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Penetration of morphine and naloxone in phosphatidylserine monolayers

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Summary

The interaction between morphine or naloxone and phosphatidylserine (PS), has been studied using monomolecular layers as membrane model. The penetration kinetics of both molecules measured at different monolayer pressures and for increasing opiate concentrations has been determined. The results show that penetration of morphine presents a maximum at 15 $mN \cdot m^{-1}$ of initial PS pressure whereas that of naloxone has a maximum at 10 $mN \cdot m^{-1}$.

Acidic phospholipids and especially phosphatidylserine (PS) have often been involved in opioid receptor function (Abood et al., 1980; Pasternak, 1974). Although initially the proteic nature of opioid receptor seemed to be well demonstrated, there is increasing evidence about the involvement of acidic lipids as structural components (Loh et al., 1974; Dennis, 1980). For this reason we considered it interesting to study the physicochemical interactions between the two opiate molecules, morphine (M) and naloxone (Nx) and PS taking lipid monolayers as a membrane model.

The present study has been carried out by measuring the penetration ability of both molecules in monolayers of PS as a function of two parameters: the initial pressure of the lipid monolayer and the concentration of morphine and naloxone in the subphase. Moreover, the amount of drugs adsorbed at the interface when lipid is present has been calculated by means of the Gibb's adsorption equation assuming that this adsorption is reversible for these two molecules.

Bovine phosphatidylserine was purchased from Supelco (PA). Purity was checked by HPTLC, using C18-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 sulphuric/chromic acid and charring. The content of phosphorous was determined after perchloric acid digestion (Barlett, 1959). Its estimated molecular mass was 790 Da.

Morphine and naloxone were kindly provided by Abello S.A. (Madrid) and were further purified by recrystallization from their hydrochlorides.

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Purity was assessed by elemental analysis. Water for the Langmuir film balance was prepared by distillation over potassium permanganate of single-distilled water in an all-glass apparatus. Unless otherwise specified, no chemicals, other than the opioids and Tris-HCl, were added to this water. Its resistance was always greater than 16 MΩ/cm, its pH was 5.5–6 and it was distilled every day. Chloroform (Merck, pro-analysis) was used as the spreading solvent. PS films were prepared from chloroform solutions of approximately 1 mg/ml concentrations. Penetration kinetics were performed on a Langmuir film balance equipped with a Wihelmy platinum plate, 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, volume 309.73 ml) was regularly cleaned with hot chromic acid; moreover, before each experiment, it was washed with ethanol and rinsed with double-distilled water. Before each experiment, the platinum plate was cleaned with chromic acid and rinsed with double-distilled water. All measurements with PS were made at a subphase temperature of 21 ± 1 °C and at a pH in the range 5.5–6 except when Tris-HCl (10 mM, pH = 7.4) was added to the subphase.

The influence of the opiates and buffer on the surface activity of water has also been considered. In the range of opiate and salt concentrations of our experiments we were unable to detect any difference in the surface tension of pure water.

Effect of initial pressure. In trying to find the best conditions to achieve a good level of penetration, we designed the experiments working with both drugs dissolved in Tris-HCl at pH = 7.4. We thought that at neutral pH, these molecules would be mainly in a non-ionic form, and consequently could penetrate better into PS monolayers. Nevertheless, the values given in Table 1 clearly show

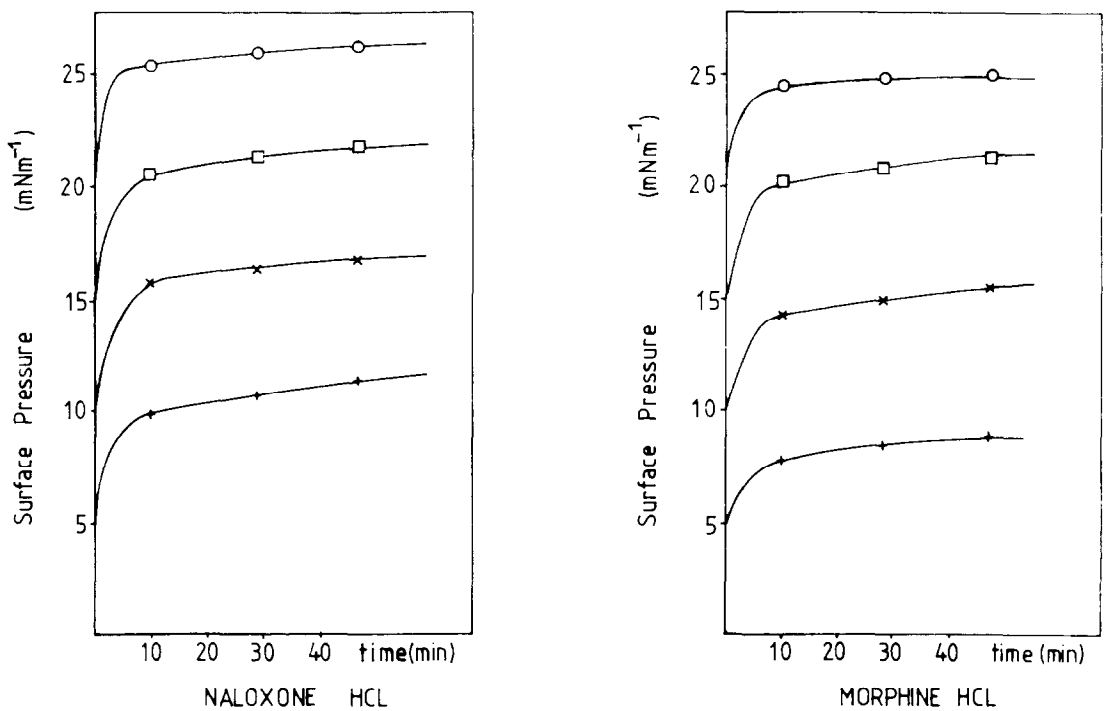

Fig. 1. Kinetics of the interaction of morphine and naloxone with monolayers of phosphatidylserine. Opiate concentration in the subphase 5×10^{-5} M.

that our assumption was not correct and that Tris molecules, having nearly the same size as the drug molecules compete with them and match their effects. For this reason the main bulk of the determinations has been done by spreading PS monolayers over subphases containing only water and drug molecules.

The kinetics of the interaction of morphine and naloxone with PS monolayers spread over non-buffered subphases at different initial pressures is given in Fig. 1.

As usual, with these kinds of compounds the penetration process is highly dependent on the initial surface pressure of the monolayer. For both molecules the penetration pattern is nearly the same. In general the process is very fast from zero to 10 min and at this time nearly 90% of the penetration was achieved. Applying the Gibb's equation to this process we can obtain the values of the superficial excess of solute in the monolayer with respect to the concentration in the subphase.

$$\Gamma = -\frac{1}{RT} \cdot \frac{\Delta P}{\Delta \ln a}$$

Table 1 summarises the values measured at 21°C 40 min after beginning the penetration.

The values summarised in Table 1 show that penetration of opioid molecules in the monolayer is not directly related to initial pressure, but, on the contrary, that there exists a specific value for which the penetration is maximum. This initial pressure is different for morphine and naloxone and it is also independent of the concentration of both molecules in the subphase. These data suggest the establishment of specific and different interactions between these two molecules and the phospholipid, highly dependent on the ordered and condensed state of the lipid molecules in the monolayer. This behaviour could be related to the specific pressure requirements of some enzymes to enable interaction and hydrolysis of phospholipid monolayers.

This idea is substantiated by the fact that the presence of Tris-HCl 10 mM in the subphase, affecting the ordering of the lipid molecules in the monolayer, nullifies this behaviour, in this case the penetration values of morphine and naloxone being nearly the same, as well as its variation at different pressures expected for a hydrophobic interaction.

The influence of the concentration of opioid molecules in the subphase on its penetration pattern, measured at 10 $mN \cdot m^{-1}$ of initial PS pressure, is shown in Fig. 2. The superficial excess (Γ) of both molecules above its concentration in the subphase is represented by the two curves nearly running parallel, the one corresponding to naloxone always being higher than the one for morphine.

It is well known that Nx binds more tightly than M to opioid receptors but apparently it does not interact with their chemical structures because there is no propagation of any effect. On the contrary, although M has a lower binding constant to the opioid receptor, a molecule interacts with it and generates a series of events. This

TABLE 1

Superficial pressure increases in phosphatidylserine monolayers, produced by morphine and naloxone, measured at different initial superficial pressures

π_i	Morphine		Naloxone	
	ΔP (w)	ΔP (Tris)	ΔP (w)	ΔP (Tris)
5	3.89	4.69	7.2	4
10	5.35	1.64	8.1	2.5
15	5.72	0.1	7	2
20	4.22	0.1	6	2

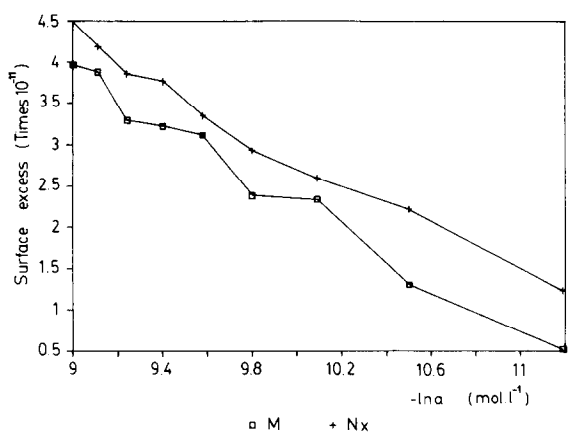

Fig. 2. Surface excess of morphine and naloxone, as a function of the concentration of both opioid molecules in the subphase.

interaction could be reflected by a change in the ordered state of the monolayer inducing a more compact array and consequently affecting the conformation and function of some of the membrane's proteins. These changes could in turn be responsible for the enzymatic activity or ion-channel regulation, phenomena that have been involved in the propagation of opioid action.

Although the complexity of the opioid–receptor interaction can not be reduced to the model of a monolayer, it seems that the differences in interactions detected in this single model can provide an interesting approach to the selection of phospholipids involved in the opioid receptor structure or function.

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