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ETHYLMORPHINE–LIPID INTERACTION STUDY BY CALORIMETRY AND MONOLAYER TECHNIQUES

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ABSTRACT

Liposomes and monolayers were used as membrane models to examine the interaction of lecithin and the opioid agonist ethylmorphine. By calorimetric measurements, the ability of the drug to increase the fluidity of the lipid has been explained by electrostatic interaction between the drug and the polar headgroup of the lipid. Compression isotherms and penetration kinetics of the lipid monolayers in the presence of the drug confirmed the previous observations.

INTRODUCTION

The chemical basis of the interaction between opioids and their membrane receptors are far from being understood. However, considering that the opioid receptor is embedded in the bilayer structure of the nerve membrane, lipid involvement in such interaction is quite likely. Thus, lipid-induced modulation of opiate receptors in mitochondrial membranes have been reported [1]. In addition there is good evidence in favour of parallel phenomena associated with some hormone, bacterial toxin and neurotransmitter receptors [2–6].

As biochemical studies on opioid-receptor binding have demonstrated, two interaction modes between opioids and membrane homogenates take place. The first one is the proper opioid-receptor binding, called stereospecific binding, produced by the anchorage of the opioid molecules to the

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receptor sites. The second one results from physical adsorption of the opioids to the membrane surface on exposure of nerve membranes to opioids. In this study we are mostly interested in the second set of events, the so called non-specific binding.

The polar headgroups of the lipid constituents are in contact with the aqueous surrounding media and therefore contribute largely to the interfacial properties of such surfaces. The predominant headgroup present in biological membranes is phosphorylcholine, which forms the polar moiety of both sphingomyelin and phosphatidylcholine. In this way, when the opioids approach the plasma membrane, they encounter mostly phosphorylcholine groups. To study such interaction we have chosen monolayers and liposomes as model membranes.

Some of the best-studied models of biological membranes are those based upon monomolecular films of lipids. Such monolayers simulate the conditions at the membrane–water interface, and for this reason are suitable for study of the interaction between opioids and phosphorylcholine groups. By using a Langmuir film balance [7] the interaction of the monolayer components with the solutes included in the aqueous subphase may be examined through measurements of the lateral compressibility of the monolayer [8]. In this way, we have prepared compression isotherms of monolayers of natural lecithin in the presence of ethylmorphine, a typical opioid agonist.

When dispersed in water and heated above the temperature of their phase transition, phospholipids will spontaneously swell and form sealed vesicles or liposomes. They are as popular as monolayers for model systems used in the studies of biological membranes. By high sensitivity scanning calorimetry the interactions between liposomes and the ionic components in the surrounding media can be examined by measurement of the main bilayer phase transition of the phospholipid liposomes. We have prepared and studied by differential scanning calorimetry (DSC), liposomes of pure dipalmitoylphosphatidylcholine in the presence of ethylmorphine.

By comparing the results obtained from both model systems we expect to gain information on how opioids interact with biological membranes by the so-called non-specific binding.

MATERIALS AND METHODS

Lipids

Dipalmitoylphosphatidylcholine (DPPC) purchased from Fluka AG was puriss. grade (approx. 99.6%). Egg phosphatidylcholine (Merck) was purified by column chromatography on alumina [9]. By phosphate analysis [10] its estimated molecular mass was 789 Dalton.

Chemicals

Ethylmorphine hydrochloride was generously supplied by UQUIFA Laboratories; its purity was checked by HPLC and elemental analysis. For monolayer work, water was twice distilled over permanganate and passed through a Milli Q filtration system (Millipore). Its resistivity was always greater than 18 MΩ cm^{-1}, its pH was 5.5–6, and it was always freshly prepared. Chloroform (Merck, pro analysi) was used as spreading solvent.

Preparation of liposomes and monolayers

Liposomes were prepared from aliquots of 6 mg of DPPC and the appropiate weight of ethyl morphine to obtain a range of molar fractions. Each mixture was dissolved in chloroform/methanol (1 : 1 v/v) and dried in a rotary evaporator under nitrogen flow. The resulting film was further lyophilized for 3 h. Liposomes were formed by adding 150 μl distilled water to the film, vortexing for 3 min above the transition temperature of the lipid (60 °C) and shaking the dispersion for 1 h at 60 °C in a water bath.

Monomolecular films were prepared by spreading 5, 10, 25 and 100 μl of $CHCl_3$ solution (1 mg/ml) on the aqueous surface of the Langmuir trough and before compression, at least 10 min were allowed for solvent evaporation. Freshly prepared films were used for each run. Subphases were either pure water or 10^{-5} M ethylmorphine hydrochloride solutions. It is worthwhile noting that this subphase concentration is not far away from the physiological concentrations at which the drug shows activity. For penetration kinetic studies, monolayers were prepared similarly by spreading enough lipid to obtain monolayers at initial pressures of 5, 10, 15 and 20 mN m^{-1}.

Differential scanning calorimetry

Thermal transitions of liposome preparations were measured on a Mettler TA 3000 calorimeter equipped with a TC 10 processor, a DSC 30 cell cooled by liquid nitrogen and a Swiss dot matrix printer. Lauric and myristic acids were used as references to calibrate the temperature and the heat flow. Samples were prepared by loading and sealing 120 μl of each dispersion in 160 μl aluminium pans. Calorimetric measurements were performed in the range 10–55 °C at a scanning rate of 2 °C min^{-1} and a sensitivity of 1.716 mW full scale. Water was always used in the reference pan. For each sample at least four heating and cooling curves were recorded. The enthalpic changes were calculated from the main DSC peak area using a planimeter and an integration program for the Apple IIe computer.

Compression isotherms and penetration kinetics

Both experiments were conducted on a Langmuir film balance equipped with a Wihelmy plate as described by Verger and Hass [11]. The output of

the pressure pickup (Beckman LM 600 microbalance) was calibrated by recording isotherms of stearic acid. The teflon trough was regularly cleaned with hot chromic acid and rinsed with double-distilled water. Films were compressed at a rate of 4.2 cm min^{-1} at a temperature of $20 \mp 1°C$. All isotherms were run at least three times in the direction of increasing pressure. The penetration kinetic experiments were conducted at a fixed area of 124 cm^2 and at initial surface pressures of 5, 10, 15 and 20 mN m^{-1}. The pressure changes were recorded during 60 min after injection in the subphase of 5 mg of the drug.

RESULTS AND DISCUSSION

The heating DSC curves of DPPC liposomes containing ethylmorphine are reported in Fig. 1. By increasing the molar fraction of the drug up to $x = 0.6$, the main lipid transition shifted towards lower values. This effect is also seen when the average temperature values of the main DSC peak for the heating and cooling modes are plotted against the drug molar fractions (Fig. 2). Liposomes of higher molar fractions did not shift their transitions further showing heating profiles identical with those for $x = 0.6$. This molar fraction represents the saturation concentration of ethylmorphine in the liposome preparation. The gradual shift of the main peak temperature T_m towards lower temperatures should be explained by the electrostatic interaction of ethylmorphine with the head polar groups of DPPC, mainly at the bilayer surface. This is confirmed by the fact that the DPPC pretransitional peak

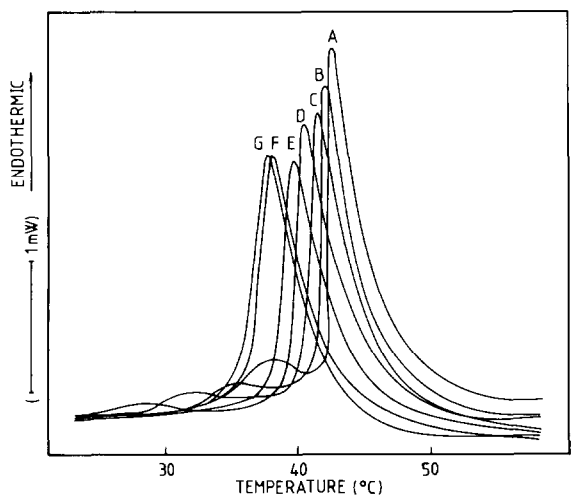

Fig. 1. Heating thermograms for DPPC samples in the presence of ethylmorphine. Molar fractions of ethylmorphine in the preparations are: A = 0, B = 0.06, C = 0.13, D = 0.24, E = 0.46, F = 0.60 and G = 0.70.

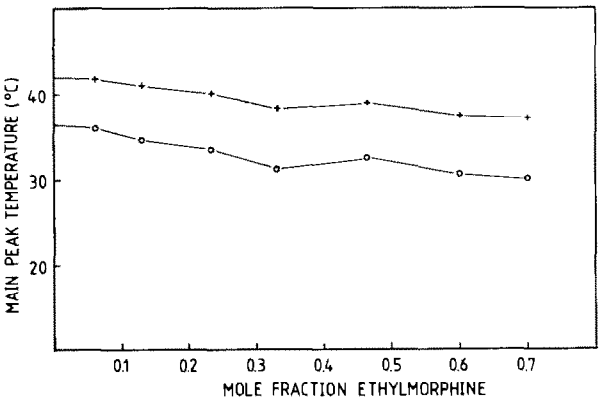

Fig. 2. Dependence of transition temperature (T_m) with the phospholipid phase transition when varying the molar fraction of ethylmorphine. Average values of four cooling (○) and heating (+) cycles are reported.

becomes gradually but noticeably reduced up to drug molar fractions of 0.23–0.33.

In addition, plots of main transition enthalpic changes ΔH (kcal mol^{-1}) as a function of the drug molar fractions for heating and cooling modes (Fig. 3) do not show significant variations, thus proving the previous hypothesis that the interaction between the ethylmorphine and DPPC does not greatly concern the bilayer. This is in good agreement with studies by Chapman and co-workers [12] conducted with other morphine-like drugs. They have shown that those drugs which penetrate the interior of the lipid bilayer and disrupt the chain packing cause a lowering of the heat of the lipid phase transition, whereas those which remain at the surface of the

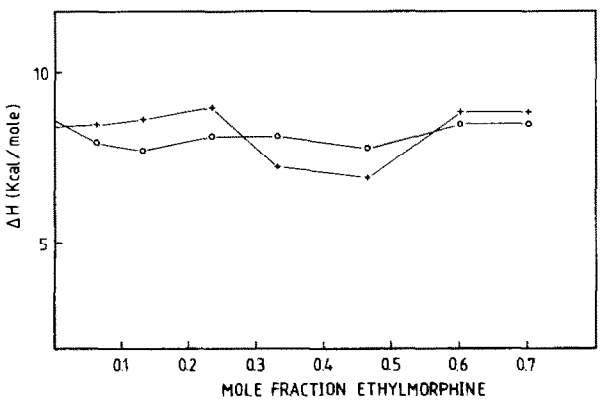

Fig. 3. Enthalpic changes of the main lipid transition for the DPPC–water systems as a function of ethylmorphine molar fractions. Average values of four cooling (○) and heating (+) cycles are reported.

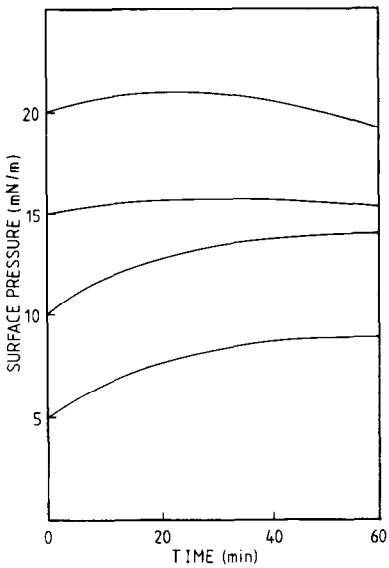

Fig. 4. Penetration patterns of ethylmorphine on phosphatidylcholine monolayers recorded at four initial pressures.

bilayer and interact electrostatically with the polar headgroups of the lipid primarily affect the transition temperature.

In summary our calorimetric experiments indicate that a major disruption of the lipid chain packing by interdigitation of the ethylmorphine is not occurring, although superficial penetration within the area of the polar headgroup of the lipid may still be possible. Lecithin monolayers either over pure water or ethylmorphine solutions, on each of the surface concentrations assayed, yielded identical compression isotherms (data not shown). These results suggest the absence of interfacial interactions between lecithin and ethylmorphine during the compression process. Previous monolayer studied with morphine and naloxone [13] led to similar results.

Examining the kinetic curves (Fig. 4), a rather slow penetration of the drug even at low initial pressure can be observed. A plateau is reached not before 40 min. The highest penetration value was $\Delta\pi = 5.2$ mM m^{-1} ($\pi_i = 5$ mN m^{-1}) after 1 h. Moreover, plots of penetration values ($\Delta\pi$) as a function of initial pressure (data not shown) exhibit a direct correlation: however, a pressure decay after 40 min for experiments conducted at $\pi_i = 15$ and 20 mN m^{-1} can be observed.

This monolayer work clearly shows interaction between the lipid monolayer and the drug at the air/water interface. The nature and extension of such an interaction can be explained on the basis that ethylmorphine is not able to modify lecithin isotherms but shows penetration during the kinetic experiments. As we have shown, in the absence of lipid, ethylmorphine (due to its hydrophilic character at the pH values of our experiments) does not

form stable monolayers and lacks surface activity. Thus, the interaction will be essentially electrostatic in nature, as the drug by itself can only approach the polar moiety of the lipid monolayer.

A weak electrostatic interaction could account for the negligible effect of the drug on the compression isotherms. However, the penetration observed could be, primarily, the result of the change in the long-range organization in the monolayer structure of the polar headgroups induced by the electrostatic bounded drug molecules. A further interaction resulting from the drug reaching the hydrophobic moiety of the monolayer, leading to a monolayer with a mixed lipid–drug packing is very unlikely to take place.

In conclusion, it is of interest to note that the monolayer and calorimetric results reported herein can reasonably be explained by the same interaction mode: this can be of help when considering the basic interaction of drugs with cell membranes which support their receptors. More precisely, since membrane fluidity is known to affect, at least, the function of membrane proteins [14] and lipid fluidization results in loss of specific binding [1], lipid microviscosity no doubt plays an important role in membrane–drug binding. In this way, the ability of the drug examined in the present study to lower the lipid transition temperature, and therefore by implication increase the fluidity of the lipid, may suggest that the membrane fluidity modulated by non-specific binding may also be important in mediating the effects of opioids upon opioid receptors.

Finally, we hope the present study will help toward a better understanding of the biochemical and biophysical nature of the drug-membrane interaction which is needed for a better understanding of the phenomenon of drug recognition and action.

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