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A kinetic investigation of the pulmonary metabolism of dopamine in rats shows marked differences compared with noradrenaline

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Abstract The aim of this study was to investigate the deamination of dopamine in the intact pulmonary circulation of isolated lungs of the rat. The first part of the study showed that dopamine is not converted to noradrenaline by dopamine-β-hydroxylase (DBH) when dopamine is perfused through isolated lung preparations with monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT) inhibited. Hence, it was not necessary to inhibit DBH in subsequent experiments.

The metabolite profile for deamination of dopamine in the lungs was examined by determining whether MAO and semicarbazide-sensitive amine oxidases (SSAO) contribute to the deamination of dopamine (and noradrenaline), and by determining the activity of MAO (k_{MAO}) for the metabolism of dopamine. Lungs were perfused with 1 nmol/l ^{3}H-dopamine or ^{3}H-noradrenaline with COMT inhibited and, in experiments to determine the contribution of SSAO to deamination, with MAO inhibited. Inhibition of MAO reduced the deamination of dopamine and noradrenaline by 99.8% and 98.6%, respectively, indicating that MAO, and not SSAO, was responsible for deamination of the catecholamines in the lungs. The k_{MAO} value for deamination of dopamine was 3.89 min^{-1}. Further experiments were carried out to determine the contributions of MAO-A and MAO-B to the deamination of dopamine in lungs perfused with 1 nmol/l ^{3}H-dopamine and 100 nmol/l lazabemide or 300 nmol/l Ro41-1049, respectively. The values of $k_{MAO\text{-}A}$ and $k_{MAO\text{-}B}$ were 3.05 min^{-1} and 0.626 min^{-1}, respectively.

It was concluded that, in rat lungs, MAO-A contributed 78–84% and MAO-B 16–22% to the total deamination of dopamine and SSAO had no significant role in its pulmonary metabolism. These relative contributions of MAO-A and MAO-B to the deamination of dopamine are very similar to those that have been determined previously for noradrenaline, but the rate constant for deamination of dopamine is 26-fold greater than that for noradrenaline in rat lungs.

Key words Dopamine · Monoamine oxidase-A Monoamine oxidase-B · Kinetics · Rat lungs

Abbreviations COMT Catechol-O-methyltransferase DBH Dopamine-β-hydroxylase · DOPEG 3′,4′-dihydroxyphenylglycol · DOMA 3′,4′-dihydroxymandelic acid · DOPAC 3′,4′-dihydroxyphenylacetic acid · DOPET 3′,4′-dihydroxphenylethanol · ECS Extracellular space · K_m Michaelis or half-saturation constant · k_{COMT} Rate constant for O-methylation by COMT · k_{deam} Rate constant for total deamination · k_{MAO} Rate constant for deamination by MAO · MAO Monoamine oxidase · MB-COMT Membrane-bound COMT · SSAO Semicarbazide-sensitive amine oxidases · S-COMT Soluble COMT · T/M Tissue to medium concentration ratio of dopamine or noradrenaline · V_{max} Maximal rate · v_{st-st} Steady-state rate of metabolite formation

Introduction

Dopamine is an endogenous catecholamine that is both a neurotransmitter and a precursor for the biosynthesis of noradrenaline and adrenaline. Dopamine plays an important role as a neurotransmitter in the central nervous system and also in the kidneys, heart, blood vessels and gastrointestinal tract (Dasta and Kirby 1986). In view of the role of dopamine release in preventing circulatory failure in conditions such as

Preliminary results of this study were presented to the December 1992 meeting of the Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists (Scarcella and Bryan-Lluka 1992)

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haemorrhagic shock and hypoxia (Chernow et al. 1982), dopamine is now an important pharmacological agent in the treatment of shock and congestive heart failure (Goldberg 1974; Horn and Murphy 1990). However, dopamine has a plasma half-life of only 1–2 min in man (Dasta and Kirby 1986) and pulmonary removal may contribute to this short half-life, as the lungs are an important site for the clearance of circulating catecholamines. We recently showed that dopamine is taken up and metabolised in perfused lungs of rats (Bryan-Lluka and O'Donnell 1992), and this is consistent with the finding that uptake of catecholamines into pulmonary endothelial cells occurs by uptake$_1$ (Bryan-Lluka and O'Donnell 1992; Bryan-Lluka et al. 1992). These results are also supported by those of other studies, including both in vitro and in vivo data, that have shown that there is pulmonary removal of dopamine in other species including rabbits (Gillis and Roth 1977; Friedgen et al. 1993), dogs (van Schaik et al. 1985, 1988; Sumikawa and Hirano 1986; Cardoso et al. 1991) and man (Russel et al. 1982). This is despite some early reports which concluded that dopamine was not removed from the pulmonary circulation of rats (Boileau et al. 1972; Nicholas et al. 1974), dogs (Boileau et al. 1972) and man (Boileau et al. 1972; Sole et al. 1979), although the lack of detection of dopamine removal in these latter studies could be due to the use of low sensitivity assay procedures or not taking into account subsequent metabolism.

After uptake in rat lungs, dopamine is rapidly metabolised to both deaminated and O-methylated metabolites, so that there is little accumulation of unchanged dopamine (Bryan-Lluka and O'Donnell 1992). A kinetic analysis of the O-methylation of dopamine showed that COMT has a much higher activity for metabolism of dopamine than for metabolism of noradrenaline in the lungs (Bryan-Lluka and O'Donnell 1992), but the kinetic properties of deamination of dopamine in intact lungs have not been investigated. The aim of the present study was to determine the contributions and activities of the two isoenzymes of MAO, MAO-A and MAO-B (Johnston 1968), and of SSAO in the deamination of dopamine in perfused lungs of the rat. Previous studies have shown that dopamine is a substrate for MAO-A and MAO-B (Bakhle and Youdim 1979) and for SSAO (Lewinsohn et al. 1978) in homogenates of rat lungs, but it was not possible from those studies to determine the quantitative contributions of the enzymes to the deamination of dopamine or to define the cell types involved since the cellular organisation of the tissue had been destroyed in homogenising the lungs. In the present study, our aim was to determine the contributions of the deaminating enzymes to the metabolism of dopamine under conditions of intact perfused lungs so that the results would reflect the fate of dopamine in the pulmonary circulation, whilst maintaining the controlled conditions possible in an in vitro preparation. The experiments were carried out using pargyline, which inhibits MAO but not SSAO (Coquil et al. 1973; Dial and Clarke 1977), and the selective and reversible inhibitors of MAO-A, Ro41-1049, and MAO-B, lazabemide (Da Prada et al. 1990).

Materials and methods

Preparation of lungs for in vitro perfusion. Adult, male or female, specific pathogen-free Wistar rats (210–265 g) were anaesthetised with sodium pentobarbitone 60 mg/kg intraperitoneally. When the rat was in deep anaesthesia, the thorax was opened and 2500 U/kg heparin sodium was injected intracardially. The trachea, pulmonary artery and left atria were cannulated in situ and the lungs were then removed from the thorax and ventilated (Bryan-Lluka et al. 1992).

Experiments to determine whether pulmonary conversion of dopamine to noradrenaline occurred. In these experiments, rats were pretreated with 75 mg/kg pargyline i.p. 18 h and 2 h prior to the experiment to inhibit MAO. The lungs were first perfused with Krebs solution containing 5% bovine serum albumin and 10 μmol/l U-0521 (to inhibit COMT) at 10 ml/min and 37° C for 20 min. The composition of the Krebs solution was (in mmol/l): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, D-glucose 11.7, (−)-ascorbic acid 0.57 and Na_2EDTA 0.04. The lungs were then perfused with aerated (95% O_2, 5% CO_2) Krebs solution containing 10 μmol/l U-0521, but no bovine serum albumin, in the absence (control experiments) or presence of 1 μmol/l dopamine for a further 20 min. At the end of this period, the lungs were quickly blotted and weighed (Westwood and Bryan-Lluka 1993) and placed in 0.31 mol/l trichloroacetic acid containing 2.7 mmol/l Na_2EDTA at 4° C for at least 1 h. The lungs were then homogenised and centrifuged at 12,000 × *g* at 4° C for 20 min. Samples of the supernatant were stored at − 80° C (for a maximum of 7 days) until the HPLC assay of dopamine and noradrenaline was carried out.

The HPLC apparatus consisted of an ICI LC1100 HPLC pump, a Rheodyne sample injector with a 50 μl sampling loop, an Activon C-130B guard column and an ICI Spherisorb C_{18} reverse-phase column (15–40 μm, 15 cm × 4.6 mm). The ICI LC1260 electrochemical detector was fitted with a glassy carbon electrode, at + 0.7 V relative to an Ag/AgCl electrode. The mobile phase contained 70 mmol/l sodium dihydrogen phosphate, 0.2 mmol/l Na_2EDTA, 0.5 mmol/l sodium octylsulphonate, 0.08% trifluoroacetic acid and 5% acetonitrile, and was adjusted to pH 3.0. The mobile phase was then filtered and sonicated, and the flow rate was 1 ml/min.

Samples of lung homogenate supernatant stored at − 80° C were defrosted and each diluted in water to a final sample volume of 500 μl, and 10 μl of internal standard (α-methyladrenaline, 0.3 μmol/l) was added. After mixing, 250 μl of 4.44 mmol/l diphenylboric acid 2-aminoethyl ester pH 8.5 (aqueous phase) and 1 ml of 2.35 mmol/l tetra-*n*-octylammonium bromide in octanol:heptane 1:1000 (organic phase) were added. After vortexing and centrifuging, the organic phase was transferred to a test tube and 500 μl octanol and 100 μl 0.16 mol/l acetic acid (aqueous phase) were added. This latter mixture was vortexed and centrifuged, the organic phase was removed and the aqueous phase was kept on ice (for a maximum of 5 h) until it was injected onto the HPLC. Reference samples contained 500 μl of water and 25 μl of a solution containing (in nmol/l): dopamine 1.32, noradrenaline 1.56, adrenaline 0.75 and internal standard 6.08, and were treated as described above. The injection volume of all samples was 50 μl. The retention times for noradrenaline, adrenaline, the internal standard and dopamine were 2.5, 4.0, 7.0 and 8.3 min, respectively.

Deamination experiments. The lungs were initially perfused at 37° C for 20 min with Krebs solution containing 5% bovine serum albumin, 10 μmol/l U-0521 and, when required, 300 nmol/l Ro41-1049 and/or 100 nmol/l lazabemide. The lungs were then perfused with aerated Krebs solution containing the inhibitors as described above plus 1 nmol/l ^{3}H-dopamine, but no bovine serum albumin, for 20 min. Perfusion solution was collected from the left atrial cannula (venous effluent) as 1 min samples at 4.5, 9.5, 14.5, 16.5 and 18.5 min during the perfusion period with ^{3}H-dopamine. At the end of the perfusion period, the lungs were treated as described above with the exception that the lungs were placed in 0.4 mol/l perchloric acid containing 2.7 mmol/l Na_2EDTA and 2 mmol/l Na_2SO_3. After the lungs had been removed from the chamber, a sample of perfusion solution was collected from the pulmonary artery cannula (arterial sample). Venous effluent, arterial solution and lung homogenate supernatant samples were used for column chromatographic separation of dopamine and its deaminated metabolites, DOPAC and DOPET (Cubeddu et al. 1979), and the ^{3}H content of the unseparated samples and the separated fractions were determined.

In experiments to investigate the pulmonary deamination of dopamine and noradrenaline by SSAO, some rats were pretreated with 75 mg/kg pargyline 18 h and 2 h prior to the experiments. When noradrenaline was used as the substrate, the lungs were perfused as described above but with 1 nmol/l ^{3}H-noradrenaline instead of ^{3}H-dopamine, and column chromatography was used to separate noradrenaline from its deaminated metabolites, DOPEG and DOMA (Graefe et al. 1973; Trendelenburg et al. 1983).

In some experiments (see Results), pellets of the lung homogenate were also analysed for ^{3}H. After samples of the lung homogenate supernatant were taken, the pellet was homogenised in 1 mol/l NaOH (Head et al. 1982) to solubilise the pellet and the homogenate was diluted 1:5 with water. Samples of the pellet homogenate were then assayed for ^{3}H.

Viability of lungs. The viability of each lung preparation was monitored by measuring the perfusion pressure throughout the experiments (0.6–1.8 kPa) and by calculating the lung weight at the end of the experiment as a percentage of the body weight (0.44–0.65%). If the perfusion pressure exceeded 2 kPa or the lung weight exceeded 0.65% of the body weight, the experiment was not included in the results so that data were not included from any lung preparation that showed evidence of oedema.

Drugs and solutions. The drugs used were: N-(2-aminoethyl)-5-(*m*-fluorophenyl)-4-thiazole carboxamide hydrochloride (Ro41-1049; F. Hoffmann-LaRoche Ltd., Basel, Switzerland); 3′,4′-dihydroxy-2-methylpropiophenone (U-0521; Upjohn Pty. Ltd., Kalamazoo, Mich., USA); dopamine hydrochloride (Sigma Chemical Company, St. Louis, Mo., USA); [7-^{3}H]-dopamine (New England Nuclear Research Products, Dupont, Boston, Mass., USA; specific activity 988 Bq/pmol); heparin sodium (as vials of 5000 U/ml; CSL, Ltd., Parkville, Australia); lazabemide (Ro 19-6327; N-(2-aminoethyl)-5-chloro-2-pyridine carboxamide hydrochloride; Hoffmann-LaRoche); (−)-noradrenaline bitartrate (Sigma); (−)-[7-^{3}H]-noradrenaline (New England Nuclear; specific activity 422 Bq/pmol); pargyline hydrochloride (Sigma; administered as a 92.5 mg/ml solution in normal saline); pentobarbitone sodium (as Nembutal vials of 60 mg/ml; Bomac Laboratories Pty. Ltd., Sydney, Australia). The ^{3}H-dopamine and ^{3}H-noradrenaline were purified over alumina before use and the final concentration of labelled amine was 1 nmol/l in all experiments. Bovine serum albumin (Cohn Fraction V, 98–99% albumin) was obtained from Sigma (Catalogue No. A7906).

Stock solutions of lazabemide (1 mmol/l) were prepared in water, and those of dopamine and noradrenaline (10 mmol/l) in 10 mmol/l hydrochloric acid. Stock solutions of Ro41-1049 (1 mmol/l) and U-0521 (1 mmol/l) and all dilutions were prepared in Krebs solution.

Calculation of results. In the experiments that were analysed by HPLC, the dopamine and noradrenaline contents of the lungs were calculated by a comparison of peak heights of the amine and the internal standard (α-methyladrenaline) in the lung homogenate supernatant and reference samples.

The T/M ratios of dopamine and noradrenaline in the lungs were calculated as:

$$\frac{\dfrac{\text{tissue amine content (pmol/g)}}{\text{medium amine concentration (pmol/ml)}} - \text{ECS}}{1 - \text{ECS}}$$

where ECS is the extracellular space of the lungs. The ECS was determined from the distribution of ^{14}C-sorbitol in previous experiments which gave a mean ECS value of 0.461 ml/g under the same conditions (Bryan-Lluka and O'Donnell 1992).

For dopamine metabolism experiments, the steady-state rate of DOPET formation was calculated as the mean of the rates of appearance of DOPET in the venous effluent at the 17th and 19th min of perfusion. As DOPAC appearance in the venous effluent had not reached steady state at the end of the 20-min perfusion period, the steady-state rate of DOPAC formation was calculated from the total DOPAC formation (Grohmann 1987). For noradrenaline metabolism experiments, the steady-state rates of DOPEG and DOMA formation were calculated as described above for DOPET and DOPAC, respectively.

The rate constants for deamination (k_{deam} and k_{MAO} values) of dopamine or noradrenaline, which are a measure of the activities of the deaminating enzymes for metabolism of these amines in the lungs, were calculated as the steady-state rate of formation of deaminated metabolites of the amine (v_{st-st}) divided by the product of the T/M ratio of the amine in the lungs and the amine concentration in the medium. Percentage inhibition of MAO activity was determined as the difference between k_{MAO} values in the presence of a MAO inhibitor and that for controls (no MAO inhibitor), expressed as a percentage of the control value.

The amount of ^{3}H remaining in the lung homogenate pellet was expressed as a percentage of the total ^{3}H in the lung homogenate supernatant and pellet.

Results are expressed as arithmetic means ± SE or as geometric means with 95% confidence limits as indicated. The significance of differences between groups was assessed, unless otherwise indicated, by Student's *t*-test (Snedecor and Cochran 1989) on absolute or log values for arithmetic or geometric means, respectively, and were regarded as significant when $P < 0.05$.

Results

Lack of metabolic conversion of dopamine to noradrenaline in rat lungs

Experiments were carried out to determine whether there was any metabolism of exogenous dopamine by DBH in the lungs, by comparing the noradrenaline contents of rat lungs perfused with dopamine and control lungs perfused with Krebs solution. MAO and COMT were inhibited in all experiments to prevent metabolism of dopamine or noradrenaline by these enzymes. Despite a marked increase in the dopamine content of the lungs when they were perfused with 1 μmol/l dopamine (3925 ± 91 pmol/g, $n = 3$) compared with controls (58.8 ± 2.7 pmol/g, $n = 3$; $P < 0.001$), there was no significant difference in the noradrenaline contents of lungs perfused with dopamine

(500 ± 23 pmol/g, $n = 3$) and controls (535 ± 27 pmol/g, $n = 3$; $P > 0.05$). These results suggested that dopamine perfused through the lungs was not metabolised by DBH to form noradrenaline, so it was not necessary to inhibit DBH in the remainder of the study.

Determination of the activities of MAO and SSAO for metabolism of dopamine and noradrenaline in rat lungs

The lungs of rats were perfused with 1 nmol/l ^{3}H-dopamine or ^{3}H-noradrenaline with COMT inhibited and MAO either intact or inhibited by (i) pretreatment of rats with pargyline or (ii) inclusion of 300 nmol/l Ro41-1049 (selective MAO-A inhibitor) and 100 nmol/l lazabemide (selective MAO-B inhibitor) in the perfusion solution. Inhibition of MAO (but not SSAO) by these treatments caused marked reductions in the steady-state rates of deamination of both dopamine and noradrenaline, with accompanying marked increases in the T/M ratios for accumulation of unchanged amine in the lungs (Table 1). Hence, the rate constants for deamination (k_{deam}), calculated from these data, were decreased by 99.8% by either MAO inhibitory protocol for dopamine and by 98.6% and 98.9% by the pargyline and Ro41-1049 + lazabemide MAO inhibitory protocols, respectively, for noradrenaline (Table 1). These results showed that deamination of dopamine and noradrenaline in rat lungs is carried out only by MAO, without any marked contribution by SSAO (i.e. $\leq 0.2\%$ of the total deamination of dopamine and $\leq 1.4\%$ of the total deamination of noradrenaline). Hence, the values for k_{deam} determined when MAO was intact (Table 1) represent the activity of MAO (k_{MAO}) for deamination of dopamine and of noradrenaline in the lungs.

The results in Table 1 with MAO intact and COMT inhibited show that the steady-state rate of deamination of dopamine was 2.4-fold greater than that of noradrenaline, and the T/M ratio of unchanged amine in the lungs was 11-fold less for dopamine than noradrenaline. Hence, the value of k_{MAO} for dopamine was markedly greater (26-fold) than that for noradrenaline in rat lungs (Table 1). The possibility that the T/M ratio of dopamine was underestimated by determining the ^{3}H content of only the supernatant after the lungs were homogenised and centrifuged, and not that remaining in the pellet, was investigated by changing the routine procedure in that the ^{3}H content of the pellet was also determined in some experiments. The percentage of the total ^{3}H content that was in the pellet was 7–9.5% for dopamine experiments and 6.5–8% for noradrenaline experiments and there were no differences in these percentages depending on whether MAO was intact or inhibited. The small amount of ^{3}H in the pellet for experiments in which the lungs were perfused with either dopamine or noradrenaline could not explain the 11-fold greater T/M ratio of noradrenaline than of dopamine in experiments in which MAO was intact and in which the ^{3}H content of the pellet was not taken into account in calculating the T/M ratios. Since the amount of ^{3}H remaining in the pellet was small and consistent, T/M ratios were calculated throughout the study from amounts of ^{3}H in the supernatant, after homogenising and centrifuging the lungs.

Contributions of MAO-A and MAO-B to the deamination of dopamine in rat lungs

The rates of appearance of the deaminated metabolites of dopamine in the venous effluent from lungs perfused

Table 1 Effects of inhibition MAO[a] on the deamination of dopamine or noradrenaline in rat lungs perfused with 1 nmol/l ^{3}H-dopamine or ^{3}H-noradrenaline, respectively, for 20 min with COMT inhibited

Experimental parameter	Dopamine			Noradrenaline		
	MAO Intact	MAO Inhibitied		MAO Intact	MAO Inhibited	
		Pargyline pretreatment	Ro41-1049 + lazabemide		Pargyline pretreatment	Ro41-1049 + lazabemide
	($n = 7$)	($n = 6$)	($n = 5$)	($n = 6$)	($n = 4$)	($n = 3$)
v_{st-st} (pmol·g^{-1}·min^{-1})[b]	4.53 (3.97; 5.17)	0.694* (0.567; 0.850)	0.502* (0.423; 0.594)	1.90† (1.73; 2.09)	0.142*,† (0.112; 0.181)	0.113*,† (0.065; 0.197)
T/M (ml/g)	1.16 (1.03; 1.32)	71.7* (56.5; 91.0)	59.1* (38.6; 90.3)	12.8† (11.3; 14.6)	66.1* (50.0; 87.3)	71.5* (53.6; 95.3)
k_{deam} (min^{-1})	3.89 (3.37; 4.49)	0.0095* (0.0072; 0.0125)	0.0085* (0.0054; 0.0133)	0.148† (0.135; 0.163)	0.0022*,† (0.0015; 0.0030)	0.0016*,† (0.0011; 0.0022)

Data are geometric means with 95% confidence limits from n rats for each group

[a] MAO was inhibited by pretreatment of the rats with pargyline or by lung perfusion with 300 nmol/l Ro41-1049 + 100 nmol/l lazabemide

[b] Steady-state rate of formation of DOPAC + DOPET for dopamine experiments or of DOMA + DOPEG for noradrenaline experiments

* Significant difference from the value for the corresponding amine with 'MAO intact' (Student's t-test; $P < 0.001$)

† Significant difference from the corresponding value for dopamine (Student's t-test; $P < 0.001$)

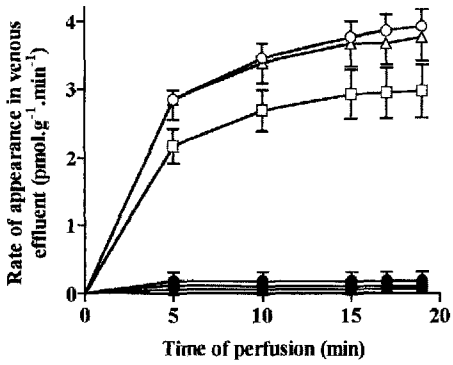

Fig. 1 The effects of Ro41-1049 and lazabemide on deamination of dopamine in rat lungs perfused with 1 nmol/l ^{3}H-dopamine for 20 min. COMT was inhibited. *Ordinate*: rates of appearance of the deaminated metabolites, DOPAC (*open symbols*) and DOPET (*closed symbols*), in the venous effluent (pmol.g^{-1}·min^{-1}) in the absence (*control, circles*) or presence of 300 nmol/l Ro41-1049 (*squares*) or 100 nmol/l lazabemide (*triangles*), shown as arithmetic means ± SE for 7 rats in the control and lazabemide groups and 8 rats in the Ro41-1049 group. *Abscissa*: time of perfusion with ^{3}H-dopamine (min)

for 20 min with 1 nmol/l ^{3}H-dopamine with COMT inhibited and in the absence or presence of 300 nmol/l Ro41-1049 to selectively inhibit MAO-A or 100 nmol/l lazabemide to selectively inhibit MAO-B are shown in Fig. 1. Although the rates of appearance of DOPAC and DOPET in the venous effluent during the perfusion period were not significantly affected by Ro41-1049 or lazabemide (two-factor analysis of variance, $P > 0.05$), the steady-state rate of formation of DOPAC, but not DOPET, was decreased by Ro41-1049, and the steady-state rates of formation of DOPAC and DOPET were unaffected by lazabemide (Table 2). However, the T/M ratio of dopamine was markedly increased by Ro41-1049 and was significantly increased by lazabemide (Table 2).

The k_{MAO} value calculated from the experiments with Ro41-1049 present (to inhibit MAO-A) represents the activity of MAO-B and that from experiments with lazabemide present (to inhibit MAO-B) represents the activity of MAO-A and the results are shown in Table 2. The activity of MAO-A was also calculated as the difference between k_{MAO} values obtained in the absence of a MAO inhibitor and those in the presence of Ro41-1049. There was no significant difference ($P > 0.05$) between this value of $k_{MAO\text{-}A}$ (3.26 min^{-1}; 95% confidence limits: 3.19, 3.33 min^{-1}; $n = 8$) and that obtained in the presence of lazabemide (Table 2). Hence, the deamination of dopamine in rat lungs could be entirely accounted for by the contributions of MAO-A (78–84%) and MAO-B (16–22%).

Discussion

The aim of this study was to determine the contributions and activities of the deaminating enzymes that are involved in the metabolism of dopamine in the pulmonary circulation of the rat. However, before these experiments were carried out, it was important to establish whether any dopamine was converted to noradrenaline by DBH when dopamine was perfused through the intact lungs of the rat. The results showed that there was no increase in the noradrenaline content of rat lungs after perfusion with dopamine, so it was not necessary to inhibit DBH in subsequent experiments. Also, these results showed that previous data on the uptake and metabolism of dopamine in perfused lungs of the rat (Bryan-Lluka and O'Donnell 1992) represented the pulmonary fate of dopamine, and not that of noradrenaline synthesised from dopamine during perfusion through the lungs. This part of the study provided evidence that uptake of dopamine is not into

Table 2 Effects of Ro41-1049 and lazabemide on deamination of dopamine in lungs perfused with 1 nmol/l ^{3}H-dopamine for 20 min with COMT inhibited

Experimental parameter	MAO Inhibitor		
	No MAO Inhibitor[a] ($n = 7$)	Ro41-1049 ($n = 8$)	Lazabemide ($n = 7$)
$v_{st\text{-}at}$ DOPAC (pmol·g^{-1}·min^{-1})	4.36 (3.92; 4.84)	3.00* (2.27; 3.96)	4.39 (3.75; 5.15)
$v_{st\text{-}at}$ DOPET (pmol.g^{-1}·min^{-1})	0.112 (0.048; 0.264)	0.073 (0.035; 0.154)	0.075 (0.061; 0.091)
T/M_{DA} (ml/g)	1.16 (1.03; 1.32)	5.01*** (3.82; 6.57)	1.46** (1.27; 1.68)
k_{MAO} (min^{-1})	3.89 (3.37; 4.49)	0.626*** (0.555; 0.706)	3.05* (2.65; 3.52)

Data are geometric means with 95% confidence limits from n rats for each group
[a] Data for 'No MAO Inhibitor' were from the same experiments as those for dopamine and 'MAO Intact' in Table 1
Significant difference from the value for the corresponding experimental parameter with 'No MAO Inhibitor': *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (Student's t-test)

noradrenergic neurones in the lungs, since dopamine taken up into the lungs was not exposed to DBH which is primarily located in the storage vesicles of noradrenergic neurones (Weinshilboum and Axelord 1971). This conclusion is important in light of the fact that there is no direct histological evidence in the literature for dopamine transport into pulmonary endothelial cells, as noradrenaline has been used in studies on the site of catecholamine uptake in the lungs (Hughes et al. 1969; Nicholas et al. 1974).

The remainder of this study focussed on determining the kinetic properties of the deaminating enzymes that metabolised dopamine in rat lungs. Previous studies have shown that dopamine is rapidly deaminated, as well as O-methylated, after uptake in intact lungs of rats (Bryan-Lluka and O'Donnell 1992). Enzymes that could have been responsible for this deamination in the intact lungs were MAO (both the MAO-A and MAO-B isoenzymes) and SSAO, since these enzymes have been shown to metabolise dopamine in homogenates of rat lungs (Lewinsohn et al. 1978; Bakhle and Youdim 1979). The results of this study show that, when non-saturating concentrations (1 nmol/l) of either dopamine or noradrenaline were perfused through the rat lungs, deamination of the catecholamines was entirely due to MAO with no significant contribution by SSAO. The lack of deamination of noradrenaline by SSAO could be due to the relatively low affinity of noradrenaline for SSAO (Coquil et al. 1973), but the lack of SSAO-mediated deamination of dopamine, which is a substrate for SSAO (Lewinsohn et al. 1978), suggests that SSAO are not present in the cells into which catecholamine uptake occurs in rat lungs, i.e. the pulmonary endothelial cells.

The deamination of dopamine in the rat lungs was further investigated using selective inhibitors of MAO-A and MAO-B. There is evidence from various tissues, including homogenates of rat lungs (Bakhle and Youdim 1979), rat liver (Fowler and Tipton 1984) and rat vas deferens (Lizcano et al. 1991), that dopamine is a substrate for both MAO-A and MAO-B. The results of the present study showed that MAO-A contributed 78–84% and MAO-B the remaining 16–22% to the deamination of dopamine in the rat lungs. The contributions of MAO-A (67–79%) and MAO-B (21–33%) to the deamination of noradrenaline in rat lungs (Westwood and Bryan-Lluka 1993) are similar to those for dopamine. It was concluded in the latter study that the results for the deamination of noradrenaline were compatible with equal amounts of MAO-A and MAO-B occurring in the lungs (Saura et al. 1992) and noradrenaline being selective for MAO-A (Johnston 1968). However, this conclusion is not compatible with additional consideration of the results from the present study with dopamine. On the other hand, the combined results are consistent with the proposal that noradrenaline, as well as dopamine, in the pulmonary endothelial cells of the rat are substrates for both MAO-A and MAO-B and that MAO-A is the predominant, but not exclusive, form of the enzyme in these cells. There is evidence, at least in human tissues, that noradrenaline is a substrate for both MAO-A and MAO-B (White and Glassman 1977; Rivett et al. 1982; Branco et al. 1992), as is dopamine in rat (see above) and human (White and Glassman 1977; Rivett et al. 1982) tissues. This conclusion is also compatible with the report that endothelial cells of the bovine adrenal medulla contain primarily MAO-A (Banerjee et al. 1984). MAO-B, which has been shown by autoradiography to be present in the same amounts as MAO-A in rat lungs (no indication of cellular location; Saura et al. 1992), would appear from the results of this study to be located mainly in cells other than the pulmonary endothelium.

The experiments in this study allowed the determination of the activity of MAO for deamination of dopamine and of noradrenaline in intact lungs of the rat ('MAO Intact' group; Table 1). Table 3 shows a comparison of the activities of MAO and COMT for metabolism of dopamine and noradrenaline in intact lungs and heart of the rat. The values for rat lungs represent deamination of 389% of the intracellular dopamine concentration per min or 6.5% per s and O-methylation of 498% per min of 8.3% per s (in experiments in which COMT and MAO, respectively, were inhibited in lungs perfused with 1 nmol/l dopamine). The corresponding values for noradrenaline are deamination of 14.8% per min or 0.25% per s and O-methylation of 35.7% per min or 0.60% per s (Table 3). Hence, the activities of MAO and COMT are, respectively, 26-fold and 14-fold greater for metabolism of dopamine than noradrenaline in rat lungs. In myocardial cells of the rat heart, on the other hand, the activities of MAO and COMT for metabolism of dopamine and noradrenaline are of the same order of magnitude (respectively, 1.1-fold and 2.4-fold greater for dopamine than noradrenaline; Table 3). Also, the activities of both MAO and COMT for metabolism of noradrenaline in rat lungs and in noradrenergic neurones or myocardial cells of rat heart are of the same order of magnitude, whereas the enzyme activities for dopamine are 12- to 16-fold greater in rat lungs than in rat heart (Table 3). In order to ensure that these high k_{MAO} and k_{COMT} values for dopamine in rat lungs were not an experimental artefact, we investigated the possibility that the T/M ratio of dopamine was underestimated by failing to measure all of the ^{3}H-dopamine that accumulated in the lungs. Thus, in some experiments, lung homogenate pellets were also analysed for dopamine or noradrenaline. Since approximately the same small amount of dopamine and noradrenaline remained in the pellets (see Results, second section, second paragraph), this possibility did not explain the 11- and 7-fold smaller T/M ratios of dopamine than noradrenaline when k_{MAO} and k_{COMT}, respectively, were

Table 3 Comparison of the activities of MAO and COMT for metabolism of dopamine and noradrenaline in intact perfused lungs and heart of the rat

Tissue	Experimental parameter	Amine	
		Dopamine	Noradrenaline
Rat lungs	k_{MAO} (min^{-1})[a]	3.89	0.148
	k_{COMT} (min^{-1})[b]	4.98	0.357
Rat heart[c]			
Noradrenergic neurones	k_{MAO} (min^{-1})	0.313	0.349
Myocardial cells	k_{MAO} (min^{-1})	0.261	0.107
	k_{COMT} (min^{-1})	0.312	0.244

[a] Present study
[b] Bryan-Lluka and O'Donnell (1992)
[c] Grohmann (1987)

determined. In addition, the fact that the T/M ratios were increased to very similar values for dopamine and noradrenaline when both MAO and COMT were inhibited (Table 1) indicates that the experimental procedure did not underestimate the amount of dopamine in the lungs.

The k_{MAO} and k_{COMT} values for dopamine and noradrenaline in rat lungs and heart (Table 3) are a reflection of the inherent enzyme activity, i.e. of the V_{max}/K_m ratio for the substrate, and of the concentration of the enzyme in the particular cell type. There is no evidence in the literature to either support or refute the possibility of a higher concentration of MAO and COMT occurring in the pulmonary endothelial cells than in the cardiac myocardial cells, and this possibility would not account for the high enzyme activities in the lungs for metabolism of dopamine, but not noradrenaline. In homogenates of rat lungs, the inherent enzyme activity value for dopamine metabolism by MAO is 1.4–fold greater than that for noradrenaline (Bakhle and Youdim 1979), but this difference is not sufficient to explain the 26-fold greater k_{MAO} value for dopamine than for noradrenaline in intact lungs of the rat (Table 3). Nevertheless, it should be noted that the lung homogenate data do not provide information specific to the pulmonary endothelial cells where the uptake and metabolism of the catecholamines occurs in the intact lungs.

The existence of different forms of MAO and COMT in lungs and heart could also be the reason for the differences in the k_{MAO} and k_{COMT} values for dopamine and noradrenaline in the intact tissues. The occurrence of MAO-A and MAO-B in different ratios in different cell types could only account for the high activity of MAO for deamination of dopamine in the lungs if MAO-B was the predominant form in the pulmonary endothelial cells - i.e. different from the situation in adult rat heart where MAO-A is the predominant form (Edwards et al. 1979; Saura et al. 1992). However, the results of the present study have shown that it is MAO-A that makes the major contribution (about 80%) to the deamination of dopamine (see Results) as well as noradrenaline (Westwood and Bryan-Lluka 1993) in rat lungs, leading to the conclusion that the pulmonary endothelial cells contain mainly MAO-A (see above). There is also evidence in the literature for two forms of COMT, MB-COMT and S-COMT, both of which have been shown to metabolise dopamine and noradrenaline (Rivett et al. 1982). It has been shown in human brain homogenates that the activities (i.e. V_{max}/K_m) of S-COMT and MB-COMT are, respectively, 7-fold and 2-fold greater for dopamine than for noradrenaline (calculated from Rivett et al. 1982). However, the high COMT activity for O-methylation of dopamine compared with noradrenaline in rat lungs is unlikely to be due to predominantly S-COMT existing in the pulmonary endothelial cells, since recent kinetic data (Bryan-Lluka 1995) was consistent with MB-COMT being the major form of the enzyme in these cells.

In conclusion, the high activities of both MAO and COMT for metabolism of dopamine in the lungs accounts for the rapid appearance of metabolites, with no accumulation of unchanged dopamine, when rat lungs with MAO and/or COMT intact were perfused with a low concentration of dopamine (Bryan-Lluka and O'Donnell 1992; present study). The high enzyme activities also account for the failure to detect any pulmonary clearance of dopamine in rat lungs when only the accumulation of total ^3H in the lungs, and not the appearance of metabolites in the venous effluent, was determined (Nicholas et al. 1974). The present study has provided kinetic data on the deamination of dopamine by MAO in the intact lungs of the rat and shown that there is not a significant contribution of SSAO to the metabolism of dopamine or noradrenaline when these amines are perfused through the pulmonary circulation. The pulmonary endothelial cells are a site of very rapid metabolism of endogenous or exogenous dopamine by MAO and COMT, and are not a site for dopamine accumulation when either of the metabolising enzymes is intact and dopamine

is present at a low concentration in the pulmonary circulation.

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