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INTERACTION OF MORPHINE AND NALOXONE WITH MIXED MONOLAYERS OF LECITHIN AND GANGLIOSIDES

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SUMMARY. Monomolecular films of lecithin, gangliosides or lecithin/gangliosides mixtures were studied on a Langmuir trough in order to examine the interactions between these lipids and opioid agonists or antagonists. Lecithin alone did not interact in a monolayer structure with opioids. However, gangliosides and lecithin/gangliosides mixtures were expanded by both morphine and naloxone. The expansion of ganglioside-containing monolayers was greater with morphine than with the antagonist, naloxone.

There have been many attempts to elucidate the chemical basis of the interactions between opiates and their membrane receptors, but it is still uncertain whether membrane lipids are involved. The distribution of lipids is asymmetric between the two membrane layers of many cells (1). Sphingolipids are located preferentially in the outer monolayer (2). Specific sphingolipids have been found to be distributed in association with the receptors for hormones, specific neurotransmitters and bacterial toxins (3-7) and may therefore be implicated in nervous function. Gangliosides, a group of acidic sialosphingolipids, are widely distributed in mammalian tissues (8), specially in brain ; there is good evidence that they are components of neuronal membranes (9,10) perhaps as constituents of the opioid receptor (11-13). In the present study, we have utilized monolayer techniques to examine the interaction between neuronal membrane lipids and

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opioid agonists and antagonists. These studies represent our initial attempts to elucidate the function of ganglioside lipids in nervous tissue.

Lipid monolayers may be arranged at an air-water interface using a Langmuir balance (14). The interactions between the monolayer components and solutes included in the aqueous subphase may be examined through measurement of the lateral compressibility of the monolayer (15). We have prepared compression isotherms for monolayers of lecithin, gangliosides and their co-mixtures in the presence and absence of morphine and its antagonist, naloxone.

MATERIALS AND METHODS

Morphine and naloxone were supplied by Abello Laboratories (Madrid) and were purified by formation of the corresponding hydrochloride and crystallization in methanol/ether. Purity was checked by thin layer chromatography.
Lecithin (L-α-phosphatidylcholine, dipalmitoyl) was obtained from Sigma Co. (St. Louis). Lecithin from soybean was also supplied by Sigma and was purified by column chromatography on alumina using $CHCl_3$/MeOH (9:1,v:v) as eluent. The molecular mass estimated from surface data using dipalmitoyl phosphatidylcholine as a standard and preparing a series of compression isotherms with both lipids in parallel, was 480 daltons.
A mixture of gangliosides, G_{M1}, G_{D1a}, G_{D1b} and G_{T1} was extracted from fresh bovine brain according to the Susuki procedure (16). Pure G_{D1a} was obtained from Supelco (Pennsylvania) and was used to assess, from surface data, the average molecular mass of the extracted gangliosides. A value of 1780 daltons was stablished. Compression isotherms were prepared at 22°C on a Langmuir film balance equipped with a Wihelmy plate as described by Verger et al. (17). Lipid monolayers were spread on three subphases: twice-distilled water and solutions of morphine hydrochloride or naloxone hydrochloride (both at 1.4×10^{-5}M). Drug concentration are similar to the ID_{50} values publised for concentration-inhibition using the *in vitro* guinea pig ileum test (18).

RESULTS AND DISCUSSION

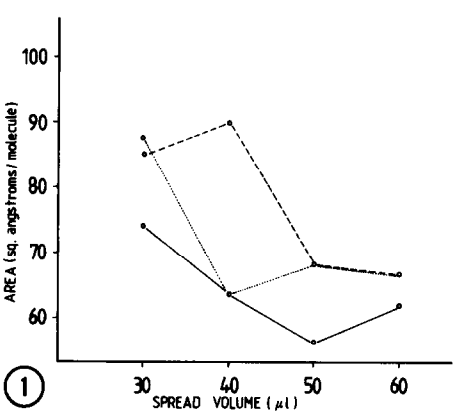
Compression isotherms were attempted for the drugs alone (in the absence of added lipid) in order to determine their surface activity. The drugs were added to the subphase as their hydrochlorides or deposited on the surface as their free bases in $CHCl_3$/MeOH (9:1,v:v). Neither morphine nor naloxone formed

detectable monolayers indicating the complete absence of surface activity or surface-active contaminants.

Monolayers of soybean lecithin were spread from $CHCl_3$ (1mg/ml) using a range of surface concentrations (from 30 to 60 μl) over each of the three subphases. No differences could be detected in the compression isotherms as a function of the subphase composition (data not shown). These results demonstrate the absence of interfacial interactions between lecithins and opioids. Lecithins are the major phospholipid components of mammalian membranes and have not been implicated in the function of opioid receptors.

In strong contrast to the behaviour of lecithin monolayers, compression isotherms for ganglioside monolayers displayed a considerable dependence on subphase composition. Ganglioside mixtures containing G_{M1}, G_{D1a},G_{D1b} and G_{T1} were spread from a stock solution (5 mg/ml) in $CHCl_3$/MeOH (2:1,v:v) over a range of surface concentrations (from 30 to 60 μl) using three different subphases. The average area per molecule is dependent upon both the surface lipid concentration and solute. As shown in Figure 1, the presence of either morphine or naloxone leads to monolayer expansion. It is worthwhile noting that the subphase concentrations are not far away from those expected for the Krebs solution supporting the perfused guinea pig ileum during *in vitro* tests.

Mixed monolayers of soybean lecithin and gangliosides prepared from the stock solutions and spread over a range of surface concentrations, yield isotherms which are dependent upon the mole fraction of each lipid. When examined at low pressures (Figure 2), the area per molecule does not exhibit ideal behaviour and consistently falls to very low values at 9.0 molar fraction of



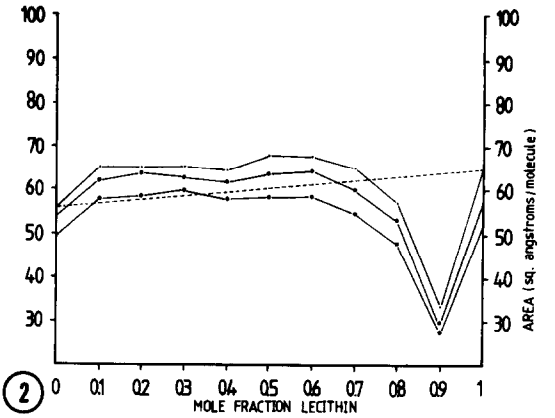


FIGURE 1.

Area per molecule in monolayers of a mixture of gangliosides at different initial surface concentrations taken at a surface pressure of 3 dyn/cm for the following subphases: twice-distilled water (solid line), 1.4×10^{-5}M morphine hydrochloride solution (dashed line) and 1.4×10^{-5}M naloxone hydrochloride solution (dotted line).

FIGURE 2.

The mean area per molecule in mixed monolayers of lecithin and gangliosides to the molecular proportions of the two components at three different surface pressures: 3 dyn/cm (solid line), 6 dyn/cm (-o-) and 9 dyn/cm (-•-) on a twice-distilled water subphase. Pure lecithin on the right, pure gangliosides on the left. The dotted line is the expected plot for the ideal mixtures.

lecithin. This condensation of the monolayer is unusually high and may represent monolayer collapse or lipid dispersion in the subphase.

Most notably, however, the inclusion of morphine or naloxone results in monolayer expansion for nearly every mixture which contains gangliosides (see Figure 3). In general, the effect is greatest for morphine. These effects occur even at the lowest levels of ganglioside tested (0.1 mole fraction), but do not increase in a simple manner with increasing mole fraction. While morphine and naloxone have opposite effects on nervous tissue function, their effects upon the ganglioside components of nervous tissue are similar though different in magnitude. The specificity of lipid expansion for gangliosides over lecithins

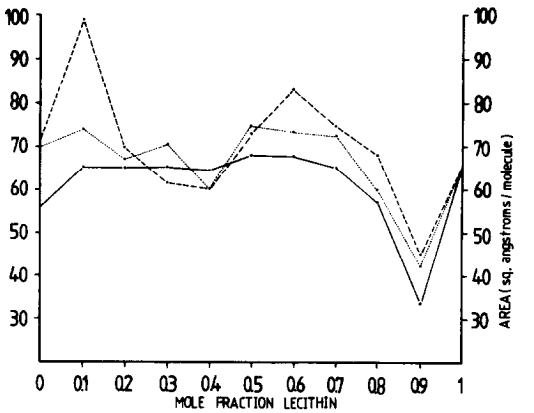


FIGURE 3.

The mean area per molecule in mixed monolayers of lecithin and gangliosides to the molar proportion of the two components at the following subphases: twice-distilled water (solid line), 1.4×10^{-5} M morphine hydrochloride solution (dashed line) and 1.4×10^{-5}M naloxone hydrochloride solution (dotted line) at a surface pressure of 3 dyn/cm.

may localize the opioid-lipid interactions to nervous tissues *in vivo*. Since membrane fluidity is known to affect the function of membrane proteins, the expansion of ganglioside lipids by opioid agonists and antagonists may be important in mediating the effects of these compounds upon opioid receptors.

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