Enantioselective Tissue Distribution of the Basic Drugs Disopyramide, Flecainide and Verapamil in Rats: Role of Plasma Protein and Tissue Phosphatidylserine Binding

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Purpose. The stereoselective distribution of three basic drugs, disopyramide (DP), flecainide (FLC) and verapamil (VP), was studied to clarify the relationships between the tissue-to-unbound plasma concentration ratio (Kpf) and drug lipophilicity and binding to phosphatidylserine (PhS), which are possible factors determining the tissue distribution of these drug enantiomers.

Methods. The drug enantiomer or racemate was administered to rats by intravenous constant infusion. Their concentrations in plasma and tissues were determined using enantioselective high-performance liquid chromatography. Plasma protein binding, and buffer-octanol and buffer-hexane containing PhS partition coefficients were also determined.

Results. The stereoselectivity of the tissue-to-plasma concentration ratio (Kp) was partly associated with that of serum protein binding. However, the Kpf value of R(+)-VP in the lung was significantly higher than that of S(−)-VP. A linear correlation was observed between the Kpf values of these drug enantiomers in brain, heart, lung and muscle, and their buffer-hexane containing PhS partition coefficients. The *in vitro* data for the binding of these drugs to PhS suggest that stereoselective binding of VP to PhS may correspond to its stereoselective tissue binding.

Conclusions. Our findings provide some evidence for a role of tissue PhS in the tissue distribution of basic drugs with respect to stereoselectivity of drug enantiomers distribution.

KEY WORDS: tissue distribution; disopyramide; verapamil; stereoselectivity; phosphatidylserine.

INTRODUCTION

In general, drug distribution is one of the determinants of drug disposition. In the absence of a specific membrane transport system, drug distribution to tissues is determined mainly by drug binding to both plasma proteins and tissue constituents. Yokogawa et al. studied the role of drug lipophilicity in the tissue distribution of basic drugs, and showed that the tissue-to-unbound plasma concentration ratio (Kpf) correlated well with log buffer-to-octanol partition coefficient for various tissues (1). Furthermore, several investigators have stressed the roles of specific substances in drug distribution to specific tissues or organs to explain the differences of drug concentration observed in various tissues. It has been reported that the different tissue distributions of vincristine and doxorubicin can be explained by differences in the concentrations of tissue tubulin and nucleus, respectively (2,3). Yata et al. studied the effects of several acidic phospholipids on the distribution of the basic drugs propranolol, imipramine and quinidine (4,5). They showed that these drugs bound to specific binding sites on phosphatidylserine (PhS) and that the tissue-to-plasma concentration ratio (Kp) was correlated with the tissue content of PhS and the binding affinity to PhS. These results indicate that binding of these basic drugs to PhS is a major factor determining their tissue distribution.

Many basic drugs have chiral centers in their structures and are available commercially as racemic mixtures. Some racemic drugs show stereoselective pharmacokinetics and pharmacodynamics, including distribution to tissues (6–8). The distribution of mexiletine enantiomers in rats shows slight stereoselectivity (9). Although the distribution of racemic drugs in various tissues has been studied, little is understood about the factors that determine enantiomeric distribution to major organs, such as the lungs, heart, brain and kidneys. Takahashi et al. observed the stereoselective distribution of propranolol in rats, and suggested that this stereoselectivity might be determined mainly by stereoselective plasma protein binding of its enantiomers (8). However, they did not establish the factors that determined tissue binding. In this study, we used the enantioselective high-performance liquid chromatography (HPLC) methods (10) described previously to determine the concentrations of basic drug enantiomers in plasma and various tissues at steady state after administering each enantiomer and racemate to rats, and tried to determine whether they showed stereoselective tissue distribution and elucidate the possible factors determining their tissue distribution from the viewpoint of drug stereoselectivity. Three anti-arrhythmic agents, disopyramide (DP), flecainide (FLC) and verapamil (VP), were selected for the investigation. These drugs are used widely for the treatment of ventricular and supraventricular arrhythmias (DP and FLC) (11,12), and supraventricular tachyarrhythmias, hypertension and angina pectoris (VP) (13) and are available commercially as racemates.

MATERIALS AND METHODS

Theoretical

The extent of tissue distribution was estimated by determining the tissue-to-plasma drug concentration ratio (Kp). In general, at steady state, Kp can be expressed by equation (eq.) (1) or (2), providing the drug is not transported by a specific membrane transport system (14):

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ABBREVIATIONS: DP, disopyramide; FLC, flecainide; VP, verapamil; PhS, phosphatidylserine; Kp, tissue-to-plasma drug concentration ratio; Kpf, tissue-to-unbound plasma drug concentration ratio; C_P^{ss}, plasma concentration at steady state; C_T^{ss}, tissue drug concentration at steady state; Q_T, tissue plasma flow; CL_{int}, intrinsic clearance; fu, unbound drug fraction in plasma; fu_T, unbound drug fraction in tissue; HPLC, high-performance liquid chromatography; PC, buffer-octanol partition coefficient; pcPhS, buffer-hexane containing PhS partition coefficient; pH 4.0 buffer, 10 mM citrate-20 mM phosphate buffer (pH 4.0); Vdssf, volume of distribution at steady state calculated from unbound plasma concentration.

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$$Kp = \frac{C_T^{SS}}{C_P^{SS}} \times \frac{Q_T + fu \cdot CL_{int}}{Q_T} \quad (1)$$

$$Kp = \frac{C_T^{SS}}{C_P^{SS}} \quad (2)$$

where C_P^{SS} and C_T^{SS} are the plasma and tissue drug concentrations, respectively, at steady state, and Q_T, CL_{int} and fu are the tissue plasma flow, intrinsic clearance and unbound drug fraction in plasma, respectively. In a non-eliminating organ, CL_{int} is zero, and Kp is expressed simply by eq. (2).

In this study, Kp was estimated using eq. (2), for tissues that do not eliminate the drugs. In the distribution equilibrium state, the concentrations of unbound drug in plasma and tissue are considered to be roughly the same, that is: fu $\times$ C_P^{SS} = fu_T $\times$ C_T^{SS}, where fu_T is the unbound drug fraction in the tissue. Therefore, Kpf (the tissue-to-unbound plasma drug concentration ratio) can be expressed by eq. (3):

$$Kpf = \frac{Kp}{fu} = \frac{1}{fu_T} \quad (3)$$

Kpf is affected only by drug binding to tissue constituents.

Materials

Racemic and ^{3}H-labeled (specific activity, 0.96 TBq/mmol) DP were gifts from Nippon Roussel K. K. (Tokyo). Both racemic FLC acetate and VP hydrochloride were gifts from Eisai Co. Ltd. (Tokyo). PhS, quinidine and propranolol were obtained from Sigma Chemical Co. (St. Louis, MO), racemic [N-methyl-^{3}H]VP hydrochloride, [4-^{3}H]propranolol, and [N-methyl-^{3}H]imipramine (specific activities, 2.2, 0.89 and 2.95 TBq/mmol, respectively) were obtained from DuPont-New England Nuclear (Boston, MA). The (+)- and (−)-enantiomers of DP, FLC and VP were separated by HPLC (10) and their stereochemical purities were ascertained by stereospecific HPLC resolution (the stereochemical purities of S(+)-DP, R(−)-DP, S(+)-FLC, R(−)-FLC, S(−)-VP and R(+)-VP were 98.4%, 99.6%, 99.0%, 100%, 98.6% and 99.3%, respectively). All the other reagents used were of analytical grade, unless stated otherwise.

Animals

Male Wistar rats (220–280 g) were maintained on a standard laboratory pellet diet with water *ad libitum* in a controlled environment. Sixteen hours prior to the experiment, they were fasted, but allowed water *ad libitum*.

Determination of Protein Binding

In this experiment, freshly isolated rat serum was used to study *in vitro* plasma protein binding.

A trace amount (5 μl) of ^{3}H-labeled S(+)- or R(−)-DP (159 GBq/mmol) was added to 0.5 ml serum, incubated for 5 min at 37°C, and ultrafiltered (Ultrafree C3-LGC, Nihon Millipore, Tokyo) at 1,700*g* for 10 min at 37°C. The radioactivities in both the filtrate and an aliquot of the serum were determined using a liquid scintillation counter (LSC; LSC-700, Aloka). In order to study the concentration dependency of DP binding to rat serum protein, 5 μl ^{3}H-labeled DP enantiomer and 5 μl unlabeled DP racemate were added to 0.5 ml rat serum (final DP concentration: 1.5–30 μM) and treated as described above.

Protein binding of FLC and VP was evaluated using equilibrium dialysis instead of ultrafiltration, because significant adsorption to the filtration device was observed (28% and 25%, respectively). A trace amount (5 μl) of ^{3}H-labeled R(+)- or S(−)- VP (2.5 GBq/mmol) was added to 0.5 ml serum and the solutions were dialyzed (Spectrapor-2, MW cut-off 12,000–14,000, Spectrum Inc.) against Sörensen buffer (0.113 M Na_2HPO_4 and 0.017 M KH_2PO_4, pH 7.4) for 6 h at 37°C. After equilibrium had been reached, the radioactivities of the solutions (inside and outside the dialysis tube) were determined by LSC. Sample volume alteration during dialysis was corrected according to the change in the protein concentration. Protein binding of FLC was determined as described for VP, except that the drug concentration was determined by HPLC. The concentration dependency of FLC and VP binding to rat serum protein was also studied (final drug concentration in serum: 0.05–0.48 μM for FLC, 9-218 nM for VP).

Animal Experiments

Male Wistar rats (210–250 g) were anesthetized with ethyl ether, and polyethylene cannulae (0.28 mm I.D. × 0.61 mm O.D.) were implanted into both the femoral vein and artery of one leg just before drug administration. Each drug was dissolved in physiological saline before administration.

Enantiomer Dosing Regimen. A loading dose of S(+)-DP, R(−)-DP, S(+)-FLC, R(−)-FLC, S(−)-VP or R(+)-VP was infused constantly at 33, 15, 9, 15, 1.1 or 1.3 μmol/h/kg, respectively, through the femoral venous cannula for 30 (DP and FLC) or 20 (VP) min, followed by maintenance infusions at 8.5, 4, 4.5, 7.6, 0.57 or 0.65 μmol/h/kg, respectively.

Racemic Drug Dosing Regimen. In order to establish whether interactions between enantiomers occurred, each drug was administered as a racemic solution. A loading dose of racemic DP, FLC or VP was infused constantly at 41, 11 or 1.2 μmol/h/kg, respectively, through the femoral venous cannula for 30 (DP and FLC) or 20 (VP) min, followed by maintenance infusions at 11, 5.5 or 0.6 μmol/h/kg, respectively.

For both experiments, blood samples (0.5 ml) were taken from the femoral artery at 2, 2.5 and 3 h and jugular vein (at 3 h) after the start of drug administration. The rats were sacrificed by total blood withdrawal from the abdominal artery at 3 h after the start of drug administration, and 50 ml ice-cold saline was injected quickly into the heart to clear the blood from the other organs. The brain, lungs, heart, liver, kidneys and muscle were excised, rinsed, blotted on filter paper, weighed

Table I. Unbound Fraction of DP, FLC and VP Under Steady-State Condition After Intravenous Constant Infusion of Each Enantiomer into Rats

		DP	FLC	VP
Unbound Fraction(%)[a]	S	59.5 ± 2.3	42.5 ± 9.5	6.2 ± 0.8
	R	72.4 ± 3.4[b]	39.4 ± 6.0	6.4 ± 1.9

Note: Each data represents mean ± SD (n = 5).
[a] Unbound fraction was determined using arterial serum.
[b] Significantly different from the S-isomer ($P < 0.05$).

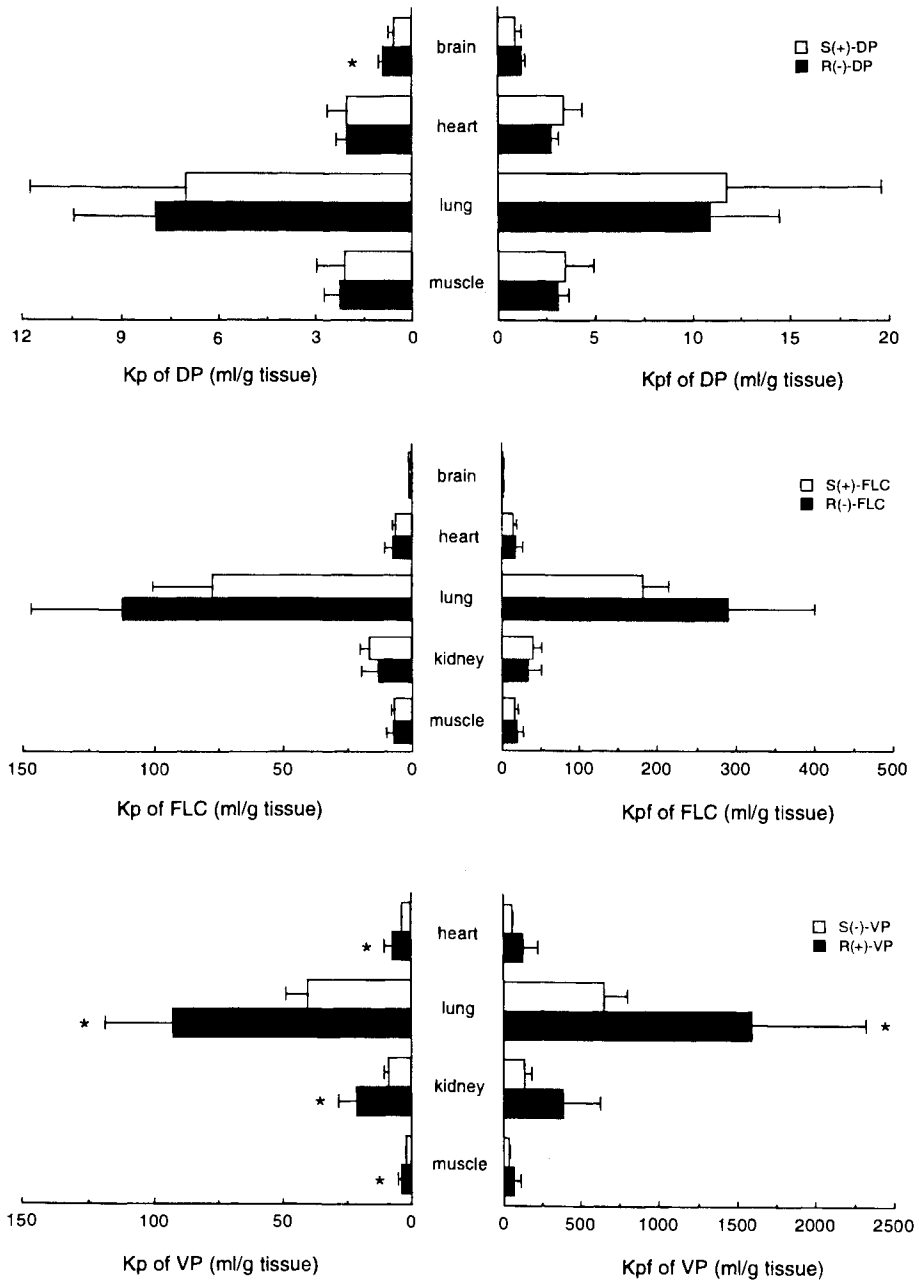

Fig. 1. Tissue distribution (Kp and Kpf) of DP, FLC and VP enantiomers under steady-state conditions after intravenous constant infusion of each enantiomer into rats. Each column represents mean ± SD (n = 5). * Significantly different from the S-isomer ($p < 0.05$).

and homogenized (1 g/10 ml) with 0.25 M sucrose-0.05 M phosphate buffer (pH 7.4) using a Potter-Elvehjem apparatus with a Teflon pestle. The blood samples were centrifuged at 1,000*g* for 10 min at 4°C and the plasma and tissue homogenates were stored at −20°C until analysis.

Determination of Buffer-Octanol Partition Coefficients (PC)

Sörensen buffer and n-octanol were used as the aqueous and organic phases, respectively. Aliquots of 1.6, 26, 0.7 or 40 ml of DP (0.5 mM), VP (0.05 mM), propranolol (0.5 mM) or imipramine (0.5 mM) in Sörensen buffer were added to 1 ml octanol and incubated for 60 min at 37°C, whereas 6 or 4 ml of octanol containing FLC (1.0 mM) or quinidine (0.1 mM) was incubated with 180 or 140 ml Sörensen buffer, respectively, for 60 min at 37°C. After equilibration, the radioactivities (DP, VP, propranolol and imipramine) and concentrations (FLC and quinidine) in both the aqueous and organic phases were determined by LSC and HPLC, respectively.

Determination of Buffer-Hexane Containing PhS Partition Coefficients (pcPhS)

In vitro binding of DP, FLC and VP to PhS was determined using the method described by Yata et al. (5). Briefly, 10 mM

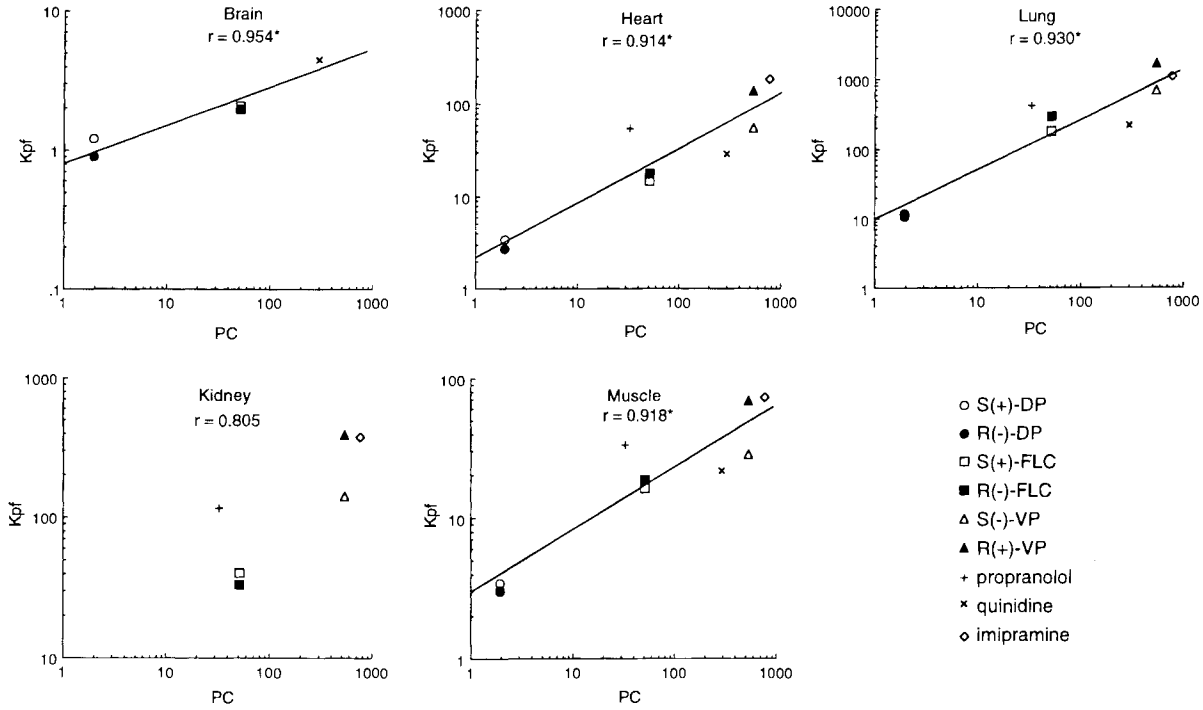

Fig. 2. Relationship between Kpf values of DP, FLC and VP enantiomers, propranolol#, quinidine#, and imipramine# and pH 7.4 phosphate buffer-octanol partition coefficients (PC) using enantiomers. The line was generated by linear regression. * $p < 0.05$. #From Refs. 5, 19–21.

citrate-20 mM phosphate buffer, pH 4.0 (pH 4.0 buffer) and n-hexane containing 0.02% (w/v) PhS were used as the aqueous and PhS phases, respectively. Each drug was dissolved in pH 4.0 buffer (DP enantiomers: 1.5 μM, racemate: 3 μM; FLC enantiomers: 0.1 μM, racemate: 0.2 μM; VP enantiomers: 5.1 nM, racemate: 10 nM), and 2 ml drug solution (the DP and VP solutions contained 10 μl ^{3}H-labeled drug) was mixed with 2 ml n-hexane containing PhS and incubated for 2 h at 25°C. After equilibration, the radioactivities (DP and VP) and concentrations (FLC) in both the aqueous and PhS phases were determined by LSC and HPLC, respectively.

Analytical Methods

The concentrations of each enantiomer in plasma and tissues were determined using the enantioselective HPLC methods reported previously (10). Briefly, the HPLC system consisted of a Shimadzu HPLC apparatus (Kyoto), a LC-6A or 10A HPLC pump and a C-R6A Chromatopac integrator. DP was detected by a SPD-6A spectrometric detector (260 nm), and both FLC (Ex. 295 nm, Em. 347 nm) and VP (Ex. 272 nm and Em. 312 nm) were detected by fluorescence detectors. Separation of DP enantiomers with a Chiralcel OF column (50 × 4.6 mm I.D., Daicel Chemical Ind., Tokyo) and FLC and VP enantiomers with Chiralpak AD columns (250 × 4.6 mm I.D., Daicel) was performed at room temperature. The mobile phases comprised hexane-isopropanol-diethylamine (82:18:0.1, 96:4:0.1 and 94:6:0.1 (v/v) for DP, FLC and VP, respectively) at constant flow rates of 0.6, 1.5 and 1.2 ml/min, respectively. These drugs were extracted from biological fluids using organic solvent (benzene or carbon tetrachloride for DP, ethyl ether for FLC and ethyl ether or n-hexane for VP) under alkaline conditions. With regard to analytical accuracy, the within- and between-day coefficients of variation were less than 6.2% and the linearities of the calibration curve were higher than 0.997. Quinidine concentrations were determined by HPLC with fluorescence detection (Ex. 357 nm, Em. 394 nm).

The protein concentration was determined by the method of Lowry using bovine serum albumin as a standard (16).

Data Analysis

The Kp value for each tissue was calculated as the tissue-to-plasma concentration ratio (ml/g tissue) at steady state using the plasma drug concentrations of arterial samples, except for the lung, when the venous sample data were used. The Kpf value was calculated as the tissue-to-unbound plasma concentration ratio at steady state.

Statistical Analysis

The values are expressed as means ± SD. The racemate and enantiomer data were compared using Student's paired and unpaired t-tests, respectively, and differences at $p < 0.05$ were considered to be significant. The linear relationships between Kpf values of all drugs studied in each tissue and the partition coefficients were analyzed using Pearson's correlation test. These analyses were performed by computer using the SPSS package (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Serum Protein Binding

Nonlinear serum protein binding of DP within its therapeutic concentration range has been observed in humans (15,17). However, within the concentration ranges studied, the fractions

Table II. Partition Coefficients Between Buffer (pH 4.0)-hexane Containing PhS of DP, FLC and VP Enantiomers and Racemate

		DP	FLC	VP
Enantiomers	S	0.28 ± 0.05	3.62 ± 0.42	3.41 ± 0.69
	R	0.38 ± 0.05[b]	3.84 ± 0.33	6.54 ± 0.58[b]
	R/S ratio[a]	1.38	1.06	1.93
Racemate	S	0.08 ± 0.01	2.52 ± 0.56	3.38 ± 0.52
	R	0.29 ± 0.04[b]	2.68 ± 0.64	2.97 ± 0.43
	R/S ratio	3.62 ± 0.53	1.09 ± 0.24	0.89 ± 0.18

Note: Each data represents mean ± SD (n = 5).
[a] R/S ratio is calculated by mean value.
[b] Significantly different from the S-isomer ($p < 0.001$).

of DP, FLC and VP bound to rat serum were virtually constant (data not shown). Significantly more S(+)-DP than R(−)-DP was protein-bound (Table I), indicating that DP binding to rat serum protein was stereoselective, as observed with human serum (15,17). However, we observed no significant differences between the unbound fractions of FLC and VP enantiomers.

Tissue Distribution (Kp and Kpf) of Enantiomers

The plasma drug concentrations of DP, FLC and VP, at 2, 2.5 and 3 h after the start of drug infusion were maintained at a steady state (data not shown). Tissue drug concentrations at steady state were determined at 3 h after the start of infusion. The concentrations of all three drugs in the liver and of DP in the kidney were not analyzed, because these are elimination organs for these drugs and DP, respectively. The concentration of VP in the brain was also not determined because VP is transferred across the blood-brain barrier by an active efflux transport system (18).

All the drugs examined in this study were distributed extensively to the lungs in comparison with other tissues (Fig. 1), as has been observed for other basic drugs (4,5). The Kp value of R(−)-DP in brain was significantly higher than that of S(+)-DP, but the values of Kpf in all the tissues did not differ significantly between enantiomers, indicating that the apparent stereoselective distribution of DP to the brain resulted from the different serum protein binding of the enantiomers, as observed for propranolol (8). Neither the Kp nor Kpf values of the FLC enantiomers differed significantly in any tissues studied. On the other hand, the Kp values of R(+)-VP were significantly higher than tbose of S(−)-VP in all the tissues studied, and the Kpf value of R(+)-VP in the lung was also significantly higher than that of S(−)-VP. The Kpf values of R(+)-VP in the heart, kidney and muscle were also larger than those of S(−)-VP, but the differences between tbem were not statistically significant. However, these findings suggest that VP may bind stereoselectively to tissue constituents.

Relationship Between Kpf and PC

The Kpf values for propranolol, quinidine and imipramine, cited in previous reports (5,19–21), were plotted against the log PC values determined in this study, in addition to the DP, FLC and VP data. As shown in Fig. 2, in all the tissues except kidney, the Kpf values were correlated significantly with log PC, suggesting that the differences in the tissue distribution of these basic drugs may be determined partly by their lipophilicity. However, drug lipophilicity cannot explain the stereoselectivity of Kpf because the PC values of the enantiomers did not differ.

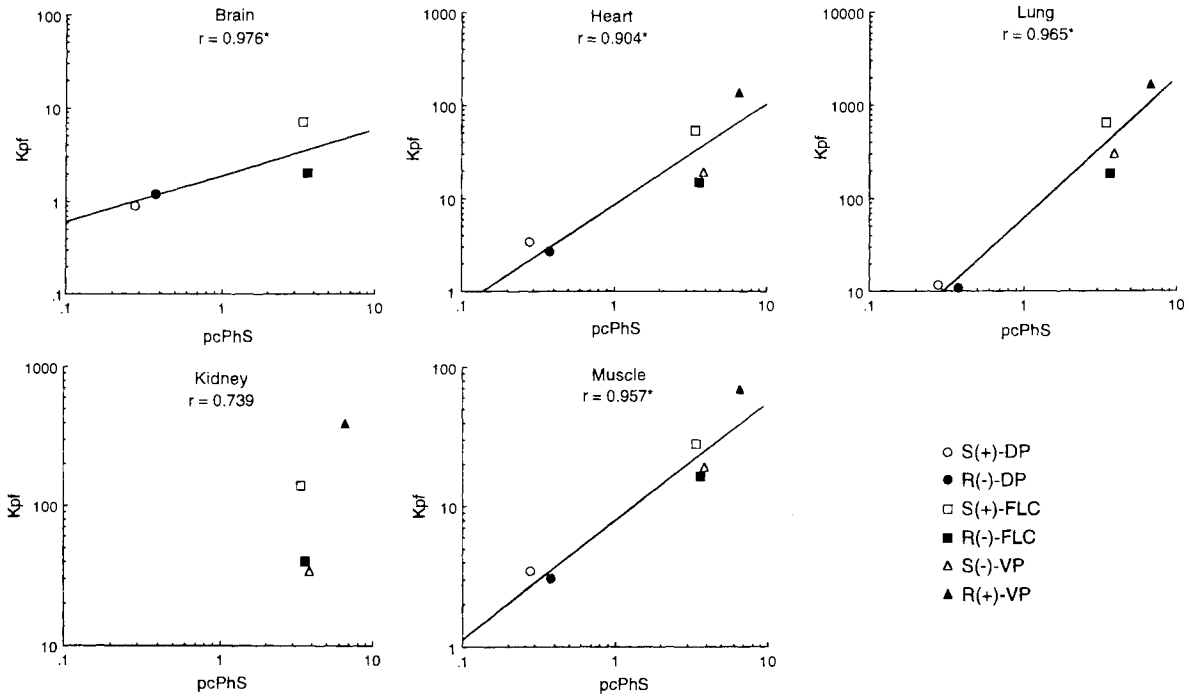

Fig. 3. Relationship between Kpf values of DP, FLC and VP enantiomers and 10 mM citrate-20 mM phosphate buffer (pH 4.0)-hexane containing 0.02% PhS partition coefficients (pcPhS) using enantiomers. The line was generated by linear regression. * $p < 0.05$.

The lack of significant correlation for the kidney may be attributable to the paucity of data compared with those for other tissues, or to the specific membrane transport system for VP in the renal proximal tubule. In an *in vitro* rat brush-border membrane vesicle study, S(−)-VP showed more potent inhibition of the uptake of tetraethylammonium, which is transportable via organic cation transport systems (22).

Relationship Between Kpf and pcPhS

In order to study drug binding to PhS, the partition of DP, FLC and VP between pH 4.0 buffer and hexane containing PhS was determined. The partitions of these drugs between pH 7.4 phosphate buffer-hexane without PhS were very high, but when pH 4.0 buffer was used, they were negligible. In this system, these drugs were not distributed into the organic phase in the absence of PhS. However, when pH 4.0 buffer and hexane containing PhS were used, the partitions of these drugs were detected, and therefore these pcPhS values can reflect the magnitude of drug binding to PhS.

The pcPhS values of DP, FLC and VP enantiomers and their R/S ratios are shown in Table II. The R/S ratio of FLC was almost unity, which corresponded to the *in vivo* Kpf (Fig. 1). The pcPhS values of R(−)-DP and R(+)-VP, however, were significantly higher than those of their S-isomers. In particular, the R(+)-VP value was twice that of S(−)-VP, indicating that VP bound to PhS stereoselectively. These differences between the pcPhS values of the VP enantiomers corresponded to their *in vivo* Kpf values (Fig. 1), suggesting that stereoselective binding of VP to PhS may account for the differences in tissue distribution.

The relationship between the values of Kpf and pcPhS for all drugs studied was then examined (Fig. 3). With the exception of the kidney, good correlation between Kpf and pcPhS was observed for all the tissues. These correlation coefficients were greater than those of PC (Fig. 2). Furthermore, the Kpf values for all the drugs studied were also correlated well with the tissue content of PhS ($r > 0.920$, data not shown). These results suggest that binding to PhS in tissues may be an important factor that determines the tissue distribution of these basic drug enantiomers, as has been reported for other basic drugs (4).

Interaction Between Enantiomers

Figure 4 shows the Kpf values of DP, FLC and VP in tissues after administration of drugs as racemate. With the exception of the lung, Kpf values for R(−)-DP in all the tissues tested were significantly higher than those for S(+)-DP, although stereoselectivity was not detected *in vivo* after enantiomer administration (Fig. 1). On the other hand, the Kpf values of the FLC and VP enantiomers did not differ significantly. The stereoselectivity of Kpf values for VP observed after enantiomer administration was not detected after administration of racemate. These results indicate that tissue binding was subject to interactions between enantiomers of both DP and VP.

The pcPhS value for S(+)-DP was significantly lower than that for R(−)-DP (Table II). These findings correspond to the *in vivo* Kpf data (Fig. 4), although quantitative analysis, such as determination of binding parameters, was not performed. The R/S ratio when racemic DP was used was also higher than that with the enantiomer. Furthermore, the absolute pcPhS values for both R(−)-and S(+)-DP decreased significantly in the presence of the other enantiomer, indicating that DP binding to PhS may be subject to competitive interaction between its enantiomers as has been observed for serum protein binding. The R/S ratio of the pcPhS for FLC was almost unity when racemic or enantiomeric FLC was used. On the other hand, the pcPhS value for R(+)-VP when racemic VP was used was significantly lower than that with the enantiomer (6.54 vs. 2.97), and the R/S ratio for VP when the racemate was used was lower than that with the enantiomer (1.93 vs. 0.89). These R/S ratios of *in vitro* drug binding to PhS correspond to the *in*

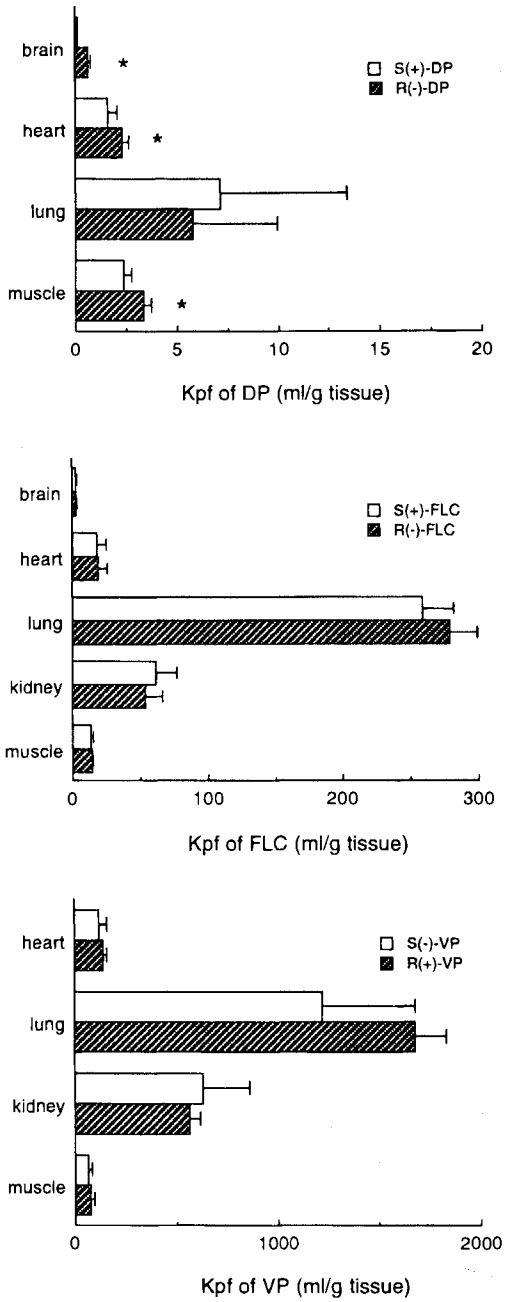

Fig. 4. Tissue distribution (Kpf) of DP, FLC and VP enantiomers under steady-state conditions after intravenous constant infusion of racemic drug into rats. Each column represents mean ± SD (n = 3). * Significantly different from the S-isomer ($p < 0.05$).

vivo Kpf values (Figs. 1 and 4), suggesting strongly that the binding of these drugs to PhS in tissues may be a major factor that determines their tissue distribution.

The volume of distribution at steady state calculated using the unbound plasma concentration (Vdssf) was almost the same between DP enantiomers after each had been administered separately to healthy subjects (23). However, the Vdssf value for S(+)-DP was less than that for R(+)-DP after racemic DP administration. The Vdssf value for S(−)-VP was higher than that for R(+)-VP (54 vs. 46 L) after enantiomer administration. This stereoselectivity was apparently abolished by administering a racemic VP solution (24). The stereoselectivity and interaction of Vdssf of DP and VP, as has been observed in humans, would correspond to the stereoselective binding to PhS examined in this study.

The effects of major metabolites on stereoselective distribution must be taken into consideration. We found that nor-verapamil, the major metabolite of VP in humans, did not affect the serum protein binding and pharmacokinetic parameters (such as total clearance) of VP enantiomers in rabbits (unpublished data).

In conclusion, the distribution of DP and VP showed stereoselectivity, which was accounted for reasonably well by the magnitudes of drug binding to serum protein and tissue PhS. Our findings indicate that the apparent tissue distribution of basic drugs will be affected by serum protein binding and tissue PhS binding.

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