A Physicochemical Study of the Interaction of Phosphatidylinositol with Buprenorphine and Naloxone[1]

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Abstract

The interactions of two opioid molecules (buprenorphine and naloxone) with phosphatidylinositol and phosphatidylcholine were studied in lipid monolayers at the air–water interface. The influence of Na^+, Ca^{2+}, and Mn^{2+} ions in these interactions has also been determined. Neither buprenorphine nor naloxone influence the ordered state of phosphatidylcholine monolayers. On the contrary, both opioid molecules interact specifically with phosphatidylinositol monolayers. The area/molecule of phosphatidylinositol spread on buprenorphine containing subphases is highly affected by this molecule and also by ions. The phosphatidylinositol/naloxone interactions are rather weak and less affected by ions.

Key Words: Monolayers; phosphatidylinositol; naloxone; buprenorphine; opioid receptor.

Introduction

It has become well established from pharmacological and membrane–ligand binding approaches that the opioid receptor system encompasses multiple subtypes (Lahti *et al.*, 1985; Miller and Shaw, 1985; Zajac and Roques, 1985), but the molecular basis of this receptor heterogeneity has remained obscure. Although recent reports have described the successful

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[1] Abbreviations: PI, phosphatidylinositol; PC, phosphatidylcholine; Bup, Buprenorphine; Nx, naloxone; ODS, octadecyl silica; k', capacity factor; log P, partition coefficient between octanol and water.

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isolation, purification, and preliminary characterization of opioid receptors, its chemical composition is still uncertain (Gioannini *et al.*, 1984; Newman and Barnard, 1984; Kullman, 1984). Like many other receptors, the opioid receptor has a protein nature; however, there is growing evidence of the involvement of phospho- and glycolipids in its structure and function (Abood *et al.*, 1978; Dennis, 1980). The role of phospholipids in the opioid system can be considered from two standpoints: the lipid as an integral part of the opioid receptor or as being in the membrane surrounding the receptor.

Supporting the first case, a preferential interaction of neutral and acidic phospholipids such as PI, PS, and gangliosides with β-endorphins and enkephalins by NMR has been reported (Behnam and Deber, 1984; Jarrell *et al.*, 1980). With regard to the second approach, it is interesting to note that Heron *et al.* (1981), when comparing physicochemical and biochemical membrane properties, found that membrane fluidity affects the binding of the opioid molecules to its receptors. Hence, the microviscosity of the membrane modifies the number of binding sites and the affinity of the opioid ligand. Moreover, PI has been related to nerve functions such as transmission of action and rapid synaptic response to neurotransmitters (Hayashi *et al.*, 1984). Thus, the cholinergic PI effect is the best known, but a number of other chemical transmitters have been shown to influence PI metabolism. Among these, many amphiphilic cationic drugs inhibit the phosphatidate phosphorylase reaction. In addition, Mulé (1966), incubating guinea pig cerebral cortex slices with ^{32}P and high concentrations of morphine, obtained increased labelling of PI. This effect was further studied by Hawthorne and Pickard (1979), who found that it could not be linked to opioid receptor involvement since the antagonist naloxone failed to prevent it. However, the same authors have found that levorphanol, a morphine-related molecule, deppresses the ^{32}P labelling of PC, PE, and PI. Considering these facts, it is worthwhile to study the type of interactions that may exist between PI and opioid molecules in order to ascertain whether they can be related to the opioid phenomena or not.

Two interaction modes between opioids and biological membranes take place. One is the proper opioid–receptor binding or stereospecific binding as a result of the anchorage of the opioids to receptor sites. The other, the so-called nonspecific binding, is produced by physical absorption of the opioids to membrane surfaces.

In the present work, we attempted to mimic what happens in biological systems by differentiating nonspecific interactions from the specific ones. In this way, we have chosen the monomolecular films of phospholipids as a membrane model to tackle this problem. The main advantage of this technique is that the characteristics of the interface can be easily modified, enabling a detailed study, at the molecular level, of opioid–lipid interactions. Thus by

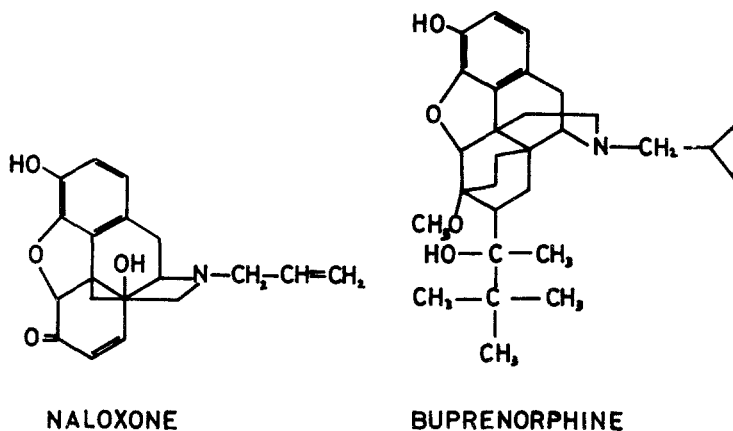NALOXONE   BUPRENORPHINE

Fig. 1. Molecular structures of the opioid molecules involved.

means of the compression isotherms, we have examined the interaction of a key phospholipid such as PI with a partial agonist like buprenorphine and the antagonist naloxone (Fig. 1). We attempted to obtain a correlation between the interactions encountered at the monolayers with the stereospecific binding characteristics of these molecules. In addition, we have conducted a parallel monolayer study with a major component of biological membranes such as PC in order to better correlate nonspecific binding features with opioid–lipid interactions. As the affinity of opioid molecules in binding assays is greatly modified by the presence of ions, we have also investigated whether the influence of Na^+, Ca^{2+}, and Mn^{2+} on this membrane model correlates with its biochemical behavior.

Materials and Methods

Plant phosphatidylinositol, potassium salt, was purchased from Supelco (Pennsylvania). Purity was checked by TLC, using coated silica gel 60 plates (Merck) and chloroform/methanol/ammonia (4 N) (9:7:2, v/v/v) as a developing system. A single spot was obtained after spraying with sulfuric/chromic acid and charring. The content of phosphorous was determined after perchloric acid digestion (Barlett, 1958). Its estimated molecular mass was 856 Daltons. Egg yolk lecithin was obtained from Merck. It was purified by column chromatography on alumina using $CHCl_3/MeOH$ (9:1, v/v) as eluent (Singleton et al., 1965). The estimated molecular mass was 787 Daltons. Buprenorphine and naloxone hydrochlorides were kindly provided by Lab. Dr. Esteve (Barcelona) and Abelló S. A. (Madrid), respectively.

Purity was assessed by elemental analysis. Premium grade NaCl, $CaCl_2$, $MnCl_2$, and Tris-HCl were obtained from Merck. Tris-buffer was 0.05 M, pH = 7.0. Water for the Langmuir film balance was prepared by distillation over potassium permanganate of single-distilled water on glass apparatus. Unless specified otherwise, no chemicals other than the opioids and the chlorides were added to this water, its resistivity was always higher than $16\,M\Omega/cm$, its pH was 5.5–6, and it was distilled every day. Chloroform (Merck, proanalysi) was used as spreading solvent. PI was spread from chloroform solutions of approximate concentrations of 0.4 mg/ml.

Surface-area measurements were performed on a Langmuir film balance equipped with a Wilhelmy platinum plate as described by Verger and de Haas (1973). The output of the pressure pickup (Beckman LM600 microbalance) was calibrated by recording the well-known isotherm of stearic acid. This isotherm is characterized by a sharp phase transition at $25\,mN \cdot m^{-1}$ for pure water at 20°C. The teflon trough (surface area $495\,cm^2$, volume 309.73 ml) was regularly cleaned with hot chromic acid and rinsed with double-distilled water. Films were spread on the aqueous surface from a micosyringe, and at least 10 min was allowed for solvent evaporation. Films were compressed at a rate of 4.2 cm/min; a change in the compression rate did not alter the shape of the isotherms. All isotherms were run at least four times in the direction of increasing pressure with freshly prepared films. The accuracy of the system under the conditions in which the bulk of the reported measurements were made was $\pm 0.5\,mN \cdot m^{-1}$ for surface pressure.

The stability of PI monolayers was assessed by preparing monolayers with different initial surface pressure (5, 10, and $15\,mN \cdot m^{-1}$) and recording the pressure decay. As the surface pressure decrease, after 60 min, was $2.5\,mN \cdot m^{-1}$ for films spread at $\pi = 10$ and $15\,mN \cdot m^{-1}$, we considered the monolayers stable. In order to verify if these pressure changes could be due to a partial solubilization of the monolayer, we have compared compression isotherms registered after 3 and 60 min of PI extension. In both cases the slopes of the isotherms were identical. Moreover, the differences detected were not significant, as found by recording compression and decompression cycles. The concentration of opioid molecules in the subphase was always $10^{-5}\,M$.

All measurements with PI were made at a subphase temperature of $21 \pm 1°C$ and at a pH of 5.5–6. As we previously determined, no further pH adjustment was necessary because PI films were not influenced by pH changes in the range 5–6. The influence of opiates and salts on the surface tension of the suphase in which they are dissolved has also been examined. In the range of opiate and salt concentrations of our experiments we were unable to detect any differences from the surface tension of pure water. In this way we can be sure that differences between lipid isotherms measured on pure

water and opiate solutions are really due to the interactions between lipid and opioid molecules.

Partition coefficients were determined as follows: 10 ml of 2 mM drug solutions (in distilled water) were shaken manually with an equal volume of *n*-octanol for 15 min. The mixture was centrifuged and the content of buprenorphine and naloxone in the aqueous layer was quantified by HPLC analysis. Analytical conditions were: column ODS 5 μm; eluent, acetonitrile/water (0.05% TFA) (45 : 55); K'(Bup) = 24; K'(Nal) = 2.07; log P-buprenorphine = 1.068; log P-naloxone = −1.121.

Results

Compression isotherms of both phospholipids PI and PC on pure water and opioid-containing subphases are reported in Fig. 2. In general, PI produced more expanded monolayers than PC. Moreover, the presence of opioid in the subphase accounts for differences between isotherms in each series. Whereas PC monolayers are slightly affected, PI monolayers are expanded by opioids. This effect is greatest for buprenorphine, specially at lower pressures. The effect of monovalent cations such as Na^+ on PI monolayers can be observed in Fig. 3. A clear expanding process is exhibited as a consequence of increasing Na^+ concentrations (10^{-3}, 10^{-2}, 10^{-1}, and 1 M) in the suphase, reaching saturation at 1 M NaCl. Similar experiments conducted with PC in the presence of Na^+ show no differences when compared to isotherms on pure water (data not shown).

The simultaneous influence of sodium and opioids on PI compression isotherms can be observed in Fig. 4. In the range of concentrations assayed (10^{-3}, 10^{-2}, 10^{-1}, and 1 M), Na^+ is able to reverse the expanding effect induced by buprenorphine. Most notably, however, Na^+ ions expand PI monolayers in the presence of naloxone.

On the same monolayer conditions, the PI effect of divalent cations of biological relevance has also been considered. First, the influence of Ca^{2+} and Mn^{2+} on PI monolayers has been examined. In contrast to Na^+, divalent cations produce a small condensing effect on PI isotherms (data not shown). Second, as Fig. 5 shows, compression isotherms of PI monolayers on subphases containing opioids and either Ca^{2+} or Mn^{2+} have also been recorded. But since Ca^{2+} and Mn^{2+} effects are very similar, we have only represented those corresponding to Ca^{2+}. The simultaneous interaction of divalent cations and buprenorphine with PI induces film condensation, which is cation concentration dependent. Although an expanding effect is the final result of the lipid–opioid interaction, in the case of naloxone the effects are not as remarkable.
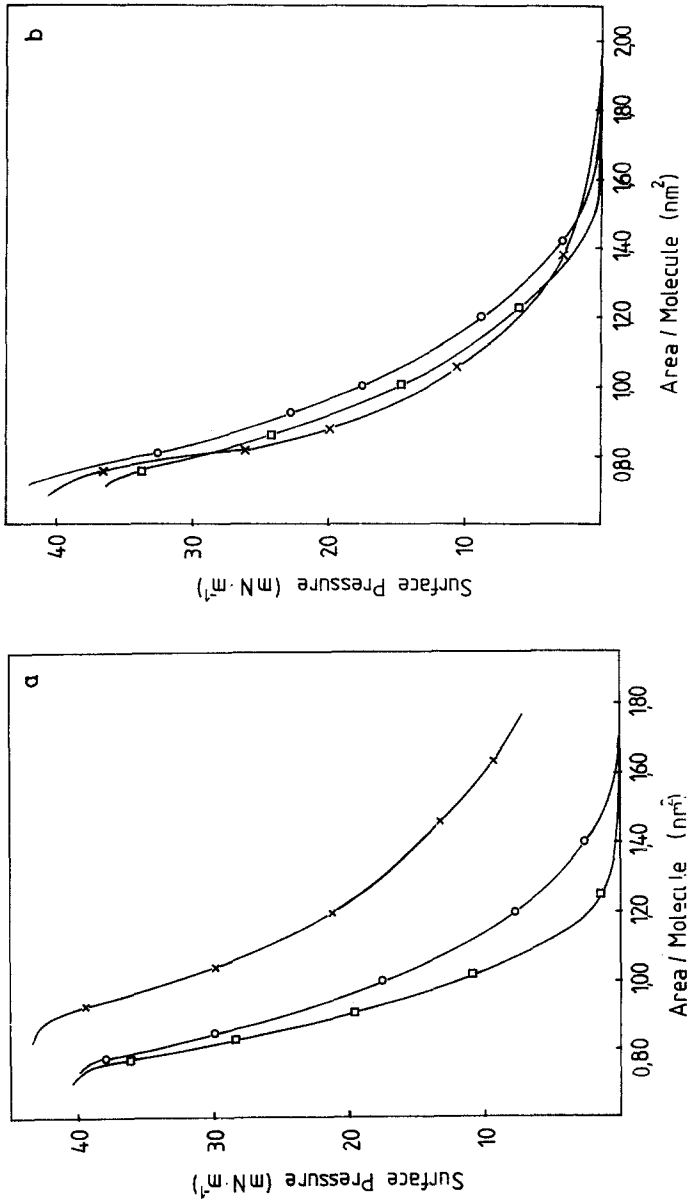
Fig. 2. Surface pressure vs. area per phospholipid molecule curves of phosphatidylinositol and phosphatidylcholine. (a) Compression isotherms of phosphatidylinositol on subphases of: (×) 10^{-5} M buprenorphine, (O) 10^{-5} M naloxone, and (□) pure water. (b) Compression isotherms of phosphatidylcholine on subphases of: (×) 10^{-5} M buprenorphine, (O) 10^{-5} M naloxone, and (□) pure water.
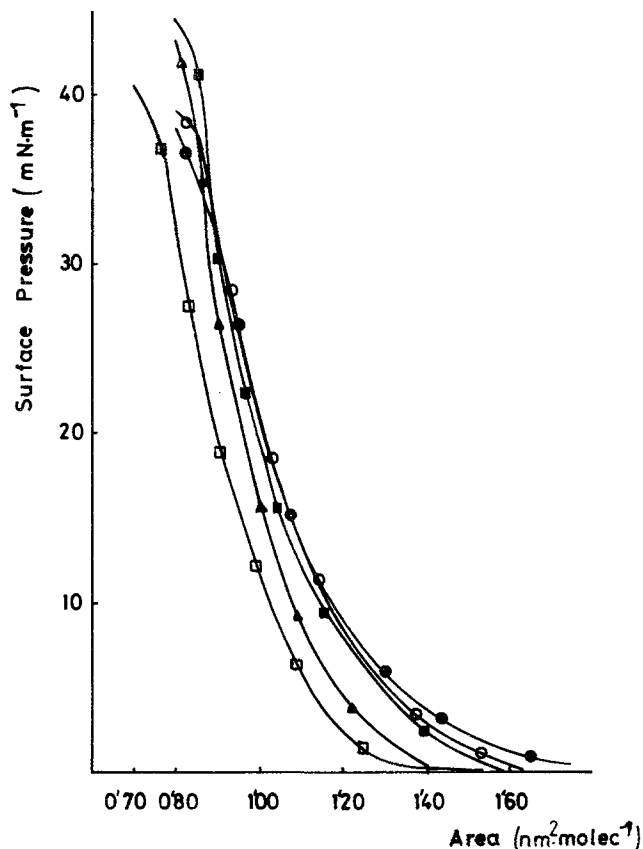
Fig. 3. Effect of sodium ion concentrations on phosphatidylinositol monolayers. The surface pressure vs. area curves are shown for the following concentrations: (●) 1 M NaCl, (○) 10^{-1} M NaCl, (■) 10^{-2} M NaCl, (▲) 10^{-3} M NaCl, and (□) pure water.

A parallel set of experiments has been performed with PC monolayers, and in all cases there were no significant differences among the compression isotherms spread on the subphases described. In order to obtain experimental conditions that more closely approximates biological conditions, we studied monolayers of PI spread over subphases including opioids plus mono- and divalent cations. Isotherms of PI monolayers spread on subphases lacking opioids but containing mixtures of Ca^{2+} or Mn^{2+} with Na^+ are practically unaffected by the ionic content (data not shown). However, the presence of opioids leads to isotherms highly dependent on the ionic composition of the subphase. Thus, Fig. 6 clearly shows the condensing effect induced by sub-phases containing a pool of ions and buprenorphine. On the contrary, under
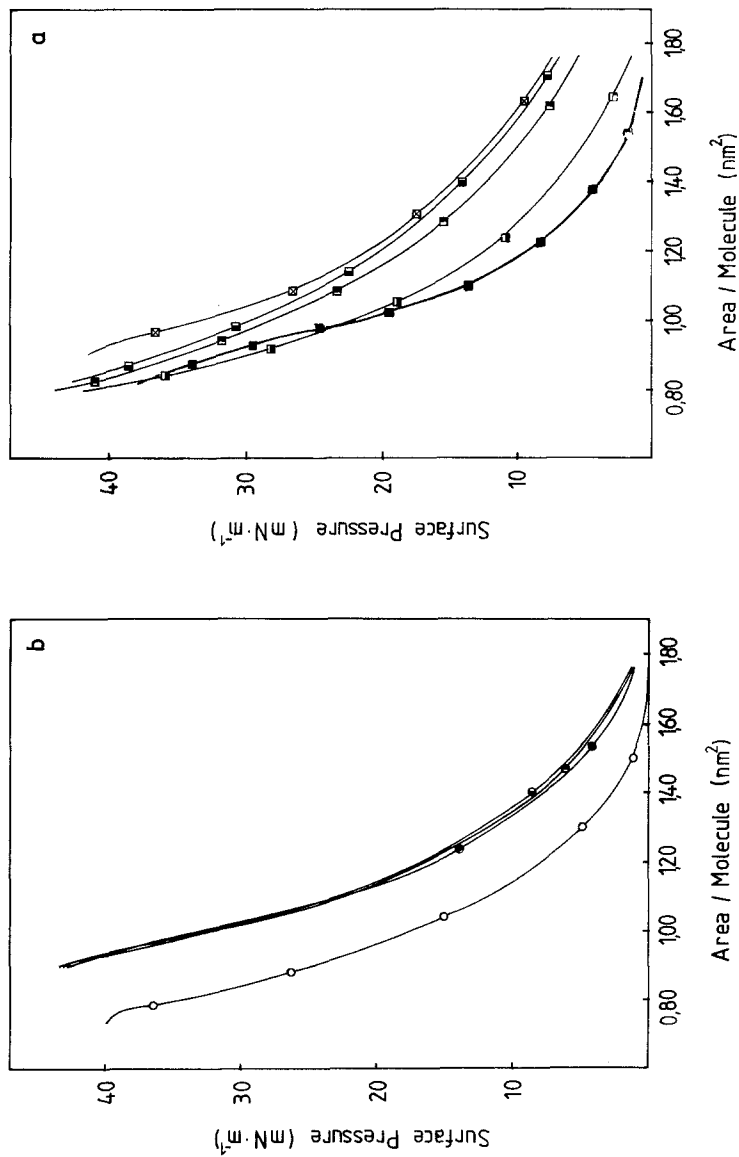
Fig. 4. Effect of sodium ion concentrations on phosphatidylinositol–opioid molecule interactions. (a) Compression isotherms of phosphatidylinositol monolayers spread on a 10^{-5} M buprenorphine solution and the following sodium chloride concentrations: (⊠) 0 M NaCl (pure buprenorphine), (■) 10^{-3} M NaCl, (▣) 10^{-2} M NaCl, (▦) 10^{-1} M NaCl, and (■) 1 M NaCl. (b) Compression isotherms of phosphatidylinositol monolayers spread on a 10^{-5} M naloxone solution and the following sodium chloride concentrations: (○) 0 M NaCl (pure naloxone), (◑) 10^{-3} M NaCl, (◐) 10^{-2} M NaCl, and (●) 10^{-1} M NaCl.
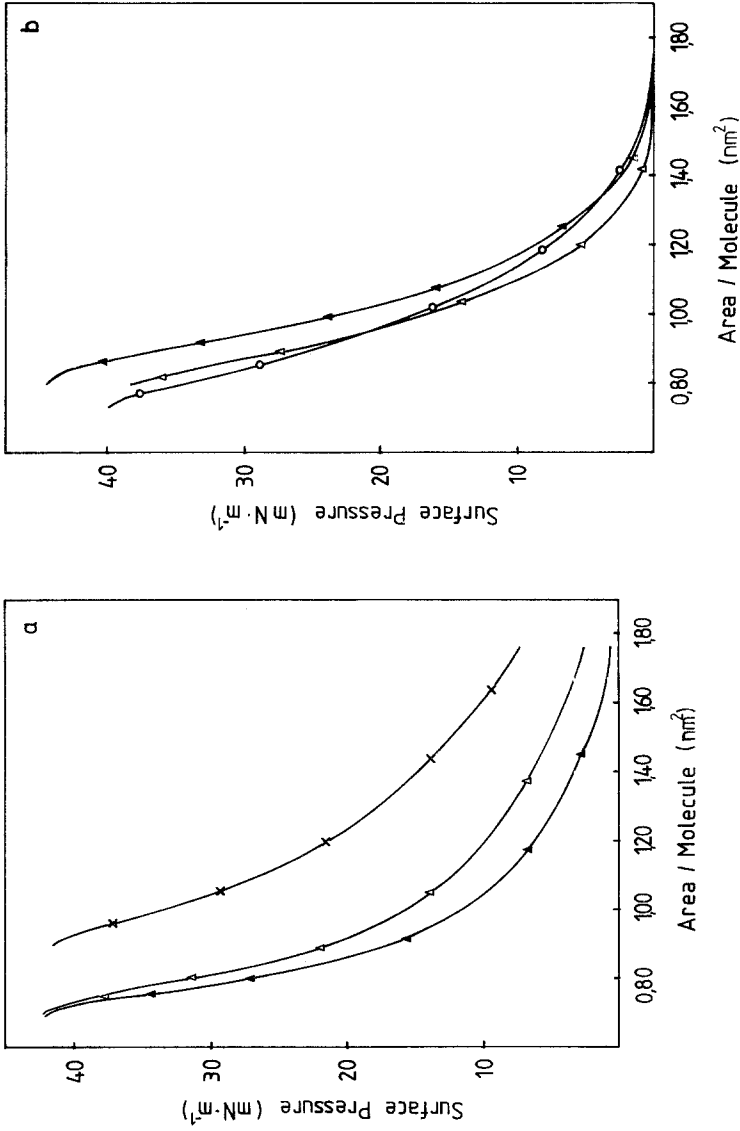
Fig. 5. Effect of calcium ion concentrations on phosphatidylinositol–opioid molecule interactions. (a) Compression isotherms of phosphatidylinositol monolayers spread on a 10^{-5} M buprenorphine solution and the following calcium chloride concentrations: ($\times$) 0 M $CaCl_2$ (pure buprenorphine, ($\triangle$) 10^{-3} M $CaCl_2$ and ($\blacktriangle$) 10^{-2} M $CaCl_2$. (b) Compression isotherms of phosphatidylinositol monolayers spread on a 10^{-5} M naloxone solution and the following calcium chloride concentrations: ($\bigcirc$) 0 M $CaCl_2$ (pure naloxone), ($\triangle$) 10^{-3} M $CaCl_2$, and ($\blacktriangle$) 10^{-2} M $CaCl_2$.
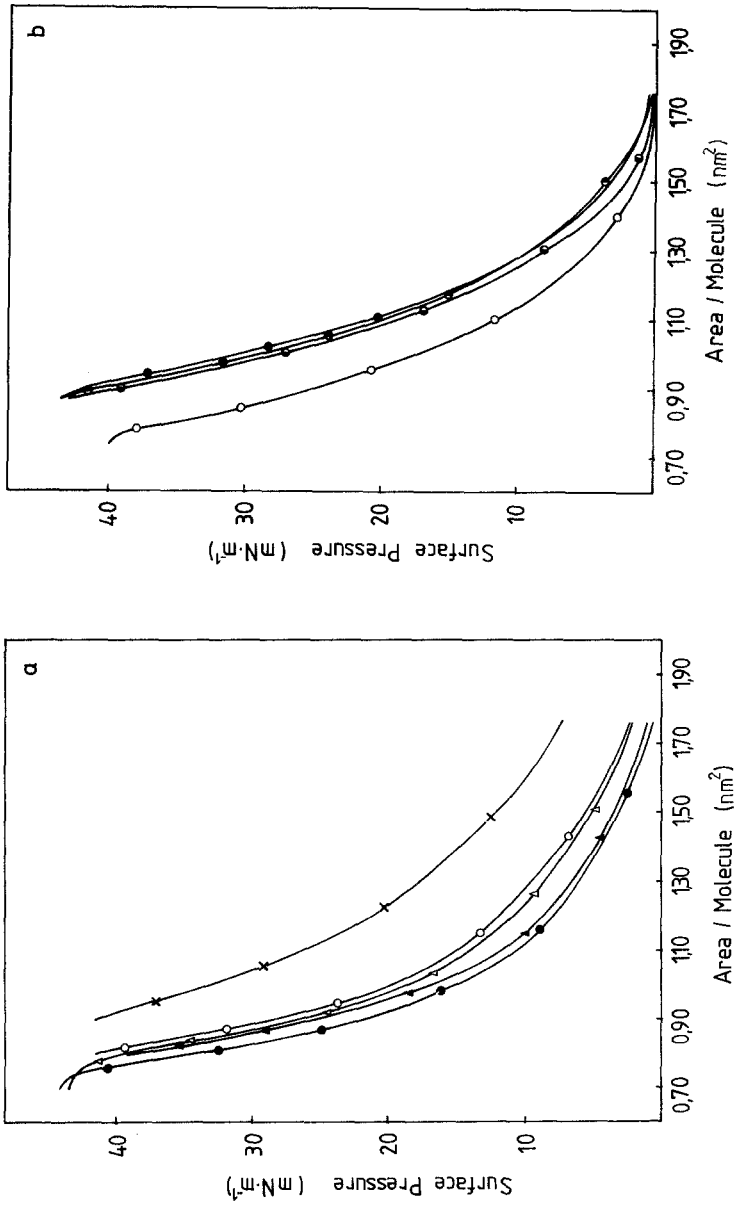
Fig. 6. Effect of mixtures of mono and divalent cations on phosphatidylinositol–opioid molecule interactions. (a) Surface pressure vs. area curves of phosphatidylinositol monolayers spread on the following subphases: (×) 10^{-5} M buprenorphine, (○) 10^{-5} M buprenorphine + 10^{-1} M NaCl + 10^{-3} M CaCl$_2$, (●) 10^{-5} M buprenorphine + 10^{-1} M NaCl + 10^{-2} M CaCl$_2$, (△) 10^{-5} M buprenorphine + 10^{-1} M NaCl + 10^{-3} M MnCl$_2$, and (▲) 10^{-5} M buprenorphine + 10^{-1} M NaCl + 10^{-2} M MnCl$_2$. (b) Surface pressure vs. area curves of phosphatidylinositol monolayers spread on the following subphases: (○) 10^{-5} M naloxone, (●) 10^{-5} M naloxone + 10^{-1} M NaCl + 10^{-3} M CaCl$_2$, (◐) 10^{-5} M naloxone + 10^{-1} M NaCl + 10^{-2} M CaCl$_2$, and (◉) 10^{-5} M naloxone + 10^{-1} M NaCl + 10^{-3} M MnCl$_2$.

the same conditions, naloxone-containing subphases differently perturb the isotherms, which exhibit expanding effects.

Although research on PI monolayers support their stability in the pH range of the present study, we wanted to determine whether the differences observed on the interactions of PI monolayers with buprenorphine and naloxone were due to rearrangements of the polar groups present in a nonbuffered medium. For this reason, a parallel series of PI isotherms on subphases containing Tris buffer, pH 7, was carried out.

Results show that buprenorphine causes a high increase in the area occupied per PI molecule related to naloxone and Tris. But, in this case, the final values at collapse pressure are the same for the three subphases; this differs from values previously shown. So it seems that in this case the buprenorphine molecules and the naloxone molecules are, during the compression process, slowly excluded from the monolayer.

The effect of the three ions Na^+, Ca^{2+}, and Mn^{2+} on monolayers spread over subphases containing Tris and naloxone is similar to that observed in subphases without buffer. It is also remarkable that the presence of Tris attenuates to some degree the effects of ions previously detected on pure water.

Partition coefficients were calculated with the molecules dissolved in distilled water, the reason being that these are the conditions under which the main bulk of the experimentation was carried out. Moreover, the differences in interaction between PI and buprenorphine and naloxone are also greater in the absence of buffer. The values obtained showed a higher hydrophobic character for buprenorphine compared to naloxone.

Discussion

The physicochemical behavior of PI monolayers has previously been studied by Papahadjopoulos (1968), Hirasawa *et al.* (1981), Pattus *et al.* (1983), and Hayashi *et al.* (1984). There is evidence of a strong interaction between Ca^{2+} and PI, which leads to more condensed phospholipid films. This interaction has been attributed to chelate formation between the PI and Ca^{2+} involving the inositol hydroxy groups. On the contrary, interactions between this lipid and monovalent cations have not been as clearly described, neither are PI and opiate drugs interactions; for this reason, no direct relationships can be established between the present work as a whole and the literature.

As an aid to interpretation, we have summarized the information gained from this work by plotting the molecular areas which correspond to condensing or compressing effects induced by the presence of ions, drugs, and buffer
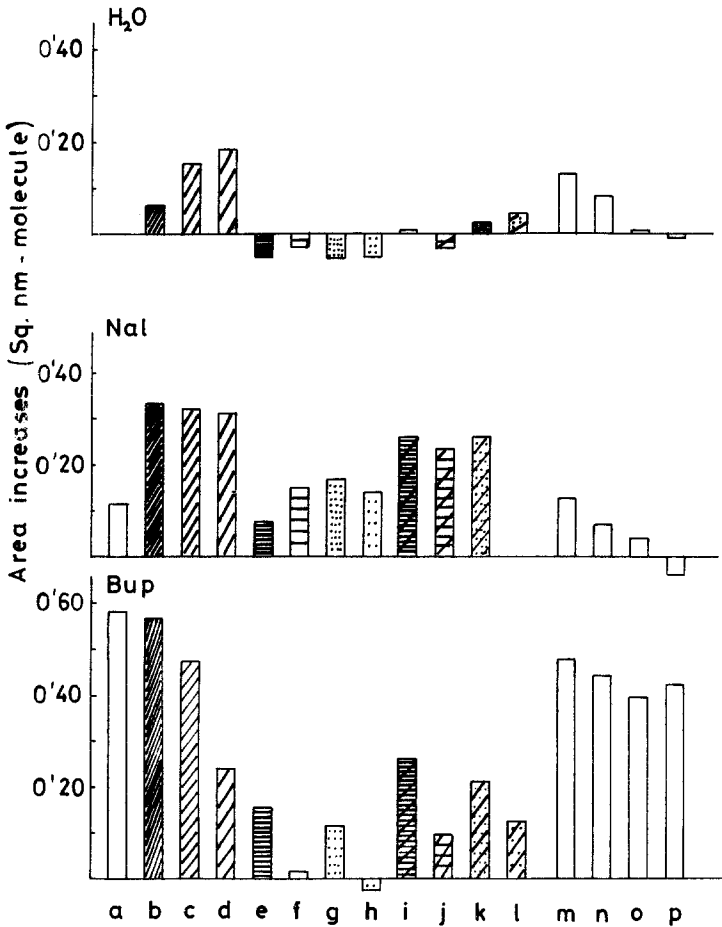
Fig. 7. Area increases experienced by phosphatidylinositol monolayers measured at $10\,mN \cdot m^{-1}$. (a) water, (b) NaCl 10^{-3} M, (c) NaCl 10^{-2} M, (d) NaCl 10^{-1} M, (e) CaCl$_2$ 10^{-3} M, (f) CaCl$_2$ 10^{-2} M, (g) MnCl$_2$ 10^{-3} M, (h) MnCl$_2$ 10^{-2} M, (i) NaCl 10^{-1} M + CaCl$_2$ 10^{-3} M, (j) NaCl 10^{-1} M + CaCl$_2$ 10^{-2} M, (k) NaCl 10^{-1} M + MnCl$_2$ 10^{-3} M, (l) NaCl 10^{-1} M + MnCl$_2$ 10^{-2} M, (m) Tris 0.05 M, (n, o, p) Tris + NaCl 10^{-1} M, or CaCl$_2$ 10^{-2} M, or MnCl$_2$ 10^{-2} M.

in the subphase. Thus, in Fig. 7, the ordinates represent differences of film area measured at $10\,mM \cdot m^{-1}$ by taking as reference the molecular area of PI monolayers on pure water.

To attempt to explain these findings, we have to consider that, in general, the interaction of a subphase molecule of solute with a phospholipid monolayer is highly dependent on the compression state of the monolayer. At very low packing densities, the molecule can completely penetrate the monolayer. At intermediate pressure, only certain hydrophobic groups of

the molecule are able to penetrate the film by leaving the more hydrophilic moiety in the subphase. Finally, at high film pressures, only phospholipid–solute ionic interactions are likely. The addition of either buprenorphine or naloxone to the subphase caused a major expansion on PI films. Such an effect is observed throughout the compression process, with the resulting pressure vs. area plots markedly different with respect to shape and slope. Since neither buprenorphine nor naloxone at concentrations of 10^{-5} M are able to modify the surface pressure of water, we can attribute the differences observed to a strong interaction between PI and these drugs. This interaction is instantaneous in the case of buprenorphine and takes place during the spreading of the film, as is seen by a rise of the initial surface pressure from 0 to 7.25 mN $\cdot$ m^{-1}. From the slope of the curves, it is also deduced that monolayers of PI on buprenorphine solutions are in an expanded state throughout the compression process, and the monolayers spread on naloxone or water are in a liquid condensed state at high pressures. Moreover, differences in behavior between buprenorphine and naloxone arise when examining the compressibility B of both series of isotherms using the equation (Nakagaki et al., 1985) $B = -1/A\,(dA/d\pi)_{\pi,T}$, which gives a value of 0.0008 mN $\cdot$ m^{-1} for buprenorphine against 0.0088 mN $\cdot$ m^{-1} for naloxone (measured at 30 mN $\cdot$ m^{-1} of compression pressure).

As said above, these films expansions can be explained by drug penetration into the hydrophobic region of the monolayer. The greater ability of buprenorphine to penetrate the monolayer is due to its bulkier and more hydrophobic molecular structure (Fig. 1). This fact is also in agreement with partition coefficients calculated for both drug molecules. Additional support of this explanation can be obtained by examining the collapse area. Such area is significantly larger in the presence of buprenorphine, indicating that the mixed PI–buprenorphine film collapses as a unit. The high affinity of the drug for PI allows buprenorphine to remain incorporated in the monolayer between the phospholipid molecules even at high surface pressure without being excluded.

Although naloxone is able to penetrate and expand the monolayer at low pressures, during the film compression a slow but complete inversion of the penetration is observed. As a result of this process, films spread over naloxone at 40 mN $\cdot$ m^{-1} show the same area as monolayers spread on water.

The interaction of PI and monovalent ions has not been described in detail. Some authors have worked with PI monolayers spread on subphases containing Na$^+$, K$^+$, or Li$^+$ chlorides (Hayashi et al., 1984; Papahadjopoulos, 1968), but there is no description of the effect of these ions on the monolayer compression state with regard to the films spread on pure water. In our case, since the compression isotherms have been obtained under the same

conditions over subphases of pure water and NaCl solutions, we can compare both diagrams. As is evident from Fig. 7, Na^+ expands the monolayer, and this effect is dependent on ion concentration in a saturable way. As the absolute quantity of Na^+ ions in the bulk of the solution is always higher than that of the phosphate ions in the monolayer (approximately 10^8), we cannot attribute the increasing expansion only to a gradual neutralization of negative charges of the phospholipid on the surface. In this case, the changes in area per molecule caused by the NaCl concentration in the subphase are probably due to the increase of the subphase ionic strength, which affects the hydroxy groups solvation equilibrium. This would be in agreement with the saturable character of the effect, as the activities of solutes will decrease inversely to the ionic concentration.

The lack of differences observed among the compression isotherms of PC spread on subphases containing NaCl at various concentrations was, as expected, due to the fact that this phospholipid has no net negative charge in its molecule nor hydroxy groups able to interact with water molecules or with each other by means of hydrogen bonding.

The combined effect of Na^+ ions and opiate molecules on PI films (Fig. 7) shows the big differences between the type of interactions that these two molecules have with PI. Considering the $PI/buprenorphine/Na^+$ inter-action, it can be seen that it is highly dependent of Na^+ concentration. This means that the ionic interaction between PI/Na^+ competes with the hydro-phobic penetration of buprenorphine molecules in the hydrophobic core of PI films; Na^+ at 10^{-1} M concentration expands and blocks the monolayer, obstructing bupenorphine penetration.

But, as the relationship between area per molecule and superficial pressure for PI over NaCl 10^{-1} M is slightly lower than that corresponding to $PI/NaCl$ 10^{-1} M plus buprenorphine, we can assume that, to a very small extent $(1.18 \, nm^2 \cdot mol^{-1}$ versus $1.27 \, nm^2 \cdot mol^{-1})$, some buprenorphine molecules could penetrate the PI films under these conditions. This assump-tion would explain the slope of the isotherm corresponding to a lipid expanded state and the high area per molecule in the collapse. Moreover, the com-pressibility of the monolayer varies steadily in the same sense.

Regarding the slope of the PI isotherms spread on naloxone/Na^+, it is clear that there exists an expansive effect that is additive. This effect is not Na^+ concentration dependent as it is for PI/Na^+ alone. Thus, it can be assumed that in this case PI/Na^+ does not prevent PI/naloxone interactions as in the case of buprenorphine, although there is no clear reason for this. The fact that sodium changes the allosteric conformation of the opioid receptor, promoting a decrease in agonist affinity and leaving the antagonist affinity unchanged (the sodium index of naloxone being equal to 1), could be related to the observations of Simon (1976).

The presence of Ca^{2+} ions in the subphase does not greatly affect the slope and compression state of the PI monolayer. Only a small compression effect is observed, which is inversely proportional to the Ca^{2+} concentration in the subphase. As proposed by Mühleisen *et al.* (1983), this small compressing effect could be due to the higher capacity of Ca^{2+} ions to be solvated ($[Ca(H_2O)_6]^{2+}$) compared to Na^+ ions; this fact would make difficult the ionic interaction among PI polar groups and the solvated cations, at least to the same extent as Na^+ ions do. On the other hand, recent studies of Hayashi *et al.* (1984) have shown that PI, having only a negative charge, has a low capacity to bind Ca^{2+}, reaching the maximum value $0.12\,Ca^{2+}$ ion per PI molecule. This low value would agree with the small effect detected in the compression isotherms. Another possible explanation could be a chelate formation between the hydroxy groups of the sugar moieties and that cation (Czarniecki and Thornton 1977).

Furthermore, this small compressing effect has been described for Ca^{2+} interactions with PS (Rojas and Tobias, 1965) and PI diphosphate (Lohdi *et al.*, 1976). Mn^{2+} ions have in general the same effects as Ca^{2+} ions, producing a nearly insignificant compression of PI monolayers.

The presence of buprenorphine or naloxone in subphases containing Ca^{2+} or Mn^{2+} ions affects the compression isotherms of PI. In general, divalent cations compress PI monolayers spread on buprenorphine- and naloxone-containing subphases, with the corresponding ones spread on naloxone less affected. In the first case there exists an inhibition of buprenorphine/PI binding due to the Ca^{2+} or Mn^{2+} ion. This phenomenon has been observed for the interaction of other drugs with the same phospholipid (Lohdi *et al.*, 1976) and proteins (Pattus *et al.*, 1983).

Since buprenorphine has a nitrogen atom with two free electrons, the formation of complexes with Ca^{2+} is possible. In this respect, since the buprenorphine concentration is a hundred times lower than Ca^{2+}, it is justified to assume that all the buprenorphine molecules would be complexed, since their interaction with PI is negligible. This situation accounts for the close values of nm^2 per molecules calculated for PI isotherms spread on buprenorphine/Ca^{2+} 10^{-2} M and Ca^{2+} 10^{-2} M alone. In any case, there must also exist a competition between Ca^{2+} and buprenorphine for the negative charges at the lipid interface. The combined effect of Na^+ and Ca^{2+} or Mn^{2+} in the subphase in addition to buprenorphine gives intermediate values for PI isotherms. The sodium ions act by partially destroying the buprenorphine/ divalent cation complexes and permitting, to some extent, the penetration of buprenorphine into the monolayer.

For naloxone-containing subphases, Ca^{2+} and Mn^{2+} ions have a smaller expanding effect than Na^+ ions, and mixtures of mono- and divalent ions give monolayers with the same degree of expansion as the corresponding ones

on NaCl alone. With regard to the Tris-containing subphases, it seems, in general, that the presence of this organic molecule has a small expanding effect on PI monolayers but, in an unexpected way, it modulates or compensates the effects of the added cations. There are no differences between naloxone- and naloxone + Tris-containing subphases. In the case of buprenorphine + Tris-containing subphases, the monolayers or PI are expanded as usual over buprenorphine, and the values of area per molecule, although lower than for buprenorphine alone, are much closer to the values of buprenorphine on pure water than those on the five ionic concentrations studied.

All these results show that monolayers, in spite of their simplicity, are a good system for studying interactions among agonists and antagonists and phospholipids. Although much work is necessary to generalize these findings, it seems clear that the way opiate molecules interact with certain phospholipids in the presence and absence of ions can be correlated with its binding mode to brain homogenates. The fact that both opioid molecules do not interact at all with PC is a good confirmation of this hypothesis.

Acknowledgments

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