Submitochondrial Distribution of Basic Drugs in the Isolated Perfused Lung

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To clarify the mechanism by which basic drugs accumulate in the lung mitochondria, the binding selectivity of drugs to different submitochondrial components of the perfused rat lung was examined. The accumulation of basic drugs was the highest in the mitochondrial outer membrane fraction. The drug accumulation in this fraction increased with lipid solubility and was dose-dependent. It appears then that selective binding sites for basic drugs are present in the mitochondrial outer membrane.

Keywords basic drug; lipid solubility; lung; mitochondria; mitochondrial outer membrane; submitochondrial fractionation; metoclopramide; quinine; imipramine; accumulation site

Introduction

It is well known that many basic drugs accumulate in the lung, making it an important reservoir of circulating drugs.[1-3] We previously found that a cationic group as well as a lipophilic group in the molecule were required for the specific accumulation of drugs in the isolated perfused lung.[4,5] An investigation of the subcellular distribution of basic drugs in the perfused lung clarified that the most selective accumulation sites were located in the mitochondrial fraction.[6] Huunan-Seppala[7] reported the binding properties of propranolol and chlorpromazine in the liver mitochondrial membrane. Bickel and Steele[8] studied the binding properties of various drugs to rat tissue subcellular fractions. On the other hand, mitochondria are known to be intracellular organellae that have outer and inner membranes and are the energy generators in a cell. Thus, clarifying the mitochondrial accumulation site of basic drugs is important for understanding their specific pharmacological actions or adverse reactions.

In this study, we examined the accumulation sites of lipophilic basic drugs in lung mitochondria with respect to their submitochondrial distribution in the perfused lung and the binding of basic drugs to the submitochondrial fraction. Imipramine, quinine and metoclopramide were used as examples of basic drugs.

Materials and Methods

Materials Quinine and ^{14}C-imipramine were purchased from commercial sources. ^{14}C-Metoclopramide was kindly supplied by Fujisawa Pharmaceutical Co., Ltd., Osaka. All other reagents were of analytical grade and were purchased from commercial sources. The pK_a values for imipramine, quinine and metoclopramide were 8.7, 8.4 and 9.0, respectively.

Animals Male Wistar rats 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*.

Isolated Lung Perfusion The perfusion method for the isolated lung was described previously.[4] The lung was ventilated with carbogen gas (95% O_2 and 5% CO_2) at a rate of 60 times/min by applying alternating negative pressure to the chamber. The perfusate (10 ml) consisted of a mixture of rat fresh blood and Krebs–Ringer bicarbonate buffer (1:1), equilibrated with carbogen gas before perfusion. The isolated lung was perfused at a rate of 8 ml/min using a peristaltic pump.

Submitochondrial Fractionation Preparation of lung mitochondria was described previously.[6] Lung homogenates were prepared with a Teflon-glass Potter–Elvehjem homogenizer in 9 parts of a medium consisting of 0.25 M sucrose and 3.4 mM Tris (S.T.) buffer, pH 7.4, at 4 °C. Mitochondrial fraction was prepared by centrifuging the 600 *g* (10 min) supernatant at 3300 *g* for 20 min (Hitachi RPR 18-3 rotor). The marker enzymes for mitochondria were concentrated approximately 7-fold in the mitochondrial fraction.[6] The submitochondrial fraction was obtained by digitonin fractionation.[9] Aliquots of ice-cold digitonin solution were added with continuous stirring to equal aliquots of mitochondrial suspension. The resulting suspension was gently stirred for 15 min and then diluted with 3 volumes of S.T. buffer. The diluted suspension was centrifuged at 12000 *g* for 12 min and the supernatant fraction was recentrifuged at 105000 *g* for 90 min: precipitates from these two centrifugations and the final supernatant were designated as fractions 1, 2, and 3, respectively. Each fraction was suspended in S.T. buffer, and the drug concentration, protein contents, and various enzyme activities in each fraction were measured.

Partition Coefficients The partition coefficients (P.C.) of each drug were obtained by our previous method.[6]

Analytical Methods Quinine was analyzed by the fluorometric method of Brodie *et al.*[10] ^{14}C-Imipramine and ^{14}C-metoclopramide were quantitatively extracted with toluene from the samples made alkaline with 0.1 N NaOH. An aliquot of the toluene extract was mixed with a scintillation cocktail, and the radioactivity was determined with a Tri-Carb liquid scintillation spectrometer. Protein was quantitated by the method of Lowry *et al.*[11] Several mitochondrial marker enzymes were assayed: cytochrome oxidase[12] for the inner membrane, monoamine oxidase[13] for the outer membrane, sulfite cytochrome c reductase[14] for the intermembrane space, and malate dehydrogenase[15] for the matrix.

Results and Discussion

Lipophilic basic drugs are rapidly accumulated against a concentration gradient by the isolated rat lung[4,5] and lung mitochondria.[6]

To determine the binding selectivity of basic drugs to the submitochondrial structures, the mitochondria were obtained from the lung perfused with medium containing 1 μM quinine, and fractionated by differential centrifugation as described in Materials and Methods. According to Greenawalt,[9] these fractions (Fig. 1 and Table I) correspond roughly to the following submitochondrial components: fr. 1, unbroken and inner membrane of mitochondria; fr. 2, outer membrane of mitochondria; fr. 3, intermembrane space and matrix of mitochondria. As shown in Fig. 1, the specific activities of cytochrome oxidase in fr. 1 and monoamine oxidase in fr. 2 are the highest among all fractions, and those of malate dehydrogenase and sulfite cytochrome c reductase in fr. 3 were higher than those of other fractions. The distribution patterns of the enzymes corresponded with Greenawalt's data.[9] No differences in these distribution patterns were observed between the mitochondrial fraction obtained from the perfused lung and the non-perfused lung. These results indicate that the

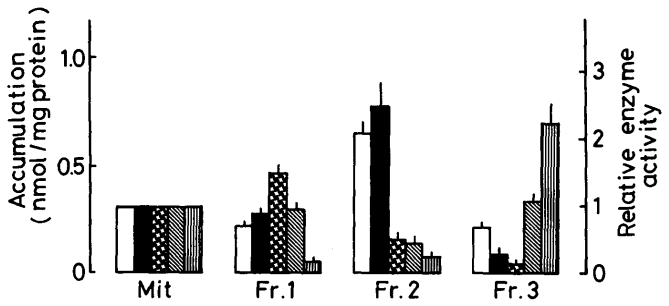

Fig. 1. Distribution of Quinine and Marker Enzymes in Mitochondria from the Perfused Lung

Relative enzyme activity: the ratio of enzyme activity in each submitochondrial fraction to that in the mitochondria (normalized to protein content). Each value represents the mean ± S.E.M. of three to five experiments. □, quinine; ■, monoamine oxidase; ⊠, cytochrome oxidase; ▨, sulfite cytochrome c reductase; ▥, malate dehydrogenase. Mit, mitochondrial fraction; fr. 1, 12000 *g* pellet; fr. 2, 105000 *g* pellet; fr. 3, 105000 *g* supernatant.

TABLE I. Submitochondrial Distribution of Basic Drugs

Fraction No.[a]	Percent distribution			Binding (pmol/mg protein)		
	IMI[b]	QUI[b]	MET[b]	IMI[b]	QUI[b]	MET[b]
1	37.1±4.1	40.3±5.0	21.9±2.8	304±34	235±29	39±5
2	46.3±4.2	40.6±4.2	10.7±2.0	1041±95	651±68	40±7
3	16.5±2.0	18.9±1.1	67.3±9.5	275±35	223±13	46±7

Mitochondria were obtained from the rat lung perfused with the medium containing 1 μM drug. Each value represents the mean ± S.E.M. of three to five experiments. *a*) Fr. 1, 12000 *g* pellet; fr. 2, 105000 *g* pellet; fr. 3, 105000 *g* supernatant. *b*) IMI, imipramine; QUI, quinine; MET, metoclopramide.

procedure used for separation of the submitochondrial components was suitable for studying the submitochondrial distribution of drugs in the lung.

Table I shows the submitochondrial distribution of basic drugs accumulated in the perfused lung. Most of the quinine in the mitochondria was associated with frs. 1 and 2, but the specific accumulation of quinine in fr. 2 was about 3 times higher than that in the other fractions. The distribution of imipramine was similar to that observed for quinine but the accumulation selectivity of imipramine in fr. 2 was greater than that of quinine. Most of the metoclopramide in the mitochondria was detected in the supernatant fraction, but about 70 percent of metoclopramide in this fraction was in an unbound form. Thus, no specific accumulation sites for metoclopramide were detected in the three fractions. The distribution of basic drugs in each fraction was coincident with the distribution of marker enzyme activity in the mitochondrial outer membrane but not with those of the other marker enzymes. These results suggest that the outer membrane contains the major binding sites for basic drugs in the lung mitochondria.

The effect of the lipid solubility on the submitochondrial distribution of the drugs in the lung was studied. The accumulation of imipramine, which is the most lipophilic of the three drugs used, in the outer membrane fraction was markedly greater than that of the other drugs (Table I). A correlation was observed between the lipid solubility and the relative drug accumulation in the mitochondrial outer membrane fraction (Fig. 2). It seems, then, that the selective accumulation of drugs in the outer membrane

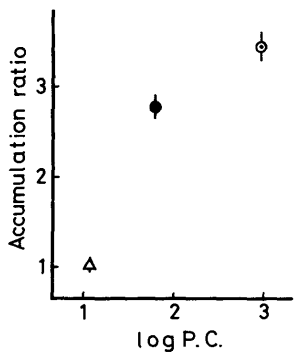

Fig. 2. Effect of Lipid Solubility on the Distribution of Basic Drugs in Lung Mitochondrial Outer Membrane

Accumulation ratio: the ratio of drug accumulation in the outer membrane fraction to drug present in the inner membrane fraction (normalized to protein content). Each point presents the mean ± S.E.M. of three to five experiments. ⊙, imipramine; ●, quinine; △, metoclopramide. P.C., partition coefficient.

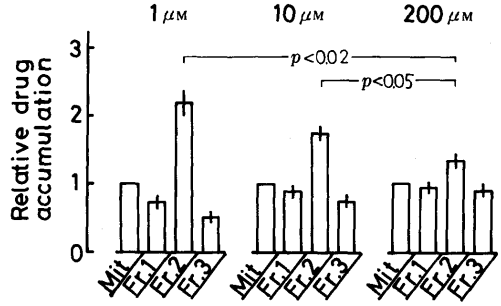

Fig. 3. Effect of Initial Drug Concentration in Perfusate on Quinine Distribution in Mitochondria from the Perfused Rat Lung

Relative drug accumulation: the ratio of drug accumulation in each submitochondrial fraction to drug present in the mitochondria (normalized to protein content). Each value represents the mean ± S.E.M. of three to five experiments. Mit, mitochondrial fraction; fr. 1, 12000 *g* pellet; fr. 2, 105000 *g* pellet; fr. 3, 105000 *g* supernatant.

fraction was regulated by their respective lipid solubilities, as proposed earlier.[4]

The effect of drug concentration on quinine distribution was also studied. As shown in Fig. 3, the most specific accumulation of quinine was found in the mitochondrial outer membrane fraction. The supernatant fraction contained a small amount of the drug. The accumulation ratio of quinine in the mitochondrial outer membrane fraction was the highest at each dose and was dose-dependent. Therefore, differences in drug concentration may affect the drug accumulation in mitochondria through influencing the binding of drugs to the lung mitochondrial components. These findings correspond to our previous observations.[4-6,16] The present investigation has shown that one of the selective binding sites for basic drugs appears to be present in the mitochondrial outer membrane, which may function as a reservoir for the drugs.

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