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Report

Contribution of Monoamine Oxidase(MAO) to the Binding of Tertiary Basic Drugs in Isolated Perfused Rat Lung

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The effect of tertiary basic drugs on mitochondrial MAO activity and the effect of MAO inhibitors (MAOIs) on basic drug accumulation in the isolated perfused rat lung were studied to clarify the role of MAO in drug binding to lung tissue. In the perfused lung preparation, the inhibition of MAO by basic drugs correlated well with their lipid solubilities and followed competitive kinetics. The inhibitory rank order (imipramine ≒ diphenhydramine > quinine > metoclopramide > procainamide) also correlated with their accumulation in the perfused lung. Moreover, MAOI treatment decreased the accumulation of basic drugs in the lung, and the potency of MAOIs to inhibit drug accumulation in the lung correlated with their MAO inhibitory activity. These results indicate that lung MAO has specific binding sites for basic drugs and may function as a drug reservoir.

KEY WORDS: tertiary basic drug; lung mitochondria; monoamine oxidase(MAO); lipid solubility; binding site; isolated perfused lung.

INTRODUCTION

Previously, we examined drug accumulation in isolated perfused rat lungs kept under artificial ventilation and demonstrated that a cationic group and a lipophilic component in the molecule were required for accumulation to occur (1). Active transport systems did not contribute to the accumulation process, and the accumulation of a basic drug was inhibited by other basic drugs in correlation with their lipid solubilities (2). The mitochondrial outer membrane was the subcellular fraction of greatest drug accumulation (3–5). A study on the effect of basic drugs on the enzymes located in the mitochondria, such as MAO, and on the effect of MAO inhibitors on the binding of basic drugs to lung mitochondria demonstrated that mitochondrial MAO has specific binding sites for basic drugs (6).

In this paper, we determine the role of mitochondrial MAO in the accumulation of basic drugs in isolated perfused rat lung by examining (i) the effect of various drugs on MAO activity in lung, (ii) the relationship of the inhibitory potency of basic drugs to MAO in lung and their lipid solubilities, (iii) the kinetics of basic drug inhibition of MAO, and (iv) the effect of MAO inhibitors on the accumulation of basic drugs by the lung. Phenylethylamine was used as a model substrate for MAO (7) since it was readily metabolized by MAO of lung tissues (6).

MATERIALS AND METHODS

Materials. Quinine, diphenhydramine, *N*-methylnicotinamide (NMN), ^{14}C-imipramine, ^{14}C-β-phenylethylamine (PEA), pargyline, semicarbazide, and iproniazid were purchased from commercial sources. Phenylbutazone, metoclopramide, and procainamide were kindly supplied by Fujisawa Pharmaceutical Co., Ltd.,Osaka. All other materials were of analytical grade.

Animals. Male Wistar rats weighing 180–220 g were used. They were housed under a constant environment (temperature, 23 ± 1°C; humidity, 55 ± 5%) and allowed water and food ad libitum.

Isolated Lung Perfusion. Isolated lung perfusion was performed by a modification of our previous method (1). Namely, the lung was ventilated with carbogen gas (95% O_2 + 5% CO_2) at a rate of 60 times/min by applying alternative negative pressure to the chamber. The perfusate consisted of Krebs–Ringer bicarbonate buffer containing 3.5% bovine serum albumin and 8 m*M* glucose (pH 7.4), equilibrated with carbogen gas before perfusion. The isolated lung was perfused at a rate of 8 ml/min using a peristaltic pump. Lung preparations were allowed to equilibrate for 10 min.

In the study of the effect of MAOIs and basic drugs on the metabolism of MAO substrates, the lungs were perfused with 1 μ*M* PEA containing 0.5 m*M* MAOIs or various concentrations of basic drugs for 30 min. The efflux of PEA and its metabolites was measured.

To examine the reversibility of the inhibitory effect of basic drugs on MAO in the lung, lungs were perfused with PEA (1 μ*M*) only for 15 min. Then the perfusate was

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changed to a mixture of PEA (1 μ*M*) and basic drug (0.5 m*M*) and the experiments were continued for a further 15 min, then the perfusate was exchanged to PEA (1 μ*M*). PEA and its metabolites in the effluent were monitored.

To clarify the effect of MAOIs on drug accumulation, the lungs were treated with a perfusate containing one of the inhibitors for 10 min and then perfused with a mixture of MAOI and 0.1 μ*M* basic drug for 15 min in order to monitor the accumulation of the drug. After perfusion the drug concentration in the lung tissue was determined.

Lungs were weighed before and after perfusion experiments. Visual examination for the development of edematous areas throughout the experiment and differences in lung weight before and after perfusion were used as criteria to assess edema (1).

Partition Coefficients. The partition coefficient of each drug was obtained by our previous method (3).

Analytical Methods. Quinine was analyzed by the fluorometric method of Brodie *et al.* (8). ^{14}C-Imipramine was quantitatively extracted with toluene from samples treated with 0.1 *N* NaOH (3). An aliquot of the toluene extract was mixed with a scintillation cocktail (Monophase-40, Packard Instrument Co.), and the radioactivity was determined with a Tri-Carb liquid scintillation spectrometer (Model 3330, Packard Instrument Co.). Diphenhydramine was analyzed by the high-pressure liquid chromatography of Jane (9). The concentrations of metoclopramide and procainamide were determined by a modified method of Bratton and Marshall (10). MAO activity was assayed radiochemically by the solvent extraction method (11). Protein was quantitated by the method of Lowry *et al.* (12).

Data Analysis. Saturation curves of PEA metabolism were analyzed by the method of Eadie and Hofstee, in which the regression lines were drawn by the least-squares method to determine the Michaelis–Menten constant (K_m) of each drug and the maximum velocity of the catalyzing reaction (V_{max}).

RESULTS AND DISCUSSION

Effect of MAO Inhibitor on PEA Metabolism

The effect of pargyline, a specific mitochondrial MAO inhibitor (MAOI) (13), on the metabolism of PEA was studied to clarify whether it was metabolized by mitochondrial MAO in perfused lung. Figure 1 shows time courses for the metabolism of PEA in the presence or absence of pargyline. The metabolism velocity gradually increased for a few minutes until reaching a steady-state level. While the velocity was effectively and quickly decreased with the addition of pargyline into the perfusate, most of the substrate was eluted in its unmetabolized form. Moreover, the inhibition potency of pargyline added at the initial period was similar to that of MAOI added after 15 min (approximately steady state). Total recovery of the substrate and its metabolites at steady state was about 100% in each experiment. These results indicate that PEA was taken up by the lung and easily metabolized by mitochondrial MAO.

Effect of Tertiary Basic Drugs on the Metabolism of PEA

To clarify the contribution of MAO to the binding site of tertiary basic drugs, the effect of basic drugs on the metabolism of PEA by perfused lung was studied. As shown in Fig. 2, an initial increase and then a steady-state situation for the metabolism velocity of PEA were observed in all cases, while the velocity differed markedly. Imipramine was one of the most effective inhibitors. The potency decreased in the following order: diphenhydramine, quinine, metoclopramide, and procainamide.

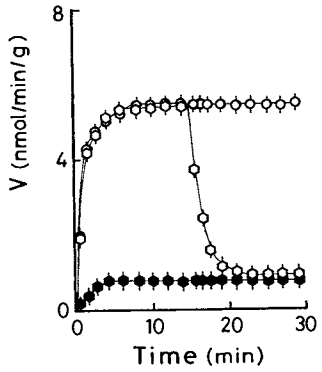

Fig. 1. Effect of pargyline (0.5 m*M*) on the metabolism of PEA (1 μ*M*) in isolated perfused rat lung. Lungs were perfused with PEA only (○), with PEA and pargyline (●), or with PEA for 15 min and then with PEA and pargyline (⊙). Values represent the mean ± SE of three experiments.

Drug inhibition of substrate metabolism at the organ level could result from interference with uptake and/or metabolic processes. The initial rates of uptake of PEA by perfused lung in the presence of each basic drug were approximately equal to each other. These results indicate that basic drugs do not affect the uptake process of substrate by the lung. Moreover, we previously recognized that MAO activity in lung mitochondrial pellet was inhibited with various basic drugs (6). Thus, it seems that basic drugs directly affect the mitochondrial MAO in the lung.

Effect of Lipid Solubility of Basic Drugs on MAO Activity

The effect of lipid solubility on the inhibiting potential of basic drugs on MAO activity was studied to clarify the fac-

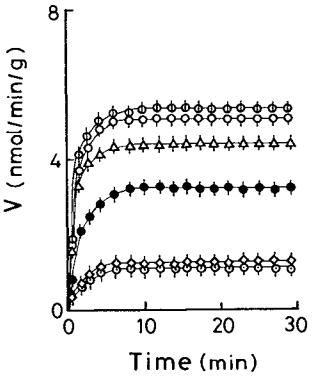

Fig. 2. Effect of basic drugs (0.5 m*M*) on the metabolism of PEA (1 μ*M*) in isolated perfused rat lung. Basic drug: ①, control; ⊙, imipramine; ◇, diphenhydramine; ●, quinine; △, metoclopramide; ○, procainamide. Values represent the mean ± SE of three to five experiments.

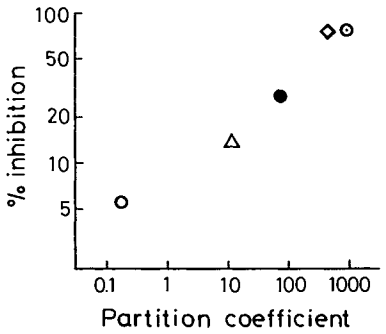

Fig. 3. Relationship between inhibition potency of basic drugs (0.5 m*M*) on MAO activity and their partition coefficients (PC). ⊙, Imipramine; ◇, diphenhydramine; ●, quinine; △, metoclopramide; ○, procainamide. Values represent the mean ± SE of three to four experiments. log (% inhibition) = 0.311 * log PC + 0.947; r = 0.981.

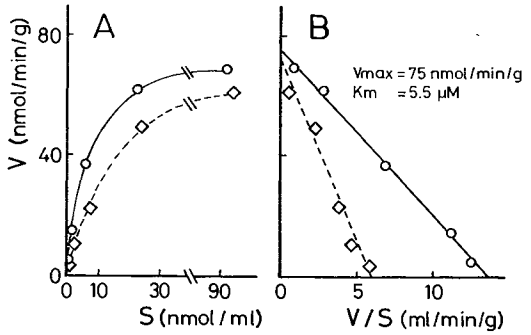

Fig. 5. Effect of diphenhydramine on MAO activity in isolated perfused rat lung. (A) Substrate concentration–velocity curve; (B) Eadie–Hofstee plot. Lungs were perfused with PEA only (○) or with PEA + diphenhydramine (0.1 m*M*) (◇). Values represent the mean ± SE of the three experiments.

tors regulating their ability. Lipid solubility was expressed as a partition coefficient between chloroform and isotonic phosphate buffer (pH 7.4) at 37°C. Figure 3 shows their relationship. The inhibitory potencies of basic drugs correlated well with their partition coefficients. The correlation coefficient calculated was 0.98 and was highly significant ($P < 0.01$). The present study on the relationship of inhibitory potency and the partition coefficient of tertiary basic drugs shows that at least MAO inhibition can be predicted according to the basicity and lipophilicity of the drugs. Moreover, the ability of basic drugs to inhibit MAO activity related well with their accumulation in the perfused lung, observed previously (1).

Effect of Drug Concentration on MAO

The effect of concentration of basic drugs on the metabolism of MAO substrates by the lung was studied. Figure 4 shows the relation between the percentage activity of MAO and the drug concentration. Although the MAO activity was scarcely affected by a low dose of diphenhydramine, the percentage activity of MAO decreased with an increase in the dose and was completely inhibited at a high dose; its dose–response curve was sigmoidal. The curves of other basic drugs were essentially the same, but the degree of inhibition varied with the drug. Diphenhydramine was one of the most effective inhibitors of all the basic drugs used and the inhibitory potencies decreased in the following order: imipramine, quinine, metoclopramide, and procainamide. On the other hand, phenylbutazone, a lipophilic acidic drug, did not affect MAO activity at any of the concentrations tested. These results indicate that strong basicity and high lipophilicity drug properties are required to inhibit MAO activity.

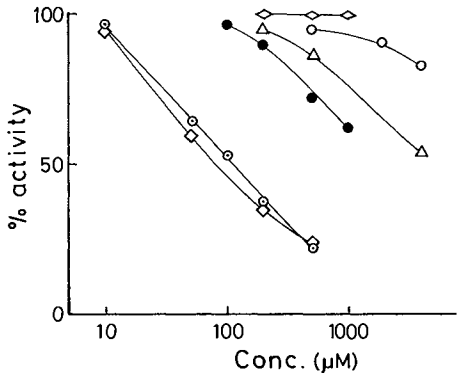

Fig. 4. Effect of drug concentration on MAO activity in isolated perfused rat lung. PEA concentration: 1 μ*M*. ⊙, Imipramine; ◇, diphenhydramine; ●, quinine; △, metoclopramide; ○, procainamide; <>, phenylbutazone. Values represent the mean ± SE of three to five experiments.

Kinetics of MAO Inhibition with Basic Drugs

Figure 5A shows the relation between metabolism velocity versus substrate concentration in the presence or absence of diphenhydramine. The velocity gradually increased and reached a maximum level with an increase in the substrate concentration. The effect of diphenhydramine on metabolism was observed at low doses of the substrate and is demonstrated using Eadie–Hofstee plots in Fig. 5B. The metabolism regression lines clearly intercept the ordinate at the same point. Therefore, the inhibition by basic drugs is competitive. Similar phenomena were also observed in the presence of imipramine, quinine or metoclopramide (data not shown). The inhibition constant (K_i) of each basic drug was calculated and compared. As shown in Table I, K_i values for diphenhydramine and imipramine were markedly smaller than those of other drugs. These values were nearly equal to the inhibitor concentration causing 50% inhibition (IC_{50}) which was derived from Fig. 4. These results indicate that tertiary basic drugs interact with the binding sites of the MAO substrate and inhibit MAO activity in the lung.

Table I. Inhibition Constants of Basic Drugs for Inhibition of MAO in the Perfused Lung

Inhibition	K_i (m*M*)[a]	IC_{50} (m*M*)[b]
Diphenhydramine	0.07 ± 0.01	0.08
Imipramine	0.08 ± 0.01	0.11
Quinine	1.5 ± 0.2	1.9
Metoclopramide	4.2 ± 0.7	4.9

[a] Values represent the mean ± SE of three experiments.
[b] IC_{50} of each drug was calculated from Fig. 4.

Reversibility of Inhibitory Potency of Basic Drugs

To confirm the characteristics of MAO, regarding the binding sites for tertiary basic drugs in the lung, the reversibility of drug binding to MAO was studied. The metabolism velocity was quickly inhibited with the addition of various basic drugs, while the velocity gradually increased with the removal of the basic drug from the perfusate and then nearly reached the initial metabolizing level (Fig. 6). These results indicate that the binding of basic drugs to MAO is reversible.

The Role of MAO to the Binding Site of Basic Drugs

For further clarification of the role of MAO to the binding of basic drugs, the effect of MAO inhibitors (MAOIs) on the accumulation of drugs by the perfused lung was studied. As shown in Fig. 7, the accumulation of quinine by the lung decreased with MAOI treatment. All MAOIs used interfered with quinine accumulation and a significant correlation was observed between their potency in this regard and their MAO inhibitory activities. As MAO activity was completely inhibited with MAOIs, drug accumulation in the lung decreased to about 50%. These results indicate that half of the accumulation sites for basic drugs in the lung were occupied by MAO. The same phenomenon was observed during the accumulation of imipramine in the lung (data not shown).

In this paper, we studied the contribution of MAO on the binding sites of basic drugs in the lung. The present results demonstrate that MAO has specific binding sites for basic drugs in the lung and that the binding affinity for MAO of drugs depends on their lipid solubility, namely, (i) MAO of the perfused lung was inhibited by various basic drugs (Fig. 2); (ii) the inhibition manner of basic drugs to MAO was competitive, reversible and dose dependent (Figs. 4, 5, and

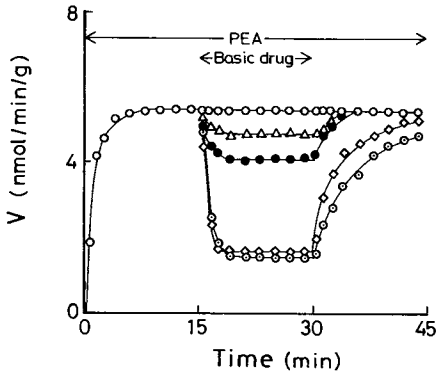

Fig. 6. Reversibility of inhibitory activities of basic drugs on MAO activity in isolated perfused rat lung. Lungs were perfused with PEA (1 μ*M*) only for 0–15 and 30–45 min and with a mixture of PEA (1 μ*M*) and basic drug (0.5 m*M*) for 15–30 min. ⊙, Imipramine; ◇, diphenhydramine; ●, quinine; △, metoclopramide; ○, control.

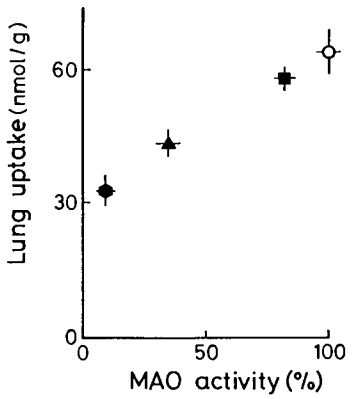

Fig. 7. Effect of MAO inhibitors on the accumulation of quinine and MAO activity in the perfused lung. Lungs were treated with 0.5 m*M* MAOI for 15 min and then were perfused with medium containing quinine (1 μ*M*) for 15 min. MAOI: ● pargyline; ▲, iproniazid; ■, semicarbazide; ○, control. Values represent the mean ± SE of four or five experiments.

6); (iii) the inhibitory potency of each basic drug to MAO correlated well with their lipid solubility (Fig. 3); and (iv) a good correlation was observed between the inhibitory potency of various MAOIs on MAO activity and their inhibition of drug accumulation in the lung (Fig. 7). From these results, we suggest that MAO may function as a reservoir for the basic drugs and that the action and/or adverse reactions of some tertiary basic drugs may be affected by inhibiting the metabolism of various biogenic amines by MAO.

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