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Accumulation Mechanism of Basic Drugs in the Isolated Perfused Rat Lung

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The mechanism of the accumulation of basic drugs was investigated by isolated rat lung perfusion. Treatment with various metabolic inhibitors or non-basic drugs did not affect the accumulation of a basic drug in the lung, but a second basic drug inhibited the accumulation of the first basic drug depending on its lipid solubility. The basic drug already accumulated was rapidly displaced by the second drug except for poorly lipid-soluble basic drugs and non-basic drugs. The ability of a second basic drug to displace the first basic drug was well correlated with its ability to inhibit accumulation. From the Scatchard plot, at least two independent sets of binding sites for basic drugs were found to be present in the isolated perfused lung. The maximum binding capacity for each basic drug was similar in both sites. These results indicate that specific common binding sites for basic drugs, which do not contribute to the active transport system, exist in the lung tissues and the affinity to the sites depends on the lipid solubility of the basic drugs.

Keywords basic drug; binding site; lung accumulation; lipid solubility; lung; isolated perfused lung; quinine; imipramine; metoclopramide; accumulation mechanism

Introduction

Some functions other than respiration have been attributed to the lung in recent years.[1,2] Various compounds are known to accumulate in the lung after administration.[3,4] The accumulation of one basic drug has been reported to be decreased by the presence of other drugs in isolated prefused lung preparations.[5,6] However, few detailed studies have been made on the relationship between the characteristics of the drug accumulation in the lung and the physico-chemical properties of the drugs.[3,4,7] The lung accumulation of various drugs has been monitored in the isolated perfused rat lung.[7,8] The kinetics of the drug disappearance from the perfusate and drug concentration ratio in the lung to the perfusate indicated that many cationic drugs could be concentrated in the lung. The partition coefficient of the basic drugs correlated well with the drug accumulation in the lung.

We determined the accumulation mechanism of lipophilic basic drugs in the lung by examining: (1) the effect of metabolic inhibitors and various drugs on basic drug accumulation by the lung, (2) the relationship between lipid solubility and the ability of other basic drugs to inhibit or to displace the accumulation of the first basic drug, (3) the binding capacity and affinity for each basic drug in the lung, and (4) the presence of common binding sites for basic drugs in the lung.

Materials and Methods

Materials Quinine, sulfanilamide, sulfisoxazole, diphenhydramine, *N*-methylnicotinamide (NMN) and ^{14}C-imipramine were purchased from commercial sources. Phenylbutazone, alloclamide, metoclopramide, procainamide and procainamide ethobromide (PAEB) were kindly supplied by Fujisawa Pharmaceutical Co., Ltd., Osaka. All other materials were of analytical grade.

Animals Male Wistar rats weighing 180—230 g (7—8 weeks old) 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 of isolated lung was performed as described previously.[7] The lung was ventilated with carbogen gas (95% O_2+5% CO_2) at the rate of 60 times/min for 60 min by periodically applying reduced pressure to the chamber. The perfusate (10 ml) consisted of a mixture of fresh rat 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 (1—2000 μM) were added to the perfusate and drug clearance from the perfusate was measured. The unbound drug concentration in the perfusate (*Pf*, nmol/ml) and the total concentration in the lung tissue (*L*, nmol/g tissue) were determined after 60 min of perfusion (approximately steady state).

To clarify the effect of metabolic inhibitors on the drug accumulation, the lung was treated with a perfusate containing each metabolic inhibitor for 15 min and the accumulation process of 0.1 mmol of basic drug was monitored for 60 min.

To determine the binding characteristics of basic drugs to the perfused lung, the lungs were perfused with the medium containing various concentrations (1—2000 μM) of basic drugs. After 60 min, the unbound drug concentration in the perfusate (*Pf*, nmol/ml) and the total concentration in the lung tissue (*T*, nmol/mg of protein in the lung tissue) were determined.

Inhibition of the accumulation process was studied by simultaneously adding the test drug and the inhibitor drug to the perfusate and following the drug concentrations in the perfusate.

In other studies, the test basic drug was first allowed to equilibrate between the lung and perfusate (generally 30 min). A second drug (2 or 0.2 μmol) was then introduced into the perfusate and the displacement of the equilibrated compound was determined.

Partition Coefficients The partition coefficient of each drug was obtained by our previous method.[9]

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 (Monophase-40, Packard Instrument Co.), and the redioactivity was determined with a Tri-Carb liquid scintillation spectrometer (model 3330, Packard Instrument Co.). Protein was quantitated by the method of Lowry *et al.*[11]

Determination of Drug Metabolite in the Perfusate and Lung Tissue Basic drugs in the lung homogenate or perfusate were extracted into chloroform. The solvents of these extracts were evaporated off and the residues were chromatographed on Silica gel HF254 (Merck Co.). Thin-layer plates were developed in chloroform–acetone–ethanol (v/v/v, 1 : 1 : 1).[7]

Data Analysis Saturation drug binding curves were analyzed by the method of Scatchard,[12] in which the regression lines were drawn by the least-squares method to determine the association constant (K_a) of each drug and the maximum binding capacity (B_{max}).

Results

Effect of Metabolic Inhibitors To clarify the contri-

bution of the active transport system to the drug accumulation in the lung, the effect of various matabolic inhibitors on this process was studied. Table I shows the percent of quinine remaining in the perfusate 15 min after treatment with or without a metabolic inhibitor. Quinine rapidly accumulated in the isolated perfused lung against a concentration gradient from the recirculating perfusate and reached a steady state after 30 min. The time course of quinine after addition of the inhibitor was similar to that in the control. Metabolites of quinine could not be detected in the extract of lung perfusate or lung homogenate in any experiment. The metabolic inhibitors had no effect on the ratio of drug concentration in the lung to unbound drug in perfusate (L/Pf) of quinine. Moreover, imipramine accumulation was also not affected by the metabolic inhibitors (data not shown).

Binding Properties of Basic Drugs The effect of drug concentration on the binding to the lung was studied to clarify the binding properties of basic drugs in the perfused lung. Figure 1 shows Scatchard plots of the binding of the basic drugs to the lung. The bound drug divided by the amount of the free drug is plotted against the bound drug per lung tissue protein. It is evident that there are at least two types of binding sites for each drug, one high-affinity/low-density site and one low-affinity/high-density site, in the perfused lung. Table II shows the binding affinity

TABLE I. Effect of Metabolic Inhibitors on Uptake of Quinine by the Isolated Perfused Rat Lung

Inhibitor[a]	Concn. (mM)	Percent remaining[b] Collecting time 5 min	15 min	30 min	60 min	(L/Pf)[c] ratio
None		31.4±2.6	19.6±2.5	12.9±1.8	12.1±1.7	264±47
2,4-DP	0.01	34.7±2.6	17.5±1.9	12.6±1.0	13.0±1.2	280±70
	0.1	29.1±4.7	14.9±2.5	12.4±1.2	10.4±0.6	289±39
NaCN	0.1	38.0±1.3	18.9±1.6	13.3±1.3	12.1±0.4	233±35
	1	37.5±3.7	22.7±2.0	15.2±2.1	14.1±1.6	275±24
Ouabain	0.01	34.3±3.5	17.3±1.5	13.3±1.2	12.0±0.7	289±44
	0.1	35.3±2.5	19.4±2.0	15.0±2.8	14.1±2.8	245±65
N_2-gas		24.7±2.2	16.5±2.5	12.6±1.1	12.9±1.0	312±62
IAA	0.01	36.5±1.2	16.0±1.1	11.2±1.8	10.8±1.5	265±26
	0.1	33.8±2.9	17.5±1.9	12.0±0.9	11.0±0.5	215±27
NEM	1	36.4±3.5	19.4±1.5	13.6±0.8	12.4±1.1	263±29
PCMPS	0.1	32.3±1.2	16.3±2.9	10.3±1.5	9.8±1.4	232±40

a) 2,4-DP, 2,4-dinitrophenol; N_2-gas, 95% N_2+5% CO_2; IAA, iodoacetic acid; NEM, *N*-ethylmaleimide; PCMPS, *p*-chloromercuriphenylsulfonic acid monosodium salt. *b*) Lungs were perfused with 0.01 mM quinine 15 min after preperfusion with metabolic inhibitors and the perfusate was collected at various times. *c*) Concentration ratio of quinine in the lung to unbound drug in perfusate at the steady state. Each value represents the mean±S.E.M. of three to seven experiments.

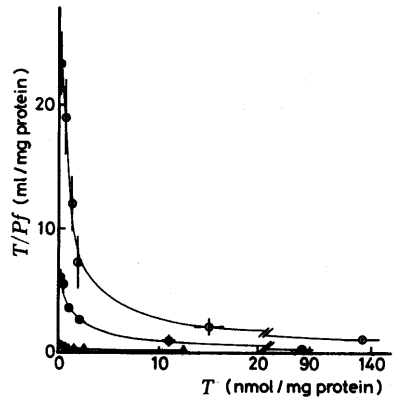

Fig. 1. Scatchard Plot of Specific Binding of Basic Drug to the Isolated Perfused Lung

⊙, imipramine; ●, quinine; ▲, metoclopramide. Each point represents the mean±S.E.M. of three to seven experiments.

TABLE II. Binding of Basic Drug to the Isolated Perfused Rat Lung

Drug	P.C.[a]	Affinity site	B_{max}[b]	K_a[c]
Imipramine	980	High	2.4	8.1
		Low	180	0.01
Quinine	64	High	2.3	1.4
		Low	130	0.007
Metoclopramide	12	High	2.0	0.06
		Low	280	0.0005

a) Partition coefficient ($CHCl_3/H_2O$, pH 7.4). *b*) Maximum number of binding sites (nmol drug per mg protein). *c*) Association constants for each class of binding site ($1/\mu M$).

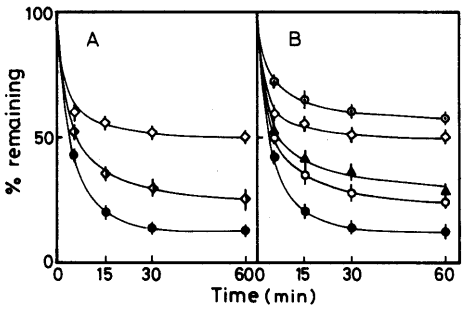

Fig. 2. Effect of Basic Drugs on Uptake of Quinine by the Isolated Perfused Rat Lung

A, lungs were perfused with a mixture of 0.01 mM quinine and none (●), 0.2 mM (◈), or 2 mM (◇) diphenhydramine. B, lungs were perfused with a mixture of 0.01 mM quinine and none (●), 2 mM procainamide (○), 2 mM metoclopramide (▲), 2 mM diphenhydramine (◇) or 2 mM imipramine (⊙). Each point represents the mean±S.E.M. of three to six experiments.

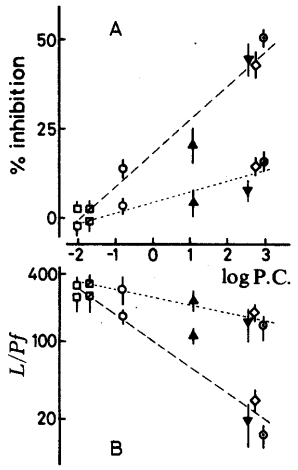

Fig. 3. Relationship between the Ability of a Second Basic Drug to Inhibit Quinine Accumulation and Logarithm of the Partition Coefficient ($CHCl_3/H_2O$, pH 7.4)

Broken line, 2 mM inhibitor; dotted line, 0.2 mM inhibitor. A, percent inhibition of quinine clearance from perfusate by a second drug. $r=0.969$ (at 2 mM), $r=0.871$ (at 0.2 mM). B, L/Pf concentration ratio of quinine in the presence of a second drug. $r=-0.963$ (at 2 mM), $r=-0.951$ (at 0.2 mM). Each point represents the mean±S.E.M. of three to four experiments. ⊙, imipramine; ▼, alloclamide; ◇, diphenhydramine; ▲, metoclopramide; ○, procainamide; ◪, *N*-methylnicotinamide; □, procainamide ethobromide.

and maximum number of binding sites. The basic drugs had similar binding capacities, while the association constants correlated with their lipid solubilities.

Competition for Pulmonary Drug Binding To clarify further the drug accumulation mechanism in the lung, the effect of a second drug on the binding of basic drugs was studied. Figure 2B reveals the time course of quinine clearance from the perfusate in the presence of various basic drugs. Although similar initial clearance and steady-state situation were obtained in all cases, the percent of quinine remaining in the perfusate differed. The inhibition by a second basic drug was dose-dependent (Fig. 2A, Fig. 3A). The concentration ratio of lung to unbound drug in the perfusate at a steady state was greatly decreased by a second basic drug. The ability of each basic drug to inhibit quinine accumulation was well correlated with lipid solubility (Fig. 3). The extent of inhibition by imipramine was the greatest. On the other hand, the acidic drug, phenylbutazone, which is lipophilic and easily binds to plasma protein, could not inhibit the accumulation of basic drugs (Fig. 5).

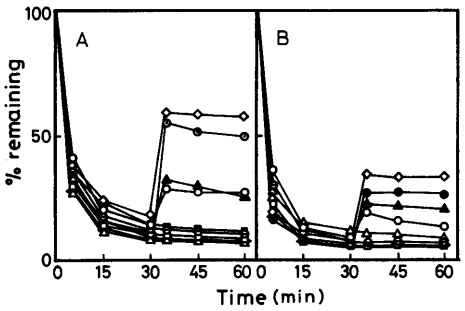

Fig. 4. The Displacement of Basic Drug Accumulated in the Lung by a Second Drug

Lungs were perfused with 0.01 mM quinine (A) or imipramine (B) for 30 min and then a second drug (2 mM) was added to the perfusate. ⊖, control; ⊙, imipramine; ●, quinine; ◇, diphenhydramine; ▲, metoclopramide; ○, procainamide; ◪, *N*-methylnicotinamide; □, procainamide ethobromide; △, sulfanilamide; ◈, sulfisoxazole.

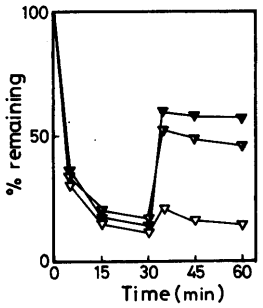

Fig. 5. The Displacement of Quinine Accumulated in the Lung by Alloclamide

Lungs were perfused with 0.01 mM quinine for 30 min and then 0.2 mM (▽), 0.8 mM (▼), or 2 mM (▼) alloclamide was added to the perfusate.

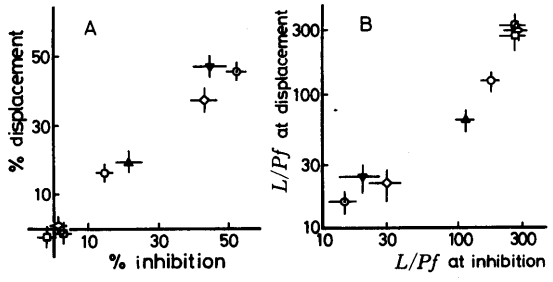

Fig. 6. Relationship of Percent Displacement and Percent Inhibition of Quinine Accumulation by a Second Basic Drug

A, horizontal and vertical lines are percent inhibition and percent displacement of quinine accumulation by a second drug, respectively ($r=0.980$). B, horizontal and vertical lines are *L/Pf* concentration ratio of quinine at inhibition and displacement by a second drug, respectively ($r=0.972$). Each point represents the mean ± S.E.M. of three to four experiments. ⊙, imipramine; ▼, alloclamide; ◇, diphenhydramine; ▲, metoclopramide; ○, procainamide; ◪, *N*-methylnicotinamide; □, procainamide ethobromide; ◇, phenylbutazone.

Displacement from the Lung of an Accumulated Basic Drug by a Second Drug To confirm the characteristics of the binding sites for basic drugs in the lung, the displacement from the lung of an accumulated basic drug by a second drug was examined. As shown in Fig. 4A, the accumulated quinine was displaced within 5 min by the addition of various basic drugs except for NMN and PAEB, which are poorly lipid-soluble, and a new steady state was established. The ability of the second basic drug to displace another already accumulated drug was well correlated with lipid solubility. The degree of displacement depended on the dose of the second basic drug (Fig. 5). Non-basic drugs did not displace an accumulated basic drug. A similar displacement phenomenon was also observed in the case of imipramine accumulated in the lung, but the percent displacement of imipramine with a second drug was lower than that of quinine (Fig. 4). Metabolites of any of the basic drugs could not be detected in the perfusate or lung tissue. Total recovery of intact drug was more than 94%.

Relation of the Abilities a Second Basic Drug to Displace and to Inhibit To determine the binding properties of basic drugs, the relationship between the extents of displacement and inhibition of quinine accumulation in the lung by various basic drugs was studied. As shown in Fig. 6, the percent displacement by various basic drugs was very closely correlated with the percent inhibition. Moreover, the *L/Pf* concentration ratio of quinine in the inhibition by a second basic drug was correlated well with that in the displacement. The correlation coefficients were 0.960 and 0.972. Similar phenomena were observed for the accumulation of imipramine (data not shown).

Discussion

We studied the mechanism of accumulation of basic drugs in the isolated perfused rat lung. The results demonstrated that the basic drugs were not accumulated through an active transport system but by specific binding to lung tissue components, that the binding sites were the same for the basic drugs tested, and that the binding affinity for drugs depended on their lipid solubility. Namely, (1) pretreatment with various metabolic inhibitors (Table I) and non-basic drugs did not affect the drug accumulation; (2) metabolites of the basic drugs could not be detected in the perfusate or lung tissue 60 min after perfusion; (3) the basic drugs were mutually inhibitory with respect to accumulation in the lung (Fig. 2); (4) a basic drug already accumulated was reversibly displaced by a second basic drug (Fig. 4); (5) in the Scatchard plot at least two independent sets of binding sites for the basic drugs were observed (Fig. 1) and the maximum binding capacity for all the basic drugs tested was similar (Table II); (6) the lipid solubility of the basic drugs was well correlated with the

binding affinity to the sites (Table II).

Previously,[7] we reported that a very small change in the clearance curves of basic drugs was observed after the exchange of perfusate from pH 7.5 to pH 8.0 or pH 7.0. The *L/Pf* concentration ratio measured was greater than the theoretical values derived from the Henderson–Hasselbach equation at each pH. Therefore, to clarify whether an active transport mechanism contributes to the drug accumulation in the lung is very important. The experimental results of treatment with various metabolic inhibitors indicate that active transport did not contribute to the accumulation of basic drugs in the lung. Thus, the drug accumulation appears to be due to the specific binding to lung tissue.

Regarding the drug accumulation, Orton *et al.*[3] reported that basic amines ($pK_a > 8.5$) were accumulated to a much greater extent than non-basic amines ($pK_a < 7.0$). Anderson *et al.*[5] stated that the drug accumulation by the lung was more dependent on the degree of protonation than on the lipophilic properties. Previously,[7] we found that poorly lipid-soluble, strongly basic drugs, such as PAEB and NMN, are not accumulated by the lung and that their *L/Pf* concentration ratios were about 1. Partition coefficients of various basic drugs were correlated well with their accumulation in the lung. The lipid solubility of a second basic drug was correlated with its ability to inhibit and to displace lung accumulation of the first basic drug. The pK_a values of basic drugs used, except PAEB and NMN, are similar. Therefore, at least among the basic drugs with similar pK_a, the lipid solubility seems to be the major factor regulating the accumulation of basic drugs by the lung. The discrepancy between their findings and ours may be attributed to the difference of the drugs used. The binding sites for basic drugs in the lung have not yet been identified, and an intensive investigation is under way in this laboratory.

In conclusion, our findings indicate that specific common binding sites for basic drugs exist in the lung and that the binding affinity to the sites depends on the lipid solubility of the basic drugs.

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