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Short Communications

Interaction of analgesics with lecithin and ganglioside monolayers

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Summary

Using monolayers as a membrane model, the interaction of dextrometorphan, codeine and phentanyle with lecithin and ganglioside was studied. The penetration kinetics of these drugs into the monolayers were measured at 5 and 20 $mN \cdot m^{-1}$ and the results show that the interaction of phentanyl is maximal with lecithin, but not with ganglioside. This fact seems to exclude GM_1 as a μ-opioid receptor.

In spite of the supposed static role of phospholipid as the "cement" in the construction of membranes and other cellular organelles, numerous lines of evidence suggest that lipids also have a dynamic role in the function of membrane-bound proteins, and in the receptor mechanism (Arienti and Parcellati, 1980; Hakamori, 1984).

Acidic lipids have frequently been suggested to play an important role in the binding affinity of opioid molecules to their receptors (Loh et al., 1974; Loh and Hitzeman, 1978). The role of lipids in the receptor mechanism can be direct, being associated with the chemical structure of the opioid receptor site, or indirectly catalyzing the opioid–receptor interactions by serving as an antena for the capture or selection of the proper orientation of the active molecule (Behnam and Deber, 1984).

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The preferential interaction and accumulation of opioid molecules on the surface of lipid membranes can easily be studied by means of its penetration ability on lipid monolayers. This interaction can be quantitatively estimated by means of the Gibbs free energy of transfer of opioid molecules from an aqueous to hydrophobic phase.

In the present paper, the interactions between opioid molecules and lipids have been studied and compared with pharmacologic data. As there is some evidence in the literature linking gangliosides to opioid receptor structure, we have employed one member of this group (GM_1) in our study. In addition, in order to detect hydrophobic interactions we carried out the same experimentation with phosphatidylcholine.

Chemicals. Codeine-free base was supplied by the Spanish Pharmaceutical Federation, and was purified by the formation and crystallization of its hydrochloride, and eventually the free base was newly liberated.

Dextrometorphan hydrobromide was kindly

supplied by Boehringer, and phentanyl citrate by Jansen-Syntex Iberica. The purity of all molecules was checked by both TLC and HPLC on C_{18} columns. Egg yolk phosphatidylcholine was supplied by Merck and purified by a repeated column chromatography process on silica gel. The eluent was chloroform/methanol (9 : 1) (Barlett, 1959).

Ganglioside GM_1 was from Supelco. TLC of both lipids in amounts of at least 10 times that required for detection showed no impurities after charring the plate with 50% (v/v) H_2SO_4.

Water was double-distilled over alkaline $KMnO_4$ in an all-glass apparatus. Chloroform used as spreading solvent was from Merck.

Penetration studies. The penetration ability of opioid molecules was measured following the description given by Schacht et al. (1978). In order not to use too much drug, a given amount of lipid was spread on the surface to obtain the desired initial surface pressure, and after that, increasing amounts of a concentrated drug solution were successively injected in the subphase, and the experimental increases recorded by surface pressure. The penetration process was achieved in 10 min, but the successive additions were made at intervals of 20 min in order to be sure that the system had reached its equilibrium after each addition. Before spreading the films, the absence of surface-active impurities from the solvents on the subphase was measured by the changes in the surface pressure that occurred on decreasing the surface area to 10% of its initial value. These changes were less than 0.1 $mN \cdot m^{-1}$. The values reported are averages of triplicate runs. Reproducibility was ± 0.5 $mN \cdot m^{-1}$ for surface pressures.

Biological assays. GPI strips were prepared according the description given by Kosterlitz et al. (1975). The strip was stimulated electrically with rectangular pulses at a frequency of 0.01 Hz for the duration of 7 ms at a constant voltage of 40 V. The values of IC_{50} were calculated from dose–response curves. Each point in the curve represented the average of 3 different animals.

Superficial activity of opioid molecules. When a solution of dextrometorphan, codeine or phentanyl

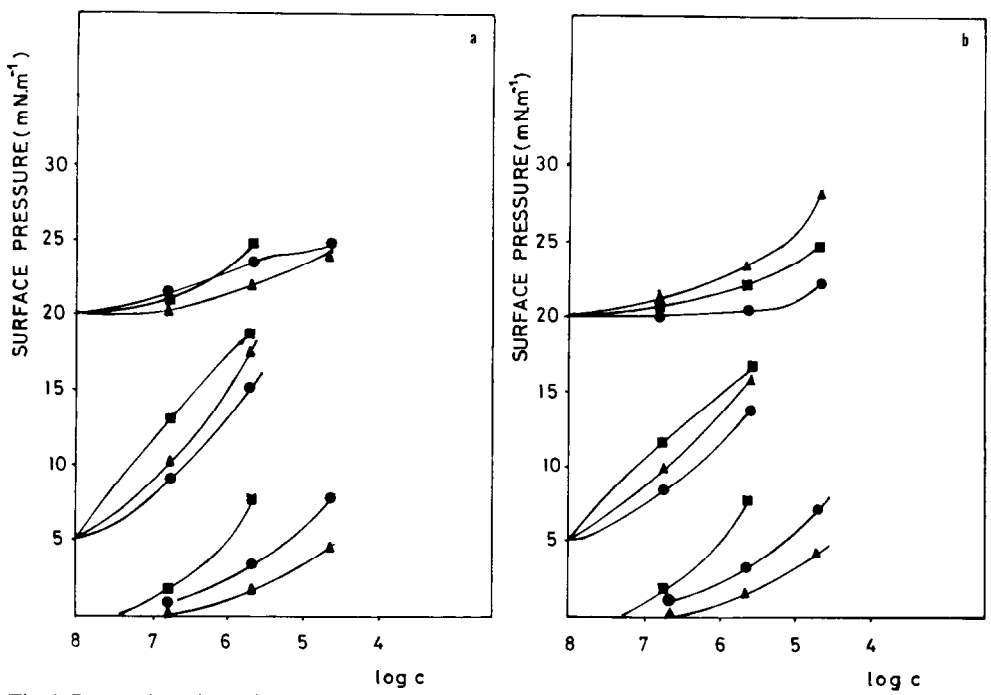

Fig. 1. Penetration of (■) phentanyl; (●) codeine and (▲) dextrometorphan in (a) PC, (b) GM_1 monolayers.

TABLE 1

Surface excess concentration of opioid molecules on monolayers of PC and GM_1, and without monolayer

	Lecithin phosphatidylcholine (π_i; mN · m^{-1})		GM_1 (π_i; mN · m^{-1})		π_i (mN · m^{-1})
Drug:	5	20	5	20	0
Dex	4.3×10^{-11}	0.796×10^{-11}	3.4×10^{-11}	1.2×10^{-11}	0.7×10^{-11}
Cod	3.36×10^{-11}	1.24×10^{-11}	2.82×10^{-11}	0.0613×10^{-11}	1.18×10^{-11}
Phen	4.33×10^{-11}	1.58×10^{-11}	3.46×10^{-11}	0.73×10^{-11}	2.5×10^{-11}

Dex = dextrometorphan; Cod = codeine; Phen = phentanyl.

in water was injected into the aqueous subphase, despite the low concentration (10^{-7} M), a significant decrease of superficial surface tension of the water could be observed. This effect is not due to the formation of a real monolayer because a reduction to the 10% of the initial area does not give a typical compression isotherm. However, it is indicative of a superficial activity of these molecules, and this activity is directly proportional to its subphase concentration.

Opiates and phosphatidylcholine monolayers. The penetration of 3 opioid molecules in phosphatidylcholine monolayers has been determined by measuring the pressure increases produced in monolayers spread at 5 and 20 mN · m^{-1}. Fig. 1 shows the maximum pressure values reached in both cases (after 30 min) and for comparative purposes the equivalent values without monolayers have been included. As usual, penetration decreases in rate and in magnitude with increasing monolayer pressure. The superficial activity codeine hydrochloride and codeine-free base, and its interaction with lipid monolayer, are of the same order of magnitude (data not shown). This fact suggests that at this level of dilution (10^{-7}–10^{-4}) the presence and chemistry of the anions do not affect the superficial behaviour of the molecules and so enables us to compare the results obtained although the anions are different.

In spite of the fact that there are no striking quantitative differences in the penetration pattern of the 3 molecules, if we assume that the higher superficial activity in absence of lipids means higher hydrophobicity, it is clear that phentanyl is the more hydrophobic molecule. As the interaction of the studied molecules with lecithin monolayers follows the same pattern at 20 mN · m^{-1}, it seems that the initial assumption is correct, namely that lecithin interactions can be considered to be due mainly to hydrophobic forces, especially when the monolayer is in a condensed and ordered state, and taken as the reference to explain non-specific interactions.

In contrast, the penetration in ganglioside monolayers does not follow the same order. The fact that dextrometorphan more intensely penetrates the ganglioside monolayers spread at initial pressures of 20 mN · m^{-1} suggests the existence of strong interactions added to the hydrophobic ones. These interactions can be due in part to the more hydrophilic character of GM_1 that interacts better with the less hydrophobic molecule, but could also be a sign of specific interactions related to the opioid receptor phenomena. By applying the Gibbs equation to the pressure increases measured, the surface excess concentration (Γ) of opioid molecules can be calculated. The values are given in Table 1.

Comparing the activities (IC_{50} values) of the 3 molecules measured in the GPI preparation (dextrometorphan 9.9×10^{-6} M, codeine 8.1×10^{-6} M and phentanyl 3.3×10^{-9} M), it may be concluded that hydrophobicity seems, at least in this case, to be directly related to the opioid activity. Moreover, gangliosides do not seem to have a relevant role in μ-opioid receptor composition or recognition.

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