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RATE-LIMITING STEP IN THE METABOLISM OF POLAR AND NON-POLAR MONOAMINES IN LUNG AND LIVER

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1 Uptake of the non-hydroxylated amines, [^{14}C]-tryptamine and [^{14}C]-benzylamine in rat lung, infused through the pulmonary circulation, was not saturable over the concentration range 2.5–1,000 μM.

2 The kinetic constants for deamination of a variety of hydroxylated and non-hydroxylated monoamines in liver, perfused via the portal circulation, with monoamine oxidase activity in homogenates of liver were similar.

3 In lung, uptake of both [^{14}C]-tryptamine and [^{14}C]-benzylamine was inhibited by the monoamine oxidase inhibitor deprenyl and competition occurred between tryptamine, benzylamine and β-phenylethylamine for uptake.

4 These results indicate that tryptamine and benzylamine metabolism in lung is not limited by uptake, unlike that of the hydoxylated amines 5-hydroxytryptamine and noradrenaline and that uptake resembles that of β-phenylethylamine in lung.

5 The selectivity of the lung in handling monoamines is not shown by the liver, suggesting that lung has a specific role in clearing certain biogenic monoamines.

Introduction

Monoamine oxidase (MAO) is a mitochondrial enzyme and consequently, metabolism of monoamines in lung comprises both uptake into the cell and deamination by MAO. The uptake in lung of β-phenylethylamine (PEA) is clearly different from that of 5-hydroxytryptamine (5-HT) and noradrenaline (NA). Both 5-HT and NA contain polar hydroxyl groups and the rate-limiting step in metabolism of these amines in the lung is their intracellular transport, perhaps by means of a carrier (Junod, 1972; Nicholas, Strum, Angelo & Junod, 1974). In contrast, PEA has no hydroxyl groups and is thus less polar than 5-HT and NA. Consequently, the rate-limiting step in metabolism of PEA in lung is deamination by MAO (Ben-Harari & Bakhle, 1980). It is possible that PEA enters lung cells without a carrier since in distinction to many other biogenic amines, it crosses the blood-brain barrier (Nakajima, Kakimoto & Sano, 1974) and the uptake process for PEA in lung includes a large diffusion component (Ben-Harari & Bakhle, 1980).

The pronounced selectivity of the uptake system in lung (Alabaster, 1977) bestows on the lung the rare ability to control the inactivation of different monoamines. Once this cellular organization of the lung is destroyed, in order to prepare cell-free preparations, the enzyme activities obtained are not always truly representative of enzymatic activity occuring in perfused lung or *in vivo* (Bakhle & Vane, 1974; Youdim & Woods, 1975; Youdim, Bakhle & Ben-Harari, 1979), where uptake exerts a crucial control.

We have studied the uptake and deamination of [^{14}C]-benzylamine (BA) and [^{14}C]-tryptamine (Tryp) in isolated perfused lung of the rat since both amines, like PEA contain no polar groups. For comparison, we have also studied the metabolism of a large number of amines in rat isolated perfused liver to determine whether the selectivity shown by uptake in isolated perfused lung in monoamine inactivation is shared by the isolated perfused liver.

Methods

Animals and preparation of isolated organs

The lungs from male rats (Sprague-Dawley strain, 150–200 g) were prepared as previously described (Alabaster & Bakhle, 1970). The perfusion medium was warmed (37°C) Krebs bicarbonate solution, gassed with 95% O_2 and 5% CO_2 and was pumped through the pulmonary circulation at 8 ml/min. The livers from female rats (Wistar strain, 150–250 g) were perfused via the portal circulation as previously

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described (Hems, Ross, Berry & Krebs, 1966) with a semisynthetic perfusion medium (Woods, Eggleston & Krebs, 1970).

Measurement of uptake and metabolism in perfused organs

The measurement of uptake in perfused lungs has been described previously (Ben-Harari & Bahkle, 1980). Uptake was calculated from the radioactivity retained in the lung after the end of the infusion together with the radioactive metabolite in the effluent. Metabolism in perfused liver was measured by varying the initial concentrations of substrates in the perfusion medium. During perfusion of liver, 0.5 ml aliquots of the medium were withdrawn at intervals and the protein precipitated with 6% (v/v) perchloric acid. The initial rate of metabolic change was calculated from the gradient of the plot of total deaminated product in the medium versus time and expressed as deaminated product produced per min and per liver.

The action of MAO was followed by determinating the deaminated metabolites by the method of Krajl (1963) for kynuramine and the method of Tipton & Youdim (1976) for the other amines.

Materials

The following radioactive amines were obtained from the Radiochemical Centre (Amersham): [1-^{14}C]-5-HT creatinine sulphate (54 mCi/mmol); [2-^{14}C]-dopamine hydrochloride (48 mCi/mmol); [2-^{14}C]-tyramine hydrochloride (41 mCi/mmol); [7-^{14}C]-benzylamine hydrochloride (50 mCi/mmol); (±)-[8-^{14}C]-noradrenaline bitartrate (50 mCi/mmol).

[2-^{14}C]-tryptamine bissucinate (51 mCi/mmol) and [1-^{14}C]-β-phenylethylamine hydrochloride (52 mCi/mmol) were obtained from New England Nuclear (Frankfurt). The unlabelled substrates were obtained from Sigma except for 5-HT (B.D.H.) Other chemicals used were of analytical reagent grade.

Statistical methods

The significance of differences between means was calculated by Students' t test for unpaired samples and values of $P < 0.05$ were accepted as significant.

Results

Uptake and metabolism in perfused lung

The rate of uptake was first studied using a single concentration of [^{14}C]-Tryp (50 μM) and [^{14}C]-BA (100 μM) and varying the duration of the infusion. The durations of infusion were 1 min, 3 min and 5 min (Table 1). Uptake of both substrates was maximal using a 1 min infusion. The longer infusion times of 3 min and 5 min resulted in no change in the uptake of BA but uptake of Tryp was significantly lower. In all further uptake studies a 1 min infusion was used.

Over the concentration range studied (2.5–1,000 μM) there was an increase in the uptake of both [^{14}C]-Tryp and [^{14}C]-BA and no plateau was attained for either amine (Figure 1).

When the amount of metabolite in lung and effluent were added together and plotted as a function of substrate concentration, saturation of metabolism was evident for Tryp (Figure 2a). From these results the apparent K_m for metabolism of Tryp was 120 μM and the V_{max} was 142 nmol min^{-1}g^{-1} lung. However for BA, over a wide range of concentrations (2.5–1,000 μM) saturation of metabolism was not evident (Figure 2b). These results have been analysed (Neame & Richards, 1972; Ben-Harari & Bakhle, 1980) to provide a linear component of metabolism and a saturable component of metabolism (Figure 2c) with a K_m of 30 μM and a V_{max} of 33 nmol min^{-1} g^{-1} lung.

Metabolism in perfused and in vitro *preparations of lung and liver*

MAO activity in mitochondrial preparations of lung has been studied previously (Bakhle & Youdim, 1979). The kinetic constants for BA and Tryp metabolism by MAO in mitochondrial preparations and in perfused lung are shown in Table 2. Included in Table 2 are the values for metabolism of PEA, another substrate like BA and Tryp, which is deaminated by type B MAO (Bakhle & Youdim, 1979) and the values for 5-HT and NA, which are deaminated by type A MAO. The K_m values for BA, Tryp and PEA in perfused lung are comparable and the K_m and

Table 1 Rate of uptake of [^{14}C]-tryptamine (Tryp) and [^{14}C]-benzylamine (BA) after different times of infusion

Duration of infusion (min)	*Uptake (nmol min^{-1}g^{-1} lung* *Tryp* (50 μM)	*BA* (100 μM)
1	52 ± 8(6)	62 ± 12(6)
3	14 ± 2(6)*	101 ± 13(5)
5	—	68 ± 9(5)

Perfused lung received a 1, 3, or 5 min infusion of [^{14}C]-Tryp or [^{14}C]-BA.
Numbers in parentheses represent the number of experiments.
Results are expressed as mean ± s.e.mean.
*Significantly different from 1 min infusion.

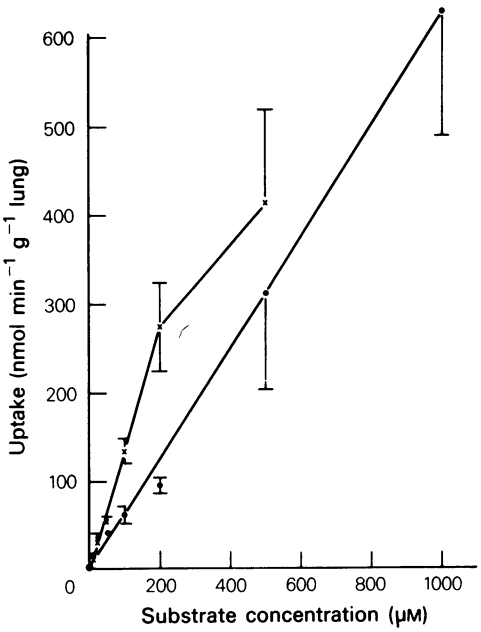

Figure 1 Uptake of [^{14}C]-tryptamine ([^{14}C]-Tryp) (×) and [^{14}C]-benzylamine ([^{14}C]-BA) (●) in isolated perfused lung of rat. Uptake was determined over the concentration range 2.5–1,000 μM and was measured as the sum of radioactivity retained in the lung and the radioactive metabolite in effluent following a 1 min infusion of ^{14}C-amine. Uptake is expressed as nmol min^{-1} g^{-1} lung. Each point represents the mean value of 4–9 experiments. The standard error is shown by the vertical bars where this is larger than the symbols used.

V_{max} values *in vitro* and in perfused lung show an overall similarity. For NA and 5-HT, the K_m values *in vitro* are much greater than in perfused lung.

MAO activity in cell-free systems derived from liver has been thoroughly studied (Housley & Tipton, 1974). The metabolism of a series of monoamines was studied in perfused liver. With all substrates the deaminated product increased with increasing substrate concentration (0.01–4.0 mM) until it reached a plateau. The result for BA and kynuramine is shown in Figure 3a. Table 3 compares the kinetic parameters for MAO oxidation in perfused liver, obtained from double reciprocal plots such as those in Figure 3b, with those *in vitro*. Values for MAO oxidation in isolated perfused livers closely resemble those calculated from experiments *in vitro*.

Effect of deprenyl and competing amines on uptake and metabolism of [^{14}C]-tryptamine and [^{14}C]-benzylamine in perfused lung

Determining the effect of a drug on metabolism of amines in perfused lung is complicated since the drug could compete both for MAO and for uptake. Inhibition of uptake and/or MAO should show itself as an inhibition of the total metabolite formed; competition for MAO alone should show itself as an inhibition of the proportion of metabolite in lung, a value shown to reflect accurately MAO activity in lung (Ben-Harari & Bakhle, 1980); and competition for uptake should show itself as an inhibition of the sum of radioactivity in lung plus radioactive metabolite in the effluent. Drugs were infused for 20 min before and during the infusion of the test drug.

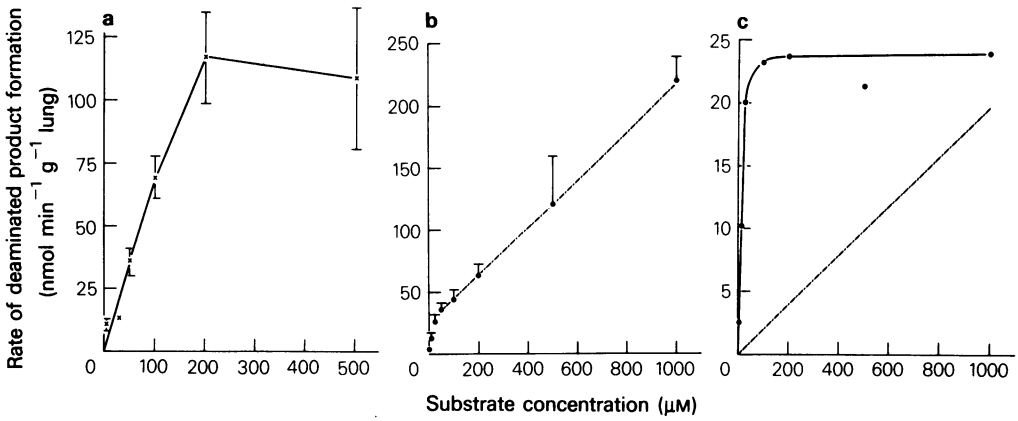

Figure 2 Metabolism of [^{14}C]-tryptamine ([^{14}C]-Tryp) (×) and [^{14}C]-benzylamine ([^{14}C]-BA) (●) in isolated perfused lung of rat. Metabolism was measured as the sum of radioactive metabolite in lung and in effluent following a 1 min infusion of ^{14}C-amine. Metabolism is expressed as nmol deaminated metabolite formed min^{-1} g^{-1} lung. Each point represents the mean value of 4–9 experiments. The s.e. is shown by the vertical bars where this is larger than the symbols used. (a) Metabolism of Tryp (2.5–500 μM). (b) Analysis of BA metabolism according to Neame & Richards (1972). The presentation of this analysis is described in Ben-Harari & Bakhle (1980). (c) Saturable component of BA metabolism.

Table 2 Kinetic parameters of monoamine deamination in perfused lung and mitochondrial preparations of lung

Substrate	K_m (μM) Perfused	K_m (μM) mitochondria*	V_{max} Perfused[a]	V_{max} mitochondria[b]
Benzylamine	30	135	33	100
Tryptamine	120	27	142	75
β-Phenylethylamine*	54	28	300	230
Noradrenaline*	1	410	3	78
5-Hydroxytryptamine*	2	330	34	88

[a]Expressed as absolute V_{max} (nmol min^{-1} g^{-1} lung); [b]expressed as relative V_{max} (V_{max} BA = 100).
*Results from Bakhle & Youdim (1979).

Table 3 Kinetic parameters of monoamine oxidation in perfused liver and homogenates of liver

Substrate	K_m (μM) Perfused	K_m (μM) Homogenate[b]	V_{max}[a] Perfused	V_{max}[a] Homogenate[b]
Benzylamine	200	245	100	100
β-Phenylethylamine*	37	21	125	118
Kynuramine	75	71	60	54
5-Hydroxytryptamine	288	187	125	124
Noradrenaline	405	416	70	71
Tyramine	270	282	208	200
Dopamine	365	405	120	112

[a]Expressed as relative V_{max} (V_{max} BA = 100); [b]homogenate results from Houslay & Tipton (1974).

Table 4 Effect of various drugs on uptake and metabolism of [^{14}C]-tryptamine ([^{14}C]-Tryp) and ^{14}C-benzylamine ([^{14}C]-BA) by rat isolated lung

Treatment	Substrate amine	% inhibition Uptake	% inhibition Metabolite	% inhibition MAO
Deprenyl (10 μM)	[^{14}C]-Tryp (50 μM)	42 ± 8*	72 ± 3*	57 ± 4*
	[^{14}C]-BA (10 μM)	68 ± 3*	66 ± 3*	0
β-Phenylethylamine (1 mM)	[^{14}C]-Tryp (50 μM)	64 ± 1*	79 ± 2*	51 ± 5*
	[^{14}C]-BA (50 μM)	49 ± 7*	79 ± 6*	76 ± 7*
Benzylamine (1 mM)	[^{14}C]-Tryp (50 μM)	39 ± 4*	58 ± 4*	39 ± 2*

[^{14}C]-Tryp and [^{14}C]-BA were infused at the concentrations shown for 1 min. Drugs were infused for 20 min before and during the infusion of substrate amine. The values shown are means (± s.e.) of between 4–11 experiments. Uptake was measured as the sum of radioactivity retained in lung and radioactive metabolite in effluent. Metabolite was measured as the sum of metabolite in lung and in effluent. MAO activity was measured as the metabolite in lung as a proportion of the total radioactivity retained in lung.
*Significantly different from control values ($P < 0.05$; unpaired t test).

Deprenyl is an inhibitor of MAO-B (Knoll, 1976) that preferentially metabolises Tryp and BA in rat lung (Bakhle & Youdim, 1979). Uptake of [^{14}C]-BA and [^{14}C]-Tryp was inhibited on the presence of deprenyl (Table 4). For [^{14}C]-BA, inhibition of total metabolite formed corresponded with inhibition of uptake, without an inhibition of MAO. However, for [^{14}C]-Tryp, inhibition of total metabolite formed (72%) appeared to be the sum of both an inhibition of uptake (42%) and an inhibition of MAO activity (57%).

Competition between monoamines in perfused lung was studied by measuring uptake of radiolabelled substrate in the presence of unlabelled alternative substrate. Uptake and MAO activity towards [^{14}C]-Tryp were inhibited by a 20 fold molar excess of unlabelled BA and PEA; inhibition of both these processes appeared to contribute to inhibition of

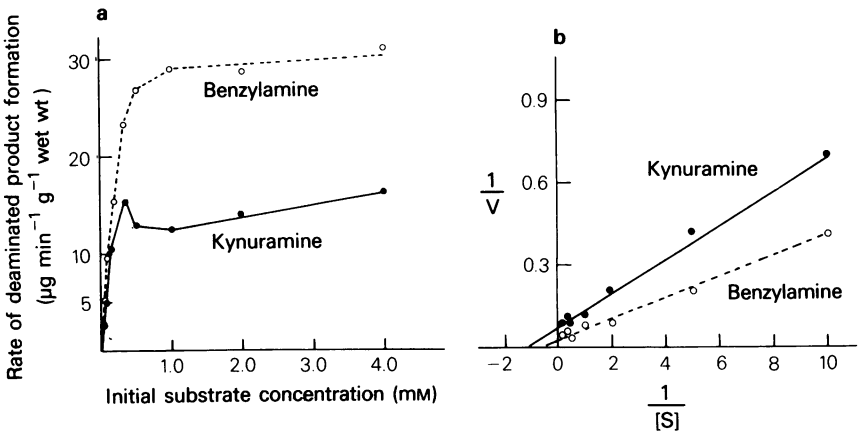

Figure 3 Metabolism of [^{14}C]-benzylamine in isolated perfused liver of rat. (a) Variation of metabolism with substrate concentration (10–4,000 μM). Metabolism was measured from the gradient of the plot of total deaminated product in the medium versus time and is expressed as deaminated metabolite formed min^{-1} g^{-1} liver. (b) Double reciprocal plot of data from (a).

total metabolite formed (Table 4). Uptake of [^{14}C]-BA was inhibited by a 20 fold molar excess of PEA. However, MAO activity towards [^{14}C]-BA was inhibited to a greater extent by PEA than was uptake and this inhibition of MAO (76%) corresponded more closely to the value for inhibition of total metabolite (79%) than did inhibition of uptake (49%).

Discussion

These experiments have clearly shown that metabolism of BA and Tryp in rat isolated lung is not limited by uptake and thus pulmonary metabolism of these amines is similar to that of their non-hydroxylated congener, PEA. The first similarity was found in the uptake experiments. Over the concentration range studied, uptake of BA and Tryp did not show saturation. Like PEA, the latter feature possibly reflects the less polar nature of these amines and is compatible with their ability to cross the blood-barrier (Nakajima *et al.*, 1969; Oldendorf, 1971; Martin, Sloan, Vaupel, Bell & Nozaki, 1976). The second similarity, the non-limiting effect of uptake, was most apparent after comparing the kinetic values for metabolism in perfused lung with those in mitochondrial preparations of lung. The K_m values for deamination in perfused lung and in mitochondrial preparations of lung were similar showing the lack of effect of uptake in limiting metabolism. Another criterion for a non-uptake-limited catabolism is that inhibition of MAO results in inhibition of uptake. This has been shown in lung after uptake of [^{14}C]-PEA was inhibited by the MAO inhibitor, deprenyl (Ben-Harari & Bakhle, 1980). In the present experiments, after deprenyl there was a decrease in the uptake of Tryp and BA in lung. In addition, deprenyl appears to inhibit directly the transfer of BA and Tryp into the lung, contributing considerably to inhibition of their catabolism. Deprenyl is known to inhibit directly the uptake of several amines in different tissues (Braestrup, Andersen & Randrup, 1975; Simpson, 1978; Finberg, Tenne & Youdim, 1981). It would appear from Table 4 that deprenyl does not inhibit MAO activity towards BA in lung. This is unlikely since deprenyl is a potent inhibitor of type B MAO in lung (Bakhle & Youdim, 1979). A likely explanation may be found in the concentration of [^{14}C]-BA employed (10 μM) which is well below the K_m for deamination of BA by MAO (135 μM; Table 2).

The resemblance between PEA uptake and BA and Tryp uptake is strengthened by the fact that uptake of each substrate was inhibited by at least one of the other substrates. In the case of [^{14}C]-Tryp, uptake appears to be inhibited directly by both BA and PEA and perhaps indirectly by their inhibition of MAO. However, in the case of [^{14}C]-BA, inhibition of uptake is most probably due to an inhibition of MAO, rather than uptake directly. The earlier uptake experiments of Ben-Harari & Bakhle (1980) demonstrated an inhibition of ^{14}C-PEA uptake in lung by BA.

All these properties contrast directly with the fate of the hydroxylated amines 5-HT and NA in lung. Uptake of the latter amines in lung is saturable at low amine concentrations and does not show a diffusion component (Junod, 1972; Nicholas, *et al.*, 1974). This rate-limiting process in perfused lung has a K_m of 2 μM, which is much less than the value for the K_m of MAO activity *in vitro* (200–400 μM; Bakhle &

Youdim, 1979); is not affected after inhibition of MAO and catechol-*O*-methyl transferase activity (Junod, 1972; Nicholas *et al.*, 1974); and is not inhibited by PEA, Tryp or BA (Youdim *et al.*, 1979). Another major difference between these amines lies in the catabolic enzyme selectivity. In lung, MAO type A selectively deaminates 5-HT and NA, whereas type B MAO selectively deaminates PEA, BA and Tryp (Bakhle & Youdim, 1979).

There was an overall similarity in kinetic parameters for MAO activity in liver and lung for both hydroxylated and non-hydroxylated amines. However, for the hydroxylated amines 5-HT and NA, the selectivity exhibited by perfused lung in metabolism of these amines (Alabaster, 1977; Youdim *et al.*, 1979) is not seen with the perfused liver. Thus the selectivity exhibited by lung must derive from the uptake of monoamines. As a result, for liver, predictions of metabolism based on *in vitro* data would be fulfilled in the perfused organ. However, for lung, this prediction would be of little use for amines such as 5-HT and NA where uptake is rate-limiting, but may serve as a reliable guide to PEA, Tryp and BA metabolism in whole lung where MAO activity appears to be the rate-limiting step. In addition, in perfused liver and perfused lung no evidence of substrate inhibition was found such as that which occurs *in vitro* (Youdim & Collins, 1965; Gabay & Valcourt, 1968; White & Wu, 1975; Ekstedt, 1976). Therefore, extrapolation from *in vitro* to *in vivo* conditions may not be justifiable.

From these experiments it can be concluded that metabolism of BA and Tryp in lung is not limited by uptake, and that uptake of these two amines is closer in several characteristics to uptake of PEA (Ben-Harari and Bakhle, 1980) and to uptake of exogenous compounds, like imipramine, propranolol and amphetamine (Anderson, Orton, Pickett & Eling, 1974; Dollery & Junod, 1976). The high affinity and large capacity for uptake of PEA in lung would suggest that this is the primary substrate for a system in lung which is capable of dealing with endogenous and exogenous drug molecules. We still do not know in which cells this uptake occurs but the presence of competition between PEA, BA and Tryp for uptake probably occurs at the same site. Competition also existed at the enzyme site which also influences the rate of amine catabolism. The selectivity of uptake in lung differentiates pulmonary inactivation from hepatic inactivation. Thus, although liver is regarded as the main metabolic organ in the body, lung plays a different and perhaps more specific role.

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References

ALABASTER, V.A. (1977). Inactivation of endogenous amines in the lungs. In *Lung Biology in Health and Disease*, ed. Bakhle, Y.S. & Vane, J.R. Vol 4, pp. 3–31. New York: Marcel Dekker.

ALABASTER, V.A. & BAKHLE, Y.S. (1970). Removal of 5-hydroxytryptamine in the pulmonary circulation of rat isolated lungs. *Br. J. Pharmac.*, **40**, 468–482.

ANDERSON, M.W., ORTON, T.C., PICKETT, R.D. & ELING, T.E. (1974). Accumulation of amines in the isolated perfused rabbit lung. *J. Pharmac exp. Ther.*, **189**, 456–466.

BAKHLE, Y.S. & VANE, J.R. (1974). Pharmacokinetic function of the pulmonary circulation. *Physiol. Rev.*, **54**, 1007–1045.

BAKHLE, Y.S. & YOUDIM, M.B.H. (1979). The metabolism of 5-hydroxytryptamine and β-phenylethylamine in perfused rat lung and *in vitro*. *Br. J. Pharmac.*, **65**, 147–154.

BEN-HARARI, R.R. & BAKHLE, Y.S. (1980). Uptake of β-phenylethylamine in rat isolated lung. *Biochem. Pharmac.*, **29**, 489–494.

BRAESTRUP, C., ANDERSEN, H. & RANDRUP, A. (1975). The monoamine oxidase B inhibitor deprenyl potentiates phenylethylamine behaviour in rats without inhibition of catecholamine metabolite formation. *Eur. J. Pharmac.*, **34**, 181–187.

DOLLERY, C.T. & JUNOD, A.F. (1976). Concentration of (±)-propranolol in isolated, perfused lungs of rat. *Br. J. Pharmac.*, **45**, 67–71.

EKSTEDT, B. (1976). Substrate specificity of the different forms of monoamine oxidase in rat liver mitochondria. *Biochem. Pharmac.*, **25**, 1133–1138.

FINBERG, J.P.M., TENNE, M. & YOUDIM, M.B.H. (1981). Tyramine antagonistic properties of AGN1135, an irreversible inhibitor of monoamine oxidase type B. *Br. J. Pharmac.*, **73**, 65–74.

GABAY, S. & VALCOURT, A.J. (1968). Studies of monoamine oxidase. I. Purification and properties of the rabbit liver mitochondrial enzyme. *Biochim. biophys. Acta*, **159**, 440–450.

HEMS, R., ROSS, B.D., BERRY, M.N. & KREBS, H.A. (1966). Gluconeogenesis in the perfused rat liver. *Biochem, J.*, **101**, 284–292.

HOUSLAY, M.D. & TIPTON, K.F. (1974). A kinetic evaluation of monoamine oxidase activity in rat liver mitochondrial outer membranes. *Biochem. J.*, **139**, 649–652.

JUNOD, A.F. (1972). Uptake, metabolism and efflux of ^{14}C-5-hydroxytryptamine in isolated perfused rat lungs. *J. Pharmac. exp. Ther.*, **183**, 341–355.

KNOLL, J. (1976). Analysis of the pharmacological effects of selective monoamine oxidase inhibitors. In *Monoamine Oxidase and Its Inhibition*. Ciba Foundation

Symposium, Vol. 89, pp. 135–161. Amsterdam: Elsevier.

KRAJL, M. (1963). A rapid fluorimetric determination of monoamine oxidase. *Biochem. Pharmac.*, **14,** 1684–1686.

MARTIN, W.R., SLOAN, J.W., VAUPEL, D.B., BELL, J.A. & NOZAKI, M. (1976). Tryptamine in the brain and the spinal cord: its role in the LSD response. In *Trace Amines and the Brain*, ed. Usdin, E. & Sandler, M. pp. 83–102. New York: Marcel Dekker.

NAKAJIMA, T., KAKIMOTO, Y. & SANO, I. (1974). Formation of β-phenylethylamine in mammalian tissue and its effect on motor activity in the mouse. *J. Pharmac. exp. Ther.*, **143,** 319–325.

NEAME, K.D. & RICHARDS, T.C. (1972). Analysis of experimental data. In *Elementary Kinetics of Membrane Carrier Transport.* ed. Neame, K.D. & Richards, T.G. pp. 41–55. Oxford: Blackwell Scientific Publications.

NICHOLAS, T.E., STRUM, J.M., ANGELO, L.S. & JUNOD, A.F. (1974). Site and mechanism of uptake of ^{3}H-L-norepinephrine by isolated perfused rat lungs. *Circulation Res.*, **35,** 670–680.

OLDENDORF, W.H. (1971). Brain uptake of radiolabelled amino acids, amines and hexoses after arterial injection. *Am. J. Physiol.*, **221,** 1629–1639.

SIMPSON, L.L. (1978). Evidence that deprenyl, a type B monoamine oxidase inhibitor, is an indirectly acting sympathomimetic amine. *Biochem. Pharmac.*, **27,** 1591–1595.

TIPTON, K.F. & YOUDIM, M.B.H. (1976). Assay of monoamine oxidase. In *Monoamine Oxidase and Its Inhibition.* Ciba Foundation Symposium, Vol. 39, pp. 393–403. Amsterdam: Elsevier.

WHITE, H.L. & WU, J.C. (1975). Multiple binding sites of human brain monoamine oxidase as indicated by substrate concentration. *J. Neurochem.*, **25,** 21–26.

WOODS, H.F., EGGELSTON, L.V. & KREBS, H.A. (1970). The cause of hepatic accumulation of fructose l-phosphate on fructose loading. *Biochem. J.*, **119,** 501–510.

YOUDIM, M.B.H., BAKHLE, Y.S. & BEN-HARARI, R.R. (1979). Comparison of monoamine oxidase activity in perfused organs and in vitro. In *Monoamine Oxidase: Structure, Function and Altered Functions* ed. Singer, T.P., von Korff, R.W. & Murphy, D.L., pp. 361–377. New York: Academic Press.

YOUDIM, M.B.H. & COLLINS, G.G.S. (1975). In: *Isozymes:* Vol. 11. *Physiological Function,* ed. Markert, C.L., p. 619. New York: Academic Press.

YOUDIM, M.B.H. & WOODS, H.F. (1975). The influence of tissue environment on the rates of metabolic processes and the properties of enzymes. *Biochem. Pharmac.*, **24,** 317–323.

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