently been shown that the energy-dependent uptake of the herbicide paraquat into rat lung slices can be inhibited by 5-hydroxytryptamine (5HT) [1]. The uptake of 5HT into isolated perfused lung has been shown to obey saturation kinetics, to be temperature- and sodium-dependent [2] and to be inhibited by metabolic inhibitors [3]. The capillary endothelial cell in the lung has been implicated as the major site of 5HT uptake [4], whereas in the case of paraquat accumulation there is no direct evidence of the cell involved. Recent studies by Sykes *et al.* (unpublished data) have suggested that it is the type I and possibly the type II alveolar epithelial cells which are in part the sites of paraquat uptake. In the present studies we have investigated the uptake of 5HT and paraquat into rat lung slices to establish the relationship between the uptake process for paraquat and that for 5HT.

MATERIALS AND METHODS

Materials

5-Hydroxytryptamine creatinine sulphate was obtained from Sigma Chemical Company, Kingston-upon-Thames, Surrey. Paraquat dichloride (99% pure) was obtained from Plant Protection Division, Jealott's Hill Research Station, Berks. Methyl-[^{14}C]paraquat (30 mCi/m-mole) and 5-hydroxy[side chain-2-^{14}C] tryptamine creatinine sulphate (56 mCi/m-mole) were purchased from the Radiochemical Centre, Amersham.

Animals. Male, Alderley Park (Wistar derived) specific pathogen-free rats (body weights 180–200 g) were used throughout.

Methods

Preparation of lung slices. Rats were killed with halothane and the lung rapidly removed and placed in either a Krebs–Ringer phosphate medium or a sodium deficient medium at room temperature. Lung slices were prepared by hand using a 'valet strip' blade (Gillette Surgical Ltd., Isleworth, Middlesex). Only slices with two cut surfaces were used.

Incubation. Slices (30–50 mg) were weighed and incubated in a modified Krebs–Ringer phosphate medium (3 ml) containing NaCl (130 mM), KCl (5.2 mM), $CaCl_2$ (1.9 mM), $MgSO_4$ (1.29 mM), Na_2HPO_4 (10 mM) and glucose (11 mM). The pH of the buffer was adjusted to 7.4 with HCl. When sodium deficient medium was required, sucrose (260 mM), was used to replace the NaCl (130 mM). To measure the accumulation of paraquat, 0.1 μCi of [^{14}C]paraquat with the required concentration of unlabelled paraquat was added to the medium. To measure the accumulation of 5HT, [^{14}C]5HT was added to the incubation medium such that the required amount of 5HT was present in the medium. Incubation was carried out under air, with shaking, at 37°. When the effect of inhibitors on the accumulation of 5HT into the lung was studied 500 μM iproniazid was added to the medium.

Measurement of paraquat and 5HT in slices with time. Slices were removed from the incubation medium and washed by transferring them to fresh Krebs–Ringer phosphate. They were carefully blotted, dissolved in 1 ml Soluene (Packard Instrument Co. Ltd.) and the radioactivity measured after the addition of 10 ml Dimilume scintillator (Packard Instrument Co. Ltd.) using a liquid scintillation spectrometer. Samples of the medium (0.1 ml) were diluted to 1.0 ml with water and the radioactivity measured after the addition of 10 ml Instagel scintillator (Packard Instrument Co. Ltd.). Counting efficiency was determined by the addition of an internal ^{14}C-standard and all counts were converted to disintegrations per minute (dpm). The ratio of ^{14}C label present per unit weight of slice to that present in an equivalent volume of medium was used to calculate the amount of paraquat or 5HT present in the slices.

Measurement of the initial rate of uptake of 5HT into slices (30 sec to 5 min). The uptake of 5HT into rat lung slices during the first 5 min of incubation was measured by taking sequential samples of the incubation medium (10 μl) (total vol of medium removed 50 μl). The disappearance of ^{14}C-label from the medium was then used as a measure of the 5HT

uptake into the lung slice and from this the amount of 5HT per unit weight of lung tissue was calculated.

RESULTS

The amount of 5HT accumulated by rat lung slices from an incubation medium containing 10^{-6} M 5HT was increased in the presence of 5×10^{-4} M iproniazid (Fig. 1). An apparent K_m of 3.3 μM and V_{max} of 6 nmoles/g wet wt/min were determined from the initial rate of uptake (between 30 sec and 5 min) of 5HT into rat lung slices in the presence of 5×10^{-4} M iproniazid. The uptake of 5HT into lung slices was reduced by the presence of imipramine and the metabolic inhibitors cyanide (10^{-3} M) and iodoacetate (10^{-3} M) in the incubation medium (Fig. 2). When the Krebs-Ringer phosphate incubation medium was replaced by a sodium-deficient medium, uptake of 5HT was also reduced (Fig. 2). The uptake of paraquat, however, was stimulated in a sodium deficient medium (Table 1).

Slices of lung taken from rats given 65 μmoles of paraquat/kg body wt i.v. 16 hr previously, accumulated 5HT in a similar fashion to lung slices from control rats (Table 2) whereas the uptake of paraquat by slices of lung from paraquat-poisoned rats was significantly reduced (Table 3).

DISCUSSION

Since the studies of Gaddum *et al.* [5] on the removal of 5HT from plasma by the cat lung, several workers, using perfused lung techniques, have shown that 5HT is taken up into the lung tissue [2, 4, 6, 7]. Although lung slices or chopped lung preparations have been used to investigate the metabolism of vasoactive compounds [8], lung slices have not been extensively used to investigate uptake processes because of their obvious non-physiological state. We have studied the uptake of 5HT into lung slices firstly to compare the uptake process in slices with that described for perfused lung preparations, and secondly to study the uptake of 5HT under conditions comparable to those described for the energy-dependent uptake of paraquat into lung slices [9].

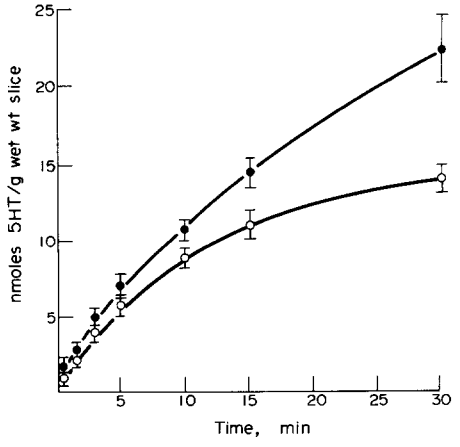

Fig. 1. The effect of iproniazid on the uptake of 5HT into rat lung slices. Slices of lung were incubated at 37° with 1 μM 5HT (○) or 1 μM 5HT plus 500 μM iproniazid (●). Results are expressed as mean ± S.E.M. with six slices per time point.

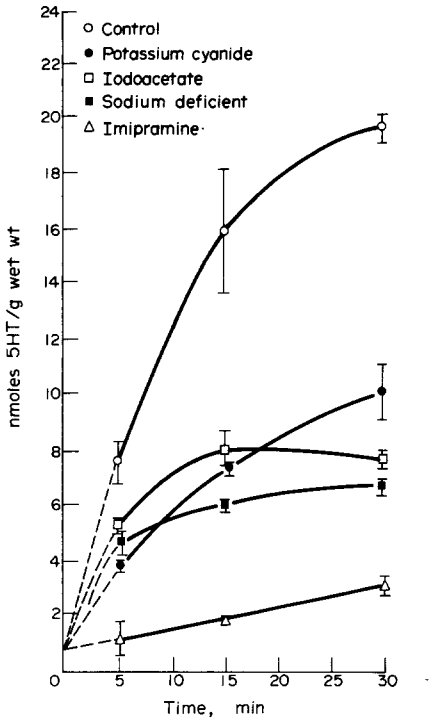

Fig. 2. The effect of inhibitors and sodium-deficient medium on the uptake of 5HT into rat lung slices. Slices of lung were incubated with 1 μM 5HT (○) or 5HT plus 1 mM cyanide (●), 1 mM iodoacetate (□) 500 μM imipramine (△) and 1 μM 5HT in a sodium-deficient medium (■). Results are mean ± S.E.M. with four slices per time point.

Characterisation of 5HT uptake in lung slices. The measurement of the accumulation of 5HT into lung slices is dependent upon the rate of uptake of ^{14}C-label into slices and the rate of efflux of ^{14}C-label from the slices. 5HT is metabolised in lung by monoamine oxidase to 5-hydroxyindoleacetic acid [10], (5HIAA) and some of the efflux of ^{14}C from slices will be due to this product of metabolism. When iproniazid, an inhibitor of monoamine oxidase, was added to the medium to prevent this metabolism there was a small increase in the amount of ^{14}C-label present in the lung at early time intervals (Fig. 1) and over a period of 30 min there was significantly more accumulation of label (Fig. 1). Since it has been shown that iproniazid inhibits the metabolism of 5HT to 5HIAA in the lung [3] we have assumed that ^{14}C label present in lung slices represents accumulated 5HT.

Although the rate of uptake of 5HT was not linear over the 30 min of incubation the initial rate of accumulation could be determined using the first 5 min of incubation in the presence of iproniazid (Fig. 1). The relationship between this initial rate and 5HT concentration in the medium was used to calculate an apparent K_m of 3.3 μM and a V_{max} of 6 nmoles/g wet wt/min for the process. These values are not very different from those obtained by Junod [2] using perfused rat lung (6.2 μM and 19 nmoles/g wet wt/min respectively). The uptake of 5HT into rat lung slices

Table 1. Paraquat uptake into rat lung slices incubated in Krebs–Ringer phosphate and sodium-deficient media

Medium	Uptake of paraquat (nmoles/g/hr)
Krebs–Ringer phosphate	40 ± 3
Sodium-deficient medium	115 ± 8
Sodium-deficient medium + KCN, 1 mM	21 ± 2

Slices of rat lung were incubated with 10 μM paraquat in media shown above at 37° for 2 hr. Results are expressed as mean ± S.E.M. with four slices per time point.

Table 2. Uptake of 5HT into normal lung slices and lung slices taken from rats given paraquat

Time (min)	Uptake of 5HT (nmoles/g wet wt slice)	
	Normal lung	Lung from paraquat-treated rats
5	4.5 ± 0.5	5.0 ± 0.6
10	7.7 ± 0.7	8.0 ± 0.6
15	8.7 ± 0.9	9.8 ± 1.2

Accumulation of 1 μM 5HT by normal lung slices and lung slices taken from rats given 65 μmoles paraquat/kg i.v. 16 hr previously. Results are expressed as mean ± S.E.M. with four slices per time point.

Table 3. Accumulation of paraquat into normal lung slices and lung slices from rats treated with paraquat

Time after paraquat (hr)	Accumulation of paraquat (nmoles/g wet wt/2 hr)
0	73.8 ± 2.9 (7)
2	71.0 ± 2.5 (10)
4	53.6 ± 1.8 (10)
8	63.1 ± 2.1 (10)
16	36.2 ± 2.2 (10)

Treated rats were given 65 μmoles/kg paraquat i.v. and killed at the times indicated. Slices were cut from the left lobe, four slices per lobe. Results are expressed as mean ± S.E.M. with number of rats used in brackets.

was inhibited by imipramine, the metabolic inhibitors cyanide and iodoacetate, and by the absence of sodium in the medium (Fig. 2) as has been demonstrated with perfused lung systems [2, 3].

Comparison of 5HT and paraquat accumulation into lung. In contrast to the inhibition of 5HT uptake in sodium-deficient medium, paraquat accumulation was stimulated (Table 1). This increase in accumulation was not a result of non-specific binding since all but a small proportion of the enhanced accumulation is energy-dependent (Table 1). Indeed, cyanide completely abolishes the uptake of paraquat into the lung [9], whereas 5HT uptake is only moderately inhibited (Fig. 2).

Following the administration of paraquat to rats, it is the type I and type II epithelial cells of the lung which are the first cells to be damaged [11–13]. Sykes *et al.* (unpublished data) have shown ultrastructural damage in type I epithelial cells of the lungs 4 hr after intravenous administration of 65 μmoles paraquat/kg body wt. This damage progresses with time such that by 16 hr there is significant destruction of both type I and type II epithelial cells in the alveoli. We have shown using the same dosing regime that lung slices taken from paraquat-treated rats progressively lose their ability to accumulate paraquat (Table 3). This can be explained as (1) the inhibition of paraquat accumulation by paraquat present in the treated lungs or (2) the progressive damage to cells responsible for the uptake of paraquat. Since the concentration of paraquat in the lungs of rats given 65 μmoles paraquat/kg i.v. peaks within 1 hr and thereafter effluxes from the lung with a half life of about 20 hr [14], it cannot be the presence of paraquat in the lung which progressively inhibits the accumulation of paraquat. We, therefore, suggest that it is the progressive destruction of the type I and type II alveolar epithelial cells which is responsible for the inhibition of paraquat accumulation (Table 3) indicating that these cells are in part sites of the accumulation of paraquat. However, lung slices from rats given the same dose of paraquat 16 hr previously accumulated 5HT in a similar manner to control rat lung slices (Table 2). The evidence presented therefore indicates that it is the epithelial cells of the alveoli which accumulate paraquat and that these cells are not a major site of accumulation of 5HT.

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