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Phospholipid–opiate interactions measured by differential scanning calorimetry and compression isotherms of monolayers

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Abstract

The interactions between synthetic lecithins (DMPC and DPPC) and meperidine, methadone and naloxone were determined by means of differential scanning calorimetry and monomolecular layers. The results of the calorimetric measurements show that only for the most hydrophobic molecules do hydrophobic interactions have a significant value. Naloxone and meperidine interact mainly electrostatically with the polar head groups of DMPC and DPPC. Similar behaviour was observed when studying the compression isotherms of PC lipid monolayers in the presence of these opiates and the penetration kinetics of the same molecules in monolayers. Moreover the differently ordered states of the molecules in monolayers, gel phase (DPPC) or liquid crystalline phase (PC) at room temperature greatly influences their interaction with opiate molecules.

INTRODUCTION

The involvement of phospholipids in the stereospecific binding of opiate ligands to brain membranes has been demonstrated by several authors [1,2]. But the role phospholipids play in this interaction has not been completely elucidated. Some evidence suggests the existence of specific interactions between phosphatidylserine [3,4], cerebroside sulphate [5,6] or phosphatidylinositol [7,8], and opiates. Nevertheless it has not been clearly demonstrated that these lipids are part of the opioid receptor. As well as this approach, other authors suggest only a static role for phospholipids. In this respect, Heron et al. [9] have found that by incubating brain membranes with PC, and thus changing their microviscosity, the binding of the opioid

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molecules was clearly reduced. In this case, the lipids would serve as regulators for the coupling between the three-dimensional structure of the receptor and the opioid molecules.

The present study was undertaken to determine the influence of opioid molecules on the ordered state and transition temperature of natural and synthetic phosphatidylcholine molecules using liposomes and monolayers as membrane models.

MATERIALS AND METHODS

Chemicals

The opiate drugs used were kindly supplied by the following laboratories: methadone hydrochloride (Laboratories Dr. Esteve), meperidine hydrochloride (Bayer) and naloxone hydrochloride (Abelló, S.A.). Their purity was checked by HPLC and elemental analysis. The water used for the surface studies was twice distilled over permanganate and passed through a Mili Q filtration system (Millipore); its resistivity was always greater than 18 M cm^{-1}, its pH was 5.5–6 and it was always freshly prepared. Chloroform (Merk, pro analysi) was used as the spreading solvent. The phospholipids DMPC and DPPC were from Sigma. Egg phosphatidylcholine (Merck) was purified by column chromatography on alumina [10], and its molecular mass determined by phosphate analysis [11] was 789 dalton.

Sample preparation

The liposomes were prepared from standard 6 mg ml^{-1} solutions of DPPC and DMPC; the samples contained 6 mg of phospholipid and different volumes of opiate solutions. The system was lyophilised, 150 μl of pure water was added and the samples were vortexed and heated at 60 °C for 1 h in a water bath.

Differential scanning calorimetry

Calorimetric studies were performed with a Perkin–Elmer Calorimeter, DSC-2 equipped with a PC processor. Scans were made at 5 °C min^{-1} against an empty sealed reference pan. All samples were heated and cooled at least three times. The temperature range was 10–55 °C with a sensitivity of 2 m cal s^{-1}, full scale. Transition enthalpies were determined from the area under the peak. Indium was used as reference to calibrate the temperature and the heat flow. Samples were prepared by loading 15–20 mg of each dispersion in aluminium pans.

Monomolecular films

The monomolecular films were prepared by spreading 25 μl of a chloroformic solution (1 mg ml^{-1}) of PC or DPPC on the aqueous surface of a Langmuir trough, as described elsewhere [12]. Before compression, at least 10 minutes were allowed for solvent evaporation. Freshly prepared films were used for each run. Compression isotherms were performed on a Langmuir film balance equipped with a Wilhelmy platinum plate. The monolayers were compressed at 4 cm min^{-1} at a temperature of 21 ± 0.5 °C. The subphases were either pure water or 10^{-4} M solutions of naloxone hydrochloride, meperidine hydrochloride or methadone hydrochloride. It should be noted that this subphase concentration is similar to physiological ones.

Penetration studies

Penetration studies were carried out as described in ref. 13, by injecting sequentially increasing amounts of opiate solutions into the subphase. Pressure changes were recorded 15 minutes after each addition.

RESULTS AND DISCUSSION

Calorimetric studies

The effect of increasing the concentration of the opioid drug on the thermotropic behaviour of DMPC appears to be highly dependent on the chemical structure of the drug molecule.

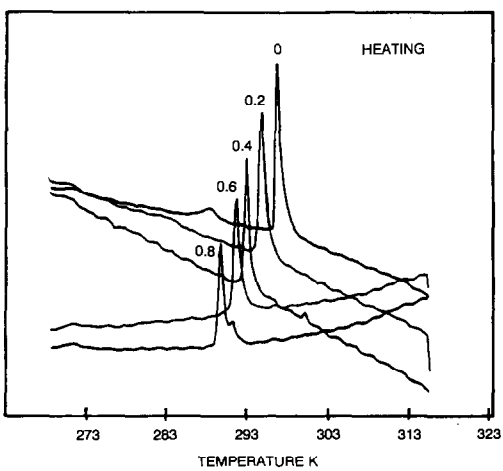

Fig. 1. Calorimetric heating curves for pure DMPC and DMPC–meperidine mixtures. Molar drug fractions are indicated on the curves.

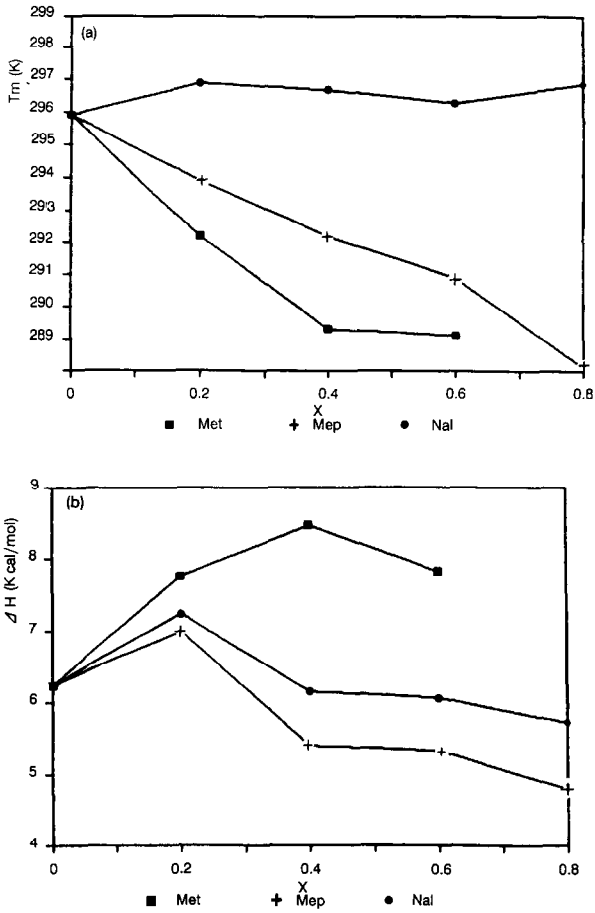

Fig. 2. Effect of opiates on: (a) T_m (°C) and (b) ΔH (kcal mol^{-1}), on the main phase transition of DMPC vesicles.

The thermograms for meperidine are given in Fig. 1. In general, for all three molecules under study, the phospholipid pretransition disappears and the peaks do not broaden significantly.

Moreover, for methadone and meperidine, a decrease in the transition temperature as a function of the drug concentration can be observed; in the case of methadone, it seems to reach a saturation level at around a molar fraction of 0.4. The presence of naloxone somewhat modifies the transition temperature. These results are given in Fig. 2a. As far as the enthalpy changes are concerned, meperidine and naloxone behave in a similar manner giving small ΔH increases or decreases at low and high drug contents, respectively. In contrast, the presence of methadone produces positive variations of enthalpy, although the absolute values are also small.

The lowering of the phase transition temperature suggests that these molecules increase the fluidity of the hydrocarbon chain region of DMPC

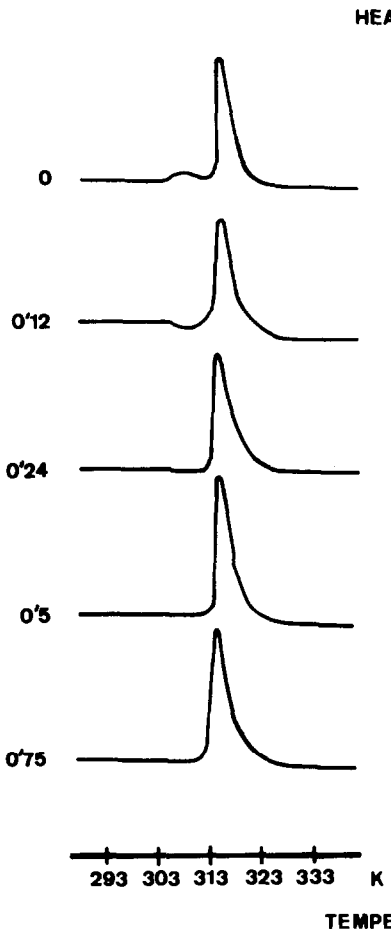

Fig. 3. Calorimetric heating curves for pure DPPC and DPPC–methadone mixtures. Molar drug fractions are indicated on the curves.

but as the transition width does not vary, the size of the cooperative unit of lipids participating in the acyl-chain phase transition has probably not been modified [14].

According to the theory proposed by Chapman et al. [15], the molecules under study will interact electrostatically with the polar head groups of the DMPC, and remain near the surface of the bilayer, thus affecting the transition temperature.

The small enthalpic changes support this suggestion; a similar weak interaction was found for ethylmorphine [16].

To determine if the length of the alkyl chain in the phospholipid affects the above cited interactions, a new set of experiments was carried out with DPPC and the same drugs. The thermograms corresponding to mixtures of DPPC and methadone are given in Fig. 3. The behaviour as far as T_m and

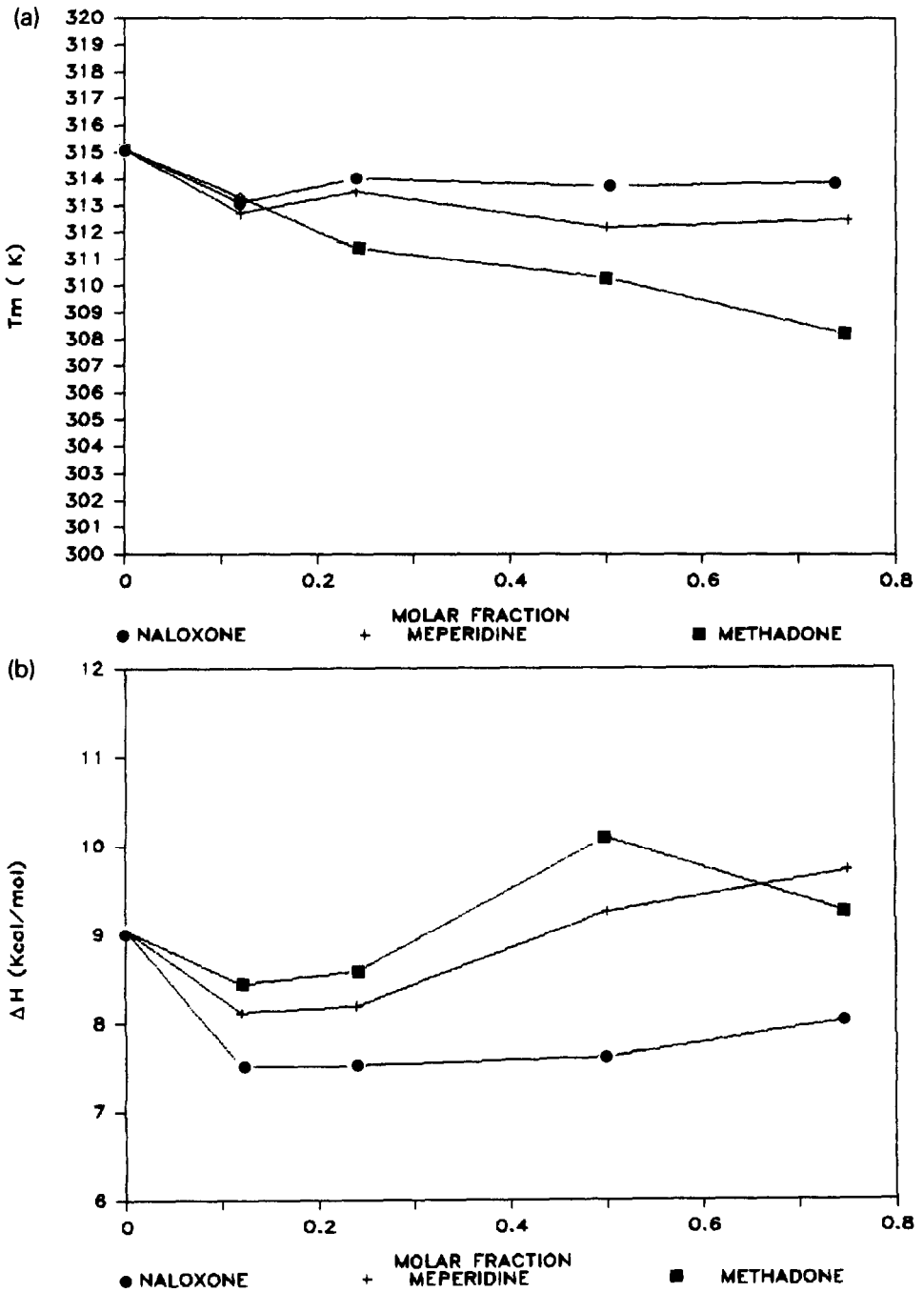

Fig. 4. Effect of opiates on: (a) T_m (°C) and (b) ΔH (kcal mol^{-1}), on the main phase transition of DPPC vesicles.

ΔH variations are concerned is similar to those previously reported for DMPC; the same is true for meperidine and naloxone (not shown).

Moreover, the differences between the extreme values obtained for T_m are of the same order, thus suggesting a predominant ionic interaction that is independent of the alkyl-chain length of the phospholipids. Methadone has the highest interaction with DMPC and DPPC, probably due to its more hydrophobic character.

All these results are summarised in Fig. 4a and 4b.

Surface studies

Compression isotherms

Compression isotherms of DPPC and PC monolayers were carried out with the opioid molecules being dissolved in the aqueous subphase. The results (Fig. 5) show that the presence of these molecules in the subphase does not modify the shape of the compression isotherms of PC. Nevertheless, the monolayers are more expanded than on pure water over the entire compression process. Methadone gives the highest expansion, this effect being more evident at low pressures. Moreover, the area/molecule values at high pressures show a constant difference of 10–15 $Å^2$ $molec^{-1}$ for the three opiates with respect to the monolayer of PC on pure water, thus suggesting a stable incorporation of opiates into the monolayer.

To better compare the results obtained using the DSC techniques with the monolayer studies, the same experiment described above was carried out with monolayers of DPPC. On compression, this phospholipid undergoes a phase change at around 8–10 mN m^{-1} when spread on pure water. The presence of opiates in the subphase decreases the area/molecule values over all the compression process. Moreover, there were no great differences among solutions of the different opiates. This behaviour is also independent of the speed of the compression process (Fig. 6). The different behaviour of the phospholipids DPPC and PC with the molecules under study is due to their different ordered states at room temperature. The transition temperature of PC is around $-5\,°C$; therefore, at $21\,°C$ the molecules of this lipid are in a fluid or liquid-crystalline phase. The permeability of monolayers in this state is very high, and the organic molecules dissolved in the subphase are able to intercalate between the phospholipid molecules.

The opposite is true for DPPC monolayers. At room temperature the molecules of this lipid are in a lamellar crystalline (lc) state or gel phase. The molecular packing properties of lipids in this phase are similar to those of anhydrous lipid crystals. In this system opiate molecules have the same effect as cholesterol when mixed with phosphatidylcholine. But in this case no changes in the compressibility of the monolayer could be observed, thus ruling out a hydrophobic interaction. The compression can be explained

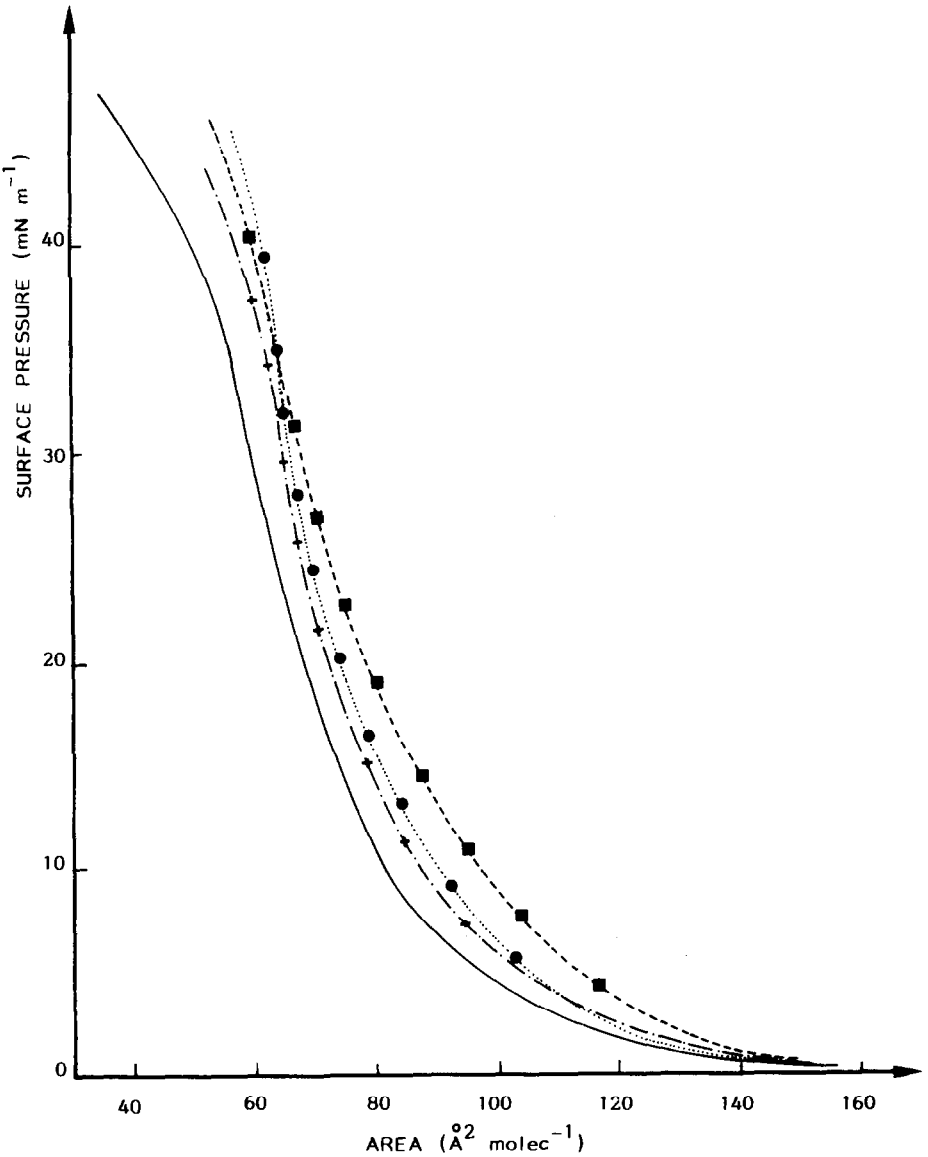

Fig. 5. Compression isotherms of PC monolayers spread on subphases containing: ——, pure water; ●, naloxone; +, meperidine; and ■, methadone.

assuming that phosphatidylcholine has a partial excess of negative charge on pure water due to the second ionization of the phosphate group; this can lead to some repulsion between the polar heads of the phospholipid. The presence of opiates that are cationic molecules could neutralise these charges and remove the repulsive forces.

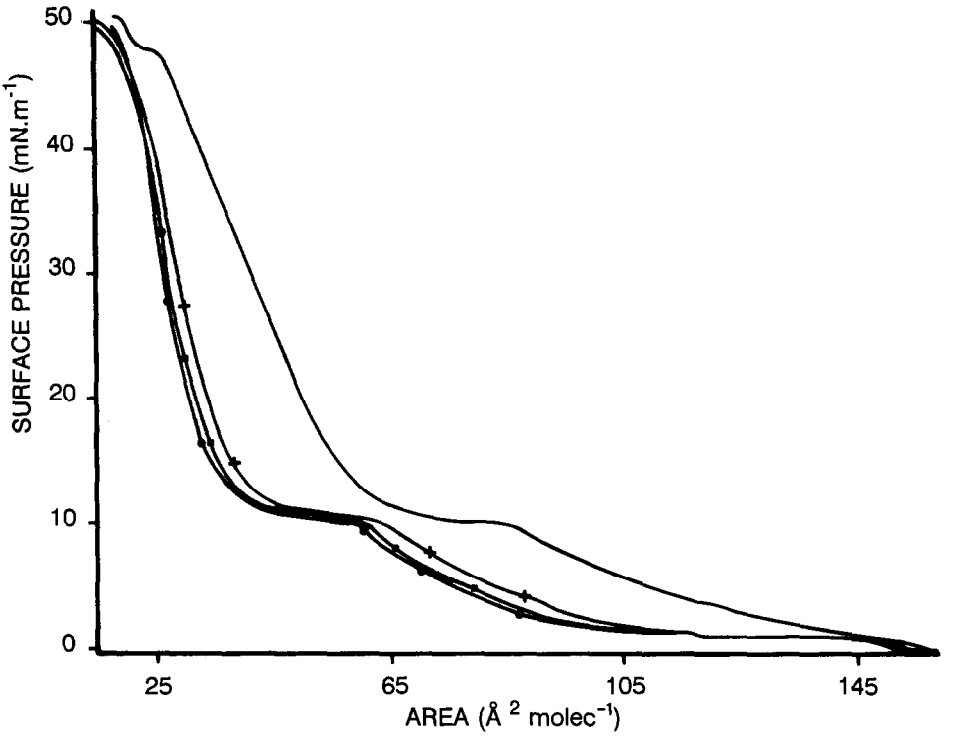

Fig. 6. Compression isotherms of DPPC monolayers spread on subphases containing: ——, pure water; ■, methadone; ●, naloxone; and +, meperidine (concentration of drugs in the subphase 10^{-4} M).

TABLE 1

Surface pressure increases measured after injection of opiates at increasing concentration under monolayers of DPPC spread at different initial surface pressures

Drug	Conc. (M)	Initial surface pressure (mN m^{-1})		
		5	10	20
Meperidine	10^{-5}	1.00	0.75	0.05
	5×10^{-5}	1.80	1.70	1.20
	10^{-4}	2.00	2.00	1.50
	2×10^{-4}	2.70	2.70	2.50
Methadone	10^{-5}	1.50	0.75	1.20
	5×10^{-5}	3.00	2.70	2.70
	10^{-4}	4.70	4.20	4.20
	2×10^{-4}	6.00	6.00	6.00
Naloxone	10^{-5}	0.50	0.50	0.50
	5×10^{-5}	2.00	1.20	1.20
	10^{-4}	2.70	2.21	2.03
	2×10^{-4}	3.12	3.01	2.71

TABLE 2

Surface pressure increases measured after injection of opiates at increasing concentration under monolayers of PC spread at different initial surface pressures

Drug	Conc. (M)	Initial surface pressure (mN m^{-1})		
		5	10	20
Meperidine	10^{-5}	0.20	0.0	0.25
	5×10^{-5}	0.80	0.20	0.25
	10^{-4}	1.25	0.40	0.30
	2×10^{-4}	2.10	0.40	0.37
Methadone	10^{-5}	3.28	1.20	0.15
	5×10^{-5}	6.40	2.45	1.10
	10^{-4}	8.63	3.30	1.65
	2×10^{-4}	10.70	4.40	2.50
Naloxone	10^{-5}	0.65	0.22	0.0
	5×10^{-5}	1.0	0.75	0.0
	10^{-4}	1.40	1.25	0.0
	2×10^{-4}	1.82	1.90	0.37

Penetration kinetics

The most easily measured parameter in lipid–molecule interactions is the change in surface pressure at constant surface area. Pressure increases are interpreted as a result of the penetration of hydrophobic residues of the molecule under study in between the lipid chains. The surface pressure increases of DPPC and PC monolayers show a linear increase with the opiate concentration in the subphase (Tables 1 and 2). Moreover, these values are dependent, to different degrees, on the initial surface pressure. This behaviour is in agreement with the above-cited two states of both molecules at 21°C. As in the thermal analysis, the most hydrophobic molecule is the one giving the highest interactions (methadone). The limiting pressure, defined as the pressure at which the molecules can no longer penetrate and at which there is no change in surface pressure, is 20 mN for meperidine and naloxone in PC.

The experimental results obtained in the present work show that the interactions of methadone, meperidine and naloxone with phosphatidylcholine are predominantly electrostatic in nature and are highly dependent on the ordered state of the molecules in the monolayer.

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