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INTERACTION OF MORPHINE WITH CHOLESTEROL MONOLAYERS

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It appears that a first step in the pharmacological response to a narcotic drug involves a stereochemical interaction of the opiate to a membrane component. The binding of narcotic drugs to a receptor substance present primarily in the synaptic membrane fraction from brains of different animal species, as first described by Pert and Snyder [1], has aroused much interest in the molecular mechanism of action of the opiates. To study the effects of morphine on a model membrane system, we investigated the interaction of morphine with cholesterol monolayers. This methodology seemed appropriate since drugs that are thought to produce their pharmacological effect through modification of membrane components have also been observed to affect artificial lipid membranes (for reviews see refs. 2 and 3).

Surface tension was measured by a Cahn recording electrobalance (model RG) with surface tension attachment and an X-Y recorder (Servogor). Throughout the experiments the temperature was maintained at 19 ± 1°C. Cholesterol monolayers were formed in a 70 ml teflon bath by adding sequentially 1-μl aliquots of freshly prepared cholesterol over a subphase of 150 mM NaCl, with a 50 μl Hamilton microsyringe. The monolayer was completed in about 2 min. Morphine was studied under two different experimental conditions. It was added as the base, dissolved in a mixture of chloroform/methanol (2:1) to the interphase, or as the hydrochloride dissolved in twice-distilled water (pH 5.5) to the subphase. In all cases, the subphase was gently stirred with a magnetic bar after the addition of morphine, and then the cholesterol monolayer was formed. Control experiments were performed adding the same volume of solvent alone to the interphase or subphase.

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All the experimental data were processed by an 1130 IBM computer connected to the X-Y recorder output. The computer program utilized regression analysis to construct area vs. pressure curves from the combined results of 3—4 experiments. Thus, each experimental determination of the area per molecule of cholesterol represents the mean value obtained from at least 3—4 monolayers performed under identical conditions. The area per molecule of cholesterol (A^2 /mol.) was calculated from the area vs. pressure curves at a constant pressure of 10 dynes/cm, assuming a molecular weight of 386 for cholesterol. The variability of the A^2 /mol. for cholesterol in different determinations was less than 6%. In 8 control experiments, the values obtained for cholesterol in a 150 mM NaCl subphase were between 46—52 A^2 /mol., with a mean of 48 A^2 /mol.

Analytical grade salts and solvents were from Merck (Darmstadt, G.F.R.); cholesterol and Tris base from Sigma (St. Louis, Mo.); morphine base and hydrochloride were from Merck (Darmstadt, G.F.R.). Distilled water was deionized and re-distilled through an all-glass Corning apparatus. Surface tension values of 72.75 dynes/cm at 20°C verified the water purity. Fresh cholesterol solutions were prepared every 2—3 days in a mixture of chloroform/methanol (2:1). Salt solutions of the subphase were prepared daily and buffered to the appropriate pH using Tris·HCl, 100 mM.

Morphine base, when applied to the interphase of a 150 mM NaCl solution (pH 7.4), strikingly increased the area per molecule of the cholesterol monolayers. As shown in Fig. 1, the effect followed a steep biphasic dose-effect relation. The maximal effect was observed at a final concentration of

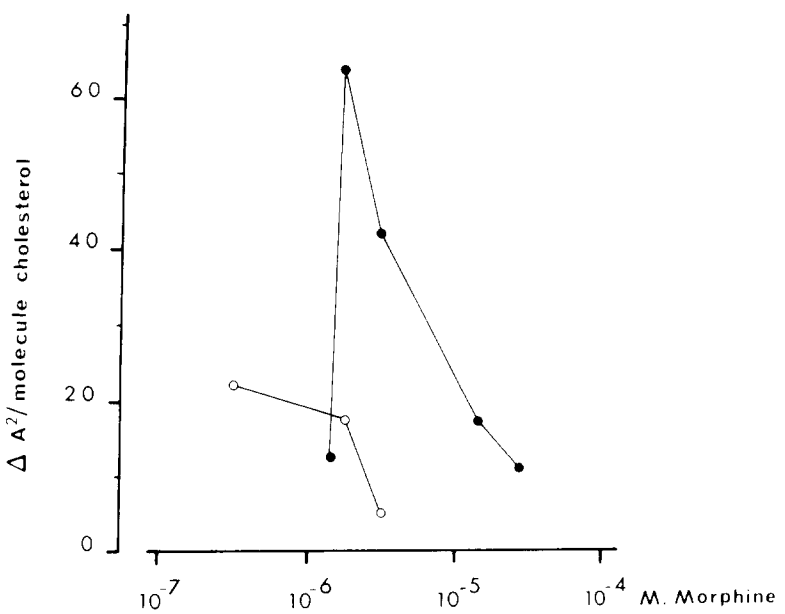

Fig.1. Effect of morphine on cholesterol monolayers. The subphase consisted of 150 mM NaCl adjusted to pH 7.4 with Tris buffer. Abcissa: final molar concentration of morphine. Ordinate: increase of area/molecule of cholesterol in relation to control (same volume of chloroform/methanol (2:1) but no morphine added). Full circles, morphine base added to interphase before application of cholesterol; Open circles, morphine hydrochloride added to the subphase.

$2.4 \cdot 10^{-6}$ M morphine base. When morphine hydrochloride was applied to the subphase, an increase in the A^2 /mol. of cholesterol was also observed but the increase was not as great. Morphine base by itself did not form monolayers.

When 50 mM $CaCl_2$ (pH 7.4) was substituted for the 150 mM NaCl subphase, the area/molecule of cholesterol was practically unaltered (46 vs. 48 A^2 /mol., respectively). However, the effect of morphine base on the cholesterol A^2 /mol. was dramatically decreased. As shown in Fig. 2, the effect of morphine was even less when 100 mM $CaCl_2$ was used in the subphase. It can be seen from these results that the effect of Ca^{2+} is not related to the ionic strength of the subphase, since solutions of 50 mM $CaCl_2$ have the same ionic strength as that of 150 mM NaCl.

If inorganic cations are completely eliminated from the subphase (monolayers formed on twice-distilled water, adjusted to pH 7.4 with Tris buffer), there was no significant difference in the area/molecule of cholesterol (46 as compared to 48 A^2 /mol. for cholesterol in 150 mM NaCl, pH 7.4), but the effect of morphine on the A^2 /mol. of cholesterol was greatly reduced (Fig. 2).

The effects of the subphase pH were also studied. As shown in Fig. 3A, decreasing the pH from 7.4 to 5.5 in a 150 mM NaCl solution produced a shift and a decrease in the effect of morphine base. When twice-distilled water (pH 5.5) was used for the subphase, no significant morphine effect was observed. However, in the presence of 100 mM $CaCl_2$ at pH 5.5, morphine increased the area occupied by cholesterol as compared to pH 7.4 (Fig. 3B). Control experiments with cholesterol alone at pH 5.5 were almost identical to that observed at pH 7.4, and showed a mean area/molecule of 47.5. The exception was 150 mM NaCl pH 5.5, whereas cholesterol showed an A^2 /mol. of 57.0.

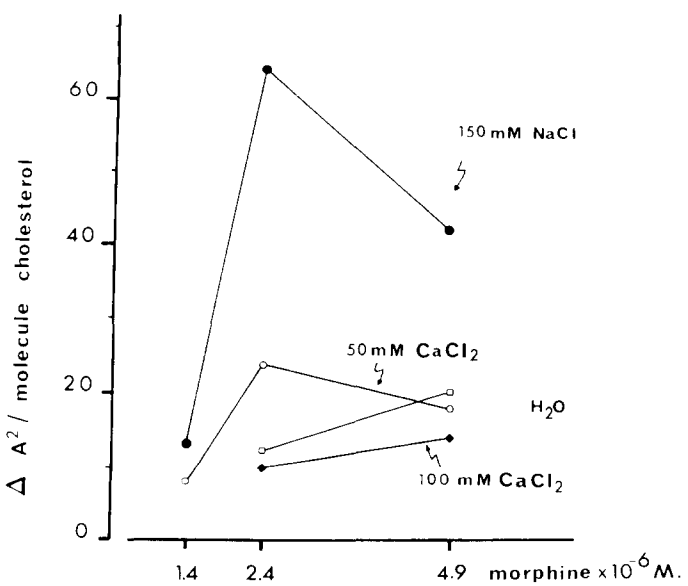

Fig. 2. Ca^{2+}/morphine interaction on cholesterol monolayers. All experiments were carried out on a subphase adjusted to pH 7.4. Abcissa: morphine base final molar concentration; ordinate as in Fig. 1. ●—●, subphase contains 150 mM NaCl; ○—○, subphase contains 50 mM $CaCl_2$; ■—■, subphase contains 100 mM $CaCl_2$; □- - -□, subphase contains twice-distilled water.

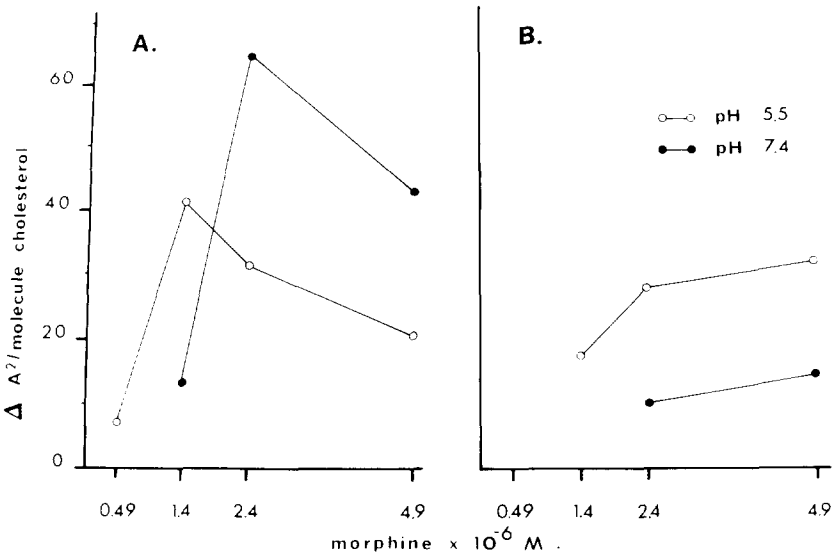

Fig. 3. Effect of pH changes on the interaction between cations and morphine on cholesterol monolayers. Abcissa: morphine base final molar concentrations; ordinate as in Fig. 1. pH changes adjusted with Tris buffer. (A) Subphase contains 150 mM NaCl; (B) Subphase contains 100 mM $CaCl_2$.

The results show that morphine consistently caused a concentration-dependent increase in the area/molecule of cholesterol monolayers. This effect indicates that morphine expanded the cholesterol monolayer, perhaps by intercalation between the cholesterol molecules at the interphase. The diminution of the effect at higher morphine concentrations could be due to a diminution of the hydration state of the interphase, causing cholesterol molecules to be more closely packed. The biphasic effect is observed when morphine is added either to the interphase or to the subphase containing 150 mM NaCl. At pH 7.4, morphine base is very insoluble in the subphase, and most of it must remain in the cholesterol phase. Lowering the pH to 5.5 increases protonation of the alkaloid, and a considerably greater proportion goes into the aqueous subphase. This can explain the diminution of the morphine effect when applied as the salt to the subphase, and the decrease effect of the alkaloid base at pH 5.5. The maximal effect of morphine base at the interphase (which represented a very significant 2.4-fold increase in the cholesterol A^2 /mol.) was seen at doses in the range of 10^{-6} M. However, a still significant 45% increase in the A^2 /mol. was observed with $4.8 \cdot 10^{-7}$ M morphine hydrochloride at the subphase. These concentrations are within the range of brain levels found after a dose of morphine that produces analgesia.

Since the area/molecule found for cholesterol was almost identical for the different cations, ionic strength or range of pH used, the changes observed in the presence of morphine are attributable to an interaction of morphine with the monolayer components. The observation that the morphine effect is greatly reduced when distilled water is used at the subphase suggests that the effect of morphine is dependent on the presence of a specific cation. The antagonistic effect of Ca^{2+} on the morphine-cholesterol interaction is partic-

ularly interesting as it is known that Ca^{2+} antagonizes the effects of morphine in vivo and in vitro [4—7]. The present results support a direct interaction of morphine and Ca^{2+} with a cholesterol membrane. This might be related to a recent report of the formation of a complex between morphine and Ca^{2+} [8].

A clear interpretation of these results is not possible at present. The localization and function of cholesterol and other lipids in nerve membranes is controversial; however, Singer and Nicolson [9] have proposed that lipids exist in a dynamic state with intermixing between different classes or families of lipids. Thus, biological membranes might contain sodium-cholesterol rich areas which could be markedly expanded by low concentrations of morphine. This membrane expansion could alter the functional properties of specialized neuronal membranes where the interaction with morphine or opiates may take place.

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