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Research Article

Phosphatidylserine as a Determinant for the Tissue Distribution of Weakly Basic Drugs in Rats

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Interorgan variation in tissue distribution of weakly basic drugs such as quinidine, propranolol, and imipramine was investigated as a function of binding to phosphatidylserine (PhS) in tissues. Tissue distributions of these drugs were determined using 10 different tissues at a steady-state plasma concentration and were expressed as tissue-to-plasma partition coefficients (K_p values). The concentration of PhS in the tissue was determined by two-dimensional thin-layer chromatography. Plotting of K_p values, except for brain, against the tissue PhS concentrations showed a linear relationship, indicating that PhS is a determinant in the interorgan variation of these tissue distributions. Further, differences in tissue distribution among the drugs was considered to be due to the difference in binding potency to PhS. Drug binding parameters to individual standard phospholipid were determined using a hexane–pH 4.0 buffer partition system. Binding was highest to PhS, and a linear relationship was found between the log nK [product of the number of binding sites (n) and the association constant (K) for PhS binding] obtained *in vitro* and K_p values of drugs in tissues *in vivo*. The empirically derived equation, $K_p = 14.3 \times (\log nK) \times (\text{PhS conc.}) - 8.09$, was found to predict K_p values *in vivo* of weakly basic drugs. Thus, a determinant of interorgan variation in the tissue distribution of the weakly basic drugs studied was the tissue concentration of PhS and the drug binding affinity to PhS.

KEY WORDS: quinidine; propranolol; desipramine; imipramine; tissue distribution; K_p value; prediction of K_p value; phosphatidylserine; binding.

INTRODUCTION

Tissue distribution of drugs is determined by physicochemical properties of the drug and physiological conditions of the experimental animals. A specific tissue constituent to which a drug binds preferentially may represent an important pharmacokinetic determinant. Terasaki *et al.* reported that nuclei binding is a determinant of the extensive tissue distribution of adriamycin and doxorubicin and that the marked variation in the tissue distribution of these drugs is due mainly to the difference in the deoxyribonucleic acid (DNA) concentration in each tissue (1–3). Further, they estimated the *in vivo* tissue-to-plasma partition coefficient (K_p) values of doxorubicin in the various organs or tissues in rats, rabbits, and guinea pigs using the *in vitro* binding parameters to DNA, concentration of DNA in each tissue, free fraction of drug in the plasma, and ratio of the unbound drug in the tissue to that in the plasma (2,3). Wierzba *et al.* studied the tissue distribution of vinca alkaloids and found that K_p values of vincristine and vinblastine for various tissues correlated with the tissue tubulin concentration (4,5).

In previous reports, we demonstrated that the tissue distribution of quinidine to the lung, liver, kidney, and heart were affected by acidic phospholipids, especially phosphatidylserine (PhS) in tissues and that interorgan variation in the tissue distribution of quinidine was accounted for by the concentration of PhS in each tissue (6,7). Similar results were also observed in the lung cellular and subcellular distributions of quinidine (8).

In the present study, the role of PhS as a determinant in the tissue distribution of weakly basic drugs were confirmed using quinidine, propranolol, and imipramine with 10 different tissues under steady-state plasma concentrations in rats. The binding kinetics of these drugs to various phospholipids were also investigated *in vitro* to confirm the role of PhS as the main binding component of the phospholipids. Predictions of desipramine tissue distribution as a model compound were successfully performed in a separate experiment.

MATERIALS AND METHODS

Materials

Quinidine sulfate and DL-propranolol hydrochloride were purchased from Wako Pure Chemical Ind., Ltd. (Osaka, Japan) and Sigma Chemical Company (St. Louis, Mo.), respectively. Imipramine hydrochloride and desipramine hydrochloride were gifts from Japan Ciba-Geigy Corp. (Takarazuka, Hyogo, Japan). These weakly basic drugs were used without further purification. The following stan-

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dard phospholipids were obtained from Sigma Chemical Company and used without further purification: L-α-phosphatidyl-L-serine (PhS, No. P-7769), DL-α-phosphatidyl-DL-glycerol (PhG; No. P-5650), L-α-phosphatidylinositol (PhI; No. P-0639), L-α-phosphatidylcholine (PhC; No. P-5388), and L-α-phosphatidylethanolamine (PhE; No. P-6386). Silica gel 60G for thin-layer chromatography (TLC) was obtained from Merck & Co., Inc. (West Germany). All other reagents were of the finest grade available.

Animal Experiments

Male Wistar rats, 250–300 g, were anesthetized with an intraperitoneal injection of sodium pentobarbital (Nembutal solution, Abbott Laboratories, U.S.A.) at a dose of 30 mg/kg and kept supine on a surface controlled at 37°C to maintain their normal body temperature above 36°C.

Intravenous Bolus Administration. The anesthetized rats were cannulated with polyethylene tubing (PE-50, Clay Adams, U.S.A.) both in the right femoral artery and in the left femoral vein, respectively. Just before the administration of a drug, the urethra of the rat was sealed with a drop of surgical adhesive cement (Aronalpha A, Sankyo Ltd., Tokyo), to prevent possible leakage of the urine. Quinidine sulfate (38.6 μmol/kg), propranolol hydrochloride (40 μmol/kg), imipramine hydrochloride (20 μmol/kg), or desipramine hydrochloride (40 μmol/kg) dissolved in a physiological saline was administered via a femoral vein cannula. Blood sample for assay of drug concentration was collected via the cannula in a femoral artery at appropriate time intervals. Three hours after the administration of a drug, the rat was sacrificed with the administration of extra amount of pentobarbital, and the bladder was removed to determine the urinary excretion of unchanged drug. Plasma was separated by centrifugation at 3000 rpm for 10 min and stored at −30°C until analysis. Pharmacokinetic parameters were estimated from the plasma concentration–time curve by a least-squares regression analysis program MULTI (9) for a two-compartment model. Some of the parameters were used to settle an infusion condition.

Determination of K_p Value at a Steady-State Plasma Concentration. Tissue-to-plasma distribution ratio of weakly basic drug except liver at a steady-state plasma concentration (C_{ss}) was determined in a similar manner as described in the previous report (6). In order to settle a steady-state plasma concentration of a drug, an amount of $C_{ss} \times V_1$ was loaded immediately followed by constant rate infusion at a rate of $C_{ss} \times V_1 \times k_{10}$, where V_1 and k_{10} denote the central volume of distribution and elimination rate constant from the central compartment. Steady-state plasma condition was assessed by determining the plasma concentration of drug at an appropriate time interval in a separate experiment. In the present study, only the liver was assumed to be metabolizing or eliminating tissue for these weakly basic drugs employed. Thus, in noneliminating tissues, the K_p value was estimated with a ratio of drug concentration in the tissue (C_t) to that in the femoral arterial plasma (C_p), that is, $K_p = C_t/C_p$. The K_p value for the liver was determined as a ratio of drug concentration in the liver to that in the hepatic vein plasma ($C_{h,v}$), that is, $K_p = C_t/C_{h,v}$. Blood in the hepatic vein was collected through a U-shaped needle inserted along the hepatic vein.

Phospholipid Binding of Weakly Basic Drugs

In vitro binding of quinidine, propranolol, imipramine, and desipramine to individual phospholipid was determined in the same manner as described previously (6) by utilizing an organic solvent–water partition system (10) with a small modification. In the present study, the binding experiments were performed by partitioning between pH 4.0 buffer solution (10 m*M* citric acid–20 m*M* Na_2HPO_4) and *n*-hexane. Briefly, 2 ml of buffer solution containing various concentrations of drug was shaken with 2 ml of the *n*-hexane solution containing individual standard phospholipids (8 μg as an inorganic phosphorus/ml) in a L-shaped tube at 37°C for 2 hr. The mixture was then centrifuged at 2000 rpm for 10 min and the separated aqueous phase and organic phase were analyzed for drugs by high-performance liquid chromatography (HPLC), respectively. For quinidine, the binding experiment was also carried out using the water phase of pH 5.0 (citric acid–Na_2HPO_4) and pH 7.4 (0.25 *M* sucrose–0.1 *M* Tris–HCl buffer) to examine the effect of pH on the phospholipid binding of quinidine.

Extraction of Phospholipids from the Tissue and Isolation of PhS

Phospholipids in the tissues were extracted according to the method of Folch *et al.* (11) with a slight modification as reported previously (6). The isolation of PhS using two-dimensional TLC was carried out according to the method of Poorthuis *et al.* (12) with a slight modification as described in the previous report (7). The recovery of standard PhS based on the amount applied on the plate was 90.0% as reported previously (7).

Analytical Method

The concentration of quinidine in the plasma and tissues was determined by HPLC in the same manner as described in the previous report (6).

The concentrations of propranolol, imipramine, or desipramine in the plasma, tissues (10% homogenates), urine, and an aqueous solution were determined by HPLC after extraction into an organic phase. The HPLC method was carried out with a LC-5A (Shimadzu, Kyoto, Japan) equipped with a fluorometric detector (RF-530, Shimadzu) or a UV detector (Unidec-100 II, Jusco, Tokyo) and a TSK-Gel (ODS-80TM, Toyo Soda) reverse-phase column, operated at ambient temperature, and the flow rate was 1 ml/min.

One milliliter of 1 *N* NaOH was added to an aliquot of plasma (50 μl), urine (50 μl), tissue homogenate (500 μl), or aqueous solution (500 μl) and the solution was mixed with 6 ml of organic solvent (cyclohexane for propranolol, *n*-hexane for imipramine, and desipramine). After centrifugation, 4 ml of the organic layer was evaporated to dryness under reduced pressure. Residues were dissolved in 100 or 500 μl of methanol containing an internal standard and 20 μl of the solution was injected onto an HPLC column. The internal standards used were salicylamide (0.15 mg/ml) for propranolol and propranolol (50 μ*M*) for imipramine and desipramine. Mobile phases were a mixture of 0.1 *M* phosphoric acid, methanol, acetonitrile, and water (10:10:20:55, v/v) for propranolol and a mixture of acetonitrile and pH 3.0,

Table I. Pharmacokinetic Parameters of Propranolol, Imipramine, and Quinidine After Intravenous Administration in Rats[a]

Parameter	Propranolol	Imipramine	Quinidine
Dose, μM/kg	40	20	38.6
A, μM	5.86 ± 0.95	5.37 ± 0.55	4.77
B, μM	6.25 ± 1.28	1.22 ± 0.11	4.02
α, min^{-1}	0.256 ± 0.037	0.242 ± 0.027	0.099
β, min^{-1}	0.014 ± 0.001	0.011 ± 0.001	0.006
k_{12}, min^{-1}	0.103 ± 0.013	0.150 ± 0.018	0.044
k_{21}, min^{-1}	0.140 ± 0.028	0.054 ± 0.006	0.049
k_{10}, min^{-1}	0.027 ± 0.003	0.049 ± 0.004	0.012
V_c, L/kg	3.49 ± 0.54	3.09 ± 0.34	4.39
V_{dss}, L/kg	6.06	11.8	8.94
CL_{total}, ml/min/kg	94.2	151.4	52.7

[a] Each parameter was calculated by use of the two-compartment model. Values except for V_c and CL_{total} represent the mean ± SE of three trials. Data for quinidine are cited from the previous report (6).

0.1 *M* phosphate buffer containing 2% $NaClO_4$ (45:55, v/v) for imipramine and desipramine.

The amount of phospholipids in tissues was determined as inorganic phosphorus as reported previously (7). Phospholipids extracted by chloroform from the tissue homogenates were converted to inorganic phosphorus according to the method of Ames and Dublin (13), and the inorganic phosphorus was assayed according to the procedure by Chen *et al.* (14). The amount of inorganic phosphorus was converted to the amount of a phospholipid by increasing the quantity 25 times according to the literature (12,14).

RESULTS AND DISCUSSION

Tissue Distribution of Quinidine, Propranolol, and Imipramine

Propranolol and imipramine administered intravenously in rats were biexponentially eliminated from plasma. The plasma concentration–time curves were analyzed according to a two-compartment model by least-squares regression analysis using MULTI (9). Pharmacokinetic parameters of propranolol and imipramine are summarized in Table I together with those of quinidine from a previous report (6). All estimated values of propranolol and imipramine were similar to the values reported by Iwamoto *et al.* (15), and Okiyama *et al.* (16), respectively.

Urinary excretions of quinidine, propranolol, and imipramine determined over 3 hr after intravenous administration were 2.6, 0.52, and 0.74% of the dose, respectively. The total clearance of these compounds is accounted for by liver metabolism (17–20). Thus, only the liver was considered an eliminating tissue.

The K_p value of quinidine, propranolol, and imipramine for various tissues was determined at a steady-state plasma concentration. In all cases, steady-state plasma concentration was obtained approximately 60 min following the initiation of the constant rate infusion, and K_p values of each drug were determined 120 min after starting the infusion (Table II). The greatest K_p value was observed in the lung, with the increasing order of quinidine < propranolol < imipramine. A marked interorgan variation in the K_p value was also observed with all drugs. These K_p values of quinidine, propranolol, and imipramine were close to those reported by Shibazaki *et al.* (21), Schneck *et al.* (19), and Bickel *et al.* (20), respectively.

In Vitro Phospholipid Binding of Quinidine, Propranolol, and Imipramine

Phospholipids in the tissues include PhS, PhG, PhI, PhC, PhE, and phosphatydic acid (PhA). However, since the concentration of PhA was below its detection limit in all tissues, the binding of these weakly basic drugs to a standard individual phospholipid *in vitro* was investigated only with PhS, PhG, PhI, PhC, and PhE. A heptane–pH 7.4 Tris–HCl buffer partition system was previously employed to determine the binding of quinidine to phospholipids (6,7). However, imipramine mostly distributes into heptane at pH 7.4 even in the absence of phospholipid in the organic phase. Therefore, to minimize the distribution of drugs into the organic phase in the absence of phospholipid, we selected an *n*-hexane–pH 4.0 buffer system in which the drugs studied

Table II. K_p Values of Quinidine, Propranolol, and Imipramine at Steady State in Rats[a]

	Kp value[b]		
	Quinidine	Propranolol	Imipramine
Lung	43.0 ± 3.2	54.2 ± 4.1	127.4 ± 16.3
Spleen	24.0 ± 2.4	14.2 ± 1.3	57.4 ± 9.3
Kidney	20.7 ± 2.1	15.3 ± 1.5	45.5 ± 3.9
Liver	16.5 ± 1.9	11.6 ± 1.6	51.9 ± 17.3
Intestine	10.1 ± 1.7	6.6 ± 0.4	23.5 ± 1.5
Pancreas	8.7 ± 0.5	11.2 ± 1.0	43.7 ± 5.9
Heart	5.8 ± 0.5	7.1 ± 0.5	21.9 ± 2.1
Muscle	4.3 ± 0.3	4.3 ± 0.5	8.8 ± 0.9
Testis	2.2 ± 0.1	8.6 ± 0.9	23.7 ± 2.0
Brain	0.9 ± 0.1	14.0 ± 1.3	23.0 ± 1.8

[a] K_p values represent the mean ± SE of four to seven trials.
[b] Steady-state plasma concentration: quinidine, 2.6 ± 0.2 μM; propranolol, 3.2 ± 0.2 μM; imipramine, 0.87 ± 0.10 μM.

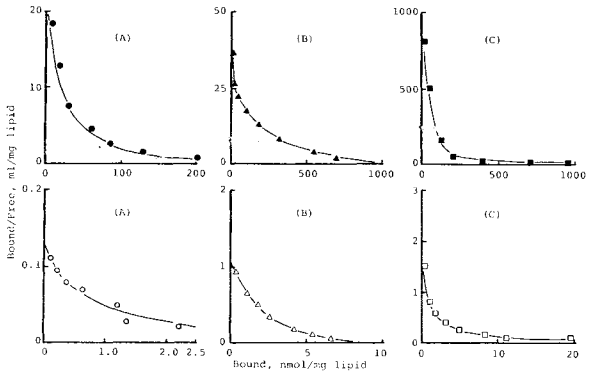

Fig. 1. Scatchard plots of the binding of quinidine (A), propranolol (B), and imipramine (C) to phosphatidylserine (filled symbols) and phosphatidylcholine (open symbols) determined at pH 4.0. Each point represents the mean of four trials.

did not partition into the organic phase in the absence of phospholipids.

The weakly basic drugs studied bound to each phospholipid except PhE with two class binding sites in a saturable manner, whereas the binding to PhE showed a one-class binding site in the drug concentration range of 1–500 μM. The Scatchard plots for the binding of three drugs to PhS and PhC are shown in Fig. 1. Binding parameters of each drug to individual phospholipids were calculated by least-squares regression analysis according to the following equation.

$$C_b/P = [(n_1K_1C_f)/(1 + K_1C_f)] + [(n_2K_2C_f)/(1 + K_2C_f)] \quad (1)$$

where n and K are the number of binding sites and the association constant, respectively. C_b, C_f, and P represent the bound drug concentration (drug concentration in the organic phase), unbound drug concentration (drug concentration in the water phase), and concentration of phospholipid in the organic phase, respectively. Subscripts 1 and 2 denote high-affinity binding and low-affinity binding, respectively. In these binding studies, the calculated curve fitted well the observed values.

The ranking of phospholipid binding of weakly basic drugs, expressed by nK (sum of n_1K_1 and n_2K_2), was in the following order: PhS > PhG > PhI > PhC > PhE (Table III). These results indicate that the drugs bind preferentially to acidic phospholipids such as PhS, PhG, and PhI. The greatest binding was observed for PhS, with values more than 30-fold that of the neutral phospholipids such as PhC and PhE. Further, a marked variation among three drugs was observed in the phospholipid binding. The nK values for imipramine were far greater than those for propranolol and quinidine.

Large differences in the binding of quinidine to acidic phospholipids, especially PhS, were observed between the heptane/pH 7.4 buffer system (6) and the hexane/pH 4.0 buffer system employed here, whereas the PhC and PhE binding was similar. This discrepancy was considered to result mainly from the pH difference. Thus, the effect of pH of the water phase on the binding kinetics of quinidine was investigated using PhS, PhG, and PhC. Plots of nk against the pH of the water phase revealed that only PhS binding is markedly influenced by pH of the water phase, whereas

Table III. Binding Parameters of Quinidine, Propranolol, and Imipramine to Individual Standard Phospholipid[a]

	Quinidine			Propranolol			Imipramine		
Affinity	K[b]	n[c]	nK[d]	K	n	nK	K	n	nK
PhS[e]			20.2			35.1			813
High	0.415	45.0		0.238	98.4		5.58	148	
Low	0.0048	309		0.0111	731		0.0161	936	
PhG[f]			9.28			14.9			287
High	0.302	20.2		0.571	10.2		1.71	158	
Low	0.0121	263		0.0073	1240		0.0150	1160	
PhI[g]			8.18			14.7			70.8
High	0.368	20.7		0.116	118		0.850	82.5	
Low	0.0015	372		0.0034	300		0.0017	389	
PhC[h]			0.13			1.10			1.31
High	0.446	0.13		0.657	1.31		0.381	3.14	
Low	0.0231	3.00		0.0372	6.68		0.0025	45.7	
PhE[i]	0.0280	0.865	0.02	0.0150	12.5	0.20			0.25
High							0.211	0.99	
Low							0.0081	5.15	

[a] Binding parameters were determined using the n-hexane/pH 4.0 buffer partition system at 25°C. Each binding parameter was calculated by a nonlinear least-squares analysis method.
[b] Association constant (μM^{-1}).
[c] Number of binding sites (nmol/mg lipid).
[d] Binding ability (ml/mg lipid); $nK = n_1K_1 + n_2K_2$.
[e] Phosphatidylserine.
[f] Phosphatidylglycerol.
[g] Phosphatidylinositol.
[h] Phosphatidylcholine.
[i] Phosphatidylethanolamine.

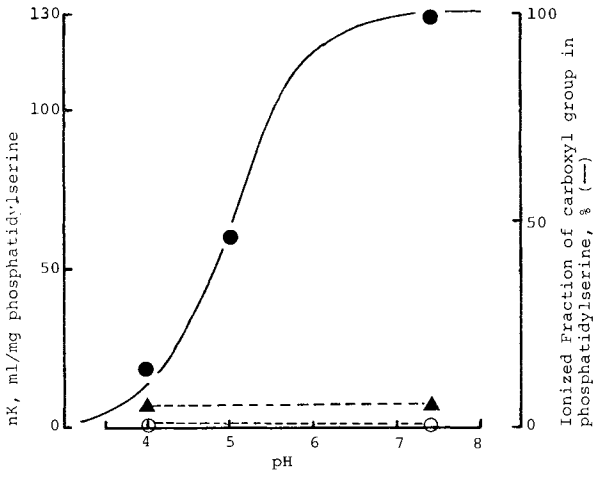

Fig. 2. Effect of pH of the water phase in the hexane–water partition system on the quinidine binding to phosphatidylserine (●), phosphatidylglycerol (▲), and phosphatidylcholine (○). Each point represents the mean of four trials. The solid line denotes the ionized fractions of the carboxyl group in phosphatidylserine. The pK_a value of the carboxyl group was assumed to be 5.0.

no effect of pH was found for PhG and PhC binding (Fig. 2).

Differences in PhS ionization at different pH's can account for the above results. PhS has three functional groups, phosphate, carboxyl, and amine, with pK_a values of 1.2, 4–5.5, and 10–11.5, respectively (22). Also, the pK_a value of phosphate group of PhG is 3–3.5, and those of PhC are 2–3 (phosphate group) and 8–11 (amine group) (22). Hence, the ionized fraction of PhS will vary markedly in the pH range from 4.0 to 7.4. At higher pH, the ionization of the carboxy group in PhS increases and thereby enhances the charge interaction between PhS and quinidine since quinidine (pK_a of 8.6) is largely protonated in these pH ranges. Therefore, the charge interaction should take place at the interface between the water and the organic phases, and it enhances PhS binding of quinidine, although the contribution of hydrophobic interactions can not be neglected. The effect of pH of the water phase on the PhG or PhC binding of quinidine was negligible.

The effect of pH on propranolol and imipramine binding to phospholipids was not examined because of their lipophilic properties. However, propranolol (pK_a, 9.6) and imipramine (pK_a, 9.5) are also highly ionized in these pH ranges. Thus, the binding of propranolol and imipramine to PhS is greater at physiological pH than at pH 4.0, as in the case of quinidine, while binding to other phospholipids will be the same at pH 4.0 and physiological pH.

Role of PhS in the Tissue Binding of Weakly Basic Drugs

Under the assumption that (1) binding of weakly basic drugs to phospholipids is accounted for by binding to PhS in the tissue and (2) drug binding to PhS is the same in all tissues, the K_p value for various tissues can be expressed as follows:

$$K_p = [(1 + 10^{pK_a - pH_i})/(1 + 10^{pK_a - pH_e})] \cdot f_u \cdot [1 + nKP/(1 + KC_f)] \quad (2)$$

where, pK_a, pH_i, and pH_e denote the dissociation constant of a drug, intracellular pH, and extracellular pH, respectively. f_u is the unbound fraction of a drug in the plasma, and C_f is the unbound concentration of the drug in plasma. n and K are the binding parameters to PhS, and P is the concentration of PhS in the tissue.

Assuming that the relation $KC_f \ll 1$ holds, Eq. (2) gives

$$K_p = [(1 + 10^{pK_a - pH_i})/(1 + 10^{pK_a - pH_e})] \cdot f_u \cdot (1 + nKP) \quad (3)$$

In Eq. (3), all parameters except P are constants at a steady-state plasma concentration of a drug. Thus, Eq. (3) indicates that the K_p value of a drug for a tissue will be determined by the concentration of P in the tissue.

The K_p values obtained *in vivo* were plotted against PhS concentration in various tissues. The concentrations of PhS in the spleen, intestine, pancreas, muscle, testis, and brain were 1.91, 1.05, 0.85, 0.57, 0.60, and 0.23 mg/g tissue (average value of three trials), respectively. The values in the lung, liver, kidney, and heart determined in the previous report were 2.80, 0.88, 1.54, and 0.57 mg/g tissue, respectively (7). A linear correlation was observed between the K_p values of quinidine, propranolol, and imipramine and the tissue concentrations of PhS except brain (Fig. 3). Brain was reported to be a special tissue for propranolol (23,24) and

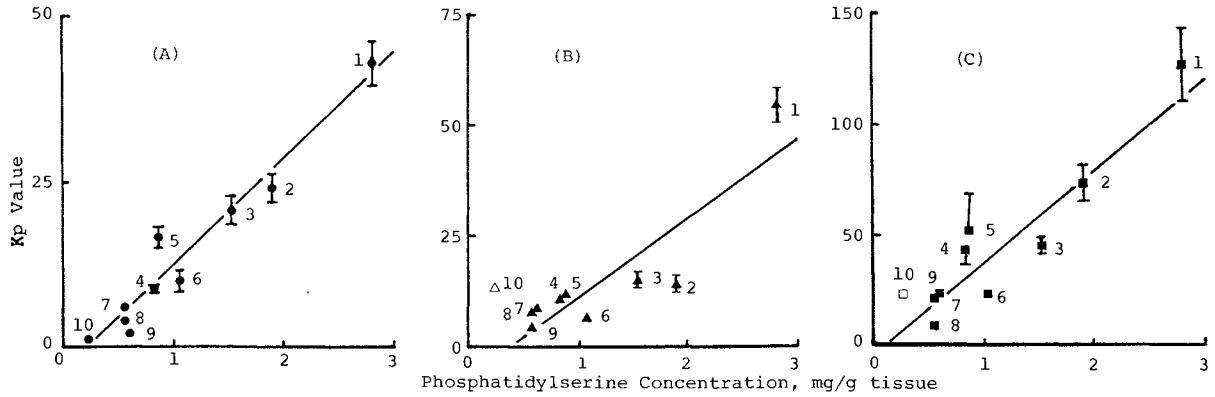

Fig. 3. Relationship between the K_p value of quinidine (A), propranolol (B), and imipramine (C) and the concentration of phosphatidylserine in the rat tissue. 1, liver; 2, spleen; 3, kidney; 4, pancrease; 5, liver, 6, intestine; 7, heart; 8, muscle, 9, testis; 10, brain. The bar represents the SE of four trials.

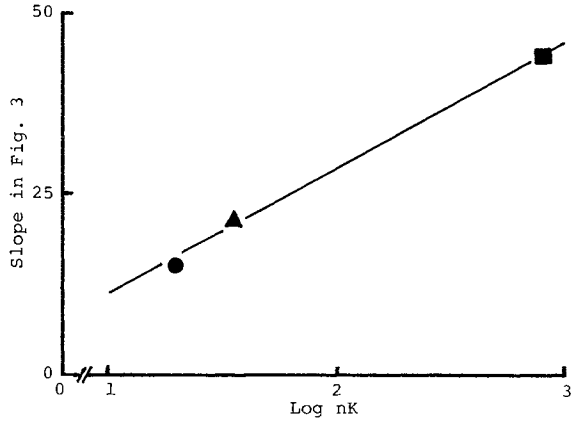

Fig. 4. Relationship between the slope of the line in Fig. 3 and the binding affinity (log nK) of quinidine (●), propranolol (▲), and imipramine (■) to phosphatidylserine determined in a hexane–pH 4.0 buffer partition system.

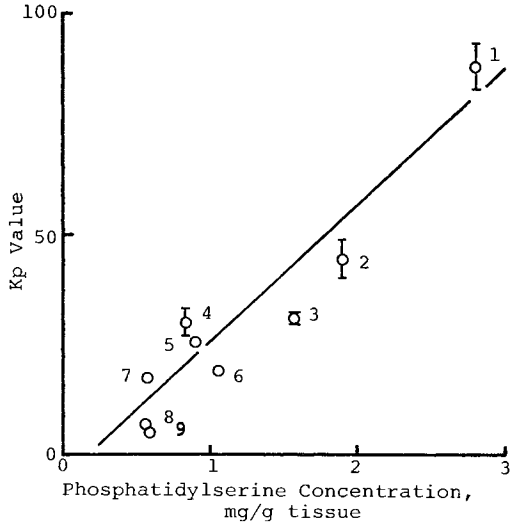

Fig. 6. Relationship between the K_p value of desipramine determined in rats and the concentration of phosphatidylserine in the rat tissue. 1, liver; 2, spleen; 3, kidney; 4, pancrease; 5, liver, 6, intestine; 7, heart; 8, muscle, 9, testis. The bar represents the SE of five trials.

imipramine (25) distribution. Thus, the K_p values of propranolol and imipramine for brain were omitted from the regression analysis. The linear correlation between K_p value and tissue PhS concentration suggests that the PhS tissue concentration is a main determinant in the tissue distribution of the weakly basic drugs studied, which preferentially binds to PhS.

There were marked differences in the slopes of the line among the three drugs shown in Fig. 3. According to Eq. (3), the slope equals $[(1 + 10^{pK_a - pH_i})/(1 + 10^{pK_a - pH_e})] \times f_u \times nK$. The calculated values of the concentration ratio of unbound fraction in the tissue to that in plasma, $(1 + 10^{pK_a - pH_i})/(1 + 10^{pK_a - pH_e})$, were 2.42 for quinidine, 2.50 for propranolol, and 2.50 for imipramine, assuming that pH_i and pH_e are 7.0 and 7.4, respectively. The free fractions, f_u, of quinidine in the plasma, propranolol, and imipramine are 0.20–0.25 (17), 0.13–0.19 (15), and 0.12–0.17 (20,25), respectively. Thus, the products between $(1 + 10^{pK_a - pH_i})/(1 + 10^{pK_a - pH_e})$ and f_u among the three drugs were similar. Therefore, the changes in the slope depend mainly on the binding affinity to PhS (nK) *in vivo*.

It was found that a linear relationship exists between the slope in Fig. 3 and the log nK for PhS obtained at pH 4.0 *in vitro* (Fig. 4). This finding suggests that the log nK obtained

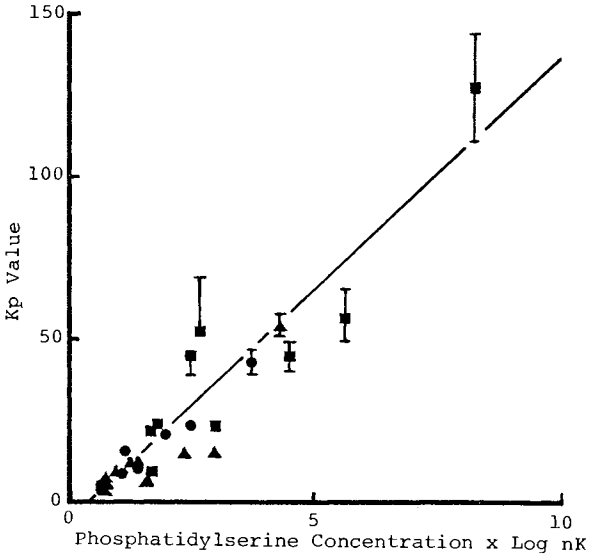

Fig. 5. Relationship between K_p values of quinidine (●), propranolol (▲), and imipramine (■) for various tissues and the product values of log nK determined at pH 4.0 and the concentration of phosphatidylserine in the rat tissue. The bar represents the SE of four trials. The regression line: $K_p = 14.3 \times (\log nK) \times (\text{PhS conc.}) - 8.08$ ($r = 0.930$).

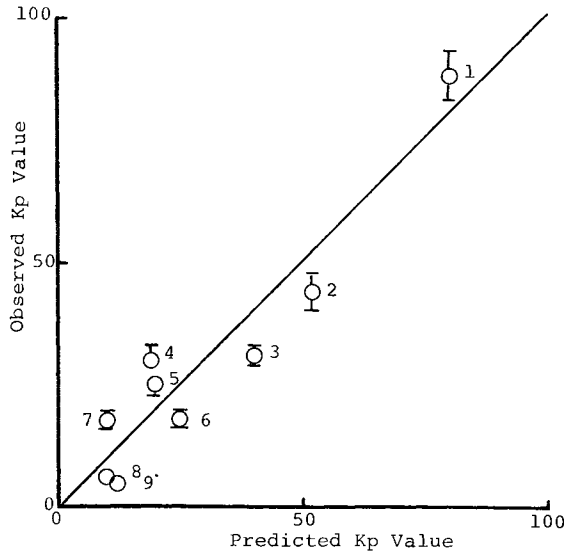

Fig. 7. Relationship between the K_p value predicted and the K_p value observed of desipramine in rats. 1, liver; 2, spleen; 3, kidney; 4, pancrease; 5, liver, 6, intestine; 7, heart; 8, muscle, 9, testis. The bar represents the SE of five trials.

in vitro is a useful index of the binding affinity of a drug to PhS *in vivo*.

In conclusion, the interorgan variation in the tissue distribution of weakly basic drugs varies with PhS concentration, and the different tissue distribution among these drugs depends on their different binding affinity to PhS *in vivo*. Further, the product of log nK and the concentration of PhS in the tissue correlates with the K_p value. As shown in Fig. 5, the following regression analysis line was obtained.

$$K_p \textit{ in vivo} = 14.3 \times (\log nK) \times (\text{PhS conc.}) - 8.09 \quad (4)$$

With Eq. (4), the K_p value for various tissues of other weakly basic drugs which bind to PhS preferentially may be predicted.

Prediction of the K_p Value of Desipramine

To examine the usefulness of the empirically derived Eq. (4) for the tissue distribution of other weakly basic drugs, desipramine was selected. The Scatchard plot for desipramine binding to PhS determined in hexane–pH 4.0 buffer system showed two binding sites: n_1 and K_1 for the high-affinity site were 402 nmol/mg lipid and 0.374 μM^{-1}, respectively, and for the low-affinity site, n_2 and K_2 were 689 nmol/mg lipid and 0.0073 μM^{-1}, respectively. Thus, the binding parameter (nK) of desipramine to PhS calculated from the sum of n_iK_i was 155 ml/mg lipid.

In a separate experiment, the K_p values of desipramine for various tissues were determined in rats. K_p values obtained at a steady-state plasma concentration of 2.60 ± 0.15 μM were as follows: 88.3 ± 5.4, lung; 44.1 ± 4.5, spleen; 30.7 ± 1.4, kidney; 30.4 + 3.1, pancreas; 25.9 ± 1.4, liver, 18.5 ± 2.0, intestine; 18.4 ± 1.9, heart; 5.8 ± 0.5, muscle; 4.7 ± 0.5, testis; and 6.1 ± 0.5, brain. As observed for quinidine, propranolol, and imipramine, desipramine showed a marked interorgan variation in its tissue distribution. Plotting of the K_p values of desipramine against the tissue PhS concentrations also showed a good linear relationship (Fig. 6).The relationship between the K_p value obtained *in vivo* and the predicted K_p value calculated using Eq. (4) is shown in Fig. 7. The good linear correlation with the slope of unity indicates that Eq. (4) can be useful to predict the K_p value of weakly basic drugs which bind preferentially to PhS.

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