METABOLISM OF PHENYLETHYLAMINE IN RAT ISOLATED PERFUSED LUNG: EVIDENCE FOR MONOAMINE OXIDASE 'TYPE B' IN LUNG

Y.S. BAKHLE
Department of Pharmacology, Institute of Basic Medical Sciences,
Royal College of Surgeons of England, Lincoln's Inn Fields, London WC2A 3PN
M.B.H. YOUDIM
MRC Unit and University Department of Clinical Pharmacology, Radcliffe Infirmary,
Oxford OX2 6HE

Phenylethylamine is inactivated in a single passage through rat lung tissue by a process of uptake and deamination by a monoamine oxidase 'type B'. This enzyme is particularly susceptible to inhibition by deprenil and less sensitive to clorgyline. The monoamine oxidase of the lung, like that of other rat tissues, can be differentiated into 'type A' and 'type B' which appear to operate independently in the organized tissue.

Introduction Monoamine oxidase (MAO) has been differentiated into MAO 'type A' (MAO-A) and 'type B' (MAO-B) on the basis of substrate and inhibitor specificity: noradrenaline and 5-hydroxytryptamine (5-HT) are substrates and clorgyline an inhibitor for MAO-A (Johnston, 1968), whereas phenylethylamine and benzylamine are substrates and deprenil an inhibitor for MAO-B (Yang & Neff, 1973; Knoll & Magyar, 1972). Although MAO-B is present in tissue homogenates, its presence in organized tissue has not yet been demonstrated (Bakhle & Vane, 1974; Youdim & Woods, 1975). The experiments to be described are the first demonstration† of MAO-B activity in an isolated perfused tissue. We have used the rat isolated lung perfused with Krebs solution since substrates for MAO-A, 5-HT and noradrenaline, are metabolized on a single passage through the pulmonary circulation (for references see Bakhle & Vane, 1974) and MAO-B activity is present in homogenates of rabbit lung (Roth & Gillis, 1974) and of rat lung (Bakhle & Youdim, unpublished experiments). By studying the fate of phenylethylamine and comparing it with that of 5-HT in rat isolated perfused lung, we hoped to demonstrate the presence of MAO-A and B and to explore the interactions between the metabolism of these two amines in the whole tissue. Since metabolism by MAO reduces the biological activity of these amines we have followed the action of MAO by bioassay as well as by radiochemical assay.

Methods The isolated lung was perfused via the pulmonary artery with warm (37°C), oxygenated Krebs solution at 8 ml/minute. For bioassay, the effluent from the lung superfused rat stomach strips (Vane, 1957) which respond to both amines with contractions, although phenylethylamine is about 1000 times less potent than 5-HT. On this tissue, phenylethylamine acts via tryptamine receptors (Vane, 1960). For the radiochemical assay we collected the effluent in 0.5 min fractions during and after the infusion of radioactive amines. In each fraction we measured the total radioactivity and separated this radioactivity into basic and non-basic materials by ion exchange chromatography (Southgate & Collins, 1969). The radioactivity associated with the non-basic material was taken to represent the metabolite(s) of 5-HT and of phenylethylamine.

Results and Discussion These experiments showed that 75% of infused radioactive phenylethylamine had emerged from the lung within 5 min, i.e. 1 min after the end of the infusion (0–4 min) and that after 30 min, 90% had left the lung. The radioactivity associated with non-basic material, i.e. metabolite of phenylethylamine, had a maximum value during the infusion of about 40% of the total but after about 6 min, nearly all radioactivity in the effluent was metabolite. The high proportion of administered radioactivity in the lung effluent and the rapidity with which it appeared, argue strongly against the binding of amine in a store, e.g. nerve granules. The same two characteristics have been observed for the removal of 5-HT in rat lung (Alabaster & Bakhle, 1970).

At the concentration used, the biological inactivation of phenylethylamine was between 50 and 60%. The radiochemical assay showed that about 55% of the infused radioactivity which emerged from the lung was metabolite. We feel that the correspondence between these two values is close enough to conclude that the loss of biological activity observed when phenylethylamine passes through the rat isolated lung is due chiefly to its metabolism by a monoamine oxidase type of enzyme.

Since phenylethylamine is the substrate, this monoamine oxidase should be of the B type and thus more susceptible to inhibition by deprenil than by clorgyline (Johnston, 1968; Knoll & Magyar, 1972). Experiments with these two monoamine oxidase inhibitors substantiated this prediction and are summarized in Table 1.

All the earlier work with 5-HT and noradrenaline

had emphasized the importance of the uptake step in the overall inactivation process and the question of competition between noradrenaline and 5-HT for uptake has received some attention (Junod, 1972; Alabaster & Bakhle, 1973; Iwasawa & Gillis, 1974; Nicholas, Strum, Angelo & Junod, 1974). We looked for competition between 5-HT and phenylethylamine uptake by measuring metabolism of one labelled substrate in presence of the other. Competition for uptake should show itself as decreased metabolism. Our experiments are summarized in Table 1 and show that there was no competition between the amines at equimolar concentrations and even when phenylethylamine was present at almost 200 times molar excess, the metabolism of 5-HT was not inhibited.

An interesting point to emerge from Table 1 is that at equimolar concentrations (1.25×10^{-5} M), phenylethylamine suffered about 50% metabolism, whereas 5-HT was less affected. Clearly the removal process for 5-HT is close to its limit whereas that for phenylethylamine seems to be well below its limit, at 1.25×10^{-5} M. For comparison, the *Km* values for the A and B type enzymes isolated from liver are 18.7×10^{-5} M and 2.0×10^{-5} M with 5-HT and phenylethylamine as substrates respectively (Houslay & Tipton, 1974). On the basis of these *Km* values neither enzyme would be expected to be saturated at concentration of substrates of 1.25×10^{-5} M; therefore either the lung enzymes have very different *Km* values from the liver enzymes, or in the perfused lung the uptake of amines into lung cells is limiting. We favour the latter possibility. The saturation of amine uptake in lung has been noticed earlier (Junod, 1972; Hughes, Gillis & Bloom, 1969); for 5-HT in rat lung the saturating concentrations should be about 10 μM (Junod, 1972) and our results would agree with this. Whether or not there is biological significance in the different capacities of the lung removal processes for 5-HT and phenylethylamine is a matter for speculation. What is more open to investigation is the difference in the uptake mechanisms for the two amines.

In conclusion these experiments strengthen our view (Bakhle & Vane, 1974; Youdim & Woods, 1975) that the enzymatic activity exhibited by the whole tissue is often strikingly different from that found in cell-free extracts. For instance, in partially purified preparations of MAO, 5-HT and phenylethylamine will compete for the enzyme site (Houslay & Tipton, 1975). This demonstration of both A and B type monoamine oxidases operating independently in an organized tissue makes it more likely that we shall find these two enzymatic activities playing a real and independent role *in vivo*, not only in lung but also in other tissues such as brain.

This work was supported by the M.R.C.

† *Note added in proof.* While this paper was in press, essentially similar findings in rabbit lungs have been reported by Roth & Gillis (1975) 'Multiple forms of amine oxidase in perfused rabbit lung'. *J. Pharmac. exp. Ther.*, **194**, 537–544.

Table 1 Effect of monoamine oxidase inhibitors on [^{14}C]-phenylethylamine deamination and metabolism of phenylethylamine and 5-hydroxytryptamine in presence of each other during transit through rat isolated lung

Labelled amine (final conc. in perfusion fluid)	*No. of experiments*	*Inhibitor*	*Competing amine*	*Radioactivity associated with metabolite(s) (% of total)**	*% Inhibition of metabolism*
[^{14}C]-Phenylethylamine (1.25×10^{-5} M)	8	None	None (control)	55.8 ± 3.6	
	5	Deprenil	None	17.4 ± 2.5	69
	3	Clorgyline	None	40.2 ± 1.5	28
	3	None	5-hydroxytryptamine (1.25×10^{-5} M)	54.7 ± 0.3	2
[^{3}H]-5-Hydroxytryptamine (1.25×10^{-5} M)	4	None	None (control)	11.6 ± 1.3	–
	2	None	Phenylethylamine (1.25×10^{-5} M)	11.0 ± 12.3	0
[^{3}H]-5-Hydroxytryptamine (7×10^{-8} M)	7	None	None (control	53.6 ± 2.6	–
	4	None	Phenylethylamine (1.25×10^{-5} M)	53.8 ± 4.1	0

The inhibitors deprenil or clorgyline were infused for 10 min before the infusion of phenylethylamine (4 min) and throughout the subsequent collection period (30 minutes). The concentration of these two inhibitors was 100 μg/ml in each case. The labelled amines [1-^{14}C]-phenylethylamine or [G-^{3}H]-5-hydroxytryptamine (approximately 60,000 d/min) were infused for 4 minutes. The competing amine, unlabelled, was infused for 5 min before, during and for 25 min after, the infusion of labelled amine.
* Mean ± s.e. mean.

References

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.

ALABASTER, V.A. & BAKHLE, Y.S. (1973). The removal of noradrenaline in the pulmonary circulation of rat isolated lungs. *Br. J. Pharmac.*, **47**, 325–331.

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

HOUSLAY, M.D. & TIPTON, K.F. (1974). A kinetic evaluation of monoamine oxidase activity in rat liver mitochondrial outer membranes. *Biochem. J.*, **139**, 645–652.

HOUSLAY, M.D. & TIPTON, K.F. (1975). Amine competition for oxidation by rat liver mitochondrial monoamine oxidase. *Biochem. Pharmac.*, **24**, 627–631.

HUGHES, J., GILLIS, C.N. & BLOOM, F.E. (1969). The uptake and disposition of dl-norepinephrine in perfused rat lungs. *J. Pharmac. exp. Ther.*, **169**, 237–248.

IWASAWA, Y. & GILLIS, C.N. (1974). Pharmacological analysis of norepinephrine and 5-hydroxytryptamine removal from the pulmonary circulation: differentiation of uptake sites for each amine. *J. Pharmac. exp. Ther.*, **188**, 386–393.

JOHNSTON, J.P. (1968). Some observations upon a new inhibitor of monoamine oxidase in brain tissue. *Biochem. Pharmac.*, **17**, 1285–1297.

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

KNOLL, J. & MAGYAR, K. (1972). Some puzzling pharmacological effects of monoamine oxidase inhibitors. *Adv. Biochem. Psychopharmac.*, **5**, 393–408.

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

ROTH, J.A. & GILLIS, C.N. (1974). Deamination of β-phenylethylamine by monoamine oxidase-inhibition by imipramine. *Biochem. Pharmac.*, **23**, 2537–2545.

SOUTHGATE, J. & COLLINS, G.G.S. (1969). The estimation of monoamine oxidase using ^{14}C-labelled substrates. *Biochem. Pharmac.*, **18**, 2285–2287.

VANE, J.R. (1957). A sensitive method for the assay of 5-hydroxytryptamine. *Br. J. Pharmac. Chemother.*, **12**, 344–349.

VANE, J.R. (1960). In *Adrenergic mechanisms*, ed. Vane, J.R., Wolstenholme, G.E.W. & O'Connor, M., pp. 356–373. London: Churchill.

YANG, H.Y.T. & NEFF, N.H. (1973). β-Phenylethylamine: a specific substrate for type B monoamine oxidase of brain. *J. Pharmac. exp. Ther.*, **187**, 365–371.

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.

(Received September 4, 1975)