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Report

Contribution of Monoamine Oxidase (MAO) to the Binding of Tertiary Basic Drugs in Lung Mitochondria

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The effects of tertiary amine-containing basic drugs on the enzymes located in the mitochondria and the effect of monoamine oxidase inhibitors (MAOIs) on drug accumulation in lung mitochondria have been studied. Various basic drugs inhibited MAO activity but not other mitochondrial marker enzymes. The potency of MAO inhibition correlated well with their lipid solubility, and the basic drugs inhibited MAO activity dose dependently and competitively. Further, MAO inhibition correlated well with binding affinity to lung mitochondria, and the binding of tertiary amine drugs to lung mitochondria was decreased by treatment with MAOIs. A good correlation was observed between the potency of MAOIs to inhibit the binding of the basic drug to the high-affinity site in mitochondria and the MAO inhibitory activity in mitochondria. These results indicate that mitochondrial MAO is one of the binding sites for tertiary basic drugs in the lung. We think that the action and/or adverse reaction of some drugs may result from inhibition of mitochondrial MAO to metabolize various biogenic amines and that mitochondrial MAO may function as a reservoir for basic drugs.

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

INTRODUCTION

Drugs administered to the body reach their target tissues via absorption and distribution processes, and their actions appear when they bind to their specific receptors. On the other hand, drug distribution to other sites occasionally produces unexpected adverse effects. Thus, physiological and pharmacological studies on drug distribution and metabolism in each tissue are important to establish an effective and safe drug administration method.

Previously, we examined the accumulation of various drugs in isolated perfused rat lungs with artificial ventilation. A cationic group as well as a lipophilic group in the molecule was required for drug accumulation in the lung (1). Active transport systems did not contribute to these accumulation processes. The accumulation of one basic drug was inhibited and displaced by a second basic drug, with a potency that correlated well with its lipid solubility (2). The subcellular distribution of basic drugs in the perfused lung indicated that the most specific accumulation sites were located in the mitochondrial outer membrane fraction (3–5).

Several enzymes, such as monoamine oxidase (MAO) (6), rotenone-insensitive NADH-dependent cytochrome *c* reductase (7), and kynurenin hydroxylase (8), are specifically located in mitochondrial outer membrane. The MAO activity of rabbit lung and brain is inhibited by imipramine (9). In this study, we examined the effect of various tertiary basic drugs on lung mitochondrial MAO and the contribution of MAO to the accumulation of tertiary amine drugs in the lung mitochondria.

MATERIALS AND METHODS

Materials. ^{14}C-Imipramine, quinine, diphenhydramine, ^{14}C-β-phenylethylamine, ^{14}C-tyramine, and ^{14}C-5-hydroxytryptamine were purchased from commercial sources. ^{14}C-Metoclopramide, alloclamide, phenylbutazone, procainamide, procainamide ethobromide (PAEB), and imipramine were kindly supplied by Fujisawa Pharmaceutical Co. Ltd., Osaka. All other materials were of analytical grade.

Animals. Male Wistar rats each weighing 170–220 g were used. They were housed in a constant environment (temperature, 23 ± 1°C; humidity, 55 ± 5%) and allowed water and food ad libitum.

Preparation of Rat Lung Mitochondria. The lung homogenate was prepared with a Potter-Elvehjem homogenizer in 9 parts of medium consisting of 0.25 *M* sucrose and 3.4 m*M* Tris buffer, pH 7.4, at 4°C. The mitochondrial fraction was obtained from the 600 *g* (10-min) supernatant of

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lung homogenate by centrifuging at 3300*g* for 20 min. The precipitate was resuspended in the same buffered medium and centrifuged again for a further 20 min at 3300*g*. This washing procedure was repeated four times, and the final mitochondria pellet was suspended in the same buffer.

MAO Inhibition Experiments. Monoamine oxidase activity was assayed radiochemically by a modification of the method of Otsuka and Kobayashi (10). The assay was carried out in 10-ml culture tubes with plastic screw caps. A mixture containing 100 μl of mitochondrial suspension (0.1 mg protein) and 100 μl of phosphate buffer (pH 7.4) was incubated at 30°C for 5 min. Then a mixture (100 μl) of the radioactive substrate and basic drugs was added to initiate the reaction, and the mixture was shaken. The reaction was then terminated by the rapid addition of 500 μl of 2 *M* citric acid solution. Blank values were obtained by the addition of citric acid before the substrate was added. Seven milliliters of the extraction solvent [phenylethylamine, tyramine, toluene:5-hydroxytryptamine, toluene, and ethyl acetate (1:1)] containing 0.5% (w/v) PPO was added to each tube, which was then capped and shaken vigorously for 5 min. After centrifugation (e.g., 2000 rpm for 15 min), 5 ml was sampled with pipetting from the organic layer. The radioactivity was then determined with a Tri-Carb liquid scintillation spectrometer. The values obtained were corrected for the efficiency of extraction of the deaminated metabolites into the organic layer to express the activity as nanomoles of substrate metabolized per milligram of protein per minute. In all cases the product formation was ensured to be linear with time up to the time period used for the assay so that values obtained corresponded to the initial velocity of the enzyme-catalyzed reaction.

Binding Experiments. Binding experiments were performed as described previously (4). Namely, the rat lung mitochondria (0.5 mg protein), which was treated with various MAOIs at 4°C for 15 min, was incubated in the drug solution (2 ml) at 37°C for 2.5 min. After centrifugation at 40,000*g* for 5 min, an aliquot of the resulting pellet-free supernatant and the precipitate were used for the determination of the free drug and the bound drug on the mitochondrial pellet, respectively.

Analytical Methods. Quinine was analyzed by the fluorometric method of Brodie *et al.* (11). ^{14}C-Imipramine and ^{14}C-metoclopramide were determined with a Tri-Carb liquid scintillation spectrometer (4). Several mitochondrial marker enzymes were assayed: cytochrome oxidase (12) for the inner membrane, rotenone-insensitive NADH-dependent cytochrome *c* reductase (7) and kynurenin hydroxylase (8) for the outer membrane, sulfite cytochrome *c* reductase (13) for the intermembrane space, and malate dehydrogenase (14) for the matrix. Protein was quantitated by the method of Lowry *et al.* [15].

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

Data Analysis. Saturation catalyzing curves were analyzed by the method of Lineweaver and Burk (16) in which the regression lines were drawn by the least-squares method to determine the Michaelis–Menten constant (K_m) of each substrate and the maximum velocity of the catalyzing reaction (V_{max}). The inhibition constant (K_i) for each inhibitor was calculated by the same method. Saturation drug binding curves were analyzed by the method of Scatchard (17), in which the regression lines were drawn by the least-squares method to determine the association constant (K_a) and the binding capacity (B_{max}).

RESULTS

Effect of Basic Drug on Mitochondrial Marker Enzymes

Table I shows the remaining activity of enzymes in the presence of imipramine and diphenhydramine, which are highly lipophilic tertiary basic drugs and selectively accumulate in lung mitochondria. MAO activity was inhibited by the basic drugs, while other enzymes were not inhibited significantly. As mitochondrial MAO has been classified into types A, B, and A + B, the metabolism of each type of MAO substrate with lung mitochondria was compared. The three substrates had a similar maximum velocity (V_{max}), but the K_m for phenylethylamine was markedly lower than that for the others (Table II).

Effect of Basic Drugs on Each MAO Type

The metabolism of each substrate was prevented by both imipramine and diphenhydramine but not by phenylbutazone, a lipophilic acidic drug (Fig. 1). At each concentration the tertiary basic drugs inhibited selectively the deamination of phenylethylamine by mitochondria, in preference over that of the other substrates tested. Thus, phenylethylamine was used as the MAO substrate in the following experiments. Table III shows the percentage activity of MAO in the presence of various drugs. The deamination activity with MAO was depressed by the basic drugs. The inhibitory potency of each drug was remarkably different, with imipramine being the most effective inhibitor. The potency decreased in the order imipramine, diphenhydramine, alloclamide, quinine, metoclopramide, and procainamide, whereas PAEB scarcely affected the activity.

Table I. Effect of Basic Drug on Rat Lung Mitochondrial Enzymes[a]

Enzyme	% activity Inhibitor	
	Imipramine	Diphenhydramine
Monoamine oxidase[b]	4.9 ± 1.2	9.0 ± 1.7
Kynurenin hydroxylase	96.5 ± 5.3	99.0 ± 4.8
Rotenone-insensitive NADH-dependent cytochrome *c* reductase	95.2 ± 3.7	97.8 ± 5.0
Cytochrome oxidase	94.8 ± 4.5	100.6 ± 3.0
Sulfite cytochrome *c* reductase	101.2 ± 5.0	99.5 ± 4.2
Malate dehydrogenase	96.7 ± 3.9	93.2 ± 6.1

[a] Enzyme activity was assayed according to methods described in the text after lung mitochondria (0.1 mg protein) was pretreated with 1 mmol of basic drug for 5 min and is expressed as the percentage of control with no inhibitor. Values represent the mean ± SE of four or five experiments.

[b] MAO substrate: β-phenylethylamine (2 μ*M*).

Table II. Michaelis–Menten Kinetic Constants for Rat Lung Mitochondria[a]

Substrate	V_{max} (nmol/mg protein/min)	K_m (μM)
β-Phenylethylamine	3.6 ± 0.3	6.1 ± 0.6
Tyramine	3.7 ± 0.3	27.4 ± 4.3
5-Hydroxytryptamine	3.5 ± 0.3	38.8 ± 3.9

[a] Lung mitochondria (0.1 mg protein) was incubated with various amounts of MAO substrate for 10 min (β-phenylethylamine) or 60 min (tyramine and 5-hydroxytryptamine) at 30°C. Values represent the mean ± SE of four experiments.

Correlation of Lipid Solubility of Basic Drugs with MAO Inhibition

The lipid solubility was expressed as the partition coefficient between chloroform and isotonic phosphate buffer (pH 7.4) at 37°C. The inhibitory activity of the drugs against MAO correlated well with their partition coefficient (r = +0.894) (Table III).

Effect of Drug Concentration on MAO Activity

Figure 2 shows the relation of the percentage activity of MAO and the drug concentration. While low imipramine or diphenhydramine concentrations had little effect, the MAO activity decreased with increasing dose and was completely inhibited at a high dose; dose–response curves were sigmoidal. The curves of other basic drugs were similar, but the degree of inhibition varied with the drug.

Figure 3A shows the double reciprocal plots for phenylethylamine deamination in the presence of imipramine. The deamination velocity was decreased with increasing dose of the inhibitor. The metabolism regression lines intercept the ordinate at the same point in each case. Similar phenomena were also observed in the presence of diphenhydramine (Fig. 3B), metoclopramide (Fig. 3C), and procainamide (Fig. 3D). Phenylbutazone, a lipophilic acidic drug, did not affect the MAO activity at any concentration tested (4–30 mM) (data not shown). The inhibition constant (K_i) of each basic drug was calculated and compared. As shown in Table IV, the K_i value of imipramine was markedly smaller than that of the other drugs. These K_i values were nearly equal to the inhibitor concentration causing 50% inhibition (IC_{50}) for all four drugs which was derived from Fig. 2.

Table III. MAO Inhibitory Activity and Partition Coefficients of Basic Drugs[a]

Inhibitor	PC[b]	% activity
Imipramine	980	4.7 ± 0.8
Diphenhydramine	442	6.2 ± 1.1
Alloclamide	582	25.7 ± 3.1
Quinine	64.2	67.9 ± 3.2
Metoclopramide	12.0	74.9 ± 1.3
Procainamide	0.17	94.9 ± 1.4
Procainamide ethobromide	0.01	100.4 ± 3.2
Phenylbutazone	770	98.7 ± 2.9

[a] Lung mitochondria (0.1 mg protein) was incubated with 10 μmol of MAO substrate and 1 mmol of basic drug for 10 min (β-phenylethylamine) at 30°C. Deaminated product formed was assayed according to the method described in the text. Enzyme activity was expressed as the percentage of control with no inhibitor. Values represent the mean ± SE of four to six experiments.
[b] Partition coefficient ($CHCl_3/H_2O$, pH 7.4).

The Role of MAO in the Binding of Basic Drugs

The effect of MAO inhibitors (MAOIs) on the binding of drugs to lung mitochondrial pellet was studied. As shown in Fig. 4A, all the MAOIs used inhibited the binding of imipramine to mitochondria in parallel with the decrease in MAO activity. The mitochondrial binding of quinine and metoclopramide was also inhibited but less effectively (Figs. 4B and C). Hence MAOIs affected strongly the mitochondrial binding of the more lipophilic basic drug, which had a higher affinity to mitochondria (Fig. 4).

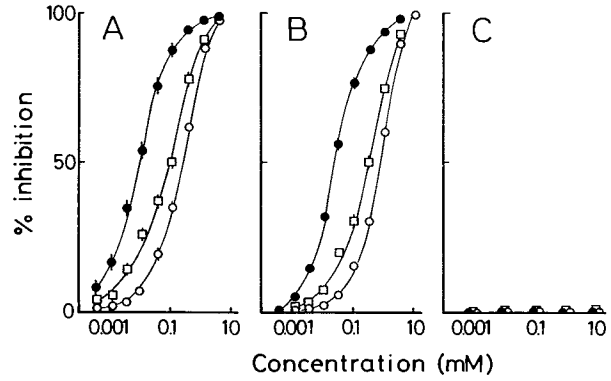

Fig. 1. Effect of imipramine (A), diphenhydramine (B), or phenylbutazone (C) on the metabolism of MAO substrates by lung mitochondria. MAO substrate (2 μM) in the presence of various concentrations of basic drug was incubated with lung mitochondria (0.1 mg protein) for 15 min at 30°C. MAO substrate: ●, β-phenylethylamine; □, tyramine; ○, 5-hydroxytryptamine. Values represent the mean + SE of four to six experiments.

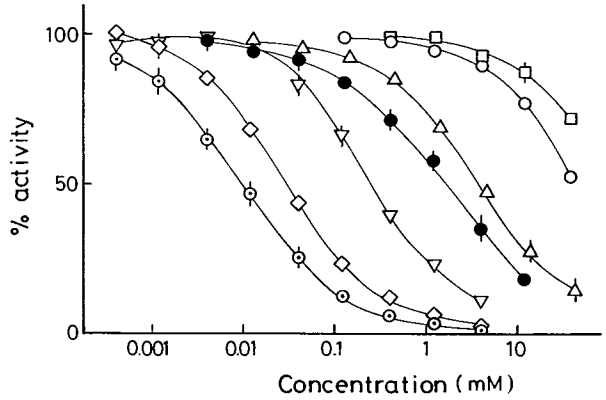

Fig. 2. Effect of concentration of basic drug on phenylethylamine deamination by lung mitochondria. Phenylethylamine (2 μM) in the presence of various concentrations of basic drug was incubated with lung mitochondria (0.1 mg protein) for 15 min at 30°C. Values represent the mean ± SE of four to six experiments. ⊙, Imipramine; ◇, diphenhydramine; ▽, alloclamide; ●, quinine; △, metoclopramide; ○, procainamide; □, procainamide ethobromide.

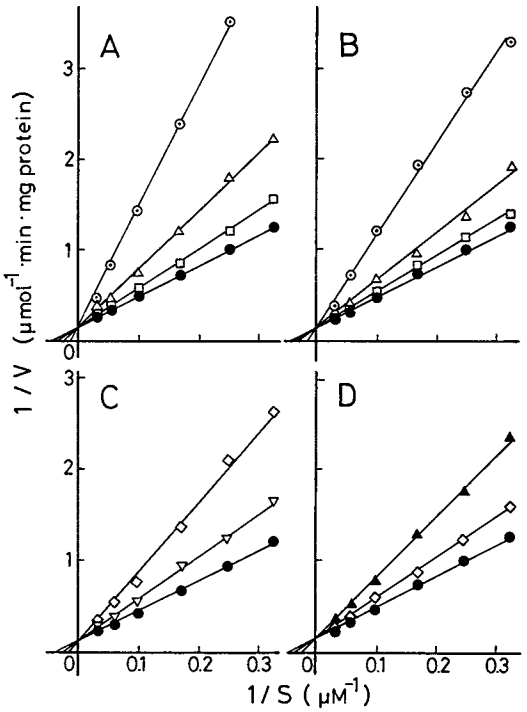

Fig. 3. Lineweaver–Burk plots for the inhibition of phenylethylamine deamination by imipramine (A), diphenhydramine (B), metoclopramide (C), or procainamide (D). Inhibitor concentration: ●, none; □, 4 μ*M*; △, 12 μ*M*; ⊙, 40 μ*M*; ▽, 1 m*M*; ◇, 4 m*M*. Values represent the mean ± SE of four to six experiments.

To clarify the effect of MAOI on the binding characteristics of basic drugs to lung mitochondria, the accumulation of basic drugs in the pellet was monitored at various drug concentrations. Figure 5A shows the Scatchard plot of imipramine binding to the pellet treated with or without MAOIs. The resultant binding parameters of imipramine are shown in Table V. The binding capacity of imipramine to the high-affinity site in the mitochondria was lowered by MAOI treatment, while the association constant was not changed. On the other hand, the binding capacity and the association constant of imipramine at the low-affinity site were not affected by MAOIs. The same phenomena were observed for the binding of quinine (Fig. 5B, Table VI).

DISCUSSION

The present findings demonstrate that MAO is one of the specific binding sites for tertiary basic drugs in the mitochondria and that the binding affinity the MAO of drugs correlates with their lipid solubility. Thus, (i) the activity of

Table IV. Inhibition Constant of Basic Drug for MAO[a]

Inhibitor	K_i (m*M*)
Imipramine	0.013 ± 0.002
Diphenhydramine	0.021 ± 0.004
Metoclopramide	2.3 ± 0.4
Procainamide	22.8 ± 3.0

[a] Values represent the mean ± SE of three or four experiments.

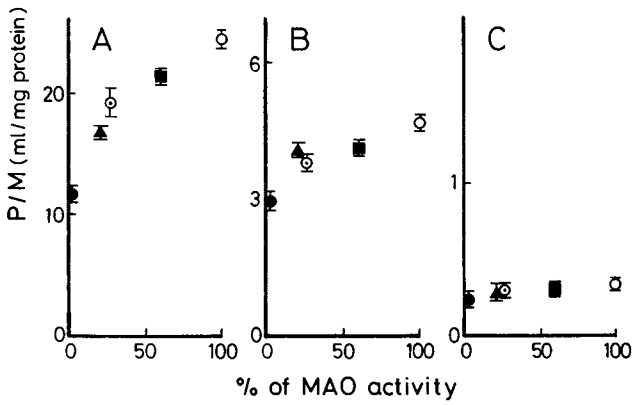

Fig. 4. Effect of MAOI on the binding of imipramine (A), quinine (B), or metoclopramide (C) to lung mitochondrial pellet. Mitochondrial pellet (0.5 mg protein) treated with MAOIs (0.5 m*M*) was incubated at 37°C for 2.5 min with basic drug (2 μ*M*). MAOI: ○, none; ■, semicarbazide; ▲, iproniazid; ⊙, aminoacetonitrile; ●, pargyline. P/M shows the concentration ratio of total drug in the mitochondrial pellet (P) to the medium (M). Values represent the mean ± SE of four to six experiments.

mitochondrial MAO was inhibited by various basic drugs, but that of other enzymes was not (Table I); (ii) the basic drugs inhibited MAO competitively and dose dependently (Figs. 1 and 2); (iii) the inhibitory activity of each basic drug correlated well with its lipid solubility (Table III); (iv) the activity of various MAOIs to inhibit MAO activity correlated well with the inhibition of basic drug binding to mitochondria (Fig. 3); and (v) MAOIs decreased selectively the binding capacity of basic drugs to the high-affinity site but not to the low-affinity site (Fig. 5, Tables V and VI). As these results correspond well with those obtained from drug binding in mitochondrial pellets and drug accumulation in the perfused lung, reported previously (1–5), we propose that mitochondrial MAO functions as a reservoir for basic drugs.

Hellerman and Erwin (18) reported that the inhibition of kidney mitochondrial MAO by pargyline was irreversible. Parkinson and Callingham (19) found that pargyline bound to

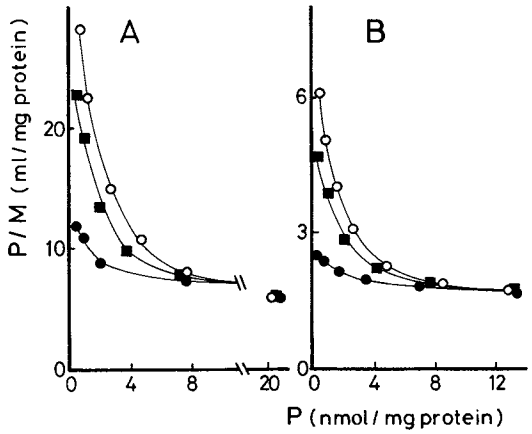

Fig. 5. Scatchard plot of specific binding of imipramine (A) and quinine (B) to rat lung mitochondria treated with 0.5 m*M* semicarbazide (■), 0.25 m*M* pargyline (●), or none (○). P/M shows the concentration ratio of total drug in the mitochondrial pellet (P) to the medium (M).

Table V. Effect of MAOIs on the Accumulation of Imipramine in the Mitochondrial Pellet[a]

Inhibitor	High-affinity site		Low-affinity site	
	B_{max}[b]	K_a[c]	B_{max}[b]	K_a[c]
None	4.0 ± 0.6	5.3 ± 1.2	94 ± 10	0.072 ± 0.008
Pargyline	1.2 ± 0.3	5.0 ± 0.8	88 ± 9	0.076 ± 0.011
Semicarbazide	3.5 ± 0.4	5.2 ± 0.7	95 ± 8	0.065 ± 0.010

[a] Values present the mean ± SE of three or four experiments. Inhibitor concentration: pargyline, 0.25 m*M*; and semicarbazide, 0.5 m*M*.
[b] Maximum number of binding sites (nmol/mg protein).
[c] Association constants for each class of binding site (1/μ*M*).

rat liver mitochondrial MAO and inhibited the deamination of MAO substrates. In this paper, MAOIs selectively inhibited the binding of basic drugs to the high-affinity site in mitochondria. As the separation and purification of intact mitochondrial MAO have not been established yet, we could not determine the MAO binding capacity of basic drugs and could not prove directly whether basic drugs occupy the high-affinity site of mitochondrial MAO. The V_{max} for MAO and the B_{max} for the high-affinity site are 3.6 nmol/mg protein/min and 4.5 nmol/mg protein (4), respectively, in mitochondrial pellets and 1 nmol/mg protein/min (submitted) and about 2 nmol/mg protein (2), respectively, in isolated perfused lung. As the dimensions are different between V_{max} and B_{max}, comparison between both values is difficult; however, both values in each experimental system are similar, and V_{max} and B_{max} are of the expected relative magnitude in mitochondrial pellets and lung tissue, as marker enzymes for mitochondria were concentrated approximately fivefold in the mitochondrial pellet. Thus, mitochondrial MAO may represent the high-affinity site for basic drugs in the lung.

Mitochondrial MAO can be separated into A and B types on the basis of differential sensitivity to inhibitors (20,21). On the basis of proteolytic digestion and peptide mapping techniques, Cawthon (22) found that the A and B types of MAO were distinct enzyme molecules. Tipton (23) stated that mitochondrial MAO in rat lung was 50% type A and 50% type B. In the present experiments, A-type (5-hydroxytryptamine), B-type (phenylethylamine), and both types (tyramine) of MAO substrate had similar V_{max} values but remarkably different K_m values (Table III). The metabolic profile of tyramine in mitochondrial MAO preparations in the presence of various inhibitor concentrations did not fit a dual sigmoidal curve (Fig. 1), and we were therefore unable to support the existence of two types of MAO. On the other hand, the lipid solubility of phenylethylamine is higher than that of 5-hydroxytryptamine, and substrate affinity to mitochondrial MAO correlates with lipid solubility. Thus, it is necessary to clarify the relationship between the enzyme affinity of substrates and their preference for MAO type.

Table VI. Effect of MAOIs on the Accumulation of Quinine in the Mitochondrial Pellet[a]

Inhibitor	High-affinity site		Low-affinity site	
	B_{max}[b]	K_a[c]	B_{max}[b]	K_a[c]
None	3.5 ± 0.5	1.5 ± 0.3	81 ± 9	0.023 ± 0.004
Pargyline	1.1 ± 0.3	1.3 ± 0.3	95 ± 11	0.020 ± 0.003
Semicarbazide	2.8 ± 0.5	1.6 ± 0.4	93 ± 10	0.019 ± 0.004

[a] Values present the mean ± SE of three or four experiments. Inhibitor concentration: pargyline, 0.25 m*M*; and semicarbazide, 0.5 m*M*.
[b] Maximum number of binding sites (nmol/mg protein).
[c] Association constants for class of binding site (1/μ*M*).

In recent years, the effects of many compounds on mitochondrial MAO have been studied (22,24,25). However, no one has demonstrated a correlation between the inhibitory activity of various compounds to MAO and their physicochemical property. The present study shows that the inhibitory MAO activity in mitochondria correlates with the basicity and lipophilicity of the drugs tested.

The results obtained in subcellular experiments cannot be readily applied to the *in vivo* conditions. Imipramine, a tricyclic antidepressant, inhibits the metabolism of biogenic amines in the central nervous system. The minimum effective concentration (MEC) of imipramine in serum is 0.3 μ*M*, at which concentration MAO activity was inhibited by 10% (Fig. 2). The MEC of the other drugs studied range from 0.1 to 20 μ*M*, and at their MECs MAO activities were barely affected. However, upon chronic administrations various psychonervous adverse reactions occur. It is therefore possible that the action and/or adverse reaction of some of these drugs may result from inhibition of mitochondrial MAO, which should be studied *in vivo*.

In conclusion, our findings indicate that in rat lung mitochondrial MAO is one of the specific binding sites for tertiary basic drugs and that the binding affinity of MAO depends on the lipid solubility of the basic drugs.

REFERENCES

1. K. Okumura, H. Yoshida, and R. Hori. *J. Pharmacobio-Dyn.* 1:230–237 (1978).
2. H. Yoshida, K. Okumura, A. Kamiya, and R. Hori. *Chem. Pharm. Bull. (Tokyo)* 37:450–453 (1989).
3. H. Yoshida, K. Okumura, and R. Hori. *Pharm. Res.* 4:50–53 (1987).
4. R. Hori, K. Okumura, and H. Yoshida. *Pharm. Res.* 4:142–146 (1987).
5. K. Okumura, H. Yoshida, A. Kamiya, and R. Hori. *Chem. Pharm. Bull. (Tokyo)* 37:1109–1111 (1989).
6. C. Schnaitman, V. G. Erwin, and J. W. Greenawalt. *J. Cell. Biol.* 32:719–735 (1967).
7. G. L. Sottocase, B. Kuylenstierna, L. Ernster, and A. Bergstrand. *J. Cell. Biol.* 32:415–438 (1967).
8. H. Okamoto and O. Hayaishi. *Arch. Biochem. Biophys.* 131:603–608 (1969).
9. J. A. Roth and C. N. Gillis. *Biochem. Pharmacol.* 23:2537–2545 (1974).
10. S. Otsuka and Y. Kobayashi. *Biochem. Pharmacol.* 13:995–1006 (1964).
11. B. B. Brodie, S. Udenfriend, W. Dill, and G. Downing. *J. Biol. Chem.* 168:311–318 (1947).
12. S. J. Cooperstein and A. Lazarow. *J. Biol. Chem.* 189:665–670 (1951).
13. S. Wattiaux-De Coninck and R. Wattiaux. *Eur. J. Biochem.* 19:552–556 (1971).
14. R. Marco, J. Sebastian, and A. Sols. *Biochem. Biophys. Res. Commun.* 34:725–730 (1969).

15. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and J. R. Randall. *J. Biol. Chem.* **193**:265–275 (1951).
16. H. Lineweaver and D. Burk. *J. Am. Chem. Soc.* **56**:658–667 (1934).
17. C. Scatchard. *Ann. N.Y. Acad. Sci.* **51**:660–672 (1949).
18. L. Hellerman and V. G. Erwin. *J. Biol. Chem.* **243**:5234–5243 (1968).
19. D. Parkinson and B. A. Callingham. *J. Pharm. Pharmacol.* **32**:49–54 (1980).
20. H. C. Kung and A. G. E. Wilson. *Life Sci.* **24**:425–432 (1979).
21. M. D. Housley, K. F. Tipton, and M. B. H. Youdin. *Life Sci.* **19**:467–478 (1976).
22. R. M. Cawthon and X. O. Breakefield. *Nature* **281**:692–694 (1979).
23. K. F. Tipton, M. D. Houslay, and T. J. Mantle. In. G. E. M. Wolstenholme and J. Knight (eds.), *Monoamine Oxidase and Its Inhibition,* Elseviers, Amsterdam, 1976, pp. 5–32.
24. A. J. Richard and L. B. Kier. *J. Pharm. Sci.* **69**:124–126 (1980).
25. C. J. Fowler and B. C. Callingham. *Biochem. Pharmacol.* **27**:1995–2000 (1978).