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Report

Subcellular Distribution of Basic Drugs Accumulated in the Isolated Perfused Lung

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To clarify the mechanism by which basic drugs accumulate in the lung, the binding selectivity of drugs to different subcellular structures of the perfused rat lung was examined. Imipramine, quinine, and metoclopramide were used as examples of basic drugs. The accumulation of basic drugs was highest in the mitochondrial fraction. The drug accumulation in the lung mitochondrial fraction increased with increasing lipid solubility and was dose dependent. These results suggest the presence of specific binding sites for basic drugs in mitochondria and that mitochondria play an important role as a reservoir for basic drugs.

KEY WORDS: basic drug; lipid solubility; lung; mitochondria; subcellular fractionation.

INTRODUCTION

The ability to accumulate drugs makes the lung a potentially important pharmacokinetic compartment (1–3). The known interactions of drugs in lung have often been assumed to result from competition for common binding sites.

In our previous studies (4,5), the accumulation of various drugs was examined in the isolated perfused rat lung. The measurement of drug disappearance from the perfusate and the drug concentration ratio in the lung and perfusate indicated that many cationic drugs are not metabolized and concentrated in the lung. Partition coefficients of these basic drugs correlated well with drug accumulation by the lung. Moreover, various metabolic inhibitors did not influence the accumulation of basic drugs in the lung, and the accumulation of drugs decreased in the presence of other basic drugs as a result of displacement, which again correlated with lipid solubility.

In this study, we examined the accumulation sites of lipophilic basic drugs in the lung, with respect to (i) their subcellular distribution in the perfused lung and (ii) the binding of basic drugs to the mitochondrial fraction.

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 materials were of analytical grade.

Animals. Male Wistar rats weighing 180–230 g were used in the experiments. 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 of isolated lung was performed as 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 alternation 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. In the drug accumulation study, drug solutions of various concentrations were added to the perfusate. After 60 min of perfusion, the concentration ratio of drug in the lung (nmol/g tissue) to unbound drug in the perfusate (nmol/ml) was determined.

Subcellular Fractionation. After 60 min of perfusion, the lung tissue was minced with scissors and homogenized in a 0.25 *M* sucrose solution, pH 7.4, containing 3.4 m*M* Tris (ST buffer) using a Teflon–glass Potter–Elvehjem homogenizer at 0–4°C. The homogenate was then filtered through two layers of gauze. The filtrate was centrifuged at 600*g* for 10 min (Hitachi Centrifuge RPR 18-3 rotor), and the supernatant fraction was recentrifuged at 3300*g* for 20 min; precipitates from these two centrifugations were designated Fractions 1 and 2, respectively. Fraction 3 was obtained from the supernatant fraction by centrifugation at 10,000*g* for 20 min. The microsomal fraction (pellet; Fraction 4) and the cytosol (supernatant; Fraction 5) were prepared by centrifuging the supernatant fraction of the 10,000*g* centrifugation at 192,000*g* (Hitachi Centrifuge RPR-55 rotor) for 45 min. Each fraction was suspended in ST buffer, and the drug concentration, protein contents, and various enzyme activities in each fraction were measured. Relative drug accumulation was expressed as the ratio of drug accumulation in each subcellular fraction to drug present in the homogenate (normalized to protein content).

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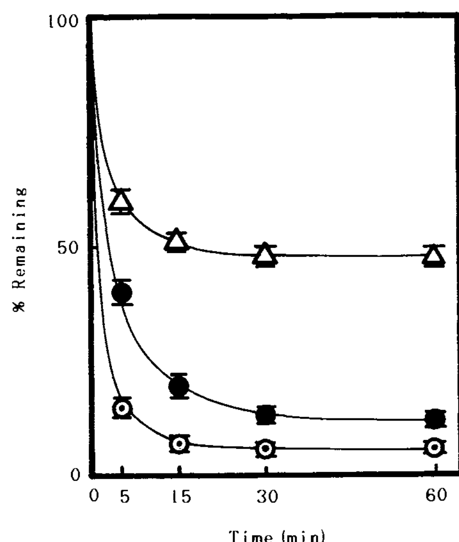

Fig. 1. Time courses of drug clearance from perfusate. Initial concentration of perfusate is 10 μ*M*. (○) Imipramine; (●) quinine; (△) metoclopramide. Each point represents the mean ± SE of four to six experiments.

Partition Coefficients. Partition coefficients were obtained by the method of Hogben *et al.* (6). That is, the buffered drug solution (pH 7.4) was added to an equal volume of chloroform in a culture tube and equilibrated at 37°C by vigorous shaking. The separated aqueous phase and organic phase were analyzed. The ratio of the drug concentration in the organic phase to that in the aqueous phase was taken as the partition coefficient of a drug.

Analytical Methods. Quinine was analyzed by the fluorometric method of Brodie *et al.* (7). ^{14}C-Imipramine and ^{14}C-metoclopramide were quantitatively extracted by toluene from the samples made alkaline with 0.1 *N* NaOH. 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.). Protein was quantitated by the method of Lowry *et al.* (8). Several marker enzymes were assayed: cytochrome oxidase for the inner membrane of mitochondria (9), monoamine oxidase for the outer membrane of mitochondria (10), 5′-nucleotidase for the plasma membrane (11), glucose-6-phosphatase for microsomes (12), and acid phosphatase for lysosomes (13).

RESULTS AND DISCUSSION

Subcellular Distribution. Basic drugs with a high lipophilicity rapidly accumulated, within 5 min, into the isolated rat lung against a concentration gradient from the recirculating perfusate and reached steady state after 30 min (Fig. 1). After perfusion, metabolites of these drugs could not be detected in the perfusate and lung tissue, and drug extracted from the perfusate was almost entirely accounted for in the perfused lung. Concentration ratios of drugs in the lung to unbound drug in the perfusate of imipramine, quinine, and metoclopramide at 10 μ*M* were 2130 ± 510, 340 ± 70, and 17 ± 0.3, respectively.

To determine the binding selectivity of basic drugs to subcellular structures, the lungs were homogenized after perfusion with the medium containing 1 μ*M* quinine and fractionated by differential centrifugation as described in Materials and Methods. According to Hook *et al.* (14), these fractions (Fig. 2 and Table I) correspond roughly to the following subcellular components: Fraction 1, unbroken and partly disrupted cells and connective tissue; Fraction 2, heavy mitochondria; Fraction 3; light mitochondria; Fraction 4, microsome and lysosome; and Fraction 5, cytosol. As shown in Fig. 2, the specific activities of cytochrome oxidase and monoamine oxidase were highest in Fraction 2, and those of 5′-nucleotidase, acid phosphatase, and glucose-6-phosphatase were highest in Fraction 4. No differences in these distribution patterns were observed between the perfused lung and the nonperfused lung. These results indicate that the procedure used for separation of the subcellular components was suitable for studying the subcellular distri-

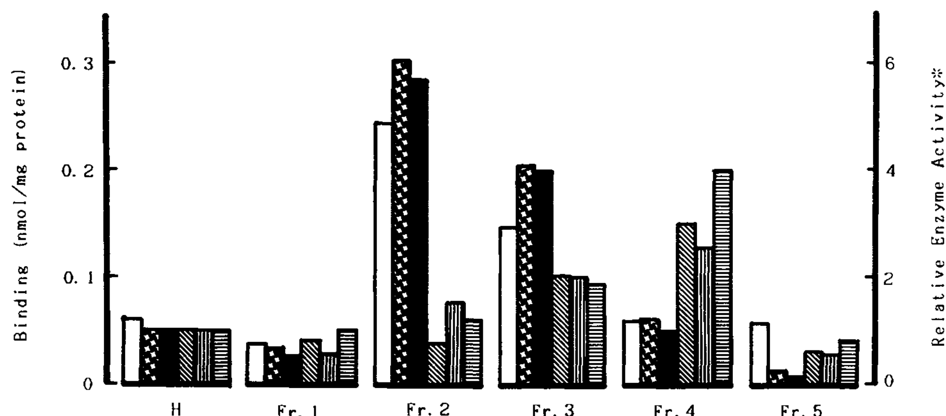

Fig. 2. Subcellular distribution of quinine and marker enzymes in homogenates from the perfused rat lung. Relative enzyme activity: the ratio of enzyme activity in each subcellular fraction to that in the homogenate (normalized to protein content). (□) Quinine; (▩) cytochrome oxidase; (■) monoamine oxidase; (▧) 5′-nucleotidase; (▥) acid phosphatase; (▤) glucose-6-phosphatase. H, homogenate; Fraction 1, 600*g* pellet; Fraction 2, 3300*g* pellet; Fraction 3, 10,000*g* pellet; Fraction 4, 192,000*g* pellet; Fraction 5, cytosol.

Table I. Subcellular Distribution of Drugs in Isolated Perfused Rat Lung

Drug conc. (μM)[a]	Fraction No.[b]	Percentage distribution[c]			Binding (nmol/mg protein)[c]		
		IMI[d]	QUI	MET	IMI	QUI	MET
1	1	28.3 ± 4.5	31.5 ± 3.9	25.7 ± 3.5	0.059 ± 0.003	0.065 ± 0.005	0.022 ± 0.001
	2	35.5 ± 3.2	31.2 ± 4.6	18.7 ± 2.0	0.436 ± 0.012	0.350 ± 0.042	0.075 ± 0.007
	3	11.9 ± 0.4	10.2 ± 1.8	5.6 ± 0.5	0.213 ± 0.021	0.216 ± 0.033	0.034 ± 0.002
	4	9.9 ± 1.0	7.8 ± 0.9	3.8 ± 0.5	0.108 ± 0.009	0.084 ± 0.008	0.015 ± 0.002
	5	9.4 ± 2.8	16.9 ± 1.1	39.2 ± 1.3	0.032 ± 0.004	0.058 ± 0.004	0.041 ± 0.001
10	1	30.3 ± 7.2	33.1 ± 3.3	27.6 ± 4.0	0.64 ± 0.03	0.60 ± 0.05	0.24 ± 0.02
	2	30.7 ± 3.5	27.4 ± 4.0	19.8 ± 2.1	3.66 ± 0.67	2.61 ± 0.34	0.78 ± 0.03
	3	12.0 ± 1.9	10.6 ± 1.2	5.4 ± 1.3	1.69 ± 0.22	1.59 ± 0.21	0.37 ± 0.01
	4	11.7 ± 2.0	10.2 ± 0.8	4.4 ± 0.4	1.11 ± 0.19	0.76 ± 0.09	0.18 ± 0.01
	5	11.5 ± 0.1	17.8 ± 1.4	44.0 ± 3.0	0.35 ± 0.01	0.46 ± 0.04	0.47 ± 0.03
200	1	31.9 ± 2.9	33.6 ± 5.5	27.3 ± 2.6	11.4 ± 1.2	9.3 ± 0.5	4.1 ± 0.3
	2	22.1 ± 2.2	21.5 ± 0.3	14.8 ± 2.2	35.4 ± 3.3	26.7 ± 1.7	9.1 ± 1.0
	3	10.7 ± 1.2	7.8 ± 0.5	4.6 ± 0.5	29.3 ± 4.0	16.0 ± 1.7	4.9 ± 0.6
	4	15.4 ± 2.0	11.2 ± 0.9	4.2 ± 0.7	22.6 ± 2.7	11.9 ± 1.5	2.8 ± 0.3
	5	15.1 ± 1.7	21.9 ± 0.2	42.4 ± 1.4	7.1 ± 1.0	8.2 ± 0.9	8.3 ± 0.4

[a] Initial drug concentration in perfusate.
[b] Fraction 1, 600*g* pellet; Fraction 2, 3300*g* pellet; Fraction 3, 10,000*g* pellet; Fraction 4, 192,000*g* pellet; Fraction 5, cytosol.
[c] Each value represents the mean ± SE of four to six experiments.
[d] IMI, imipramine; QUI, quinine; MET, metoclopramide.

bution of drugs in the lung. Table I shows the subcellular distribution of basic drugs accumulated in the perfused lung. At 1 μ*M*, 83% of the quinine and 91% of the imipramine remaining in the lung after perfusion were associated with Fractions 1 to 4. Most of the quinine in the homogenates was associated with Fractions 1 and 2, but the selective accumulation of quinine in Fraction 2 was two to six times higher than that in the other fractions. Fraction 5 contained approximately 17% of the total drug dose, but its drug accumulation relative to protein content was the lowest among all fractions. The distribution of imipramine was similar to that observed for quinine but the selective accumulation of imipramine in Fraction 2 was greater than that of quinine. Most of the metoclopramide in the homogenates was detected in the cytosol fraction. However, the accumulation of metoclopramide in Fraction 2 was twice that in the cytosol fraction. The distribution of basic drugs in each fraction was coincident with the distribution of mitochondrial marker enzyme activity but not with those of the other marker enzymes. These results suggest that the mitochondria are the major binding site for basic drugs in the lung.

Effect of Concentration and Lipid Solubility on Subcellular Drug Distribution. The subcellular distribution of basic drugs was studied in the perfused lung as a function of the drug concentration. As shown in Figs. 3 and 4A, the greatest accumulation of all drugs was found in the mitochondrial fraction, while the cytosol and microsomal fractions contained only small drug amounts. The extent of imipramine accumulation in the mitochondria was higher than that of other drugs at each dose and was dose dependent. The distribution of quinine was similar to that observed for imipramine and was also dose dependent; however, the metoclopramide distribution was independent of the drug concentration. Differences in concentration effects on drug accumulation in mitochondria may be related to binding phenomena to components of lung mitochondria.

The effect of the lipid solubility on the subcellular distribution of the drugs in the lung was also studied. Figure 4B shows the relationship between lipid solubility and relative drug accumulation in the mitochondrial fraction. Lipid solubilities are expressed as the partition coefficient between chloroform and isotonic phosphate buffer (pH 7.4). The ac-

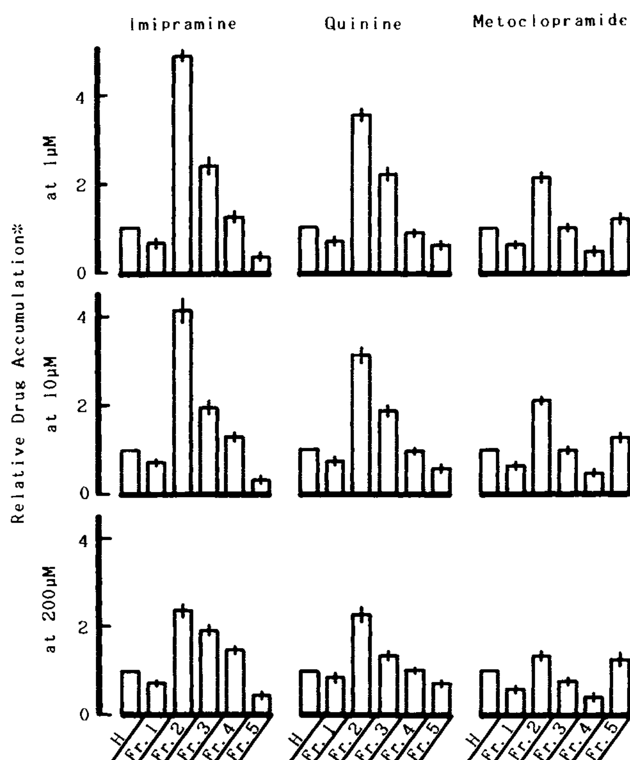

Fig. 3. Effect of initial drug concentration in the perfusate on subcellular distribution of basic drugs in homogenates from the perfused rat lung. Relative drug accumulation: the ratio of drug accumulation in each subcellular fraction to drug present in the homogenate (normalized to protein content). H, homogenate; Fraction 1, 600*g* pellet; Fraction 2, 3300*g* pellet; Fraction 3, 10,000*g* pellet; Fraction 4, 192,000*g* pellet; Fraction 5, cytosol.

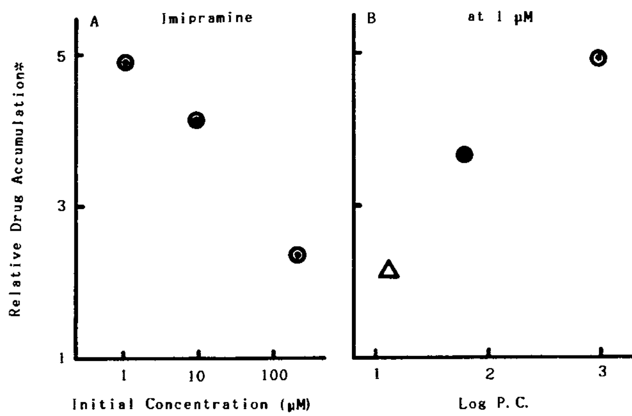

Fig. 4. Effect of initial drug concentration (A) and lipid solubility (B) on subcellular distribution of basic drugs in the perfused lung. Relative drug accumulation: the ratio of drug accumulation in each subcellular fraction to drug present in homogenate (normalized to protein content). (⊙) Imipramine; (●) quinine; (△) metoclopramide.

cumulation of imipramine, which is the most lipophilic of the three drugs used, in the mitochondrial fraction was markedly greater than that of the other drugs. It seems, then, that the selective accumulation of drugs in the mitochondrial fraction correlates with lipid solubility, as proposed earlier (4). The lipid content in Fractions 4 and 5 is known to exceed that in the other fractions. The lack of selective drug accumulation in Fractions 4 and 5 suggests that basic drugs may not bind to lipid but to other lung components such as protein, and the binding sites in mitochondria may function as a reservoir.

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