MOLECULAR PHARMACOLOGY, 16, 154-162

Effect of Opiate Agonists and Antagonists on Lipid Bilayer Fluidity

DAVID A. JOHNSON, ROGER COOKE, AND HORACE H. LOH[1]

Langley Porter Neuropsychiatric Institute and Departments of Pharmacology, Biochemistry/Biophysics and Psychiatry, University of California, San Francisco, San Francisco, California 94143

(Received August 18, 1978)
(Accepted January 10, 1979)

SUMMARY

JOHNSON, DAVID A., ROGER COOKE, AND HORACE H. LOH: Effect of opiate agonists and antagonists on lipid bilayer fluidity. *Mol. Pharmacol.* **16**, 154-162 (1979).

To determine whether opiates increase the fluidity of lipid bilayers that contain opiate-binding lipids, multilamellar bilayers composed of dipalmitoylphosphatidylcholine and 1 mol % cerebroside sulfate, phosphatidylserine, 1-phosphatidylinositol, or 1-phosphatidyl 4,5-bisphosphate were formed. The effect of morphine, naloxone, levorphanol, and dextrophan on the main phase transition temperature was determined by measuring the partition of the spin label, 2,2,6,6-tetramethylpiperidine-1-oxyl, between the aqueous and fluid hydrophobic phases. Although the addition of 1 mol % cerebroside sulfate rendered the bilayers sensitive to the fluidizing effects of drugs, no correlation was observed between the fluidizing and analgesic effects. To show that this lack of correlation was not dependent on the lipid concentration, the experiments with cerebroside sulfate were repeated by measuring the main phase transition temperature with the fluorescence depolarization of 1,6-diphenylhexatriene incorporated into the bilayers. The results of these experiments also showed no correlation between fluidizing and analgesic effects. Hosein *et al.* (*Biochem. Biophys. Res. Comm.*, 78:194-201, 1977), using differential scanning calorimetry, showed that opiates specifically affected a phase transition in brain lipid bilayers and that this effect required an ether precipitate rich in cerebroside sulfate. Since we found no specificity in the ability of opiates to increase lipid fluidity of bilayers that contained cerebroside sulfate, the work of Hosein *et al.* was repeated by monitoring fluidity with 1,6-diphenylhexatriene incorporated into the bilayers. Even 1 mM morphine failed to alter the polarization of 1,6-diphenylhexatriene, indicating that there were no alterations in bulk hydrocarbon fluidity. Because the depolarization of 1,6-diphenylhexatriene monitors the bulk hydrocarbon region and differential scanning calorimetry monitors the whole system, our results suggest that the alterations in the phase transition observed with differential scanning calorimetry do not occur because of "melting" of the bulk hydrocarbon regions of lipids.

INTRODUCTION

Recently there has been renewed interest in the molecular mechanism of action of opiates in producing analgesia, tolerance, and physical dependence. One focus of research has been on the identification of those biomolecules directly involved in the action of opiates (1-4). A number of observations implicate an endogenous lipid, cerebroside sulfate (CS)[2], in opiate activity: (a)

This study was supported in part by USPHS grants DA-00006 and DA-01583 and by the Earl C. Anthony Fund.

[1] Recipient of Career Research Scientist Development Award K2-OA-70554.

[2] The abbreviations used are: CS, cerebroside sulfate; DPH, 1,6-diphenylhexatriene; DSC, differential scanning calorimetry; TEMPO, 2,2,6,6-tetramethylpi-

0026-895X/79/070154-09$02.00/0

it is the major binding component in one putative receptor isolated from mouse brain (5); (b) the binding of opiates to CS is saturable and stereoselective, and the affinity of a large number of opiates for CS correlates remarkably well with their pharmacological potencies (6, 7); (c) azure A, a dye which has high affinity for CS, competitively inhibits opiate receptor binding and increases the ED_{50} for morphine when injected intraventricularly (8); (d) jumpy mice, which have low CS levels, are resistant to the effects of morphine and show a decrease in the number of opiate binding sites (8); and (e) cerebroside sulfatase (EC 3.1.6.8) treatment of rat brain synaptic membranes abolishes about 50% of the stereospecific opiate binding (9).

Because opiates bind to CS and because membrane fluidity has been linked to many membrane processes (10, 11), we hypothesized that opiates effected analgesia by binding to CS in membranes and in turn altering membrane fluidity. Hosein *et al.* (12), using differential scanning calorimetry (DSC), reported that opiates specifically increase brain lipid fluidity and that this effect required an ether precipitate rich in CS (5). We, therefore, attempted to determine whether a small amount of CS (comparable to the amount in the synaptic and microsomal membranes (14) incorporated into lipid bilayers would render lipid membranes sensitive to the fluidizing action of opiates. Since the main phase transition temperature of lipid bilayers is sensitive to changes in fluidity (16), we studied the main phase transition temperature of lipid bilayers composed of dipalmitoylphosphatidylcholine (DPPC) and 1 mol % CS with two methods: (a) the partitioning of the spin label, 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), between the aqueous and fluid hydrophobic phases (16), and (b) the steady state fluorescence depolarization of 1,6-diphenylhexatriene (DPH) incorporated into the bilayers (13, 17, 18). We found that when CS was incorporated into bilayers, opiate agonists and antagonists could fluidize the bilayers; however, there was no correlation between their analgesic activity and their ability to fluidize the lipid bilayers.

Since we could not show any specificity in the ability of opiates to increase lipid fluidity of bilayers that contained CS, we attempted to verify the work of Hosein *et al.* (12) using another method, fluorescence depolarization of DPH incorporated into the brain lipid bilayers. However, when we examined the ability of as much as 1 mM morphine to alter the polarization of DPH, no changes were observed, indicating that there were no alterations in the bulk hydrocarbon fluidity.

Because the measurement of the fluorescence depolarization of DPH monitors the bulk hydrocarbon region and the DSC monitors the whole system, including lipid-lipid, lipid-protein and protein-protein interactions, our data suggest that the alterations in the phase transitions observed with DSC may be due to changes in the melting of proteins or in proteins interacting with lipids, and not to the melting of the bulk hydrocarbon regions of lipids.

MATERIALS AND METHODS

Materials. 1,2-Dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC) was purchased from Calbiochem; 1-phosphatidylinositol was obtained from Sigma Chemical Co.; phosphatidylserine was purchased from Miles Laboratories; and 1,6-diphenyl 1,3,5-hexatriene was obtained from Aldrich. Cerebroside sulfate was purified from bovine brain following the method of Fluharty *et al.* (19) and 1-phosphatidylinositol 4,5-bisphosphate by the method of Johnson *et al.* (20). 2,2,6,6-Tetramethylpiperidine-1-oxyl (TEMPO) was synthesized following the method of Rozantezev and Neiman (21). Dextrophan and levorphanol tartrate were generously donated by Hoffman-LaRoche; naloxone hydrochloride by Endo Laboratories. Morphine sulfate was purchased from Mallinckrodt. All other reagents were reagent grade and were used without further purification.

Preparation of lipid bilayers. Lipids dissolved in an organic solvent were rotary

peridine-1-oxyl; DPPC, dipalmitoylphosphatidylcholine; Tm, main phase transition temperature; Tp, pretransition temperature; EPR, electron paramagnetic resonance.

evaporated on the walls of 25 ml pear-shaped flasks. The flasks were then placed under high vacuum for one hour. After removal from vacuum, the flasks were filled with nitrogen and sealed. Multilamellar bilayers were formed by adding glass beads plus buffer and by gently swirling the beads in the flask in a 50° water bath until the lipid was dispersed.

EPR measurements. Fifty μM TEMPO was prepared by adding 100 mM TEMPO in ethanol to sodium phosphate buffer, 100 mM, pH 7.4. The lipid bilayers were prepared by mixing 14.85 μmol DPPC and 0.15 μmol of one of the acidic lipids or 15 μmol of DPPC in the absence of acidic lipids in an organic solvent prior to evaporation. Just before the EPR spectra were taken, 0.3 ml of the buffer with or without the drug was added to the flask, and the multilamellar bilayers were prepared as described above.

EPR spectra were recorded on a Varian E-3 spectrometer equipped with a variable temperature accessory. The temperature of the sample was measured by placing a thermister just above the microwave cavity inside the sample cell. The thermister was connected to a Cole-Palmer 8202-20 centigrade thermometer. The high field hyperfine line of TEMPO was recorded two minutes after the temperature in the sample cell had equilibrated to the new temperature setting.

Fluorescence depolarization measurements. Steady state fluorescence depolarization of DPH as a function of temperature or time was measured with a fluorimeter, a schematic drawing of which is shown in Fig. 1. Photomultiplier tubes were placed to the right and left of the sample cell: one of the emission beams was polarized parallel and one perpendicular to the plane of the 360 nm polarized excitation beam. Corning 3-74 cutoff filters were placed in the path of the emission beams. The signals from the photomultiplier tubes were fed into Pacific Instrument amplifiers and the outputs of these were fed into an Ithaco 351 Ratiometer. The ratiometer was operated in the polarization mode

$$P = \frac{I_\parallel - I_\perp}{I_\parallel + I_\perp}$$

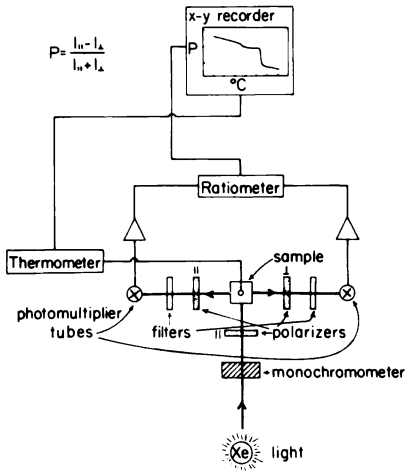

FIG. 1. *Schematic diagram of fluorimeter*

where $I_\parallel$ is the fluorescence intensity parallel to and $I_\perp$ is the intensity perpendicular to the plane of polarization of the excitation beam. The output of this was fed into the x axis of a Hewlett-Packard 7035B x-y recorder. Prior to running of each sample, the outputs of the photomultiplier amplifiers were balanced using a horizontally polarized excitation beam to correct for the difference in the detection efficiency of the two emission beams.

Temperature was regulated by circulating water which heated or cooled the sample cell holder. The sample was mixed with a magnetic stirrer. A thermister was placed in the sample cell just above the excitation beam, and the temperature was monitored with a Cole-Palmer 8502-20 centigrade thermometer modified to allow an analogue signal proportional to the temperature to be fed into the x axis of the recorder. When polarization was monitored as a function of time, a Hewlett-Packard 17108 AM time base unit was plugged into the x axis of the recorder.

After the bilayers were prepared, sufficient 2 mM DPH in tetrahydrofuran was added to the buffer to make it 0.75 μM with respect to DPH. The samples were incubated at 50° for 90 min prior to use so that the dye would be completely absorbed into the bilayers. Drugs were added just prior to use when temperature scans were performed, or injected directly into the sample cell while polarization was recorded and

temperature held constant. Temperature scans were performed at a rate of approximately 1° per min.

Preparation of crude mitochondrial lipids. Following the method of French and Tudoroff (22), brain mitochondria from male Sprague-Dawley rats were isolated and lipid extracted according to the method of Bligh and Dyer (23).

RESULTS

EPR measurements. For the EPR experiments, the phase transition temperatures were determined initially by plotting the natural log of the TEMPO spectral parameter (f) vs the reciprocal of the temperature (°K) (16). The midpoint of the phase transition was designated as the midpoint between the upper and lower transitions.

Figure 2 shows a plot of the spectral f parameter vs temperature of DPPC plus 1 mol % CS in the presence and absence of naloxone and morphine. Even 2 mM morphine had little effect on the phase transition temperature, while 1 mM naloxone, the highest concentration we could get in our buffer, shifted the phase transition down about 1.3°. Although the effect of naloxone was seen only at pharmacologically absurd concentrations, we thought the effect might reflect a qualitative difference in the interaction of opiate agonists and antagonists with CS. To determine whether the effect was due to the presence of CS, the experiment was repeated both in the absence of CS and in the presence of several other acidic lipids that have been shown to bind opiates (24–26). The results of these experiments are shown in Table 1.

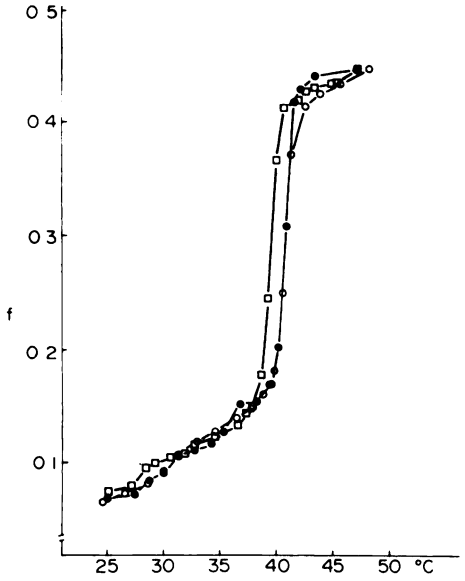

FIG. 2. *Plot of the spectral parameter (f) vs temperature*

50 μM TEMPO in 100 mM sodium phosphate buffer, pH 7.4, in presence of lipid bilayers composed of 50 mM DPPC and 1 mol % CS in the absence (●——●) and presence of 1 mM naloxone (□——□) and 2 mM morphine (○——○).

In the absence of CS, neither morphine nor naloxone had a significant effect on the main phase transition temperature. This was also true when 1 mol % of 1-phosphatidylinositol was incorporated. Naloxone (1 mM) but not morphine (2 mM) had small but detectable effects (−0.3°) when 1 mol % phosphatidylserine was incorporated. When 1-phosphatidylinositol 4,5-bisphosphate was incorporated, both morphine and naloxone lowered the main phase transition temperature, though the effects were small, 0.3° and 0.8°, respectively (Table 1).

Since it appeared that morphine and naloxone might interact in different ways with DPPC-CS bilayers, other opiates were tested in order to determine whether the DPPC-CS bilayers could discriminate between opiate agonists and antagonists. Un-

TABLE 1

The effect of opiate agonists and antagonists on the midpoint of the main phase transition temperature (°C) of bilayers composed of DPPC and 1 mol % of various acidic lipids (~50 mM total lipid) measured by the partitioning of the spin label TEMPO (average standard error on the measurements was ±0.1°)

Composition	No Drug	Morphine (2 mM)	Naloxone (1 mM)
DPPC	40.8°	40.8°	40.8°
DPPC and cerebroside sulfate	40.6°	40.7°	39.3°
DPPC and phosphatidylserine	40.5°	40.4°	40.2°
DPPC and 1-phosphatidylinositol 4,5-bisphosphate	39.7°	39.4°	38.9°
DPPC and 1-phosphatidylinositol	40.5°	40.4°	40.4°

fortunately, levorphanol (mM), an agonist, and dextrophan (1 mM), its inactive enantiomer, both lower the phase transition temperature about 0.7° and 0.3°, respectively. Thus, with this method it was not possible to discriminate between the interaction of opiate agonist and antagonist with CS.

Fluorescence measurements. Because the TEMPO partitioning experiments required approximately 50 mM lipid and because drug effects were seen only at about a one-to-one ratio of drug to CS, we thought that effects might be seen only when nearly all the CS was bound or, in other words, with at least 0.5 mM drug. At this concentration of drug, it would be difficult to discriminate by pharmacologic measures opiate agonists from antagonists (27). So, the phase transition temperatures were measured using steady state fluorescence depolarization of a very hydrophobic dye, DPH. With fluorescence depolarization, it is possible to measure transitions with 100 to 1000 times less lipid than is minimally needed in the EPR experiment.

Based on preliminary experiments, we chose to use 500 μM lipid because this concentration appeared to provide an optimal signal-to-noise ratio. Figure 3 shows the typical polarization vs. temperature plot observed for DPPC plus 1 mol % CS. The main phase transition was determined by visually fitting straight lines through the linear portions of the curve above and below the main phase transition and then fitting a line through the transition. The temperature half-way between the intersections of lines a, b, and c (Fig. 3) was designated *Tm*, the main phase transition. Repeated determinations of *Tm* over many days showed that the values never differed by more than 0.3° from one another. Also apparent in the polarization vs. temperature scans was the presence of the thermal "pretransition" characteristic of multilamellar DPPC bilayers. Since the pretransition provides additional information on possible structural changes in the bilayers, the pretransition temperature, *Tp*, was recorded for each sample, if present, as the point where the polarization vs. temperature curve departed from line *a* (Fig. 3). The values for *Tp* were not as reproducible as the *Tm* values, but varied no more than 1° from one another from day to day with the same lipid mixture.

Table 2 summarizes the effects of mor-

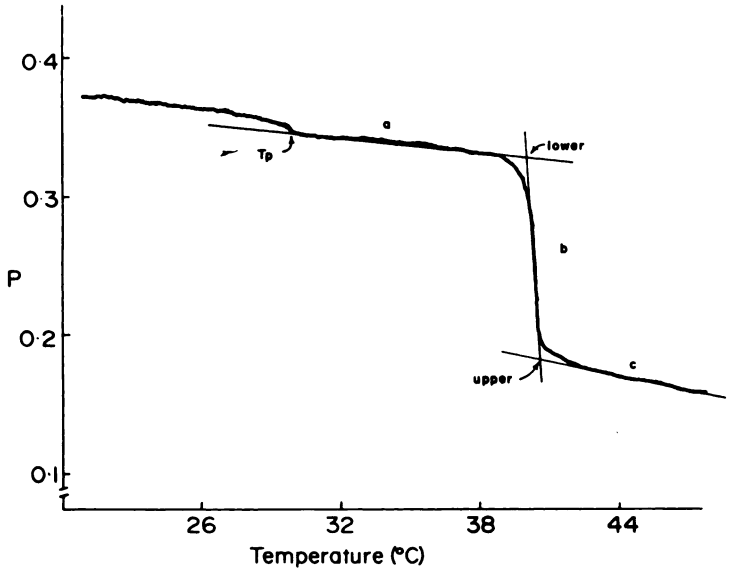

FIG. 3. *Fluorescence polarization (P) of 500 μM DPPC and CS*

Fluorescence polarization of 0.75 μM DPH in 100 mM sodium phosphate buffer, pH 7.4, incorporated into lipid bilayers composed of 500 μM DPPC and 1 mol % CS as the temperature was scanned at approximately 1°/min.

TABLE 2

The effect of opiate agonists and antagonists on the midpoint of the main phase transition and pretransition temperatures (°C) of bilayers

Bilayers are composed of DPPC plus 1 mol % CS (500 μM total lipid) in 100 mM sodium phosphate buffer, pH 7.4. Transition temperatures were obtained by measuring the fluorescence depolarization of incorporated DPH (average standard error of measurements for the main transition ±0.1° and ±0.4° for the pretransition).

Drug (mM)	DPPC and CS (1 mol %)	
	Pretransition[a]	Main Transition
	(°C)	(°C)
Control	30.3	40.3
Morphine		
1.0	N.D.	40.2
Naloxone		
1.0	N.D.	39.0
0.1	29.1	40.3
0.01	30.2	40.3
Levorphanol		
1.0	N.D.	40.0
Dextrorphan		
1.0	29.6	40.1

[a] N.D. = transition not detectable.

phine and naloxone, levorphanol, and dextrophan on DPPC-CS bilayers. As with the EPR experiments, 1 mM morphine appeared to have no detectable effect on the main phase transition temperature, while 1 mM naloxone lowered the *Tm* about 1.3°. Levorphanol (1 mM) lowered the *Tm* slightly as well as dextrophan (1 mM) (0.2°). Although 1 mM morphine did not appear to affect the main phase transition, at concentrations above 0.5 mM, it eliminated the pretransition. Levorphanol but not dextrophan also eliminated the pretransition.

To test for the possibility that opiate might have an effect on membranes which contained more than 1% CS, we examined the effect of morphine on the phase transition of DPPC bilayers that contained 10 or 50% CS and of bilayers composed of 100% CS. Pure CS bilayer has a broad but distinct phase transition from approximately 38 to 51°. As CS is added (<1%), the DPPC pretransition disappears, and the main transition is decreased and broadened. The phase transitions generated by mixtures of DPPC and CS appear to arise from homogeneous mixtures of lipids and not two lipid domains because the transitions are single smooth transitions. Also, the first time the temperature is scanned (down then up) through the phase transition, a small amount of hysteresis is seen; however, this does not occur after subsequent scans. Consequently, phase transitions were measured after the temperature was scanned through the phase transition once. As in the above experiments with 1% CS, morphine (1 mM) had no detectable effect on the phase transition of any DPPC-CS mixture or pure CS bilayer we prepared (data not shown).

Hosein *et al.*, using DSC, showed that opiates specifically affected a phase transition in brain lipid bilayers and inferred that opiates altered lipid fluidity. However, because we had never observed morphine to significantly alter lipid fluidity, we attempted to verify their work using another method; namely, fluorescence depolarization. Crude brain mitochondrial lipids were isolated following the procedures used by Hosein *et al.* (12). The lipids were suspended in sodium phosphate buffer at a concentration of 100 μM with respect to lipid phosphorus. Because no sharp transitions were observed when the temperature was scanned from 2° to 50°, the temperature of the samples was held constant at 37° and various drugs were injected. When we added as much as 1 mM of morphine, we could not detect any change in polarization (Fig. 4A). To show that opiates could depolarize the DPH in bilayers when injected into the sample cuvette, naloxone was injected in the bilayers composed of DPPC and 1 mol % CS at 39.9°. The results of this experiment are shown in 4B; naloxone depolarized the dye within 20 seconds.

To show that fluidizing agents could affect bilayers composed of the crude mitochondrial lipids, various amounts of ethanol were injected. The results of this experiment are shown in Fig. 4C. As little as 1% ethanol decreased the polarization 4–5%, and when 5% ethanol was added, a 12–13% decrease was observed.

DISCUSSION

The primary goal of this work was to determine whether an endogenous lipid,

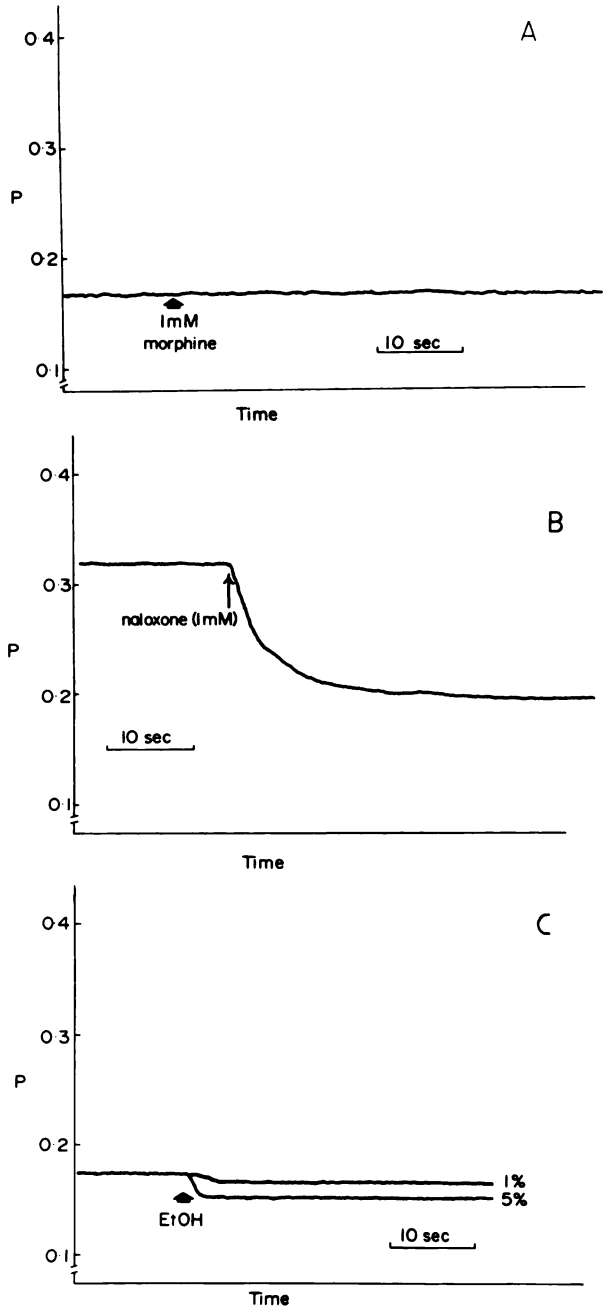

FIG. 4. *Fluorescence polarization (P) of crude mitochondrial lipids or 500 μM DPPC + CS in the presence of opiates*

Fluorescence polarization of 0.75 μM DPH in 100 mM sodium phosphate buffer, pH 7.4, incorporated into lipid bilayers (A) composed of crude mitochondrial lipids (100 μM with respect to total lipid phosphorus) at 37° while 1 mM morphine was injected into sample cuvette, (B) composed of 500 μM DPPC and 1 mol % CS at 39.9° while 1 mM naloxone was injected, and (C) composed of crude mitochondrial lipid (100 μM with respect to total lipid phosphorus) at 37° while 1% and 5% (v/v) of ethanol were injected into the sample cuvette.

CS, which has been implicated in opiate activity, could make lipid membranes responsive to possible fluidizing effects of opiates at pharmacologically reasonable concentrations. In our various attempts at this problem, we found no evidence that opiate agonists or antagonists interacted with CS to increase lipid membrane fluidity at pharmacological concentrations. Very high concentrations of certain opiate agonists and antagonists lowered the phase transition temperatures of bilayers due to the presence of CS; however, these effects were not correlated with the analgesic activity of the drugs. Our results, therefore, suggest that opiates do not interact with CS to cause an increase in lipid membrane fluidity. This conclusion, of course, does not exclude the possibility that some opiate-CS interaction occurs that may then affect protein(s) in such a way that lipid membrane fluidity is altered.

To our knowledge, there have been only two reports examining the effect of opiates on lipid membrane fluidity, one by Hosein *et al.* (12) and one by Cater *et al.* (28). Hosein and his associates, using DCS of crude mitochondrial lipids from control and morphine-treated rats, observed that morphine treatment caused a decrease in the temperature range and enthalpy of the phase transition. This effect was dose dependent and reversible both *in vivo* and *in vitro* by naloxone. Moreover, the ether precipitable fraction of total lipid extract was required for the opiate effect. They concluded that morphine disrupts the packing of the lipids and, thus, increases the lipid fluidity.

When we attempted to repeat their *in vitro* experiments by monitoring the mobility of a fluorescent membrane probe, DPH, incorporated into the membranes, no changes in mobility of DPH were observed even when as much as 1 mM morphine was added. These findings indicated no increase in lipid fluidity. One explanation for the discrepancy between our results and those of Hosein *et al.* is that the phase changes observed by Hosein *et al.* are due to changes in the melting of proteins or proteins interacting with lipids, and not to the melting of the bulk hydrocarbon regions of lipid. Another explanation would be that we did not add enough morphine. The concentration of morphine in their *in vitro* experiments was somewhere between 25 and 50 mM (depending on the final volume); concentrations not obtainable with our experimental protocol. The fact that in their *in vivo* experiments morphine had apparently the same effect as the *in vitro* experiments may have been due to the induction of some long term change in the receptor conformation unrelated to the *in vitro* effects seen only at extremely high concentrations of morphine. If the results of Hosein *et al.* are only obtainable at extremely high concentrations of morphine, the relevance of their work to the analgesic activity of opiates must be questioned, since the ED_{50} concentration of morphine in the brain is about 0.3 μM (29). Cater *et al.* (28) examined the effect of opiate agonists and antagonists on phosphatidylcholine-water model membranes using DSC, NMR and ESR. Their results were consistent with ours insofar as they observed that morphine had no marked effect on the fluidity of the phosphatidylcholine bilayers and while other opiate agonists and antagonists alter the lipid fluidity, this action was not related to their analgesic activity.

ACKNOWLEDGMENTS

The authors wish to acknowledge the editorial and typing assistance of Kaye Welch.

REFERENCES

1. Simon, E. J. & Hiller, J. M. (1978) *In vitro* studies on opiate receptors and their ligands. *Fed. Proc.*, **37**, 141–146.
2. Snyder, S. H. & Simantov, R. (1977) The opiate receptor and opioid peptides. *J. Neurochem.*, **28**, 13–20.
3. Lowney, L. I., K. Schultz, Lowery, P. J. & Goldstein, A. (1974) Partial purification of an opiate receptor from mouse brain. *Science*, **183**, 749–753.
4. Terenius, L. (1973) Stereospecific interaction between narcotic analgesics and a synaptic plasma membrane fraction of rat cerebral cortex. *Acta Pharmacol. Toxicol.*, **32**, 317–320.
5. Loh, H. H., Cho, T. M., Wu, Y. C. & Way, E. L. (1974) Stereospecific binding of narcotics to brain cerebrosides. *Life Sci.*, **14**, 2231–2345.
6. Cho, T. M., Cho, J. S. & Loh, H. H. (1976) H^3-Cerebroside sulfate redistribution induced by

cation, opiate or phosphatidylserine. *Life Sci.*, **19**, 117–124.

7. Cho, T. M., Cho, J. S. & Loh, H. H. (1976) A model system for opiate-receptor interactions: Mechanism of opiate-cerebroside sulfate interaction. *Life Sci.*, **18**, 231–244.
8. Law, P. Y., Harris, R. A., Loh, H. H. & Way, E. L. (1978) Evidence for the involvement of cerebroside sulfate in opiate receptor binding: Studies with azure a and jimpy mutant mice. *J. Pharmacol. Exp. Therap.*, **207**, 458–468.
9. Law, P. Y., Fischer, G., Loh, H. H. & Herz, A. (1979) Inhibition of specific opiate binding to synaptic membrane by cerebroside sulfatase. *Biochem. Pharmacol.*, in press.
10. Wilson, G. & Fox, C. F. (1971) Biogenesis of microbial transport systems: Evidence for coupled incorporation of newly synthesized lipids and proteins into membranes. *J. Mol. Biol.*, **55**, 49–53.
11. Chapman, D. & Urbina, J. (1971) Phase transitions and bilayer structure of *mycoplasma laidlawii B. FEBS Lett.*, **12**, 169–172.
12. Hosein, E. A., Lapalme, M. & Vadas, E. B. (1977) Monitoring the stereospecificity of morphine action *in vivo* and *in vitro* through brain membrane lipid fluidity. *Biochem. Biophys. Res. Comm.*, **78**, 194–201.
13. Andrich, M. P. & Vanderkooi, J. M. (1976) Temperature dependence of 1,6-diphenyl-1,3,5-hexatriene fluorescence in phospholipid artificial membranes. *Biochemistry*, **15**, 1257–1261.
14. Cotman, C., Blank, M. L., Moehl, A. & Snyder, F. (1969) Lipid composition of synaptic plasma membranes isolated from rat brain by zonal centrifugation. *Biochemistry*, **8**, 4606–4612.
15. Chapman, D. (1973) Physical chemistry of phospholipids, in *Form and Function of Phospholipids* (G. B. Ansell, J. N. Hawthorne and R. M. C. Dawson, eds.), Elsevier, New York, 117–142.
16. Shimshick, E. J. & McConnell, H. M. (1973) Lateral phase separations in phospholipid membranes. *Biochemistry*, **12**, 2351–2360.
17. Shinitzky, M. & Inbar, M. (1974) Difference in microviscosity induced by different cholesterol levels in the surface membrane lipid layer of normal lymphocytes and malignant lymphoma cells. *J. Mol. Biol.*, **85**, 603–615.
18. Lentz, B. R., Barenholz, Y. & Thompson, T. E. (1976) Fluorescence depolarization studies of phase transitions and fluidity in phospholipid bilayers. 1. Single component phosphatidylcholine liposomes. *Biochemistry*, **15**, 4521–4528.
19. Fluharty, A. L., Davis, M. L., Kihara, H. & Kritchevsky, G. (1974) Simplified procedure for preparation of ^{35}S-labeled brain. *Lipids*, **9**, 865–869.
20. Johnson, D. A., Merlone, S. C., Loh, H. H. & Ellman, G. L. (1978) Binding of 5-hydroxytryptamine to acidic lipids in an aqueous medium. *J. Neurochem.*, **31**, 713–717.
21. Rozantzev, E. G. & Neiman, M. B. (1964) Organic radical reactions involving no free valence. *Tetrahedron*, **20**, 131–137.
22. French, S. W. & Todoroff, T. (1971) Effect of chronic ethanol ingestion and withdrawal on brain mitochondria. *Res. Comm. Chem. Pathol. Pharmacol.*, **2**, 206–215.
23. Bligh, E. G. & Dyer, W. J. (1959) A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.*, **37**, 911–917.
24. Abood, L. G. & Hoss, W. (1975) Stereospecific morphine adsorption to phosphatidylserine and other membranous components of brain. *Eur. J. Pharmacol.*, **32**, 66–75.
25. Hoss, W. & Smiley, C. (1977) Immobilization of opiates by membrane lipids. *Mol. Pharmacol.*, **13**, 251–258.
26. Wu, Y. C., Cho, T. M., Loh, H. H. & Way, E. L. (1976) Binding of narcotics and narcotic antagonists to triphosphoinositide. *Biochem. Pharmacol.*, **25**, 1551–1553.
27. Frank, G. (1976) Opiate drug receptors on excitable cell membranes. *Arch. Int. Pharmacodyn.*, **217**, 4–17.
28. Cater, B. R., Chapman, D., Hawes, S. M. & Saville, J. (1974) Lipid phase transition and drug interactions. *Biochim. Biophys. Acta*, **363**, 54–69.
29. Patrick, G. A., Dewey, W. L., Spaulding, T. C. & Harris, L. S. (1975) Relationship of brain morphine levels to analgesic activity in acutely treated mice and rats in pellet implanted mice. *J. Pharmacol. Exp. Ther.*, **193**, 876–883.