Partition of Dopamine Antagonists into Synthetic Lipid Bilayers: the Effect of Membrane Structure and Composition

ANA BELA SARMENTO*, M. C. PEDROSO DE LIMA*† AND C. R. OLIVEIRA*

*Center for Cell Biology, Laboratory of Biochemistry, Faculty of Medicine and †Department of Biochemistry, University of Coimbra, 3049 Coimbra Codex, Portugal

Abstract—Partition coefficients, K_p, of four dopamine antagonists (pimozide, fluspirilene, haloperidol and domperidone) between the aqueous phase and lipid bilayer vesicles were determined as a function of lipid chain length, unsaturation and temperature encompassing the range of the lipid phase transition. Model membranes of egg phosphatidylcholine (PC), dimyristoyl (DMPC)-, dipalmitoyl (DPPC)-, distearoyl (DSPC)- and dioleoyl (DOPC)-phosphatidylcholines were studied. K_p values of the drugs are different in the various membranes under study and depend on temperature, aliphatic carbon chain-length and on the presence of unsaturation in the aliphatic lipid chain. First-order transition of membrane lipids from the gel to the liquid crystalline state is accompanied by a sharp increase of the partition coefficient of pimozide and fluspirilene in DMPC, DPPC and DSPC bilayers. For domperidone, K_p values are maximal within the mid-point of phase transition of DMPC and DPPC, while for DSPC K_p values increase progressively with increasing temperature. Haloperidol K_p values display a maximum at the mid-point of phase transition of DMPC, while a progressive increase of K_p is observed in DPPC and DSPC. The four drugs are easily accommodated in bilayers of short aliphatic chain lipids (DMPC), the partition coefficients being 17 137 for pimozide, 18 700 for fluspirilene, 686 for domperidone and 722 for haloperidol, at temperatures 10°C below the mid-point of the lipid phase transition. Except for haloperidol, the partition of the drugs in DOPC (18:1) is higher than that in DSPC (18:0) bilayers at a temperature above the phase transition temperature of both lipids. From our experiments we can conclude that artificial membranes are useful models to understand the physicochemical mechanisms involved in the interaction of dopamine antagonists with biological membranes.

Dopamine antagonists produce a wide variety of effects on biological membranes. In addition to their specific drug-receptor interaction (Seeman 1981) they have been shown to induce membrane expansion, membrane fluidization, alteration of transmembrane fluxes and inhibition of membrane excitability (Seeman 1972). Although it is generally thought that neuroleptics produce such effects by interacting with membranes, the molecular events accompanying changes in membrane properties are still largely unknown. In many classes of neuroleptics there is a good correlation between some of the non-specific membrane activities and their octanol/water partition coefficients, which are related to drug lipophilicity (Seeman 1972). It would be of interest to determine the partition coefficients of dopamine antagonists in pure lipid bilayers used as a model membrane system.

Dopamine antagonists are chemically diverse compounds that exhibit an amphipathic character, having two distinct domains, one hydrophobic and the other hydrophilic (Seeman 1972). It is assumed that the amphipathic molecules dissolve in the membranes by intercalation of their hydrophobic domains into the hydrophobic interior of the membrane, while the hydrophilic domains are positioned at the membrane aqueous interface (Conrad & Singer 1981). Attending to the pK_a of dopamine antagonists (Leysen & Gommeren 1981; Pauwels et al 1986), they are mostly positively charged at physiological pH. Therefore, their incorporation into the membranes will be affected by

electrostatic interactions with phospholipid polar head groups and hydrophobic interactions with fatty acid carbon acyl chains. The role of hydrophobic interactions in the partitioning of the drugs into the lipid bilayer can be clarified by using synthetic pure lipids which contain the same polar head group but differing in the characteristics of the aliphatic carbon chain.

In this study we determined the partition coefficients of four dopamine antagonists belonging to different chemical groups, diphenylbutylpiperidines (pimozide and fluspirilene), butyrophenones (haloperidol) and benzilamides (domperidone) into lipid bilayers formed from the synthetic pure lipids, which differ in carbon chain length and unsaturation.

Materials and Methods

Chemicals

Phosphatidylcholine (PC), was obtained from Avanti Polar Lipids (Birmingham, AL), and stored in a chloroform solution under nitrogen at −70°C. Dimyristoyl-, dipalmitoyl-, distearoyl- and dioleoylphosphatidylcholines (DMPC, DPPC, DSPC and DOPC, respectively), were obtained from Sigma Chemical (St Louis, MO). [³H]Haloperidol (sp. act. 15 Ci mmol⁻¹) was obtained from New England Nuclear; [³H]domperidone (sp. act. 60 Ci mmol⁻¹), [³H]pimozide (sp. act. 16·5 Ci mmol⁻¹) and [³H]fluspirilene (sp. act. 8·59 Ci mmol⁻¹) were kindly donated by Dr J. E. Leysen, Department of Biochemical Pharmacology, Janssen Pharmaceutics (Beerse, Belgium). Haloperidol, domperidone, pimozide and fluspirilene were obtained from Janssen Pharmaceutics

Correspondence: C. R. Oliveira, Laboratório de Bioquímica, Faculdade de Medicina, Universidade de Coimbra, 3049 Coimbra Codex, Portugal.

(Beerse, Belgium). The lipids had a purity of 99% except PC (purity 99·9%) and the drugs had a purity higher than 98·7%.

Preparation of liposomes

Multilamellar liposomes were prepared from pure synthetic lipids according to the method described by Bangham et al (1967).

Synthetic lipids were dissolved in chloroform and the solution was dried in a rotatory evaporator followed by high vacuum for at least 2 h. The dried mixture was hydrated with 15 mM Tris, pH 7·4 and stirred by vortex mixing six times for 30 s each time, at a temperature above the phase transition temperature of the lipids. The liposomes were allowed to equilibrate under nitrogen for 12 h at room temperature (21°C). The final phospholipid concentration after hydration was 130 μM.

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 (Antunes-Madeira & Madeira 1984).

[^{3}H]Haloperidol, [^{3}H]domperidone, [^{3}H]pimozide and [^{3}H]fluspirilene were incubated with liposomes (130 μM in lipid). ^{3}H-labelled drugs were added to unlabelled drugs to obtain the final concentration (1 μM for pimozide and fluspirilene, 15 μM for domperidone and 20 μM for haloperidol). Drug and lipid concentrations were selected according to drug solubility in order to obtain less than 10% of drug incorporation into the lipid phase. The samples were allowed to equilibrate at various temperatures for 30 min, this time being found sufficient for the maximal incorporation of the drugs into the lipid bilayer. Samples (0·5 mL) were rapidly filtered through Whatman GF/B filters, under vacuum. The filters were washed with 10 mL ice-cold buffer (15 mM Tris, pH 7·4). Radioactivity retained in the filters and total radioactivity in the samples were determined by liquid scintillation spectrometry (Packard 2000 Tri-carb). Automatic quenching correction was 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 40°C in 8·0 mL Triton X-100 scintillation fluid (Pasternak & Snyder 1974). The degree of retention in the filters was determined by measuring the amount of phospholipid retained, by first digesting the filters at 180°C in the presence of 1 mL 70% HClO$_4$ for 2 h (Bötcher et al 1961). The released inorganic phosphate was measured by the method of Bartlett (1959). The retention capacity of the filters was about 70% for liposomes. The fraction of lipid recovered in the filter was measured at each temperature and this value was used for the calculation of the partition coefficients. The partition coefficients were calculated from the fraction of drug retained in liposomes (p), according to the following equation (Connors 1967):

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

where K_p is the partition coefficient, V_L and V_A are the volumes of the lipid and aqueous phases, respectively. 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^{-1}). V_A is the volume of filtered sample (0·5 mL). In our experimental conditions, the equation can be rewritten as:

$$K_p = \frac{p}{1·526 \, L \, (1-p)} \times 10^6$$

were L is the amount of lipid in nmol.

Results

The effect of temperature on the incorporation of the drugs in model membranes of DMPC, DPPC and DSPC is shown in Fig. 1. These lipids have the same polar head group but differ in aliphatic chain lengths. For pimozide and fluspirilene, the values of partition coefficient (K_p) increase as the temperature increases, exhibit a maximum close to the phase transition temperature (T_c) and for DMPC and DPPC they decrease sharply above this temperature. For pimozide, maximal partitions were: 36 796 ± 1739, 14 607 ± 778 and 14 267 ± 768 for DMPC ($T_c = 24°C$), DPPC ($T_c = 42°C$) and DSPC ($T_c = 54°C$), respectively. Pimozide incorporation decreases below and above the mid-point temperature of the phase transition. If we compare pimozide partitioning into DSPC and DOPC at temperatures above the transition temperature, we observe that this drug incorporates better in the bilayers of DOPC. The partition coefficient for fluspirilene is also dependent on temperature, a maximal value being obtained within the temperature range of cooperative phase transition. Maximal partitions were: 49 208 ± 1091, 41 529 ± 1637 and 9363 ± 818 for DMPC, DPPC and DSPC, respectively.

Independently of the temperature, pimozide incorporates better in short aliphatic chain lipid bilayers. The correlation between the partition of pimozide (K_p) and the chain length of phospholipids (n) at temperatures 10°C below the mid-point of their phase transition can be described by the equation, $K_p = -2232n + 47142$. Similarly, fluspirilene incorporates better in bilayers of short aliphatic chain lipids and the dependence between K_p and the number of carbon atoms of aliphatic chains (n) is found to be linear ($r = -0·982$) according to equation $K_p = -2678n + 56666$. A higher incorporation of pimozide in DOPC than in DSPC is observed.

The incorporation of domperidone in the lipid bilayers of DMPC and DPPC is maximal around the phase transition temperature ($K_p = 826 ± 31$ for DMPC and $K_p = 420 ± 34$ for DPPC), and lower values are found below and above this temperature. For DSPC an increase in K_p values is obtained with increasing temperature ($K_p = 150 ± 14$ at 37°C and $K_p = 357 ± 24$ at 65°C).

For haloperidol the effect of temperature on its partition coefficient in DMPC is similar to that for domperidone. A direct correlation between the increase in K_p values and the increase of temperature is found for DPPC ($K_p = 450 ± 50$ at 32°C and $K_p = 1886 ± 125$ at 60°C) and DSPC ($K_p = 233 ± 73$ at 37°C and $K_p = 616 ± 75$ at 65°C). As for the drugs previously described, the partition of domperidone and haloperidol has the highest value in short aliphatic chain lipids. A good correlation ($r = -0·956$) was found between the partition of domperidone and the lipid chain length (n)
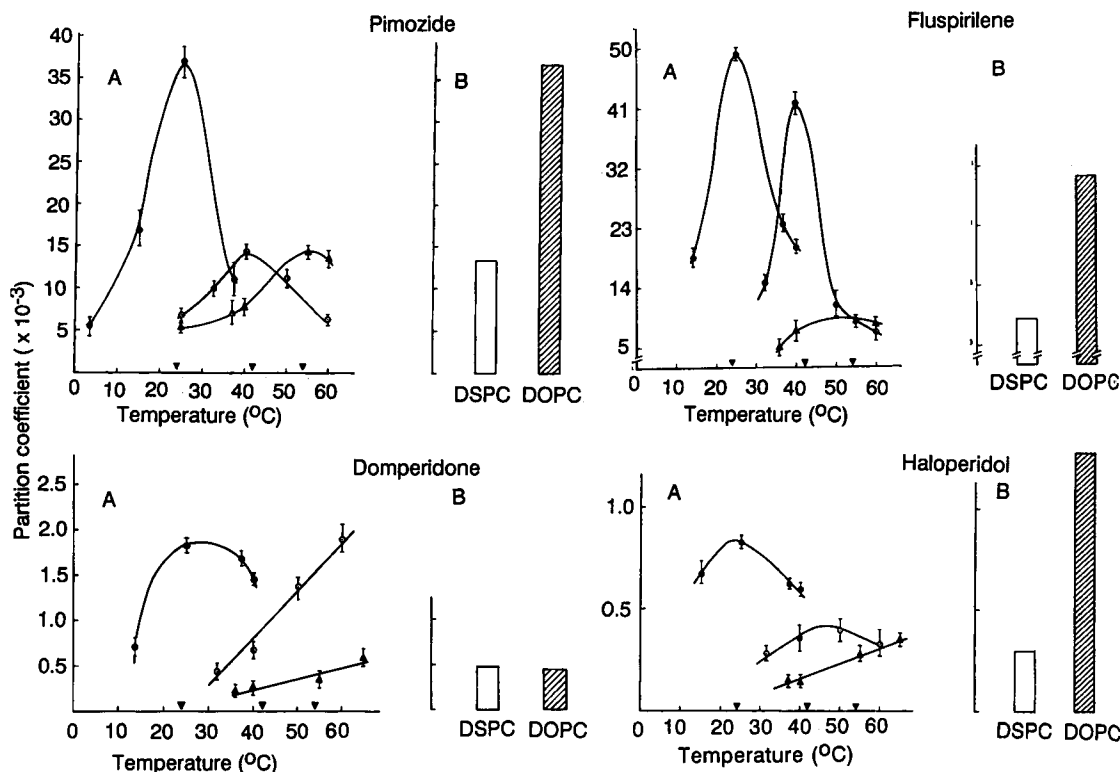
Fig. 1. A. Partition coefficients (K_p) of drugs into bilayers differing in aliphatic chain length, DMPC (●), DPPC (○) and DSPC (△), as a function of temperature. Maximal values of K_p are reached around the mid-point temperatures of the thermotropic phase transitions, 24, 42 and 54°C for DMPC, DPPC and DSPC, respectively, as indicated by the arrowheads. B. Partition coefficients of drug into bilayers differing in aliphatic chain unsaturation, DSPC ($T_c = 54°C$) and DOPC ($T_c = -22°C$) at 60 and 0°C, respectively. Partition coefficients were determined following a 30-min incubation of the samples containing lipid (130 μM), pimozide (1 μM), fluspirilene (1 μM), domperidone (15 μM) or haloperidol (20 μM). K_p values were calculated as described in experimental procedures. The values are the means ± s.d., of three independent experiments, each one run in triplicate.

according to equation $K_p = -118n + 2267$. For haloperidol, a linear dependence ($r = -0.999$) between K_p and n is observed as described by equation $K_p = -123n + 2461$.

Domperidone incorporates better in DOPC than in DSPC, while for haloperidol, partition coefficients in these two lipids do not change significantly.

The incorporation of the four dopamine antagonists in egg phosphatidylcholine bilayers relative to the buffer phase over the temperature range from 4 to 40°C is shown in Fig. 2.

For pimozide, fluspirilene and haloperidol, the partitioning of the drugs increases with increasing temperature, a slight optimum being observed for pimozide at about 35°C. In contrast, the partition of domperidone decreases as the temperature increases.

The K_p values over the referred temperature range vary from 23626 ± 1500 to 39343 ± 1967 for pimozide; from 23241 ± 1500 to 31283 ± 1250 for fluspirilene; from 2253 ± 250 to 3931 ± 257 for haloperidol; and from 2411 ± 236 to 1750 ± 237 for domperidone.

Discussion

In the present study, the membrane/buffer partition coefficients of the dopamine antagonists, pimozide, fluspirilene, haloperidol and domperidone were determined in artificial bilayers prepared from synthetic phosphatidylcholines, DMPC, DPPC, DSPC, DOPC and egg PC. The dopamine antagonists studied in this work belong to different chemical classes, their structure and some of the physicochemical properties being summarized in Table 1. The results show that drug incorporation is dependent on the lipid bilayer composition, membrane order and temperature.

The physical parameters of bilayers that determine the partition coefficients of the drugs are related to their lipid composition and they are modulated by temperature (Luxnat & Galla 1986). The results obtained with the synthetic saturated phosphatidylcholines, having the same polar head group but differing in aliphatic chain length, suggest that the drugs incorporate better in short aliphatic chain lipids. The K_p values are reduced in long-chain lipids (DSPC), which is easy to accept in the light of an increased chain-chain interaction that prevents amphipathic incorporation. Apparently, as the length of the fatty acid hydrocarbon chain increases, the voids which accommodate drug molecules decrease in number. Since short-chain lipids produce more fluid membranes as compared with those formed with long-chain lipids (Lenaz 1979), it appears that membrane order is one of the parameters which controls drug incorporation. Therefore, lipid chain length strongly affects amphipath solubility.
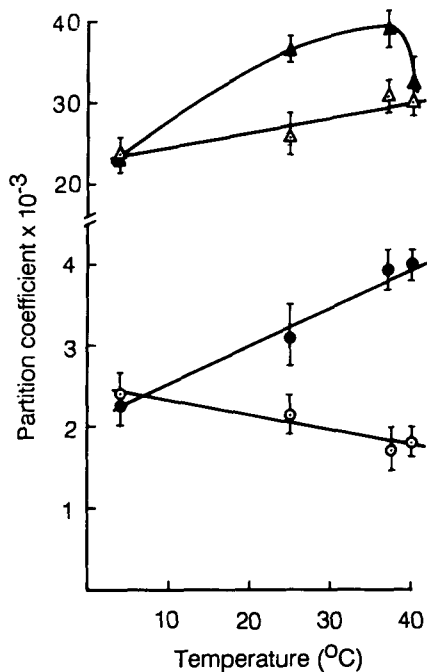
FIG. 2. Partition coefficients of pimozide (▲), fluspirilene (△), haloperidol (●) and domperidone (○) into phosphatidylcholine lipid bilayers as a function of temperature. Partition coefficients were determined following a 30-min equilibration period as described under Materials and Methods. The results are means±s.d. of triplicate determinations in three independent experiments.

The results obtained on the effect of temperature on K_p indicate that for all the drugs tested, pimozide, fluspirilene, domperidone and haloperidol, the incorporation is dependent on temperature. For the drugs studied, partitioning in DMPC is maximal within the temperature range of cooperative transition from the gel to the liquid crystalline stage (Fig. 1). This can be explained by the fact that within this temperature range, gel and liquid crystalline domains coexist in the bilayer and oscillation between the two phases appears to promote the incorporation of drugs (Antunes-Madeira & Madeira 1984, 1985, 1986; Müller et al 1986). Ordering oscillation in adjacent phases may create transient defects between disordered and ordered domains (Papahadjapoulos et al 1973), which would favour incorporation of drug molecules. Additionally, an enhancement of lateral compressibility and extensibility of the lipid at the phase transition may also contribute to the increased incorporation of the drugs (Shimshick & McConnell 1973; Phillips et al 1975). Above and below the mid-point temperature of the phase transition the partitioning of the drugs decreases. An explanation for this temperature-related effect may be due to the thermally-induced changes in the conformation of the phospholipid head group. It has been suggested that the N^+-end of the phosphocholine dipole of PC, which is parallel to the bilayer surface, becomes increasingly submerged in the hydrocarbon core with increased temperature. This results in lateral head-group repulsion and decreasing surface pressure (Dill & Stigter 1988). We have to consider that the drugs studied exist predominantly in the protonated form at the experimental pH (see Table 1). Thus, the association of drugs

Haloperidol

Domperidone

Pimozide

Fluspirilene

pK_a	% ionized
8·66[a]	94·79[a]
7·90[c]	75·97[c]
8·63[b]	94·44[b]
8·70[c]	95·23[c]

[a] Leysen & Gommeren (1981), [b] Pauwels et al (1986), [c] J. E. Leysen, Janssen Pharmaceutics, Beerse, Belgium.

with liposomes may be due, in part, to electrostatic interactions. Although a reduced surface pressure may be expected to favour drug incorporation into the bilayer, it may be that the altered conformation of the head group now predominates in terms of drug partitioning. Therefore, electrostatic repulsion may then occur between the protonated drug molecules and the N^+-end of PC submerged in the lipid bilayer. The importance of head group conformation is also evident through pre-transition temperature, an event known to involve head group rearrangements (Dill & Stigter 1988).

In DPPC a similar partitioning profile to that in DMPC is observed for pimozide, fluspirilene and domperidone. Although in DSPC the incorporation of pimozide and fluspirilene also increases with temperature, the sharp decrease in partitioning above the transition temperature does not occur. However, the partition of domperidone and haloperidol in DSPC shows a clear linear dependence on

temperature. These experimental data suggest other parameters than membrane order are determining the incorporation of drugs into the lipid bilayers. A comparison of the structures of the four dopamine antagonists (Table 1) suggests that thermotropic geometrical factors imposed by the molecular structure of the lipid and the molecular geometry of the drugs are also modulating drug incorporation.

The partition of pimozide, fluspirilene and haloperidol in egg phosphatidylcholine bilayers increases with increasing temperature (Fig. 2) while domperidone incorporates poorly at higher temperatures. As the phase transition temperature of egg PC is centred around 5°C, the lipid bilayers remain in the fluid state over the temperature range under study (4–40°C). We would expect that for all drugs, their incorporation in the PC bilayers would be larger at higher temperatures, since a more fluid state of the membranes would then be formed. This is clearly not the case for domperidone, which suggests once more that the geometry of drug molecules may also be involved in the accommodation of the drugs into the lipid bilayers.

If we compare the incorporation of the dopamine antagonists in DSPC (18:0) and DOPC (18:1) at temperatures above the mid-point phase transition temperature, we conclude that, except for haloperidol, their partitioning is higher in unsaturated bilayers. Since membrane fluidity increases with the degree of hydrocarbon unsaturation (Houslay & Stanley 1982), a potential relationship between partitioning and membrane order is evident.

We can conclude that partition coefficients are strongly influenced by the physical state of the membrane which may be modulated by temperature, and membrane composition. However, other physical parameters such as drug and lipid geometry may also contribute to drug accumulation and retention into the lipid bilayer.

Acknowledgements

References

Antunes-Madeira, M. C., Madeira, V. M. C. (1984) Partition of parathion in synthetic and native membranes. Biochim. Biophys. Acta 778: 49–56

Antunes-Madeira, M. C., Madeira, V. M. C. (1985) Partition of lindane in synthetic and native membranes. Biochim. Biophys. Acta 820: 165–172

Antunes-Madeira, M. C., Madeira, V. M. C. (1986) Partition of DDT in synthetic and native membranes. Biochim. Biophys. Acta 861: 159–164

Bangham, A. D., De Gier J., Greville, G. D. (1967) Osmotic properties and water permeability of phospholipid liquid crystals. Chem. Phys. Lipids 1: 225–248

Bartlett, G. R. (1959) Phosphorus assay in column chromatography. J. Biol. Chem. 234: 466–468

Bötcher, C. J. F., Van Gent, C. M., Pries, C. A. (1961) Rapid and sensitive submicrophosphorus determination. Anal. Chim. Acta 24: 203–204

Connors, K. A. (1967) In: Connors, KA. (ed.) A Textbook of Pharmaceutical Analysis. John Wiley & Sons, New York, pp 277–294

Conrad, M. J., Singer, S. J. (1981) The solubility of amphipathic molecules in biological membranes and lipid bilayers and its implications for membrane structure. Biochemistry 20: 808–818

Dill, K. A., Stigter, D. (1988) Lateral interactions among phosphatidylcholine and phosphatidylethanolamine head groups in phospholipid monolayers and bilayers. Biochemistry 27: 3446–3453

Houslay, M. D., Stanley, K. K. (1982) In: Houslay, M. D., Stanley, K. K. (eds) Dynamics of Biological Membranes. John Wiley & Sons, New York

Lenaz, G. (1979) The role of lipids in the structure and function of membranes. Subcell. Biochem. 6: 233–343

Leysen, J. E., Gommeren, W. (1981) Optimal conditions for [3H]apomorphine binding and anomalous equilibrium binding of [3H]apomorphine and [3H]spiperone to rat striatal membranes: involvement of surface phenomena versus multiple binding sites. J. Neurochem. 36: 201–219

Luxnat, M., Galla, H.-J. (1986) Partition of chlorpromazine into lipid bilayer membranes: the effect of membrane structure and composition. Biochim. Biophys. Acta 856: 274–282

Müller, H.-J., Luxnat, M., Galla, H.-J. (1986) Lateral diffusion of small solutes and partition of amphipaths in defect structures of lipid bilayers. Biochim. Biophys. Acta 856: 283–289

Papahadjapoulos, D., Jacobson, K., Nir, S., Isac, T. (1973) Phase transitions in phospholipid vesicles. Fluorescence polarization and permeability measurements concerning the effect of temperature and cholesterol. Biochim. Biophys. Acta 311: 330–348

Pasternak, G. W., Snyder, S. H. (1974) Opiate receptor binding: enzymatic treatment discriminate between agonist and antagonist interactions. Mol. Pharmacol. 10: 183–193

Pauwels, P. J., Leysen, J. E., Laduron, P. M. (1986) [3H]Bactrachotoxinin A20 -α-benzoate binding to sodium channels in rat brain: characterization and pharmacological significance. Eur. J. Pharmacol. 124: 291–298

Phillips, M. C., Graham, D. E., Hauser, H. (1975) Lateral compressibility and penetration into phospholipid monolayers and bilayer membranes. Nature 254: 154–155

Seeman, P. (1972) The membrane actions of anesthetics and tranquilizers. Pharmacol. Rev. 24: 583–655

Seeman, P. (1981) Brain dopamine receptors. Pharmacol. Rev. 32: 230–309

Shimshick, E. J., McConnell, H. M. (1973) Lateral phase separation in phospholipid membranes. Biochemistry 12: 2351–2360