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PARTITION COEFFICIENTS OF DOPAMINE ANTAGONISTS IN BRAIN MEMBRANES AND LIPOSOMES

C. R. OLIVEIRA,* M. C. P. LIMA, C. A. M. CARVALHO, J. E. LEYSEN† and A. P. CARVALHO

Center for Cell Biology, Department of Zoology, University of Coimbra, P-3049 Coimbra Codex, Portugal and † Department of Biochemistry and Pharmacology, Janssen Research Foundation, B-2340 Beerse, Belgium

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Abstract—Partition coefficients, K_p of dopamine antagonists, spiperone, haloperidol, domperidone and pimozide were determined in caudate nucleus microsomal membranes and in liposomes from membrane lipids. K_p values were measured as a function of temperature and the thermodynamic parameters for the transfer of the drugs from the aqueous medium to the lipid bilayer were evaluated. Partition in native membranes or in liposomes formed from the membrane lipids is not strongly dependent on temperature over the range from 8 to 37°. The K_p values for spiperone, haloperidol and domperidone in membrane are 32 ± 6, 192 ± 11 and 308 ± 40 respectively, whereas the equivalent values in liposomes are much higher: 195 ± 12, 558 ± 16 and 316 ± 16. In contrast, for pimozide, the K_p values in membranes are higher than in liposomes: 1097 ± 11 for microsomes and 662 ± 10 for liposomes. Partition values in natural membranes decrease sequentially as follows: pimozide > domperidone > haloperidol > spiperone. Membranes rich in cholesterol show lower partition coefficients for haloperidol. The interaction of the antagonists with the bilayer is associated with small enthalpy changes and large increases in entropy, as expected for hydrophobic interactions. We conclude that the partition coefficients of the drugs studied for membranes and membranes lipids are very different from those reported for octanol/water and the latter values should not be used to estimate drug partition into membranes.

Dopamine antagonists exhibit non-specific as well as specific membrane effects. In addition to their specific drug–receptor interaction, which results in dopamine receptor blockade [1], they possess a wide range of non-specific membrane activities. These include expansion of the membrane, membrane fluidization, alteration of transmembrane fluxes and inhibition of membrane excitability [2]. The ability of these drugs to interact with, and cause functional perturbations of several biological membrane systems suggests that their site of non-specific action may be the lipid component of these membranes, since some of the non-specific membrane activities correlate with octanol/buffer partition coefficients of the drugs [2]. Neuroleptic drugs are highly fat-soluble and surface-active and they are very soluble in model lipid membranes [1].

Relevant information regarding possible interaction of amphipathic drug molecules with biological membranes, especially with the lipid component of these membranes, can be obtained in studies of pure lipid membrane systems [3]. However, certain limitations for such systems, as models of protein-containing biological membranes, must be taken into account [4]. Compositional differences exist between the real membranes and simple bilayers. The former generally contain large concentrations of integral proteins, and in some cases neutral lipids, such as cholesterol, as well as attached peripheral proteins, along with the phospholipids. Integral membrane proteins and cholesterol can affect fluidity and the structural order of the membrane [5] and modulate drug incorporation into the lipid bilayer.

Considering only non-specific drug–membrane interactions, the incorporation of dopamine antagonists may influence membrane function in three different ways. First, attending to the pK of the drugs [6, 7], they are mostly positively charged at physiological pH. They change the surface membrane potential proportionally to their membrane concentration [8] and they are surface-active [2]. Second, the drugs may affect protein structure and, thus, perturb their function, as has been suggested for local anesthetics [9]. Third, dopamine antagonists can incorporate into the lipid part of biological membranes, influencing its dynamic and/or structure, and thus they may secondarily affect membrane proteins [3].

In this work we studied the third mode of non-specific action of four dopamine antagonists: two butyrophenones, spiperone and haloperidol, a benzimidazolone, domperidone, and a diphenylbutylpiperidine, pimozide. Incorporation of the drugs into the lipid bilayer of native membranes and liposomes prepared from membrane lipid extracts is expressed by a partition coefficient which is the ratio of the equilibrium concentrations of the drugs between the lipid and the aqueous phase. Since in biological systems a complex equilibrium situation exists, in the present study we took into consideration the effect of temperature, membrane lipid composition, cholesterol content, lipid chain length, lipid–protein-interaction leading to a domain structure, as well as the physico-chemical characteristics of the drugs. The thermodynamic parameters for the interaction of the drugs with bilayer molecules were calculated to provide insight into the nature of drug/bilayer complexes.

* Correspondence should be addressed to C. R. Oliveira.

MATERIALS AND METHODS

Drugs and chemicals. ^{3}H-Spiperone with a specific activity of 19 Ci/mmol was obtained from the Radiochemical Centre (Amersham, U.K.); ^{3}H-haloperidol with a specific activity of 14.6 Ci/mmol was obtained from New England Nuclear; ^{3}H-domperidone with a specific activity of 8.4 Ci/mmol and ^{3}H-pimozide with a specific activity of 22.7 Ci/mmol were kindly donated by Dr J. E. Leysen, Department of Biochemical Pharmacology, Janssen Pharmaceutica (Beerse, Belgium). Spiperone, haloperidol, domperidone and pimozide were obtained from Janssen Pharmaceutica (Beerse, Belgium). All other reagents used were analytical grade.

Preparation of native membranes. The microsomal fraction from sheep brain caudate nucleus was isolated by the method described by Hájos [10]. The caudate nuclei were removed within 1 hr after death and added to cold 0.32 M sucrose, 15 mM Tris pH 7.4 (9 ml sucrose solution per 1 g wet wt tissue) and homogenized in a glass homogenizer. The homogenate was centrifuged at 1500 *g* for 10 min, and the resulting supernatants were centrifuged at 9000 *g* for 20 min. The supernatants obtained were centrifuged at 39,000 *g* for 30 min. The pellets (microsomal fraction) were resuspended in 15 mM Tris, pH 7.4, 12.5 μM nialamide (TN buffer) at 4°, and washed twice with the same buffer. The "washed" pellets were resuspended in TN buffer and the protein concentration was determined by the biuret method [11]. The microsomal fraction was stored at −80° until used.

Preparation of liposomes. Multilamellar liposomes were prepared with lipids extracted from the microsomal fraction of the caudate nucleus [12]. The desired amount of lipid (0.4 mg/ml), determined by a phosphorus assay according to Bartlett [13], was taken from the chloroform solution and the solvent was evaporated first in a nitrogen stream and then under vacuum for 3 hr. The hydration buffer (TN, pH 7.4) and two glass beads were added and vortexed six times for 30 sec each time at room temperature. The liposomes were allowed to equilibrate under N_2 atmosphere for 12 hr at room temperature. The final phospholipid concentration after hydration was 500 μM. Cholesterol in lipid extracts was assayed by the Lieberman–Büchard method [14] and expressed in relation to the phospholipid content.

Liposome size and distribution were analysed in a Malverne autosizer Model 4700, by dynamic light scattering as described by Aurora *et al.* [15]. The samples were placed in a temperature controlled cell (25°) and illuminated with a laser beam. The light scattered by the suspended particles was detected at 90° by a photomultiplier. The output signals from the photomultiplier were analysed in a digital correlator with associated microcomputer. Results were presented in both graphical and numerical form. The diffusion coefficient, the mean size and the polydispersity index of the particles were determined.

Lipid composition of the membranes. Total lipids were extracted from microsomal membranes by the method of Reed *et al.* [16]. Aliquots of lipid extracts dissolved in $CHCl_3$ were then analysed for total lipid phosphorus [13], cholesterol content [14], phospholipid and fatty acid composition. Lipid samples were separated on thin-layer plates of silica gel G (Merck Darmstadt, F.R.G.) using as solvent a mixture of $CHCl_3$:CH_3OH:H_2O (65:25:4, v/v). Individual phospholipids were identified using group-specific spray reagents [17] and by co-chromatography with lipid standards (Supelco, PA, U.S.A.). For quantitative estimation of resolved phospholipids the thin-layer plates were exposed to iodine vapor, the yellow stained spots were marked, the iodine was evaporated and the individual spots were scraped from the plate. The amount of each phospholipid was determined by measuring the amount of inorganic phosphate in the scraped spots previously digested in 70% $HClO_4$ [18].

Derivatives of fatty acids of the total lipid extract were prepared by the method of Morrison and Smith [19], after an acid hydrolysis with HCl 2N. Fatty acid methyl esters were prepared using 10% boron–trifluoride (BF_3) in methanol and analysed on a Perkin–Elmer 900 gas–liquid chromatograph equipped with a flame ionization detector and interfaced with a Hewlett–Packard 3380 A integrator. Fatty acid analysis was performed by temperature-programmed GLC using two stainless steel columns

Table 1. Effect of various ethanol concentrations on liposome size and distribution

	Ethanol					
MLV	0%	0.1%	0.3%	0.5%	1.0%	3.0%
Size (nm)	625.7	639.9	634.5	610.3	647	684
Peak (nm)	268	329	327	292	324	374
Polydispersity	0.49	0.48	0.50	0.47	0.47	0.51
Dif. coefficient	7.84 E-9	7.66 E-9	7.73 E-9	8.05 E-9	7.58 E-9	7.21 E-9

Liposomes prepared from total extracted microsomal membrane lipids were prepared in TN buffer at pH 7.4, in the presence of increasing concentrations of ethanol. Ethanol in a percentage of 3% was present in the experimental assays, since it was used to solubilize the dopamine antagonists. Multilamellar liposomes (MLV) size and distribution were determined in a Malvern autosizer Model 700 as described under Materials and Methods.

(1.86 m × 0.125 cm inner diameter) packed with 20% DEGS on Chromosorb AWDNCS 80–100 mesh. The initial and final oven temperatures were 130 and 170°, respectively, and the rate of increase was 1°/min. Injector and detector temperatures were 210 and 230°, respectively, and N_2 (35 ml/min) was the carrier gas. Authentic fatty acid methyl esters were used to identify retention times [20]. During all procedures (lipid extraction, solvent evaporation, liposome preparation and the described derivatizations and reactions) the lipids were kept in an N_2 atmosphere.

Determination of partition coefficients. Partition coefficients were measured by a filtration procedure developed in our laboratory to measure the partition of a substance into a lipid phase [21].

For studies of drug incorporation, ^{3}H-spiperone (19 Ci/mmol s.a.), ^{3}H-haloperidol (14.6 Ci/mmol s.a.), ^{3}H-domperidone (8.4 Ci/mmol s.a.) and ^{3}H-pimozide (22.7 Ci/mmol s.a.) were incubated with the membrane suspensions in buffer (TN buffer, pH 7.4) or with liposomes (500 μM in lipid). ^{3}H-Labelled drugs were added to unlabelled drugs to obtain the desired concentration (30×10^{-6} M). Drug and lipid concentrations were selected according to drug solubility in order to obtain less than 10% of drug incorporation into the lipid phase. After the samples had equilibrated for 1 hr, aliquots of 0.5 ml were rapidly filtered through Whatman GF/B filters, under vacuum. The filters were washed with 10 ml of ice-cold buffer. Radioactivity retained in the filters was counted by liquid scintillation spectrometry in a Packard 460 scintillation counter, programmed for automatic quenching correction computed from an efficiency correlation determined for ^{3}H-quenched standards by the external standardization method. The radioactivity measurements were taken after overnight equilibration at 4° in 8.0 ml Triton X-100 scintillation fluid [22]. The partition coefficients were calculated from the fraction of drug retained in membranes or liposomes (p), according to the Connors equation (1967) [23]:

$$p = \frac{K_p (V_L/V_A)}{K_p (V_L/V_A) + 1}$$

where K_p is the partition coefficient, V_L is the volume of the membrane lipid compartment, V_A is the volume of the aqueous phase. In our experimental conditions V_L was calculated from the amount of lipid recovered in the filters expressed in nmol and from the specific volume of phospholipid (0.984 μl/mg PL). V_A is the volume of filtered sample (500 μl). The retention capacity of the filters is 88% for microsomal membranes and 65% for liposomes, as determined by measuring the amount of phospholipid retained in the filters. After treating the filters at 180° with 1.0 ml 70% $HClO_4$ [18], released lipid phosphate was measured according to Bartlett [13]. Since the drugs are present predominantly in the protonated form at the pH value of the assay, the partition coefficients determined must be labelled as apparent partition coefficients which reflect the overall distribution behaviour of protonated and unprotonated species [6, 7].

RESULTS

Effect of temperature on partition coefficients of dopamine antagonists in native membranes and liposomes

The partition coefficients of spiperone, haloperidol, domperidone and pimozide were studied in caudate nucleus microsomal membranes and in model membranes obtained from total extracted lipids, over a temperature range from 8° to 37°. Spiperone incorporates poorly in microsomal membranes as compared to liposomes (Fig. 1), and this incorporation is not strongly dependent on temperature. In microsomal membranes the partition coefficient of spiperone decreases slightly from 44 ± 3 at 8° to 32 ± 6 at 37°, whereas in membrane lipid liposomes it increases from 151 ± 10 at 8° to 195 ± 12 at 37°. The incorporation of haloperidol, both in caudate nucleus membranes and in liposomes, is higher than that observed for spiperone, but is observed in a similar distribution to spiperone in membranes and in liposomes over the range of temperatures studied. The K_p values decrease from 288 ± 20 at 8° to 192 ± 11 at 37° in membranes and from 585 ± 10 at 8° to 558 ± 16 at 37° in liposomes.

The incorporation of domperidone is almost the same in membranes and in liposomes. The partition coefficient for domperidone in caudate nucleus membranes is 359 ± 11 at 8° and decreases to 308 ± 40 at 37°. In liposomes a similar distribution to that for native membranes was observed. The K_p value determined at 8° is 344 ± 33 and it decreases to 316 ± 16 at 37°. In contrast, pimozide partitioning in membranes is higher than in liposomes. The K_p for pimozide in microsomal membranes increases from 799 ± 20 at 8° to 1097 ± 11 at 37°, and in liposomes it increases from 575 ± 16 at 8° to 662 ± 10 at 37°. The K_p values do not change with varying lipid concentrations between 0.18 and 0.4 mg/ml (data not shown). As we can observe in Table 1, the size and distribution of multilamellar liposomes do not change significantly with ethanol concentrations from 0.1 to 3% (an ethanol concentration of 3% was used to prepare the drug solutions). In our experimental conditions liposomes were shown to maintain their properties (size and distribution) till 10 hr after being prepared.

Thermodynamic analysis of the transfer of dopamine antagonists from buffer to microsomal membranes and/or to liposomes

The thermodynamic parameters for the transfer of spiperone, haloperidol, domperidone and pimozide were calculated from the data of Fig. 1. The van't Hoff plots for the temperature dependence of the partition coefficients of these drugs appear to be linear over the range of examined temperatures.

The data from Table 2 show that the values for the enthalpy of transfer ($\Delta H°$) of spiperone, haloperidol and domperidone to the membranes are small and negative (−1.8, −2.1, −0.9 kcal/mol, respectively), but a small and positive value for $\Delta H°$ (+1.7 kcal/mol) was determined for pimozide. Low values for the $\Delta H°$ of transfer of these dopamine antagonists were also found by direct calorimetric measurements in a LKB batch microcalorimeter (data not shown).

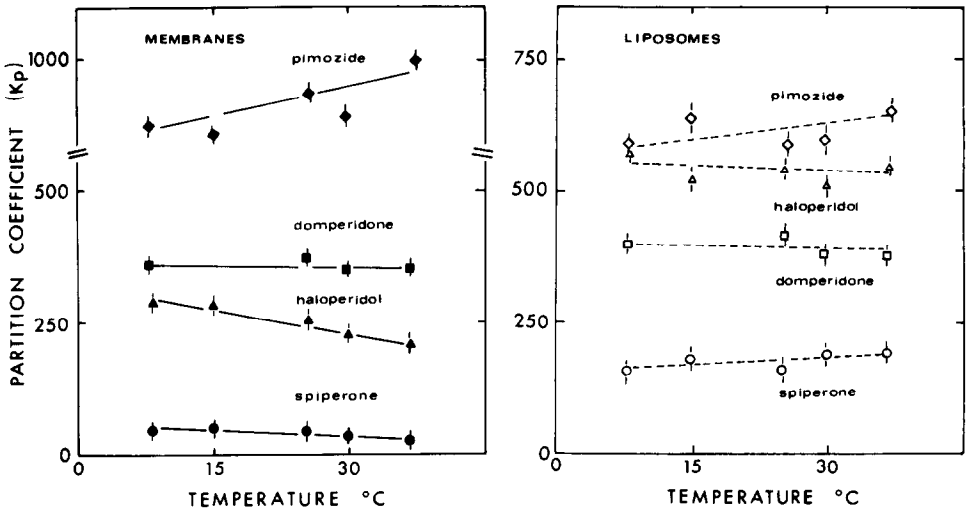

Fig. 1. Effect of temperature on partition coefficients of dopamine antagonists in microsomal membranes (solid symbols) and in liposomes (open symbols). Spiperone (○) and (●), haloperidol (△) and (▲), domperidone (□) and (■) pimozide (◇) and (◆), at 30 μM concentration, were incubated with caudate nucleus microsomal membranes and/or liposomes prepared from total extracted membranes lipids (0.4 mg PL/ml) in TN buffer at pH 7.4, for 60 min at different temperatures. K_p values were calculated as described in Materials and Methods. The values are the means ± SD of three independent experiments, each run being in triplicate at each temperature, in three individual membrane and/or liposome preparations.

Table 2. Equilibrium thermodynamic parameters for the transfer of dopamine antagonists from buffer to microsomal membranes or to liposomes

		$\Delta G°$ (kcal/mol)	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/mol deg)
Spiperone	Membranes	−2.3	−1.8	+1.2
	Liposomes	−3.2	+1.4	+14.6
Haloperidol	Membranes	−3.2	−2.1	+3.8
	Liposomes	−3.9	+0.2	+13.2
Domperidone	Membranes	−3.5	−0.9	+14
	Liposomes	−3.5	−0.4	+13
Pimozide	Membranes	−4.3	+1.7	+19.4
	Liposomes	−3.9	+0.4	+14.2

The $\Delta G°$ and $\Delta S°$ values were calculated at 37° using the following equations: $\Delta G° = -RT \ln K_p$ where R is the gas constant (1.987 cal/mol deg), T is the incubation temperature in degrees Kelvin and K_p is the partition coefficient calculated at 30 μM drug concentration as shown under Materials and Methods. The $\Delta H°$ values were determined from the slope of van't Hoff plots ($\ln K_p$ vs $1/T$) equal to $-\Delta H°/R$ obtained in each individual membrane or liposome preparation (0.4 mg PL/ml) over all the temperature range. The values shown are from a representative of three experiments performed at each temperature, each one done in triplicate.

The incorporation of these dopamine antagonists in native membranes and liposomes is associated with significant positive entropy changes. For spiperone and haloperidol, the incorporation into membranes is associated with positive but smaller entropy changes ($\Delta S°$ = +1.2 cal/mol deg and +3.8 cal/mol deg, respectively) than those observed for domperidone ($\Delta S°$ = +14 cal/mol deg) and for pimozide ($\Delta S°$ = +19.4 cal/mol deg).

Effect of cholesterol and membrane lipid composition on K_p *values*

Cholesterol strongly reduces the K_p value. Haloperidol partitioning, either in native membranes, or in liposomes prepared from total lipid extracts, decreases as the relative content in cholesterol increases (Fig. 2). When the cholesterol content increases (from 1:2 to 1.5:2 molar ratio), the partition coefficient for haloperidol, determined at 25°, decreases from 253 ± 20 to 51 ± 4 in caudate nucleus microsomal membranes and it decreases from 546 ± 40 to 82 ± 6 in liposomes from total membrane lipid extracts.

These results prompted us to analyse the lipid composition of microsomal membranes. As we can observe from Table 3, a high content in phospha-

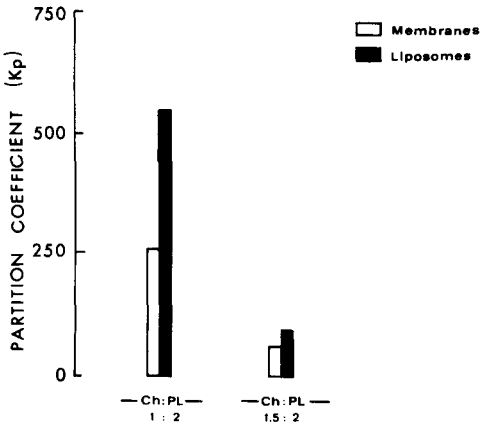

Fig. 2. Dependence of haloperidol partition coefficient on the intrinsic cholesterol content of microsomal membranes and liposomes. Caudate nucleus microsomal membranes and liposomes prepared from total extracted membrane lipids, were incubated with haloperidol (30 μM) at 25°, for 60 min. Cholesterol/phospholipid molar ratio (CH : PL) were 1 : 2 and 1.5 : 2, respectively. Partition coefficients (K_p) were obtained as described in Materials and Methods. The results are means of triplicate determinations in a representative of three experiments with standard errors varying less than ±8%.

tidylcholine (32 ± 6%) and phosphatidylethanolamine (31 ± 2.8%) is found in microsomal membranes. The acyl composition of microsomal membrane phospholipids shows a high percentage of short acyl groups (C-16 and C-18), when compared to long acyl groups (C-20, C-22 and C-24). However, a high content in polyunsaturated fatty acids is observed (C-20 : 4 and C-22 : 6) in microsomal membranes.

DISCUSSION

We investigated the membrane/buffer partition coefficients of four dopamine antagonists, spiperone, haloperidol, domperidone and pimozide in caudate nucleus microsomal membranes and in liposomes prepared from total lipid extracts. The results show that the partition coefficient of these drugs in biological structures is lower than that estimated on the basis of the classical octanol/water partitioning (Table 4) [1, 6, 7]. Our data indicate that the partitioning of the drugs into the lipid bilayer is not strongly dependent on temperature but, at least in the case of haloperidol, it depends on the cholesterol content of the bilayer. Temperature influences the incorporation of drugs in lipid bilayers [24–26] and modulates membrane fluidity [27–29]. However, we

Table 3. Phospholipid (A) and fatty acid composition (B) of caudate nucleus microsomal membranes

A. Phospholipid composition

Type of phospholipid	% of total content
Phosphatidylcholine (PC)	32 ± 6.0
Phosphatidylethanolamine (PE)	31 ± 2.8
Phosphatidylserine (PS)*	24 ± 3.6
Sphingomyelin	10 ± 2.0
Other **	3 ± 1.5

B. Acyl groups and fatty acid composition

Acyl group	% of total fatty acid content
16:0	25.9 ± 2.1
18:0	26.4 ± 4.0
18:1	19.3 ± 3.0
20:2	1.4 ± 0.7
20:4	8.1 ± 0.9
22:6	14.0 ± 2.2
24:1	4.9 ± 1.5

* Phosphatidylinositol contamination of the PS fraction is less than 5%.

** Includes phosphatidic acid, phosphatidylinositol and polyphosphatidylinositols.

Lipids were extracted from caudate nucleus microsomal membranes (7 mg/ml protein). Thin-layer chromatograms of membrane lipids (about 100 μg of lipid applied in each track) were run as described in Materials and Methods. Total phospholipid was determined by measuring the amount of Pi in the scraped spots [18]. Fatty acids were analysed by GLC and authentic fatty acid methyl esters were used to identify retention times as described under Materials and Methods. The results are means of duplicates.

Table 4. Partition coefficients, pK_a-values and drug ionization at pH 7.4 (37°)

Drug	Octanol/water Papp*	Liposomes	Membranes	pK_a*	% ionized*
Spiperone	490	195	32	8.31	89.05
Haloperidol	1047	558	192	8.66	94.79
Domperidone	1900	316	308	7.90	75.97
Pimozide	20417	662	1097	8.63	94.44

* For spiperone, haloperidol and pimozide, Papp, pK_a values and % of drugs ionized are referred by Leysen and Gommeren [6] and by Pauwels *et al.* [7]. The values for domperidone were kindly provided by Dr J. E. Leysen, Janssen Pharmaceutics, Beerse, Belgium.

observed that the incorporation of spiperone, haloperidol, domperidone and pimozide into microsomal membranes and related lipid bilayers is not significantly dependent on temperature over the temperature range of 8° to 37° (Fig. 1). The thermodynamic parameters for drug transfer from the aqueous medium to the membrane were calculated by the van't Hoff analysis of the temperature dependence of the apparent K_p of the drugs. The data summarized in Table 2 show that the transfer of the drugs to the lipid phase is entropically driven, which suggests that ionic and/or hydrophobic forces can be responsible for complexes formed by membrane lipids and drugs.

We have to consider that the drugs studied are derivatives of amines which exist as mixtures of charged and uncharged species at the experimental pH (7.4). From the pK values of the drugs reported by Leysen [6] and by Pauwels [7], we calculated for each drug the relative concentrations of the two forms of the drugs, at pH 7.4. Since they exist predominantly in the protonated form, ionic interactions with polar lipids with a negative net charge (PS and gangliosides) are expected, while the neutral form of the drug binds to zwitterionic polar lipids (PC, PE, SM). As we can observe in Table 3, microsomal membranes have a high content in phosphatidylserine (PS = 24 ± 3.6%), a negatively charged phospholipid. These data reinforce the importance of charge–charge interactions even in nonspecific membrane activity of dopamine antagonists.

The results of Fig. 1 and Table 2 also show that the apparent K_p values for the dopamine antagonists, either in microsomal membranes or in liposomes, are lower than the classical partition coefficients of these drugs determined in octanol/water (Table 4) [6, 7]. Furthermore, for three dopamine antagonists, spiperone, haloperidol and domperidone, the apparent K_p is lower in native microsomal membranes than in liposomes. In contrast, microsomal membranes incorporate more pimozide than do the lipids extracted from the membranes (K_p = 1097 ± 11 at 37° in membranes; K_p = 662 ± 10 at 37° in liposomes).

In an attempt to interpret these data, we analysed the effect of cholesterol on the partition coefficient of haloperidol in native membranes and in liposomes (Fig. 2), and looked at the lipid composition of the membranes (Table 3). Our data summarized in Fig. 2, show that when the cholesterol content increases from 33 mol % to 43 mol %, the amount of haloperidol incorporated in the lipid bilayer decreases (K_p in membranes decreases from 235 ± 20 to 51 ± 4; K_p in liposomes decreases from 546 ± 40 to 82 ± 6). Other studies also show that cholesterol decreases the K_p values of several drugs [26, 30, 31]. Apparently, cholesterol prevents drug entry into the bilayer either by inducing a tighter packing of fatty acid acyl chains, which increases membrane order [30, 32], or by displacing the drug from its binding sites in the cooperative region of the membrane [33].

The lipid composition of microsomal membranes (Table 3), shows a high content in phosphatidylethanolamine (PE = 31% ± 2.8%) and in long-chain polyunsaturated fatty acids (22:6 = 14.0 ± 2.2%). Long-chain lipids have been shown to reduce the K_p values probably by increasing chain–chain interactions [34].

As we can observe in Fig. 1, preferential transfer of spiperone, haloperidol and domperidone to liposomes, as compared to that observed to native membranes, is consistent with the effect of intrinsic membrane proteins in preventing drug incorporation. However pimozide, the most lipophilic of the drugs studied [7] incorporates more in microsomal membranes than in liposomes prepared from membrane lipids, which indicates that for this drug the presence of proteins in the membrane contributes to the accumulation of the drug. Although a direct interaction of the drug with the membrane proteins cannot be ruled out, the results suggest that an extra free volume is available for pimozide incorporation at the lipid–protein interface, from where cholesterol appears to be excluded [35–37]. The distinct structure and geometry of pimozide molecules as compared to that of butyrophenones (spiperone and haloperidol), has also to be considered when analysing the incorporation of the drugs into the lipid bilayer.

It is interesting to note that pimozide is a neuroleptic of the diphenylbutylpiperidine series which are potent calcium channel inhibitors [38, 39]. It was recently demonstrated in our laboratory that the incorporation of other calcium channel antagonists is larger in membranes than in liposomes prepared with extracted membrane lipids [40]. These results are in agreement with those reported by other investigators [25], and suggest that partitioning of calcium channel acting drugs into the lipid bilayer may be a component of the pathway by which these drugs reach specific protein receptor sites in the membranes. In contrast, for the D_2 dopamine receptor antagonists studied, integral membrane proteins inhibit drug incorporation into the lipid bilayer, suggesting that the cationic form of the antagonist is the predominantly active form of the drug molecule.

We conclude that partition coefficients of dopa-

mine antagonists determined for biological membranes are very different from those obtained for liposomes formed from the lipids extracted from the membranes and in both cases the partition coefficients are substantially lower than for octanol/water. High cholesterol content of the membranes is associated with a significant reduction in the partition coefficient for one of the drugs tested (haloperidol). The partition coefficients of drugs in octanol/water cannot be utilized as a model for evaluating drug lipophilicity in biological membranes. The small amount of the drug that dissolves in the membrane interior may have important consequences in the functional properties of the membrane if it concentrates in the boundary regions, where the lipid fatty acyl chains interact with the hydrophobic surfaces of the integral membrane proteins.

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