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INTERACTION OF BUPRENORPHINE WITH PHOSPHATIDYLCHOLINE

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ABSTRACT

The interaction between phosphatidylcholine and buprenorphine have been studied by means of differential scanning calorimetry of liposome, compression isotherms and penetration kinetics of monolayers. The results show that the interactions are highly dependent on the ionized state of the buprenorphine molecules, being higher in buffered solutions.

INTRODUCTION

Buprenorphine is a member of the oripavine series that exhibits both opioid agonist and antagonist effects in laboratory animals, depending on the dose administered [1,2]. Due to this dual character and to the fact that it does not induce physical dependence, it has been proposed as an effective pharmacotherapy for heroin addiction [3].

Structure–activity relationship studies on opioid molecules have shown that hydrophobicity is an important factor in determining their opioid activity. This fact correlates with the presence of lipids in the opioid receptor structure [4–6]. Moreover, several authors have shown that membrane microviscosity affects the affinity of opioid molecules for their receptors [7]. These facts had given support to the idea that membrane fluidity may play an important role as a catalyst for drug–receptor interactions, serving as an antenna to orientate active molecules to better interact with their receptors [8,9]. In this respect, it would be worthwhile to investigate the interactions between opioid molecules and those lipids commonly found in biological membranes.

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The present work was undertaken in order to determine the interactions between buprenorphine and phosphatidylcholine. The study covers differential scanning calorimetry (DSC) of dipalmitoyl phosphatidylcholine (DPPC) liposomes containing different drug levels. As a complementary membrane model, the penetration process of this molecule on PC monolayers and the compression isotherms of PC spread on subphases containing buprenorphine have been determined.

EXPERIMENTAL

Chemicals

DPPC obtained from Sigma was puriss. grade and used without further purification. Egg phosphatidylcholine (Merck) was purified by column chromatography on alumina [10], and its molecular mass (789 Daltons) was determined by phosphorus analysis [11].

Buprenorphine hydrochloride was kindly supplied by Lab. Esteve (Barcelona). The purity was checked by elemental analysis and HPLC.

Water employed for surface studies was distilled twice over permanganate and passed through a MilliQ filtration system. Its resistivity was always greater than 18 $M\Omega\ cm^{-1}$. The pH was 5.5–6, and it was always freshly prepared.

Acetic acid and sodium acetate were obtained from Merck. The corresponding buffer has pH 7.4 and conductivity 17.9 $mS\ cm^{-1}$.

Chloroform (Merck, pro analysis) was used as the spreading solvent.

Methods

Preparation of liposomes

From a standard solution of DPPC in chloroform of 6 $mg\ ml^{-1}$, samples were prepared containing 6 mg of phospholipid and different volumes of buprenorphine solution. The system was freeze dried and samples were rehydrated by adding 150 μl of distilled water. The system was gently shaken and heated at 60 °C for 1 h.

Calorimetric analysis

Calorimetric analyses were performed with a differential scanning calorimeter (Perkin–Elmer DSC-2 with intracooler). Weighed amounts of the liposomal samples were sealed in stainless steel pans. For each sample several scans were performed, in both heating and cooling modes between 0 and 50 °C with heating rates of 5 °C min^{-1}. Indium was used as the calibration standard.

Monomolecular films

Monolayers were prepared by spreading 25 μl of a lipid/chloroform solution of 1 mg ml^{-1} on the water surface using a Hamilton syringe. Before compression at least 10 minutes were allowed for solvent evaporation. The monolayer was then compressed by a Teflon barrier and the pressure increases were recorded. The measurements were done at $21 \pm 1°C$.

The subphases were pure water or buprenorphine solutions (10^{-5}M–2×10^{-4} M).

Compression isotherms

Compression isotherms were carried out using a Wilhelmy-type film balance, described by Verger and de Haas [12]. Force measurements were made with a Sartorius balance and standardized against stearic acid isotherm. The dimensions of the Teflon trough were $28.4 \times 17.45 \times 0.625$ cm. Before each measurement the Teflon trough was thoroughly cleaned with methanol and distilled water. The platinum plate was cleaned by immersion in sulphochromic acid and rinsed with methanol and distilled water. Every determination was carried out in at least triplicate.

Penetration kinetics

Penetration kinetics were performed by spreading the necessary amount of lipids to obtain monolayers of initial pressures of 5, 10 and 20 mN m^{-1}. The surface of the Teflon trough was 124 cm^2. Different volumes of a 24 mM buprenorphine solution were injected into the subphase with a Hamilton syringe to attain buprenorphine concentrations in the range of 10^{-5}–2×10^{-4} M. Subphases were constantly stirred with a small magnet to ensure a homogeneous distribution of the drug molecules.

For comparative purposes the same experiments were carried out in the absence of lipid monolayer to determine the surface activity of buprenorphine.

RESULTS AND DISCUSSION

The effect of increasing buprenorphine concentration on the thermotropic phase transition of DPPC for heating and cooling processes is shown in Fig. 1 (a and b).

Both exothermic and endothermic transitions exhibit a small broadening process compared with pure phospholipid, but this effect does not increase with the amount of drug present. The transition temperatures, T_m, when plotted as a function of molar buprenorphine content (Fig. 2a), show a slightly decreasing trend. The pretransition is completely abolished.

The enthalpy change of the transition (Fig. 2b) and the increase in the half-height width ($\Delta T_{1/2}$) (Fig. 2c) seem to be nearly independent of the

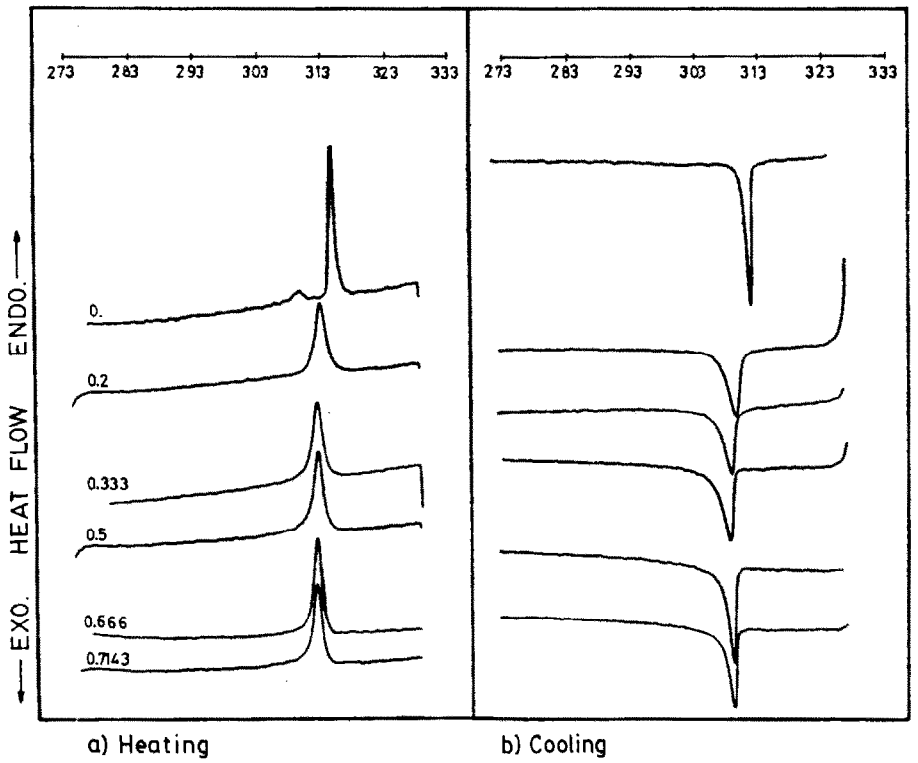


Fig. 1. DSC scans of DPPC liposomes containing varying amounts of buprenorphine. Molar fractions for buprenorphine are indicated on the curves.

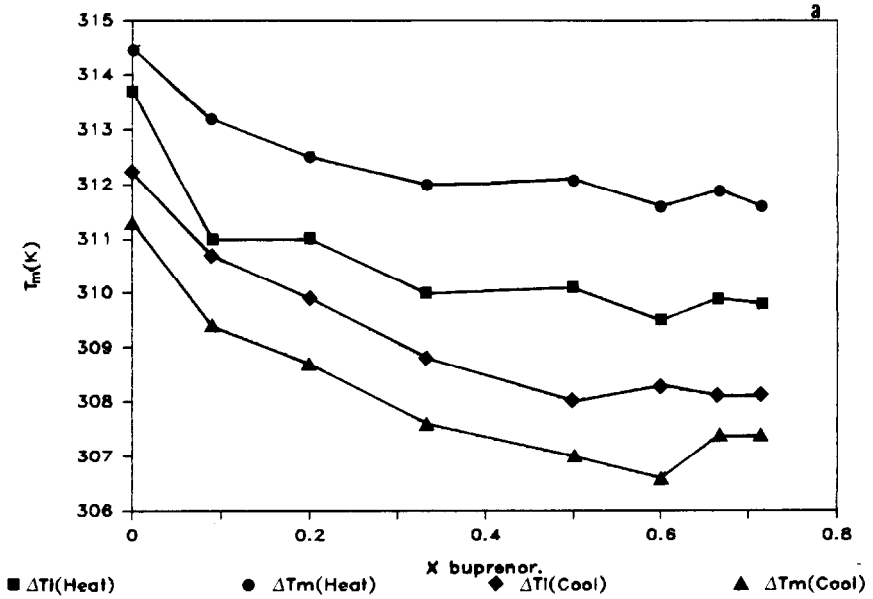
amount of drug added. If the absolute values associated with these changes are compared to those described in the literature for other molecules, it is clear that bupernorphine interacts only very slightly with DPPC molecules.

Classical thermodynamic theory predicts that a solute in a bulk solvent will change its T_m. Thus, by applying Raoult's second law to two different concentrations, C_1 and C_2 of the solute in the same solvent, the change in melting temperature can be expressed as

$$T \approx \frac{RTm^2}{H}(C_1 - C_2)$$

where H is the enthalpy change of the transition from gel to liquid crystalline and C_1 and C_2 are the concentrations of the solute in the liquid crystalline and gel phases respectively. In the case of lipid bilayers, T_m will increase or decrease depending on the partitioning of the solute in the gel or liquid crystalline phases. In the present case, as T_m decreases, it can be assumed that buprenorphine partitions preferentially in the liquid crystalline domains. Considering the chemical structure of buprenorphine, the two hydroxyl groups, tertiary amine and four methyl groups give the molecule an amphiphilic character. It has been established that those compounds that

penetrate the interior of the lipid bilayer and disrupt the chain packing, result in a lowering of the enthalpy of the lipid phase transition, and in contrast, compounds that remain near the surface of the bilayer interact



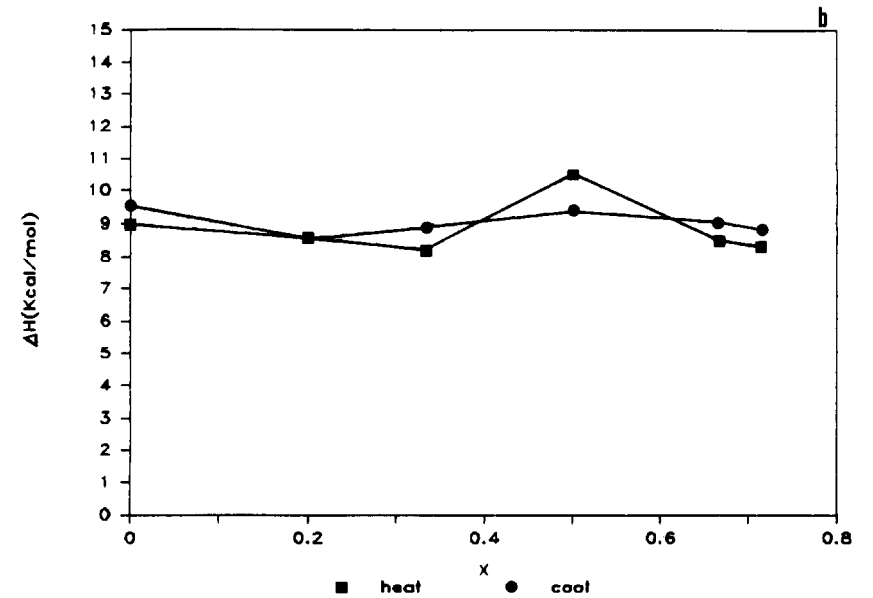


Fig. 2. Dependence of (a) transition temperature (T_m); (b) enthalpic changes (ΔH); and (c) height/half height width (H/HHW), associated with the phospholipid phase transition for different molar fractions of buprenorphine in the liposome preparations. (continued over page.)

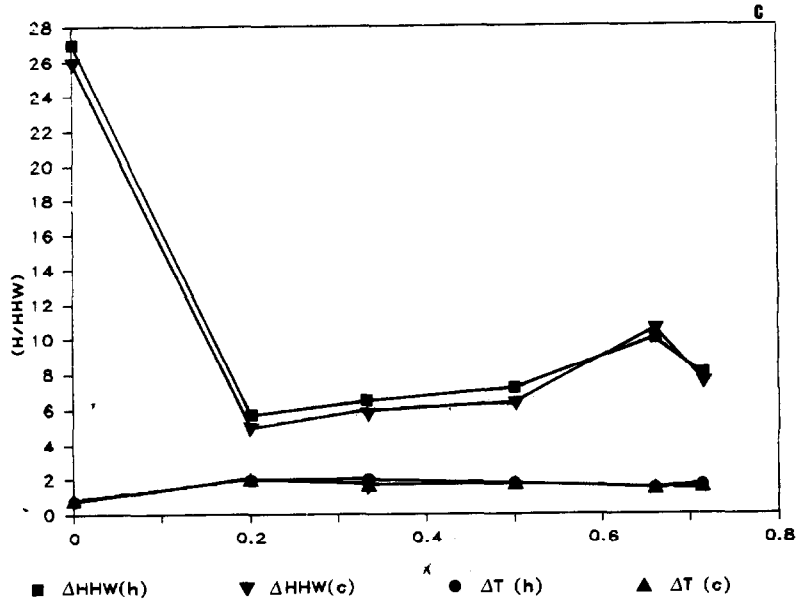


Fig. 2 (continued).

electrostatically with the polar head groups of lipids and primarily affect the transition temperature. The results reported here would suggest that buprenorphine interacts with polar head groups by hydrogen bonding, thus lowering the transition temperature. This kind of interaction has also been postulated for the hydroxyl groups of vitamin E [13], and of general and local anaesthetics. The increase in the width of the transition would probably indicate that a reduction in the size of the cooperative unit of lipids participating in the acyl-chain phase transition, had occurred. Nevertheless as the magnitude of these changes is very small ($\Delta T_{1/2\max} = 2°$ C) we can hypothesise that buprenorphine molecules, as in the cases of cholesterol [14] and dehydroergosterol [15], may form buprenorphine-enriched domains. These domains are laterally separated from the phospholipid domains and most of the buprenorphine molecules are not in physical contact with the phospholipid molecules, their influence on the transition temperature being smaller than theoretically expected.

Monolayers

The pressure/area isotherms of pure DPPC monolayers spread on distilled water compared with those spread on buprenorphine solutions are given in Fig. 3. Drug lipid ratios ranged from 100 : 1 to 2000 : 1. Pure DPPC monolayers exhibit a change in slope, corresponding to the expanded–condensed liquid phase transition at about 10 mN m^{-1}. The presence of increasing amounts of buprenorphine in the subphase modifies the slope of

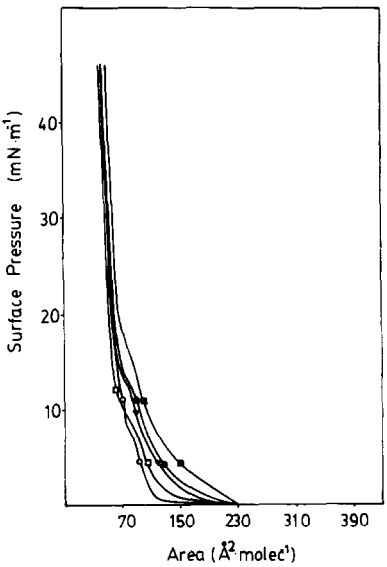


Fig. 3. Compression isotherms of DPPC monolayers spread on subphases containing different amounts of buprenorphine: ○, 0; □, 10^{-5}; ▽ 5×10^{-5}; ●, 10^{-4}; ■, 2×10^{-4}.

the isotherms moving this transition to higher pressures and higher areas/molecule. Moreover, the effect of buprenorphine when the surface pressure increases to 20 mN m^{-1} becomes insignificant except for the highest buprenorphine concentration. In this case, the isotherm corresponding to a Bup/PL ratio of 2000:1 has an area/molecule value approximately 15 $Å^2$ higher. This behaviour suggests that at low surface pressures buprenorphine hydrochloride molecules can interact electrostatically and hydrophobically with DPPC monolayers, a certain percentage of them being included in the monolayer, but as the pressure increases these molecules are gradually excluded from the monolayer. When there is a very great excess of buprenorphine with respect to phospholipid, a small percentage of the molecules remain in the monolayer thus rendering the high pressure isotherms parallel but not superimposable.

This low interaction level is in agreement with the results previously described from DSC experiments.

Penetration kinetics

The adsorption of buprenorphine to the air/water interface is reflected in an increase of surface pressure with time and with an increase of drug concentration in the subphase. This process is non-linear, reaching a plateau within several minutes (not shown). Nevertheless, a delay of 15 min was left between two consecutive additions of drug solution into the subphase in order to be sure that equilibrium had been reached.

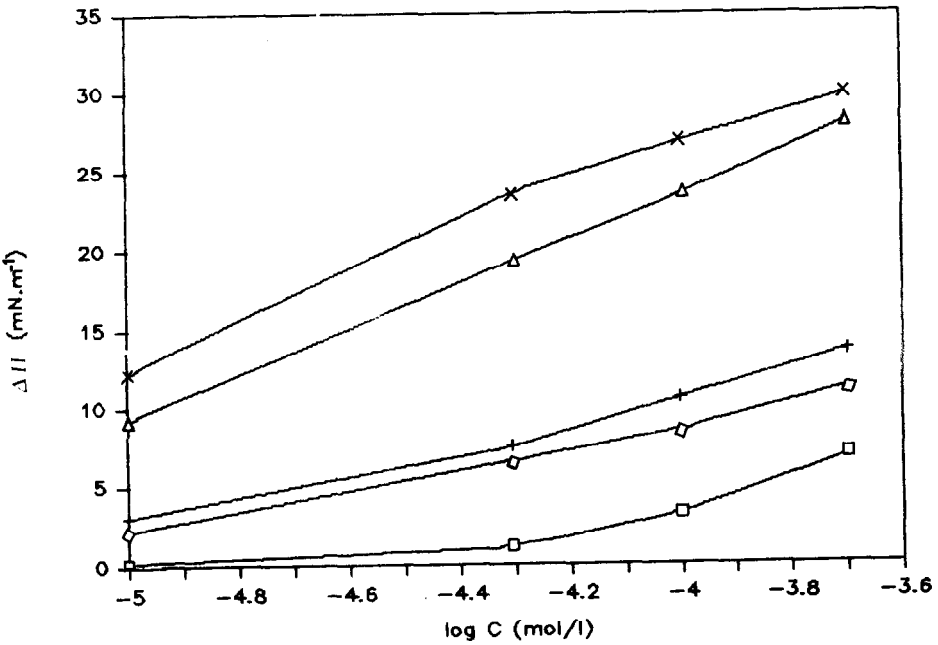


Fig. 4. Increase in maximum value of surface pressure as a function of initial film pressure: □, 0 mN m^{-1}; +, 5 mN m^{-1}; and ◇, 20 mN m^{-1} (subphase pure water); △, 0 mN m^{-1}; and ×, 5 mN m^{-1} (subphase acetate buffer pH 7.4).

The penetration of buprenorphine molecules into a lipid film of phosphatidylcholine was accompanied by a notable increase of surface pressure. This increase was a function of the initial film pressure. The kinetics of the process were similar to those in previous experiments without a monolayer. Figure 4 represents the pressure increases as a function of buprenorphine concentration in the subphase. The values are highly dependent on the initial pressure of lipid, being lower for more compressed monolayers.

The binding of water-soluble substrates to a lipid monolayer may be due to hydrophobic interactions for non-charged and partially hydrophobic molecules, and to electrostatic interactions for polar or ionizable groups. Buprenorphine in the hydrochloride form contains a charged tertiary amine and can establish electrostatic interactions, but due to its structure it can also behave as a hydrophobic molecule and penetrate into the hydrophobic core of the lipid monolayer.

Moreover, phosphatidylcholine is a neutral phospholipid having in its polar head both negatively and positively charged groups that can interact with ionized molecules, but that mainly neutralize each other. For this reason, the interactions between molecules and phosphatidylcholine are primarily of a hydrophobic character.

On the other hand, determinations of hydrophobicity parameters carried out in our laboratory, showed important differences between octanol/water

coefficients of buprenorphine hydrochloride depending on the pH of the aqueous solutions. Therefore the penetration studies were repeated using buffered solutions as subphases. The penetration pattern of buprenorphine in lipid monolayers and its surface activity represented as a function of drug concentration is given in Fig. 4. It can be clearly appreciated that suppressing the ionization of the amine group increases the hydrophobic character of the molecule. The pressure increases due to the charged buprenorphine molecules suggest an intercalation of the hydrophobic part of the molecules between the fatty acyl chains while the tertiary ammonium group remains in the lipid–water interface. The higher values obtained for buffered buprenorphine solutions agree with this idea as these solutions have much more intense hydrophobic interactions.

The low interaction level detected between phosphatidylcholine/buprenorphine molecules contrasts with previous results obtained in our laboratory for phosphatidylinositol/buprenorphine [16], suggesting that monolayers can provide a valuable model for the detection of specific and non-specific interactions.

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