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THE METABOLISM OF 5-HYDROXYTRYPTAMINE AND β-PHENYLETHYLAMINE IN PERFUSED RAT LUNG AND *IN VITRO*

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1 Metabolism of 5-hydroxytryptamine (5-HT) and β-phenylethylamine (PHE) by monoamine oxidase (MAO) was investigated in rat isolated lungs and in mitochondrial preparations from rat lung.

2 In perfused lungs 5-HT metabolism had an apparent *K*m of 2 μM and PHE metabolism a *K*m of 54 μM, whereas *in vitro* the *K*m values were 330 μM and 28 μM respectively.

3 *In vitro*, MAO activity had substrate and inhibitor specificities compatible with the presence of A and B types of MAO.

4 In perfused lung, metabolism of 5-HT but not that of PHE was inhibited by desmethylimipramine.

5 These results show that PHE metabolism in perfused lung, unlike that of other metabolized amines, is not limited by transport and the transport process for PHE is unlike that of 5-HT or noradrenaline.

6 These results also show that the kinetic parameters obtained for MAO activity *in vitro* do not generally apply to the isolated lung where transport of substrate can be the deciding factor. This discrepancy emphasizes that the enzymic properties of the whole organ cannot reliably be deduced from its enzymic content.

Introduction

Although the ability of rat isolated lung to metabolize 5-hydroxytryptamine (5-HT) and phenylethylamine (PHE) through the action of monoamine oxidase (MAO) has been recognised for some time (Alabaster & Bakhle, 1970; Junod, 1972a; Bakhle & Youdim, 1976), the role of MAO in lung has not been investigated as fully as that from other tissues, e.g. liver and brain (Youdim & Holzbauer, 1976). We therefore decided to study the kinetics of 5-HT and PHE metabolism by MAO in rat lung using both isolated lungs and cell-free mitochondrial preparations, since *in vitro* studies do not necessarily reflect the *in vivo* conditions (Bakhle & Vane, 1974; Youdim & Woods, 1975).

Some of this work has been presented to the British Pharmacological Society (Bakhle, 1977).

Isolated perfused lung

The lungs from male rats (Wistar strain, 150 to 250 g) were prepared as previously described (Alabaster & Bakhle, 1970). Oxygenated, warmed (37°C) Krebs solution was perfused through the pulmonary circulation at a constant rate (8 ml/min) and the effluent either collected in timed fractions (for radiochemical assay) or superfused over smooth muscle strips of rat stomach (Vane, 1957) for bioassay.

Bioassay procedure Both 5-HT and PHE contract the rat stomach strip and the products of MAO action on these substrates are at least a thousand fold less active. Thus, amine metabolism results in a loss of contractor activity. The amines were infused for 3 min either into the pulmonary circulation (referred to as 'al') or directly over the bioassay tissues ('dir'). By comparison of the response to a concentration of amine infused al with those produced by dir infusions, the concentration of amine surviving passage through the pulmonary circulation could be determined. For instance, if 100 ng of 5-HT/ml infused al caused a contraction of the assay tissues equivalent in height to 3 ng 5-HT/ml infused dir, this was taken as $(100 - 3) \div 100 = 97\%$ metabolism.

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Radiochemical assays We used ^{14}C-labelled 5-HT and PHE diluted with non-radioactive substrate infused at a rate of 0.4 ml/min for 3 min into the Krebs flow through the lung. The final concentrations of amine ranged from 0.1 to 150 μM and at each infusion 2×10^5 d/min was used. Previous work had shown that 30 min after the start of the infusion, radioactivity in the effluent had fallen to background levels and 95% of infused radioactivity had been recovered in the effluent (Alabaster & Bakhle, 1970). Two subsequent infusions of radioactive amine 30 min apart gave results identical with the first infusion. After this time, i.e. 1.5 h, the lungs usually became oedematous (judged visually) and we therefore made only three infusions in each preparation. These infusions were of the same amine but of widely different concentrations. For instance, with 5-HT, nine concentrations were chosen: 0.1, 0.15, 0.5, 1.5, 5, 10, 20, 30 and 50 μM and each lung received a low concentration, an intermediate and one high concentration infusion, avoiding the border-line concentrations. For instance, if 0.5 μM was the 'low' value, then 5 or 10 μM was chosen as the 'intermediate' value. Effluent was collected from the start of the infusion for 30 min in a single fraction, mixed thoroughly and a sample taken to determine total radioactivity. The amine metabolites were separated from unchanged substrate in the effluent by ion exchange chromatography on Amberlite CG 150 (Southgate & Collins, 1969). None of the drugs used interfered with the separation procedure. All results from radiochemical experiments were corrected for radioactive quenching and expressed as d/min.

Preparation of lung mitochondrial monoamine oxidase

Freshly obtained rat lungs were washed with ice cold 0.32 M sucrose to remove blood. They were minced and homogenized in 0.32 M sucrose (4°C) with a motor driven Teflon pestle having a clearance of 0.25 mm between mortar and pestle. The homogenate was diluted with 0.32 M sucrose to give a final preparation of 20% (w/v) and mitochondria were prepared according to the method described by Youdim (1975). The mitochondria thus obtained were suspended in 0.32 M sucrose to give the original concentration (20% w/v). Samples (5 ml) were placed in plastic tubes and frozen at −20°C. MAO activity could be retained for almost 3 months.

In vitro *assay of monoamine oxidase*

All assays of MAO were carried out at pH 7.4 in potassium phosphate buffer (final concentration 0.05 M) at 37°C. With the exception of kynuramine, MAO activity against different substrates was measured by the radioassay techniques described by Tipton & Youdim (1976). When kynuramine was the substrate, the fluorimetric procedure of Krajl (1963) as adapted by Youdim (1975) was used.

Substrate specificity of monoamine oxidase in vitro

The substrate specificity of lung mitochondrial MAO was determined with a range of substrates from 0.01 to 3 mM. It must be noted that over this wide range of substrate concentrations, no evidence of non-linearity was observed in double-reciprocal plots (Lineweaver & Burk, 1934). With kynuramine, a range of concentrations of 0.01 to 0.2 mM was used, since at higher concentrations the enzyme was inhibited (Youdim, Holzbauer & Woods, 1974).

Protein concentrations were determined by the colorimetric procedure of Lowry, Rosebrough, Farr, & Randall (1951) with bovine serum albumin as standard.

Materials

The following radioactive amines were used: [1-^{14}C]-5-HT creatinine sulphate (54 mCi/mmol); [1-^{14}C]-dopamine hydrochloride (48 mCi/mmol); [2-^{14}C]-dopamine hydrochloride (48 mCi/mmol); [2-^{14}C]-tyramine hydrochloride (41 mCi/mmol); (±)-[7-^{14}C]-adrenaline bitartrate (57 mCi/mmol) (all from the Radiochemical Centre, Amersham); [1-^{14}C]-β-phenylethylamine hydrochloride (10.5 mCi/mmol) and [2-^{14}C]-tryptamine bisuccinate (51 mCi/mmol) (from New England Nuclear, Frankfurt); [1-^{14}C]-benzylamine. hydrochloride (40 mCi/mmol) (from I.C.N., Cleveland, U.S.A.). The unlabelled substrates were obtained from Sigma. We acknowledge gratefully the gifts of (−)-deprenyl hydrochloride (Professor J. Knoll), clorgyline hydrochloride (May & Baker) and desmethylimipramine hydrochloride (Geigy).

The significance of differences between means was calculated by Student's t test for paired or unpaired samples and values of $P \leqslant 0.05$ were accepted as significant.

Results

Kinetic parameters of amine metabolism in perfused lung

In isolated lungs, we infused radioactive amines through the pulmonary circulation for 3 min and collected the lung effluent for 30 min. The amount of metabolite in this 30 min period was taken to represent the velocity (V) of the metabolic reaction and the variation of V with substrate concentration (S) is shown in Figure 1 which also demonstrates the

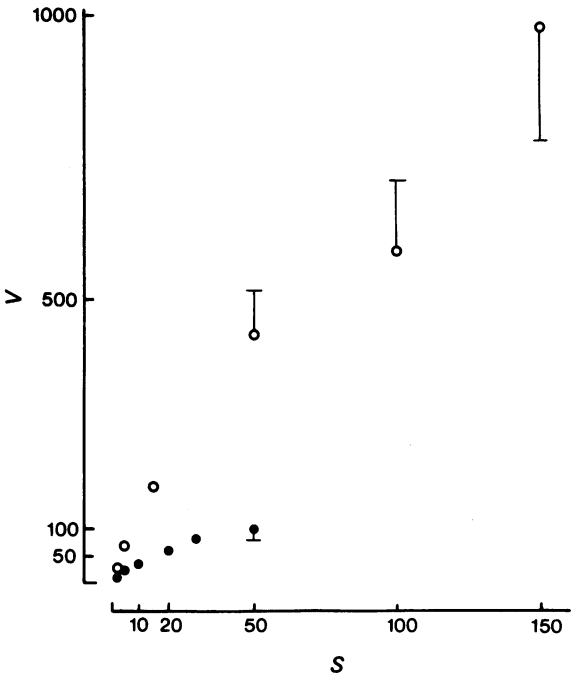

Figure 1 Metabolism of 5-hydroxytryptamine (5-HT) and phenylethylamine (PHE) in rat isolated lungs at different substrate concentrations. Amine was infused for 3 min and the lung effluent collected for another 27 min. Total amine metabolites in the effluent collected over this time is *V* (nmol). The substrate concentration (*S*; μM) is shown and contained 2×10^5 d/min per infusion. The values shown are the mean of 4–8 experiments; vertical lines show s.e. mean. The standard errors are not shown where they were smaller than the symbols drawn.

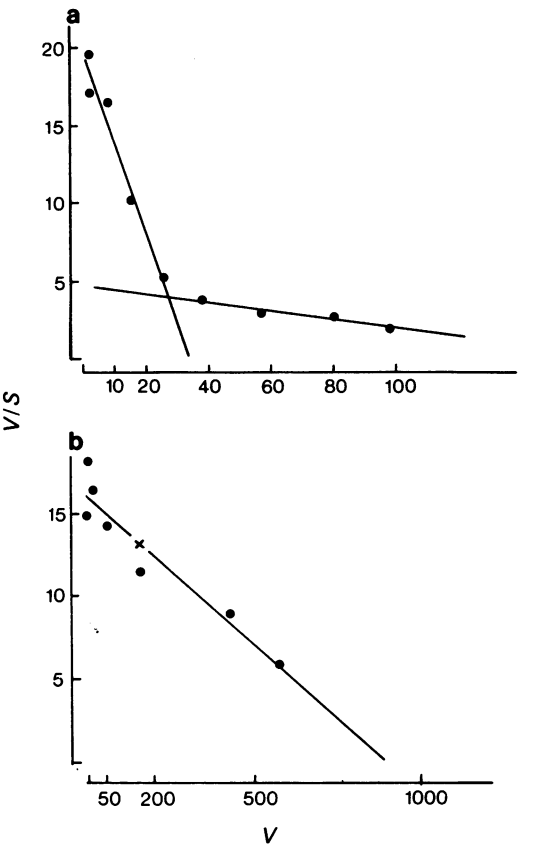

Figure 2 Analysis of metabolism of 5-hydroxytryptamine (5-HT, a) and phenylethylamine (b) in isolated perfused lung. *V* is expressed as nmol and S as μM. The slopes of the lines are 1/Km and the intercept on the *V* axis represents *Vmax*. For 5-HT in (a) the lines have been drawn by eye and as though each process were independent (see text). For phenylethylamine (b), the line drawn is a computed best fit for the values represented by filled circles. The value represented by X is from earlier work (Bakhle & Youdim, 1976) and was included for comparison.

greater capacity of the perfused lung to metabolise PHE compared with 5-HT. When these results were analysed by the method of Eadie & Hofstee (Figure 2a & b), another difference was apparent. Whereas the values for PHE (Figure 2b) fell on one computed line, suggesting a single process with apparent *K*m of 54 mM, those for 5-HT metabolism were best fitted by two lines as shown (Figure 2a). The steeper line relating to the low substrate concentration (0.1 to 10 μM 5-HT) represents a process with *K*m of 2 μM. The kinetic analysis at the higher concentration (10 to 50 μM) is less reliable, as the lowest estimate of *K*m over this range (if the low *K*m process is totally neglected) is 50 μM, i.e. equal to the highest concentration studied. If allowance is made for the low *K*m process, computed estimates of the higher *K*m are in excess of 50 μM.

Substrate specificity of mitochondrial monoamine oxidase in vitro

The kinetic constants for 5-HT and PHE metabolism by MAO in mitochondrial preparations are shown in Table 1, along with those of other substrates. Although the *K*m values for PHE *in vitro* and in perfused lung are comparable (28 compared with 54 μM), those for 5-HT are markedly different (330 compared with 2 μM). The results in Table 1 also show that

the hydroxylated substrates, 5-HT, adrenaline, noradrenaline, dopamine and tyramine, have higher *Km* values than the non-hydroxylated substrates. With liver MAO, this difference is less apparent. Another peculiarity of lung MAO is that it has a larger capacity for PHE deamination than liver MAO. Apart from PHE, 5-HT and tryptamine, the kinetic parameters for the six substrates are virtually identical in the two preparations.

Variation of MAO activity with pH was studied in the mitochondrial preparations from lung with four substrates (Figure 3). The optimum pH for 5-HT, about 9.0, was almost 1.5 pH units higher than that for PHE and dopamine. Kynuramine, a substrate for either type of MAO, exhibited a much wider maximum, extending over nearly two pH units from 7.5 to 9.5.

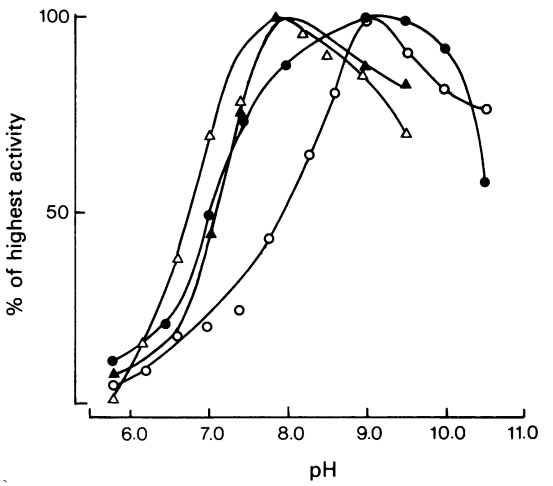

Figure 3 Activity-pH curve of lung mitochondrial monoamine oxidase; pH 5.8 to 7.8, Na-K phosphate buffer (0.05 M), pH 8.0 to 10.5 borate buffer (0.05 M). (△) Phenylethylamine; (▲) dopamine; (○) 5-hydroxytryptamine; (●) kynuramine.

Inhibitor studies in perfused lung and in vitro

In isolated lungs, deprenyl but not clorgyline inhibits PHE metabolism (Bakhle & Youdim, 1976). In the present experiments we have shown that clorgyline is a more potent inhibitor of 5-HT metabolism. Inhibition of 5-HT metabolism without an effect on uptake in isolated lungs can readily be detected by bioassay (Alabaster & Bakhle, 1970). The characteristic response is a prolonged contraction of the assay tissues caused by the persistence of unmetabolized and thus biologically active 5-HT in the lung effluent. This effect is illustrated in Figure 4 where 5-HT inactivation was measured in the presence of increasing amounts of either clorgyline or deprenyl infused through the pulmonary circulation. In Figure 4b, the response to 5-HT which had passed through the pulmonary circulation (5-HT al) was prolonged after infusion of 0.1 μg/ml clorgyline whereas, in Figure 4a, at least 10 μg/ml of deprenyl were needed to produce any prolongation. Similar results were obtained in four other experiments.

Inhibition by clorgyline of 5-HT inactivation in perfused lung was quantified by use of [^{14}C]-5-HT. In these experiments clorgyline was either given to the animal at different times before the lungs were removed or, in lungs from untreated animals, infused before and during the infusion of [^{14}C]-5-HT. In two

Table 1 Substrate specificity of the rat lung mitochondrial monoamine oxidase

	Lung		*Liver**	
	Km (μM)	*Relative Vmax*	*Km* (μM)	*Relative Vmax*
Benzylamine	135	100	245	100
Phenylethylamine	28	230	21	118
5-Hydroxytryptamine	330	88	187	124
Kynuramine	52	61	71	54
Adrenaline	445	65	400	70
Noradrenaline	410	78	416	71
Dopamine	400	108	405	112
Tyramine	305	215	282	200
Tryptamine	27	75	185	81

MAO activity was determined according to the radioassay method described by Tipton & Youdim (1976) except when kynuramine was the substrate when Krajl's method (1963) was used. * Results from Houslay & Tipton (1974).

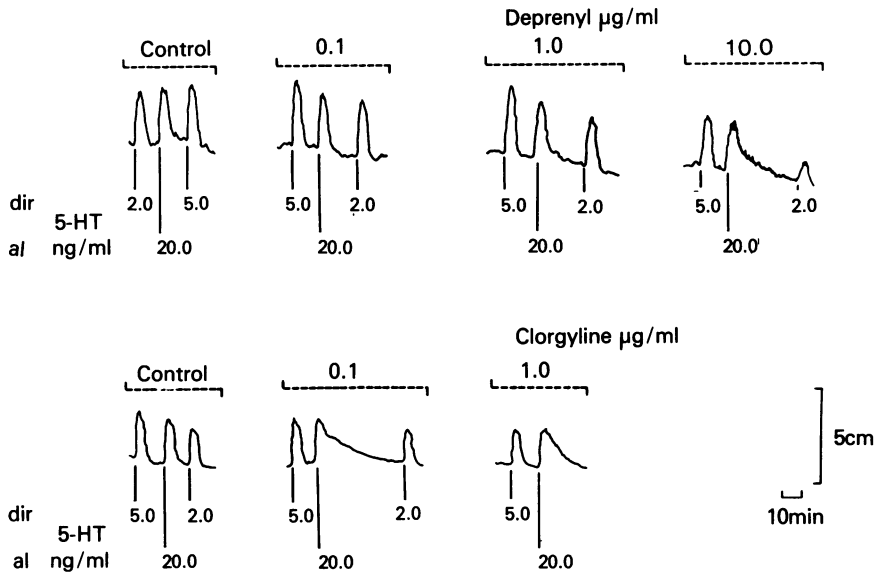

Figure 4 Effect of monoamine oxidase (MAO) inhibitors on 5-hydroxytryptamine (5-HT) inactivation in rat isolated lungs. The records of two experiments are shown; each record is continuous but has been separated for clarity. The responses shown are of rat stomach strips superfused in effluent from rat lung perfused with Krebs solution. Infusions (4 min) of 5-HT were given directly (dir) or through the lung (al). When required, the MAO inhibitors were infused continuously at the concentrations shown through the lung. The middle response of each panel is to an al infusion of 5-HT; in (a) note than deprenyl at 10 μg/ml caused a small prolongation of response to al infusion. In (b), from another lung, a marked prolongation to the al infusion was seen with clorgyline at a much lower concentration (100 ng/ml).

animals 5 mg/kg clorgyline given 2 h before death caused only 19% inhibition of 5-HT metabolism (0.15 μM) but the same dose 3 h before gave 37 ± 7% inhibition (mean ± s.e. mean, 6 animals). Infusion of clorgyline (500 ng/ml) was also effective in decreasing 5-HT metabolism giving 53 ± 6% (n = 3) inhibition. In the mitochondrial preparations more extensive studies were undertaken. The results shown in Figures 5 and 6 compare the properties of clorgyline and deprenyl added to the enzyme *in vitro* as inhibitors of four amine substrates. Here also deprenyl is a more effective inhibitor of PHE, and clorgyline of 5-HT, metabolism. This specificity was also shown when the enzyme was prepared from animals pretreated with inhibitors (Table 2).

The metabolism of 5-HT and noradrenaline in perfused lungs is inhibited by tricyclic antidepressants (see Gillis & Roth, 1976; Alabaster, 1977). In a series of experiments in which the metabolism of 5-HT and PHE (both at 0.15 μM) were measured before and after infusion of desmethylimipramine (10^{-5} M), whereas 5-HT metabolism was significantly inhibited (40 ± 9%, n = 4), that of PHE was unaffected (8 ± 3% inhibition, n = 4).

Table 2 The *in vivo* effect of clorgyline and (−)-deprenyl on rat lung monoamine oxidase (MAO) activity

Dose of inhibitor (mg/kg)	*% Inhibition of MAO activity* 5-HT	DA	PHE
Clorgyline			
1	58 ± 9	49 ± 7	17 ± 12
2.5	79 ± 11	62 ± 8	28 ± 14
5.0	97 ± 5	83 ± 5	34 ± 8
10.0	99 ± 3	99 ± 3	53 ± 10
Deprenyl			
1	25 ± 9	35 ± 7	61 ± 11
2.5	33 ± 10	39 ± 9	79 ± 12
5.0	37 ± 11	49 ± 12	95 ± 5
10.0	51 ± 13	68 ± 7	100 ± 3

The animals were injected with either drug as indicated, and 2 h later the rats were killed and lung mitochondrial MAO was prepared. Enzyme activity was determined with 5-hydroxytryptamine (5-HT), dopamine (DA) or phenylethylamine (PHE) as substrates (Tipton & Youdim, 1976). The results are expressed as % inhibition and are the means ± s.e. mean from 6 animals in each group.

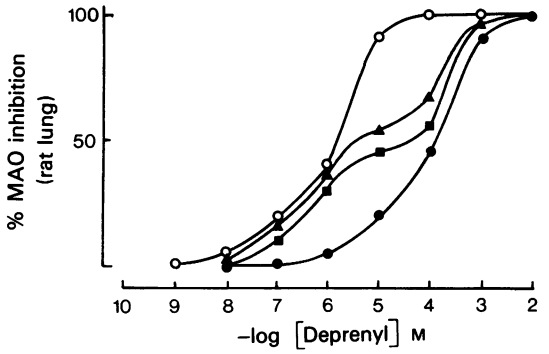

Figure 5 The *in vitro* inhibitory effect of (−)-deprenyl on lung mitochondrial monoamine oxidase activity. Mitochondrial preparations were incubated at 37°C for 20 min with 50 µl of a (−)-deprenyl solution in water to give the final concentration indicated. The activity of enzyme used was determined with dopamine (■), tyramine (▲), phenylethylamine (○) and 5-hydroxytryptamine (●) (Tipton & Youdim, 1976).

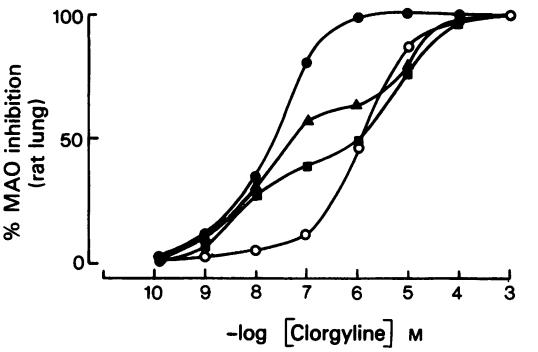

Figure 6 The *in vitro* inhibitory effect of clorgyline on lung mitochondrial monoamine oxidase activity. Mitochondrial preparations were incubated at 37°C for 20 min with 50 µl of a clorgyline solution in water to give the final concentration indicated. The enzyme activity was determined with dopamine (■), tyramine (▲), phenylethylamine (○) and 5-hydroxytryptamine (●) as in Figure 5.

Discussion

The purpose of this work was two-fold: to define the MAO activity in lung more closely, particularly with 5-HT and PHE as substrates, and to compare the properties of MAO *in vitro* with those exhibited by the whole perfused lung.

The MAO activity demonstrable in the mitochondrial preparations includes both A and B types of the enzyme as deduced from the substrate specificities and the inhibitor studies. Comparison of lung MAO with preparations from other tissues, liver (Houslay & Tipton, 1974, as in Table 1) and brain (Tipton & Mantle, 1977), shows that the binding of hydroxylated amines, for instance noradrenaline or 5-HT, is less than that of the non-hydroxylated amines, benzylamine and phenylethylamine, in all tissues, suggesting that the types of MAO are the same molecular species in each tissue. However, the proportion of A and B types may vary from tissue to tissue and this would lead to varying *Vmax* values. We have therefore used relative *Vmax* values, i.e. relative to benzylamine, in order to reduce the source of variation and the comparison in Table 1 discloses a relatively high *Vmax* for PHE in lung. This large metabolizing capacity may be related to the presence of PHE (4 ng/g) (Durden, Philips & Boulton, 1973) and phenylethanolamine (87 ng/g) (Saavedra & Axelrod, 1973) in rat lung, but there is much more 5-HT (2.3 µg/g) also present (Sadavongvivad, 1970). The significance of this large metabolic capacity for PHE in lung is therefore still unclear. Comparison of the pH optima and inhibitor susceptibility of rat lung MAO with those from other sources emphasizes that the components of lung MAO are very similar to those from liver or brain and are fully compatible with the criteria already established for A and B types of the enzyme (Houslay, Tipton & Youdim, 1976).

Although there is much qualitative agreement between the MAO activity *in vitro* and in perfused lung, both 5-HT and PHE are metabolized and the inhibitors show the same specificity, there are important differences arising from the uptake step necessary in the organised tissue and absent in the *in vitro* preparation. This difference is well illustrated in the metabolism of 5-HT. In the perfused lung at low concentrations the rate-limiting process in 5-HT metabolism had a *Km* of about 2 µM and was clearly less than that of the MAO activity *in vitro*, about 300 µM. Thus, 5-HT inactivation was controlled by some factor other than the efficiency of substrate binding to the enzyme and this factor is most probably the process of 5-HT uptake in lung. The present *Km* value of 2 µM is comparable to the *Km* values for uptake of 5-HT in rat and rabbit lung determined previously by other methods (Junod, 1972a; Iwasawa, Gillis & Agahajanian, 1973).

It is also relevant to point out that although noradrenaline has, *in vitro*, kinetic parameters for MAO action very similar to those of 5-HT, it is always less well metabolized than 5-HT in perfused lung (Hughes, Gillis & Bloom, 1969; Alabaster & Bakhle, 1973; Nicholas, Strum, Angelo & Junod, 1974). Furthermore, although they are substrates for MAO *in vitro*,

dopamine in rat isolated lungs (Nicholas *et al.*, 1974) and dopamine and adrenaline *in vivo* pass through the pulmonary circulation without loss of activity, i.e. without metabolism (Ginn & Vane, 1968; Boileau, Crexells & Biron, 1972). For these amines also, transport to intracellular MAO seems rate-limiting.

On the other hand, PHE metabolism in the perfused lung has an apparent *Km* of 54 μM which is very close to that measured *in vitro*. In rabbit isolated lung, PHE removal was anomalous in that alone out of six substrates the removal of this amine showed no saturation up to 830 μM, whereas 5-HT and noradrenaline in particular reached saturation at about 3 to 6 μM (Gillis & Roth, 1977). The lack of effect of desmethylimipramine on PHE metabolism was unexpected for two reasons. Firstly, this and other tricyclic antidepressants inhibit PHE metabolism by rabbit lung *in vitro* more effectively than that of 5-HT (Roth & Gillis, 1974). It was therefore surprising that desmethylimipramine, at a concentration capable of significant inhibition of PHE metabolism *in vitro*, did not affect PHE metabolism in the perfused lung. This discrepancy may be due to species difference but an alternative cause could lie in the extensive binding of imipramine in rat perfused lung to a site which is clearly extracellular (Junod, 1972b). Thus, despite seemingly adequate concentrations of drug in the perfusate, the *intracellular* concentration of drug might not reach the levels required to inhibit MAO. Secondly, even if drugs like imipramine are bound extracellularly, this does not prevent them from decreasing metabolism of 5-HT and noradrenaline in perfused lung by inhibiting uptake (Alabaster & Bakhle, 1970; Junod, 1972a; Nicholas *et al.*, 1974). We had therefore expected desmethylimipramine to decrease PHE metabolism, if not by inhibiting MAO, then at least by inhibiting uptake. The resistance of the PHE uptake mechanism to desmethylimipramine and to cocaine (Bakhle & Pepper, unpublished experiments), both potent inhibitors of 5-HT uptake, underlines the difference between the inactivation mechanisms of the two amines in whole lung.

In summary, MAO activity in rat lung is comparable in many essential characteristics to that in other tissues. The A and B types of MAO in lung have similar properties to those demonstrated elsewhere. However, the properties of MAO activity *in vitro* cannot reliably be extrapolated to predict the metabolic properties of the whole organised lung which are strongly affected by the properties of amine transport processes. This work, therefore, provides another example of the way in which environment crucially modifies enzymic activity (Bakhle & Vane, 1974; Youdim & Woods, 1975).

The rat lung, like that of rabbit, exhibited *in vitro* and in perfusion, a remarkable ability to metabolize large amounts of PHE. This property is remarkable because the substrate is unlikely to occur in high concentrations in pulmonary arterial blood, because metabolism in whole lung is not obviously controlled by uptake and because the uptake mechanism is different from those already described of other amines in lung. The significance of this metabolic function of lung remains unclear, but its existence re-emphasizes the wide variety of biochemical reactions occurring between the lung cells and substrates in the pulmonary vascular bed.

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