Toda et al (1982) showed that the H_1 receptor-mediated relaxation of canine mesenteric arteries could be mediated through PGI_2. More recently (Ea Kim et al 1992), we demonstrated that the H_3-receptor-mediated relaxation could involve the endothelial release of both nitric oxide and PGI_2, since it was reduced by tranylcypromine (a PGI_2-synthesis inhibitor) and L-NMMA or L-NAME inhibitors of nitric oxide synthesis. The present data suggest that the methylhistamine-induced fall in MAP of guinea-pigs might be mediated via the L-arginine nitric oxide pathway. The pressor effect induced by the agonists tested was not a urethane-dependent mechanism since similar reponses were obtained with these agonists in our own preliminary experiment in pentobarbitone-anaesthetized guinea-pigs. Further studies are needed to explain the molecular mechanism responsible for activation of H_3-receptor sites at this level.

The authors wish to thank Dr F. X. Galen for his generous help.

References

Arrang, J. M., Garbarg, M., Lancelot, J. C., Lecomte, J. M., Pollard, H., Robba, M., Schunack, W., Schwartz, J. C. (1987) Highly potent and selective ligands for histamine H_3-receptors. Nature 327: 117–123

Black, J. W., Owen, D. A. A., Parsons, M. E. (1975) An analysis of the depressor responses to histamine in the cat and dog: involvement of both H_1 and H_2-receptors. Br. J. Pharmacol. 54: 319–324

Ea Kim, L., Javellaud, J., Oudart, N. (1992) Endothelium dependent relaxation of rabbit middle cerebral artery to a histamine H_3-agonist is reduced by inhibitors of nitric oxide and prostacyclin synthesis. Br. J. Pharmacol. 105: 103–106

Harvey, C. A., Owen, D. A. A. (1980) Time-course of depressor responses to histamine. Br. J. Pharmacol. 68: 187P

Ishikawa, S., Sperelakis, N. (1987) A novel class (H_3) of histamine receptors on perivascular nerve terminals. Nature 327: 158–160

Knowles, R. G., Palacios, M., Palmer, R. M. J., Moncada, S. (1990) Kinetic characteristics of nitric oxide synthase from rat brain. Biochem. J. 269: 207–210

Moore, P. K., Al-Swayeh, O. A., Chong, N. W. S., Evans, R. A., Gibson, A. (1990) L-N^G nitroarginine (L-NOARG) a novel L-arginine reversible inhibitor of endothelium-dependent vasodilatation in vitro. Br. J. Pharmacol. 99: 408–412

Palmer, R. M. J., Rees, D. D., Ashton, D. J., Moncada, S. (1988) L-Arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. Biochem. Biophys. Res. Commun. 153: 1251–1256

Rees, D. D., Palmer, R. M. J., Schulz, R., Hodson, H. F., Moncada, S. (1990) Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. Br. J. Pharmacol. 101: 746–752

Toda, N., Konishi, M., Miyazaki, M. (1982) Involvement of endogenous prostaglandin I_2 in the vascular action of histamine in dogs. J. Pharmacol. Exp. Ther. 223: 257–262

Van de Voorde, J., Leusen, I. (1982) Vascular endothelium and the relaxation effect of histamine on aorta of the rat. Arch. Int. Pharmacodyn. 256: 329–330

J. Pharm. Pharmacol. 1993, 45: 931–933
Communicated November 23, 1992

Partitioning and thermodynamics of dipyridamole in the *n*-octanol/buffer and liposome systems

G. V. BETAGERI, S. R. DIPALI, *Department of Pharmacal Sciences, School of Pharmacy, Auburn University, Auburn, AL 36849-5503, USA*

Abstract—The thermodynamics of partitioning (K) of dipyridamole has been determined in *n*-octanol/buffer and liposome-buffer systems at pH 7·4. Dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC) were used to prepare multilamellar liposomes. Partitioning of dipyridamole did not depend on the amount of *n*-octanol employed, however, partitioning was dependent upon the quantity of DMPC employed to prepare liposomes. Plots of log K vs $\frac{1}{T}$ were linear in the *n*-octanol and liposome systems. Partitioning was generally greater in liposomes than in the *n*-octanol/buffer system. Among liposomes, the partitioning was greater in DMPC liposomes at all temperatures. The values of enthalpy (ΔH) and entropy (ΔS) were positive in both the *n*-octanol and liposome systems. These values were lower in DMPC liposomes and were comparable in the *n*-octanol and DPPC liposomes. Thus, the interaction of dipyridamole depends on the rigidity of lipid bilayers and liposomes constitute a more selective partitioning system than the *n*-octanol/buffer system.

Dipyridamole is a widely used coronary vasodilator and antithrombic drug (FitzGerald 1987). In the past few years, attention has also been focused on its ability to potentiate the cytotoxic effects of various antitumour drugs (Grem & Fischer 1989). Recently, a further possible use of dipyridamole emerged in the treatment of human immunodeficiency virus type-1 (HIV-1) infections. Dipyridamole potentiates the antiviral effects of 3′-azido-3′-deoxythymidine (AZT) and 2′,3′-dideoxycytidine (ddC) in HIV-infected human monocyte/macrophages in-vitro (Szebeni et al 1989).

The distribution of drugs in membranes was first quantitatively described by Collander (1951) to be related to their partition coefficients in bulk oil/water systems. Among the many oils which have been investigated, semipolar solvents yield better correlations with the partitioning of solutes in model and biological membranes than non-polar solvents (Leo et al 1971; Diamond & Katz 1974). In particular, *n*-octanol has been a useful reference system for extrathermodynamic studies on a variety of systems (Hansch & Dunn 1972). Although the water-saturated *n*-octanol/water system presumably possesses structural characteristics as a result of the formation of water/*n*-octanol clusters (Smith et al 1975), it has been suggested that the bulk oil lacks sufficient structural similarities to biological membranes to account for the role of steric influences of drug molecular structure on membrane partitioning, transport, drug-receptor interactions and, hence, biological activity (Rogers & Wong 1980). The present study compares the partitioning behaviour of dipyridamole in *n*-octanol/buffer and buffered liposome systems using a thermodynamic approach and, from this, evaluates the interaction of dipyridamole with lipid bilayer systems in comparison with the *n*-octanol/buffer system.

Correspondence: G. V. Betageri, Department of Pharmacal Sciences, School of Pharmacy, Auburn University, Auburn, AL 36849-5503, USA.

Materials and methods

Chemicals. Dipyridamole and cholesterol were purchased from Sigma Chemical Co. (St Louis, MO, USA). Dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC) were obtained form Avanti Polar Lipids Inc. (Alabaster, AL, USA). *n*-Octanol and chloroform (Fisher Scientific Co., NJ, USA) were reagent grade.

Distribution studies in n-octanol/buffer systems. Convenient volumes of aqueous phase (5 mL isotonic phosphate buffer, pH 7·4) containing the appropriate concentration of drug (0·02 mM) and *n*-octanol (0·1 mL) were weighed into 25-mL scintillation vials and equilibrated for 4 h at constant temperature (±0·5°C) in a shaking water-bath (Dubnoff metabolic shaker bath). Both phases had been mutually pre-equilibrated. Concentrations of dipyridamole in the aqueous phase were determined by UV spectrophotometry (Beckman DU-65) at λ_{max} (290 nm). The concentration of dipyridamole in the *n*-octanol phase was determined by mass balance. The distribution of dipyridamole was obtained from the average of duplicate determinations at each temperature over the range 23–55°C.

Distribution studies in liposome systems. Phospholipid films (DMPC or DPPC) were formed in 50-mL round-bottom flasks following rotary evaporation of a chloroform solution (6 mg mL^{-1}). The resulting dried films were dispersed in 5 mL aqueous, isotonic, phosphate buffer (pH 7·4) containing 10 μg mL^{-1} dipyridamole. The flasks were vortex mixed for 10 min to form multilamellar liposomes (MLVs). The distribution of the drug was determined in 24 h temperature-equilibrated MLVs following centrifugation (35 000 rev min^{-1} for 30 min, Beckman L3-50 Ultracentrifuge) at the desired temperature from UV analysis and mass balance calculations over the range 23–55°C. Determinations were made in duplicate and the results averaged. Repeated analysis of stock solutions of dipyridamole confirmed its stability under the experimental conditions.

Calculation of partition coefficients

The apparent molal partition coefficients, K′, were calculated from the distribution results by:

$$K' = \frac{(C_T - C_w)w_1}{C_w w_2} \quad (1)$$

where C_T = the total initial concentration of dipyridamole in the aqueous buffer phase before equilibration, C_w = final aqueous phase concentration of dipyridamole, w_1 = weight of aqueous phase, and w_2 = weight of *n*-octanol or phospholipid in the sample. Ion-corrected partition coefficients were calculated from

$$K = K'(1 + 10^{pK_a - 7.4}) \quad (2)$$

using a pK_a value for dipyridamole of 6·4.

The standard change in free energy, $\Delta G^\circ_{w\to L}$, due to partitioning is:

$$\Delta G^\circ_{w\to L} = -2.303\ RT \log K \quad (3)$$

The temperature dependence of partitioning was employed to obtain data on the enthalpy of the process based on the relationship (Katz & Diamond 1974):

$$\Delta H^\circ_{w\to L} = -2.303\ Ra_2 \quad (4)$$

The values for a_2 are obtained from the slopes of log K vs 1/T (Fig. 1), and assume that $\Delta H^\circ_{w\to L}$ and $\Delta S^\circ_{w\to L}$ are approximately independent of temperature over the range of interest (Cratin 1968).

Since

$$\log K = \frac{\Delta H^\circ_{w\to L}}{2.3RT} + \frac{\Delta S^\circ_{w\to L}}{2.3R} \quad (5)$$

a linear plot of log K vs 1/T enables calculation of $\Delta H^\circ_{w\to L}$ from the slope and $\Delta S^\circ_{w\to L}$ from the intercept. However, the change in entropy of partitioning, $\Delta S^\circ_{w\to L}$, was conveniently obtained from

$$\Delta S^\circ_{w\to L} = \frac{\Delta H^\circ_{w\to L} - \Delta G^\circ_{w\to L}}{T} \quad (6)$$

where $\Delta H^\circ_{w\to L}$ and $\Delta S^\circ_{w\to L}$ have the physical meaning of the change in enthalpy and entropy, respectively, when one mole of solute is transferred from water to lipid at infinite dilution (Katz & Diamond 1974).

Results and discussion

The dependence of the apparent partition coefficient (log K′) as a function of *n*-octanol and DMPC concentration is shown in Table 1. The partitioning of dipyridamole was independent of *n*-octanol concentration. In DMPC liposomes the partitioning of dipyridamole increased up to 6 mg mL^{-1} DMPC. With further increases in DMPC the partitioning of dipyridamole tended to decrease. Similar results were reported for propranolol (Betageri & Rogers 1987). However, they are in marked contrast to that observed for chlorpromazine in the *n*-octanol/phosphate buffer system (Ahmed et al 1985). This indicates the partitioning of a single species of dipyridamole in the *n*-octanol/buffer system. However, under similar conditions, chlorpromazine partitioned as an ion pair. Therefore, for temperature dependency studies, 0·1 mL *n*-octanol and 6 mg mL^{-1} phospholipids were employed.

Temperature dependence of partitioning. The temperature dependence of log K for dipyridamole in the *n*-octanol/buffer and liposome systems and the associated thermodynamic parameters of free energy of transfer (ΔG), enthalpy (ΔH) and entropy (ΔS) are given in Fig. 1 and Table 2, respectively. The values of log K increased uniformly with temperature in both the *n*-octanol/buffer and liposome systems. The magnitudes of $\Delta G^\circ_{w\to L}$ are indicative of the spontaneity of the partition process. These values seem to be more favourable for the accommodation of dipyridamole in bilayers compared with the *n*-octanol/buffer system. However, these values were lower for DMPC liposomes and are of a similar magnitude for *n*-octanol and DPPC liposomes. Positive enthalpies and entropies have been found for phenols in a cyclohexanol/aqueous system (Anderson et al 1983), phenothiazine in the *n*-octanol/aqueous phase system (Ahmed et al 1985) and β-blockers in the *n*-octanol/buffer system (Betageri & Rogers 1987). In contrast, negative enthalpies of partitioning of phenols in the *n*-octanol/0·15 M NaCl system

Table 1. Effect of the amount of lipid phase on partitioning of dipyridamole in phosphate-buffered saline (pH 7·4) at room temperature (21°C).

n-Octanol (mL)	log K	DMPC (mg mL^{-1})	log K
0·1	2·22 (±0·03)	2	3·04 (±0·005)
0·5	2·24 (±0·28)	4	3·09 (±0·002)
1·0	2·24 (±0·11)	6	3·12 (±0·017)
		8	2·95 (±0·015)
		10	2·92 (±0·015)

The results are mean ± s.d.

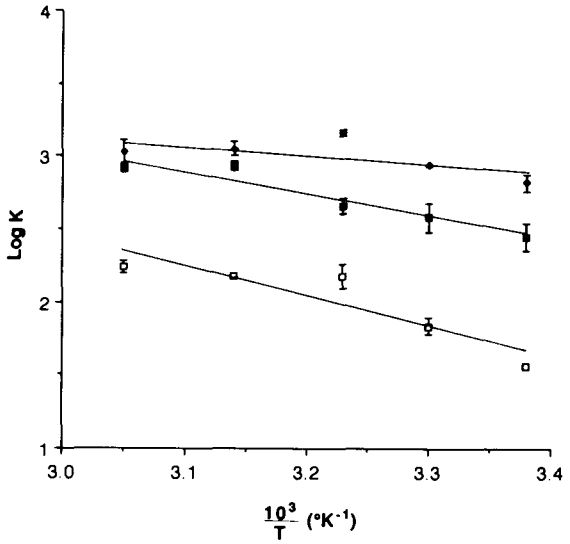

FIG. 1. Van't Hoff plots of the partition coefficient of dipyridamole in the *n*-octanol (□), DMPC (◆) and DPPC (■) liposome-phosphate buffer system at pH 7·4.

Table 2. The thermodynamics of partitioning of dipyridamole in the *n*-octanol or phospholipid/phosphate buffer systems at pH 7·4 and 37°C.

Lipid phase	log K	$\Delta G^{\circ}_{w\to L}$ (kJ mol^{-1})	$\Delta H^{\circ}_{w\to L}$ (kJ mol^{-1})	$\Delta S^{\circ}_{w\to L}$ (J mol^{-1} K^{-1})
n-Octanol	2·17	−12·88	26·88	126·32
DMPC	3·16	−18·76	10·97	95·90
DPPC	2·66	−15·79	24·79	130·90

DMPC = dimyristoylphosphatidylcholine, DPPC = dipalmitoylphosphatidylcholine.

have been observed (Rogers & Wong 1980) indicating the presence of significant hydrogen bonding between molecules of phenol and *n*-octanol. The present results indicate that dipyridamole does not significantly hydrogen bond with *n*-octanol.

The enthalpies (ΔH) and entropies (ΔS) of partitioning are greater in the *n*-octanol/buffer and DPPC liposomes. Similar results were observed with β-blockers (Betageri & Rogers 1987) and phenothiazines (Ahmed et al 1985). However, the magnitude was much higher with β-blockers and phenothiazines compared with dipyridamole. These results suggest a different mechanism of interaction of dipyridamole with model membrane systems. When the membrane exists in a fluid state ($> T_c$ of DMPC), the interaction of dipyridamole is primarily by hydrophobically-controlled partitioning (i.e. entropy-dominated). However, in a membrane of a more rigid structure ($< T_c$) as with DPPC liposomes, the interaction of dipyridamole with liposomes is predominantly through polar group association of the membrane surfaces and partitioning becomes enthalpy dominated. These results suggest that the interaction of dipyridamole depends upon the composition of the membrane and that the more rigid the phospholipid composition of the membrane the less favourable the distribution of dipyridamole. Such information cannot be obtained by measuring the distribution of drug molecules in *n*-octanol due to its inability to alter structural properties.

We wish to thank Daniel L. Parsons for useful discussions and critical reading of the manuscript.

References

Ahmed, A. M. S., Farah, F. H., Kellaway, I. W. (1985) The thermodynamics of partitioning of phenothiazines between phosphate buffer and the lipid phases of cyclohexane, *n*-octanol and DMPC liposomes. Pharm. Res. 1: 119–124

Anderson, N. H., Davis, S. S., James, M., Kojima, I. (1983) Thermodynamics of distribution of *p*-substituted phenols between aqueous solution and organic solvents and phospholipid vesicles. J. Pharm. Sci. 72: 443–448

Betageri, G. V., Rogers, J. A. (1987) Thermodynamics of partitioning of β-blockers in the *n*-octanol/buffer and liposome systems. Int. J. Pharm. 36: 165–173

Collander, R. (1951) The partition of organic compounds between higher alcohols and water. Acta Chem. Scand. 5: 774–780

Cratin, P. D. (1968) Partitioning at the liquid-liquid interface. Ind. Eng. Chem. 60: 14–19

Diamond, J. M., Katz, Y. (1974) Interpretation of non-electrolyte partition coefficients between dimyristoyl lecithin and water. J. Membr. Biol. 17: 121–154

FitzGerald, D. (1987) Dipyridamole. N. Engl. J. Med. 316: 1247–1257

Grem, J. L., Fischer, P. H. (1989) Enhancement of 5-flurouracil's anticancer activity by dipyridamole. Pharmacol. Ther. 40: 349–371

Hansch, C., Dunn, W. J. (1972) Linear relationship between lipophilic character and biological activity of drugs. J. Pharm. Sci. 61: 1–18

Katz, Y., Diamond, J. M. (1974) Thermodynamic constants for nonelectrolyte partition between dimyristoyl lecithin and water. J. Membr. Biol. 17: 101–120

Leo, A., Hansch, C., Elkins, D. (1971) Partition coefficients and their uses. Chem. Rev. 17: 525–615

Rogers, J. A., Wong, A. (1980) The temperature dependence and thermodynamics of partitioning of phenols in the *n*-octanol-water system. Int. J. Pharm. 6: 339–348

Smith, R. N., Hansch, C., Ames, M. (1975) Selection of a reference partitioning system for drug design work. J. Pharm. Sci. 64: 599–606

Szebeni, J., Wahl, S. M., Popovic, M., Wahl, L. M., Gartner, S., Fine, R. L., Skaleric, U., Friedman, R. M., Weinstein, J. N. (1989) Dipyridamole potentiates the inhibition by 3′-azido-3′-deoxythymidine and other dideoxynucleosides of human immunodeficiency virus replication in monocyte-macrophages. Proc. Natl. Acad. Sci. USA 86: 3842–3846