International Journal of Pharmaceutics, 76 (1991) 145–149

ADONIS 037851739100341P

IJP 02545

Interactions of naloxone with lipid monolayers

C. Mestres [1], F. Reig [2], M.A. Busquets [1], I. Haro [2] and M.A. Alsina [1]

[1] *Physicochemical Unity, Faculty of Pharmacy, University of Barcelona, Av. Joan XXII, s/n. 08028 Barcelona (Spain)* and [2] *Peptide Laboratory, CID-CSIC, Jordi Girona Salgado 18-26, 08034 Barcelona (Spain)*

(Received 17 May 1991)
(Accepted 5 June 1991)

Key words: Monolayer; Naloxone; Sulfatide; HPLC

Summary

The interaction between an opioid antagonist, naloxone, and several lipids and lipid mixtures was studied using monomolecular layers as a membrane model. No specific interactions could be detected. The main factor affecting the penetration of naloxone in monolayers seems to be the initial ordered state of the molecules instead of the chemical composition of the monolayer. A new membrane model was assayed based on the retention times in high-pressure liquid chromatography working with hydrophobically modified columns.

Introduction

The ability of bioactive compounds to elicit a biological action requires that an interaction takes place between the compound and the biological membrane. This fact involves the distortion of the ordered structure of the bilayer membrane and the characterization of such an interaction is the first step to understand their overall mechanism of action.

It has long been recognized that lipids are involved in opiate-opioid receptor interactions. A preferential interaction between opiates and acidic phospholipids and glycolipids has been described, thus suggesting that the opioid receptor could be a lipoprotein (Cho et al., 1986; Valette et al., 1987; Ott et al., 1988).

Although naloxone cannot be classified as an opioid molecule, its ability to compete with opiates for their receptors (especially the μ subtype) demonstrates the high affinity of this molecule for such receptors.

In this paper, we describe a systematic study of the effects of naloxone on the surface pressure and monomolecular area of lipid monolayers spread at an air/water interface. Moreover, we have assayed a new membrane model by saturating an HPLC column with different lipids and studying the retention times before and after modification.

Correspondence: F. Reig, Peptide Laboratory, CID-CSIC, Jordi Girona Salgado 18-26, 08034 Barcelona, Spain.

Experimental

Chemicals

Phosphatidylcholine (PC) was purchased from Merck and purified by column chromatography (Singleton et al., 1965). The content of phosphorus was determined after perchloric acid digestion (Bartlett, 1959). Its estimated molecular mass was 790 Da. Sulphatides (S) and cholesterol (Ch) purchased from Sigma were puriss. grade (approx. 99%). The purity of phospholipids was checked by thin-layer chromatography. Water for the Langmuir film balance was prepared by passing simply distilled water through a Milli Q filtration system. The resistivity was always greater than 16 MΩ/cm and the pH 5.5. Naloxone hydrochloride was a gift from Laboratorios Abelló Spain. Its purity was controlled by HPLC. No chemicals other than naloxone were added to this water. Chloroform (Merck) was used as spreading solvent. Lipids were spread from solutions of mg/ml. Monolayers were prepared from the following compositions: PC, S, Ch, PC/S (0.8 : 0.2), Ch/S (0.6 : 0.4), PC/S/Ch (0.16 : 0.04 : 0.8), PC/S/Ch (0.48 : 0.12 : 0.4). These mixtures were selected according to previous miscibility studies. The compositions giving the maximum deviations from ideality were those used throughout this work.

Methods

Compression isotherms The film balance assembly has been described elsewhere (Verger et al., 1973). The output of the pressure pick-up was calibrated by recording the isotherms of stearic acid and DPPC. The Teflon trough (495 cm^2, 309.7 ml), was regularly cleaned with hot chromic acid. Moreover, before each experiment, it was washed with ethanol and rinsed with double-distilled water. Films were spread on the aqueous surface using a Hamilton microsyringe and at least 10 min allowed for solvent evaporation. Films were compressed continuously at a range of 4.2 cm/min. All the isotherms were run at least three times in the direction of increasing pressure with freshly prepared films.

Penetration studies The surface activity of naloxone and its interaction with lipid monolayers were determined according to techniques which have been previously reported (Reig et al., 1988a). In these experiments a cylindrical Teflon trough of 50 ml was used. Lipids were spread from chloroform solutions until the desired initial film pressure was obtained (5, 10 or 20 mN/m).

High-pressure liquid chromatography (HPLC) experiments The chromatographic determinations were carried out in an Applied Biosystems apparatus. The analytical column (300 × 3.9 mm) was packed with 10 μm Bondapack C18. The effluent from the cells was connected to a UV detector operated at 210 nm. The flux speed was 2 ml/min. The mobile phase consisted of acetonitrile/ammonium acetate 0.2% (60 : 40) and 20 μl of each sample (0.33 mg/ml) were injected in each run. When necessary, the column was saturated by recirculating a 0.1% lipid solution in methanol for 30 min. After this time the column was disconnected and the circuit carefully washed in order to eliminate any traces of lipids from the system. The retention times of naloxone in standard columns, and after saturation with the different lipid mixtures were determined.

Results and Discussion

Compression isotherms

The interactions between PC and naloxone have been previously described (Reig et al., 1988b). In general, the presence of naloxone in the subphase does not modify strongly the shape and compressibility of the different isotherms, the phospholipids being in the same ordered state as in the absence of this molecule. As an example, the compression isotherms of PC/sulphatides (0.8 : 0.2) on aqueous subphases containing this molecule are represented in Fig. 1.

Nevertheless, the molecular area values show small variations according to the compositions of the monolayer. The calculated values at 10 and 20 mN/m are given in Fig. 2. These two pressures have been selected, since 20 mN/m is close to the pressures found in biological membranes and at about 10 mN/m most of the monolayers involved in this study undergo the transition from the liquid expanded to liquid condensed state.

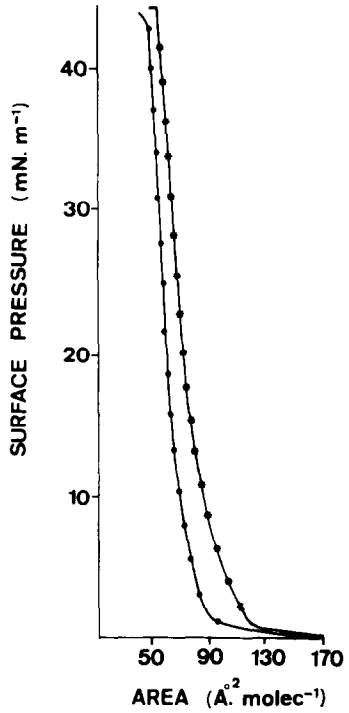

Fig. 1. Surface pressure/area isotherms of mixed monolayers PC/S (0.8:0.2). Subphase: (●) pure water and (∗)10^{-5} M naloxone.

The compressibility of these monolayers was calculated at the same surface pressure and no significant differences between samples with or without naloxone were found. Values ranged between $11-12 \times 10^{-3}$ m/mN at 10 mN/m and $7.0-7.7 \times 10^{-3}$ m/mN at 20 mN/m.

Penetration kinetics

Although naloxone does not form an insoluble monolayer at the air/water interface, its aqueous solutions showed some surface activity giving a maximum equilibrium surface pressure of 2.25 mN/m when injected into the subphase at a concentration of 2×10^{-4} M. From these data and applying the Gibbs equation, the superficial excess of naloxone was found to be 21.9×10^{-12} mol/cm^2. The interaction between naloxone and the lipids of the monolayer was determined as follows. A fixed amount of lipid was spread on the surface to obtain the desired initial surface pressure and, thereafter, increasing amounts of the concentrated drug solution were successively injected into the subphase.

The penetration process was achieved in 5 min but an interval of 15 min was left between two

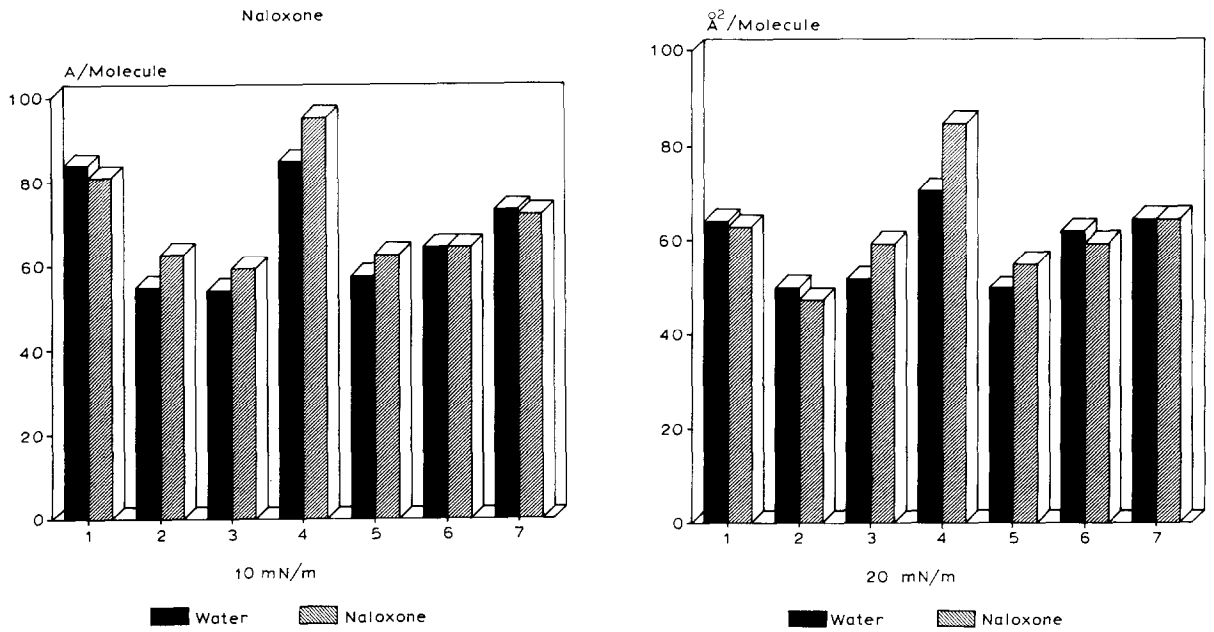

Fig. 2. Molecular area values measured at (a) 10 and (b) 20 mN/m in monolayers of: (1) PC; (2) S; (3) Ch; (4) PC/S, 0.8:0.2; (5) Ch/S, 0.6:0.4; (6) PC/S/Ch, 0.16:0.04:0.8; (7) PC/S/Ch, 0.48:0.12:0.4 spread on an aqueous subphase with and without naloxone (10^{-5} M).

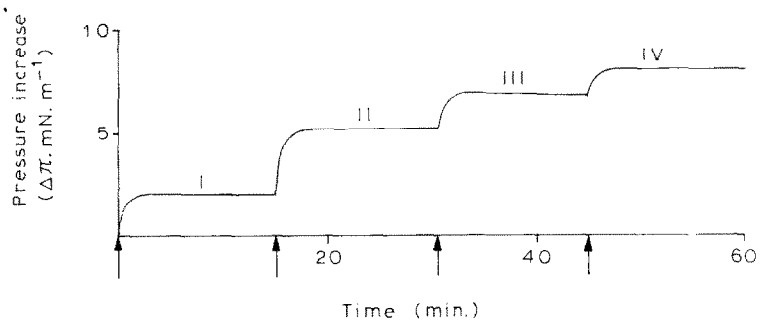

Fig. 3. Increase of pressure with time for sulphatides in monolayer at 5 mN/m following additions of a concentrated naloxone solution. Arrows indicate the time of injection. Naloxone concentration in the subphase: (I) 10^{-5} M, (II) 2×10^{-5} M, (III) 10^{-4} M, (IV) 2×10^{-4} M.

consecutive injections. The register corresponding to the sulphatides' monolayer at 5 mN/m is given in Fig. 3. There is a direct relationship between the drug concentration in the subphase and the pressure increases produced as demonstrated in Fig. 4. It is interesting to note that pressure increases produced when injecting naloxone under monolayers at 5 or 20 mN/m are very similar. This result is in agreement with the small differences in compressibility described before. The dependence of the pressure increases on the initial surface pressure at a fixed concentration is given in Fig. 5. Here, some anomalous behaviour can be appreciated. Considering the shape and the compressibility of the isotherms, one can see that when cholesterol is the main component of the monolayer, molecules are arranged in a solid state, nearly since the start of the compression and for this reason the initial pressure of the monolayer exerts little influence in the pressure increases achieved.

Monolayers composed of pure lecithin and mixtures with a low content of cholesterol show normal behaviour, giving lower pressure increases at higher initial pressures. In contrast, monolayers rich in sulphatides give a discontinuity at Π_1 10 mM/m. Although differences in compressibility are very small from one composition of monolayer to another, those rich in sulphatides show a smooth change in the slope of the curve at about

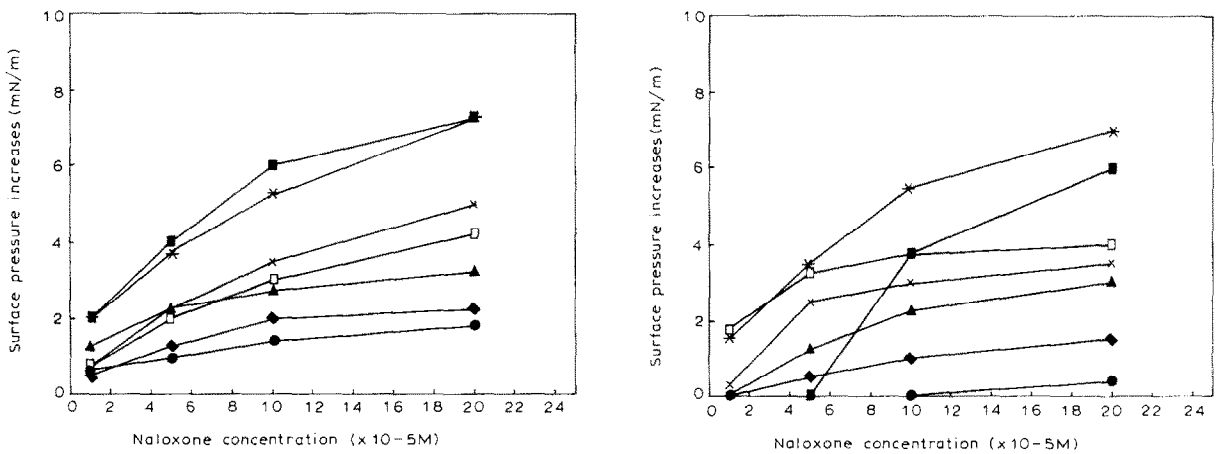

Fig. 4. Surface pressure increases (mN/m) in (●) PC; (■) S; (*) Ch; (□) PC/S, 0.8:0.2; (★) Ch/S, 0.6:0.4; (◆) PC/S/Ch, 0.48:0.12:0.4; (▲) PC/S/Ch, 0.16:0.04:0.8 monolayers spread at Π (a) 5 and (b) 20 mN/m as a function of the naloxone concentration in the subphase.

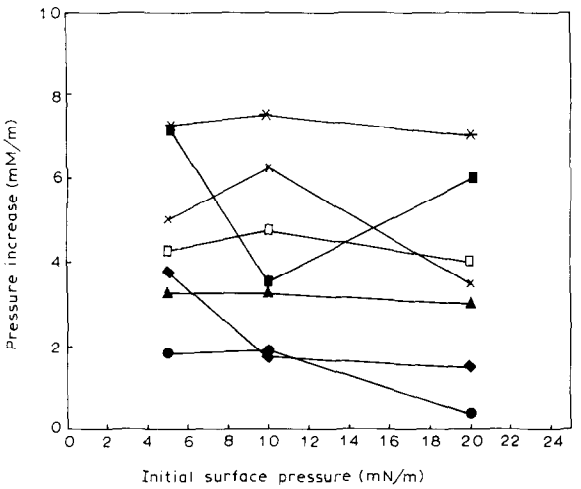

Fig. 5. Dependence of the pressure increases on the initial surface pressure for (●) PC; (■) S; (*) Ch; (□) PC/S, 0.8:0.2; (★) Ch/S, 0.06:0.4; (◆) PC/S/Ch, 0.48:0.12:0.4; (▲) PC/S/Ch, 0.16:0.04:0.8 monolayers spread on 2×10^{-4} M naloxone solutions.

10 mN/m. This fact could explain the discontinuity found for the penetration of naloxone at this specific surface pressure.

Chromatographic determinations

The main reason for attempting to assay this new model membrane was the knowledge that HPLC retention times could be used as a measure of hydrophobicity for different types of molecules (Reig et al., 1989). We assumed that if there were specific interactions between naloxone and some of the lipids, it should be possible to detect them by using this technique. The parameters determined in this study were the retention time and K values of naloxone after saturating the columns with the different lipid mixtures. These two parameters remained unchanged except when the saturating lipids were pure sulphatide or lecithin/sulphatide (0.8:0.2). In both cases, an increase in retention time of 5% was detected, thus suggesting the existence of a very small increase in the affinity of this molecule for the modified stationary phase of the column.

These unexpected results can be due to the intrinsic characteristics of the chromatographic process, especially as far as the equilibrium time is concerned. The short times left for interaction between the opiates and the stationary phase are probably the main reason for the lack of modification in the retention times. This experiment was redesigned, allowing a longer interaction time by reducing the flux of the mobile phase, but in this case the peaks flattened and diffusion phenomena occurred. From these results, it appears that this experiment is not suited to the detection of such interactions.

References

Barlett, G.R., Phosphorus assay in column chromatography. *J. Biol. Chem.*, 234 (1959) 466–468.

Cho, T.M., Hasegawa, J.I., Ge, B.L. and Loh, H.H., Purification to apparent homogeneity of a type opioid receptor from rat brain. *Proc. Natl. Acad. Sci. USA*, 83 (1986) 4138–4142.

Ott, S., Costa, T. and Herz, A., Sodium modulates opioid receptors through a membrane component different from G-proteins. *J. Biol. Chem.*, 263 (1988) 10524–10533.

Reig, F., Espigol, C., García Antón, J.M., Valencia, G. and Alsina, M.A., A physicochemical study of the interaction of phosphatidylinositol with Buprenorphine and Naloxone. *J. Bioenerg. Biomembr.*, 20 (1988a) 533–549.

Reig, F., Busquets, M.A., García Antón, J.M., Valencia, G., and Alsina, M.A., Interaction of analgesics with lecithin and ganglioside monolayers. *Int. J. Pharm.*, 44 (1988b) 257–260.

Reig, F., Alsina, M.A., Busquets, M.A., Valencia, G. and García Antón, J.M., Preparation and 'in vitro' activity of liposome encapsulated opioids. *J. Microencapsulation*, 6 (1989) 277–283.

Singleton, W.S., Gray, M.S., Brown, M.L. and White, J.L., Chromatographically homogeneous lecithin from egg phospholipids. *J. Am. Oil. Chem. Soc.*, 42 (1965) 53–56.

Valette, A., Rouge, P., Coulais, E., Potonnier, G., Cros, J. and Simon, E.J., Interaction with lecithin of kappa opioid binding sites solubilized from human placenta. *Life Sci.*, 40 (1987) 535–540.

Verger, R. and De Haas, G.H., Enzyme reactions in a membrane model. *Chem. Phys. Lipids*, 10 (1973) 127–135.