MOLECULAR PHARMACOLOGY, 22:721-724

Increased Fluidity of a Model Membrane Caused by Tetrahydro-β-Carbolines

PEKKA PEURA,[1] PETER MACKENZIE,[2] ULLA KOIVUSAARI,[3] AND MATTI LANG[4]

Department of Pharmaceutical Chemistry, University of Kuopio, Kuopio, Finland, Developmental Pharmacology Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20205, Department of Physiology, University of Kuopio, Kuopio, Finland, and Eflab Oy, 08100 Helsinki 81, Finland

Received June 22, 1981; Accepted July 7, 1982

SUMMARY

Alterations in membrane fluidity caused by alcohols and tetrahydro-β-carbolines (THBCs) have been studied. Dipalmitoylphosphatidylcholine vesicles were used as a membrane preparation, and changes in the fluidity were revealed by two fluorescent probes: 1-anilinonaphthalene-8-sulfonic acid (1,8-ANS) and *N*-phenylnaphthylamine (NPN). It was found that THBCs, which are condensation products of tryptamine and formaldehyde or acetaldehyde, were at least 2 orders of magnitude more potent in causing fluidity changes than the comparable alcohols (methanol and ethanol). Both 1,8-ANS (binding close to the polar end of the phospholipid molecules) and NPN (binding to the hydrophobic region of the membrane) were able to reveal changes in membrane fluidity, although there were differences between the behavior of the two probes. The condensation product of acetaldehyde—the primary metabolite of ethanol—and tryptamine were found to be 200–300 times more potent in causing fluidity changes than ethanol itself (as determined with both 1,8-ANS and NPN).

INTRODUCTION

Interference with the function of nerve cells is thought to be the mode of action of a wide range of chemical compounds. Among these compounds are general and local anesthetics (1) as well as hallucinogenic agents (2). It seems that the molecular mechanism of action of these compounds depends upon their chemical structure. They can either bind to receptors (3, 4) or—as lipophilic compounds—they can concentrate unspecifically in the phospholipid bilayer, thereby causing changes in the fluidity or charge of the membrane (5–9).

The psychopharmacological action of ethanol has been studied by several researchers (10). Its alterations of the membrane structure are known to some extent (11, 12), but the detailed mechanism of its action is largely unknown (10).

One interesting hypothesis suggests that it is not the ethanol molecule itself but rather the condensation products of acetaldehyde, the initial metabolite of ethanol, and biogenic amines which are responsible for the biological effects of ethanol (13). This hypothesis is based, for example, on the following facts: (a) acetaldehyde and biogenic amines react *in vitro*, forming condensation products (14), and (b) the structures of some condensation products are very similar to those of hallucinogenic *Harmala* alkaloids (15).

The formation of condensation products from acetaldehyde with indolalkylamines, the tetrahydroharmans, or with catecholamines, tetrahydroisoquinolines, has been repeatedly proposed (16–20). Quite recently, our group has been able to identify 1-Me-THBC[5] in human plasma and platelets after ethanol consumption (21). After verifying the formation of 1-Me-THBC *in vivo*, our goal was to study the biological effects of this compound *in vivo* and *in vitro*.

In this report we describe the effects of 1-Me-THBC and THBC on the fluidity of a model membrane. Changes in membrane fluidity as determined with the fluorescent probes 1,8-ANS and NPN are compared with those caused by comparable alcohols.

MATERIALS AND METHODS

THBC derivatives were synthesized with the Pictet-Spengler reaction by refluxing tryptamine with aldehydes (22) (formaldehyde, acetaldehyde, propionaldehyde, and butyraldehyde). The NMR and mass spectrometry spectra were consistent with the assigned structures of synthesized compounds (23). Prior to use the compounds

[1] Department of Pharmaceutical Chemistry, University of Kuopio.
[2] Developmental Pharmacology Branch, National Institute of Child Health and Human Development.
[3] Department of Physiology, University of Kuopio.
[4] Eflab Oy, Helsinki.

[5] The abbreviations used are: 1-Me-THBC, 1-methyl-tetrahydro-β-carboline (tetrahydroharman); THBC, tetrahydro-β-carboline; 1,8-ANS, 1-anilinonaphthalene-8-sulfonic acid; NPN, *N*-phenylnaphthylamine; DPPC, dipalmitoylphosphatidylcholine; 1-Et-THBC, 1-ethyl-tetrahydro-β-carboline; 1-Pr-THBC, 1-propyl-tetrahydro-β-carboline.

0026-895X/82/060721-04$02.00/0

were stored in the dark at −20°. No detectable changes in the structures of the compounds took place.

As a membrane preparation, DPPC vesicles were used, since changes in their phase transition temperature (about 42°) are easily monitored (24). DPPC was sonicated (MSE, ultrasonic disintegrator) above its phase transition temperature in 10 mM Tris-HCl buffer (pH 7.4) containing 100 mM KCl, 0.1 mM dithiothreitol, 0.1 mM EDTA, and 0.05% sodium azide for about 10 min until the suspension was clear. The sonicated preparation was then centrifuged at 100,000 × *g* for 30 min and the supernatant was used as the source of DPPC vesicles. This method produces unilamellar vesicles which have the typical bilayer structure of biological membranes (25).

To express changes in the fluidity of the membrane preparation we used two fluorescent probes, 1,8-ANS (as the magnesium salt, from Serva, Heidelberg, West Germany) and NPN (Merck, Darmstadt, West Germany, recrystallized twice from hot methanol).

The fluorescence intensity of a suspension containing DPPC vesicles [0.1 mg of DPPC/ml of 10 mM Tris-HCl buffer (pH 7.4)], a fluorescent probe, and a THBC derivative or an aliphatic alcohol was determined with a Perkin-Elmer MPF 3A spectrophotofluorometer. The cuvette temperature was increased at a constant rate of 2°/min. Emission was recorded at 472 nm (excitation 382 nm) for 1,8-ANS and at 422 nm (excitation 348 nm) for NPN. A 5-μl sample of 1 mM 1,8-ANS in suspension buffer or of NPN in acetone was dissolved in vesicle suspension. The effect of THBC, 1-Me-THBC, 1-Et-THBC, or 1-Pr-THBC on the fluidity of the membrane preparation was studied. The compounds were dissolved in acetone, and 5-μl aliquots were added to vesicle suspensions. THBC and 1-Me-THBC were added at 1.0 mM, 0.5 mM, 0.1 mM, and 0.05 mM concentrations and investigated with both fluorescent probes. The effects of 1-Et-THBC and 1-Pr-THBC on DPPC vesicle fluidization were determined at 0.5 mM and 1.0 mM with both fluorescent probes. A control experiment was carried under similar conditions without THBC derivatives in the suspension. Methanol, ethanol, propanol, and butanol were used at 75 mM concentrations in the experiments. The partition coefficients of THBC derivatives between 1-octanol and Tris-HCl buffer (pH 7.4) and their solubility in the buffer were analyzed.

RESULTS

The effects of THBC and 1-Me-THBC on the phase transition temperature of DPPC vesicles is shown in Fig. 1. The maximum of the fluorescence intensity in the model membrane shifted into lower temperature with increasing concentrations of THBC and 1-Me-THBC. The fluorescence intensity of a membrane-bound probe reaches its maximum at the phase transition temperature (6, 26, 27).

The maximal fluorescence of membrane-bound 1,8-ANS was reached at 41.5° in the absence of THBC derivatives and at 39.8° with NPN. At 0.05 mM concentration, THBC and 1-Me-THBC had only a slight effect on the phase transition temperature recorded with both

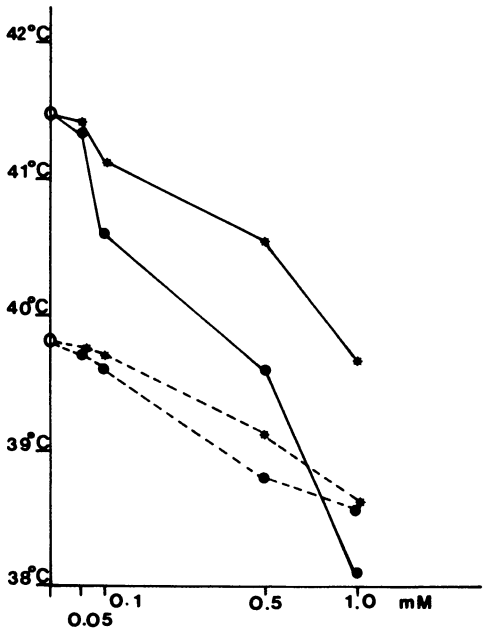

FIG. 1. *Phase transition temperatures of DPPC vesicles*
Phase transition temperatures of DPPC vesicles in increasing concentrations of THBC (●), 1-Me-THBC (★), and the controls (○) determined with 1,8-ANS (——) and NPN (– – –).

fluorescent probes. An increase in the concentration of 1-Me-THBC from 0.1 mM to 1.0 mM produced a change in the phase transition temperature: 1.5° determined with 1,8-ANS and 1.0° with NPN. THBC changed these temperatures by 2.5° and 1.0°, respectively. The THBC derivatives with a longer alkyl side chain fluidized the model membrane more efficiently.

The fluidization effects of various concentrations of methanol, ethanol, propanol, and butanol (comparable to the aldehydes used in synthesizing the THBC derivatives) were also studied. Aliphatic alcohols were found to have the same kind of effect, although it was much weaker on the membrane preparation than the comparable THBCs. Accordingly, a 75 mM alcohol concentration (Table 1) changed the phase transition temperature approximately as much as 0.1 mM THBC (Fig. 1). Formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, and tryptamine had no measurable effects on membrane fluidity even at 25 mM concentrations.

The solubility of all of the THBC derivatives studied in the Tris-HCl buffer was in the same range, 3.2–5.1 mM/liter. However, those compounds with a longer alkyl

TABLE 1

Shifts in phase transition temperatures of DPPC vesicles caused by 75 mM methanol, ethanol, propanol, and butanol as determined by 1,8-ANS and NPN

	Phase transition temperature	
	1,8-ANS	NPN
Control	41.8°	40.6°
Methanol	41.2	40.8
Ethanol	40.7	40.6
Propanol	40.5	40.5
Butanol	38.1	39.5

TABLE 2

Partition coefficients of THBC, 1-Me-THBC, 1-Et-THBC, and 1-Pr-THBC at 22° between 1-octanol and Tris-HCl buffer (pH 7.4) and the solubilities of the compounds in the buffer solution

	Partition coefficient	Solubility
		mM/liter
THBC	2.8	5.1
1-MeTHBC	3.1	4.6
1-Et-THBC	6.1	3.8
1-Pr-THBC	12.3	3.2

side chain showed increased solubility in the 1-octanol phase (Table 2).

DISCUSSION

The four THBC derivatives investigated shifted the phase transition temperature of DPPC vesicles toward lower temperatures. This effect was found with concentrations of THBC and 1-Me-THBC as low as 0.1 mM (Fig. 1). A longer alkyl side chain in the THBCs increases the fluidity effect of THBCs. This may be due to the increased lipophilic character of those compounds. The two fluorescent probes used are known to bind at different sites in the membrane (26). 1,8-ANS binds close to the polar heads of the lipid molecules and NPN to the inside of the membrane, among the hydrophopic fatty acid tails (27, 28). By using those probes we tried to localize THBC derivatives in the membrane preparation. Both fluorescent probes revealed changes in the membrane fluidity. This indicates that the compounds tested affect the membrane structure both close to the polar head and in the deeper layers. However, it is interesting to note that the shift in the membrane fluorescence caused by THBC and 1-Me-THBC was somewhat stronger in the case of 1,8-ANS than in the case of NPN.

By carrying out measurements with several concentrations we were able to estimate that THBC derivatives were 100–200 times more potent in causing membrane changes than were the comparable alcohols when 1,8-ANS was used as a probe. Only butanol at a concentration of 75 mM caused a considerable change in NPN fluorescence, whereas methanol, ethanol, and propanol had only a very weak effect or no effect (Table 1).

The acute effects of ethanol are suggested to result from interactions of ethanol with membranes (29). The chronic consumption of ethanol results in tolerance and dependence, which is suggested to arise from alterations in the membrane components of the nervous system (30, 31). Feeding ethanol to mice has been reported to increase the cholesterol content in mitochondrial and synaptosomal membranes (32) and to change the ratio between saturated and unsaturated fatty acids in synaptosomal membranes (33), which results in decreased fluidization of membranes. Low concentrations (20–40 mM) of ethanol have been reported to increase the fluidity of erythrocyte, mitochondrial, and synaptosomal membranes of mice *in vitro* (11). A high concentration of propanol (1 M) was needed to change the fluidity of human erythrocyte membranes *in vitro* (34). However, those experiments were carried out by ESR techniques. In our study, the concentration of ethanol required for fluidization of the model membrane was quite high (75 mM), but not lethal to mammals.

THBC and 1-Me-THBC forms *in vitro* easily from tryptamine and formaldehyde or acetaldehyde, respectively (35). In ethanol intoxication this reaction may occur, producing 1-Me-THBC, which has been identified in human plasma after ethanol intake (21). However, the concentration of the compound in plasma is low compared with the concentration of 1-Me-THBC needed to change the fluidity of the model membrane, but the concentration of this compound in the central nervous system is unknown.

In conclusion, 1-Me-THBC, which appears in plasma after alcohol consumption, and three of its close analogues were found to be at least 2 orders of magnitude more potent in causing fluidity changes throughout a model membrane than were the comparable alcohols. This should be taken into account in studies concerning the mode of action of ethanol, especially since several earlier studies have suggested that ethanol itself expresses its biological effects by changing the fluidity of the nerve membrane.

REFERENCES

1. Roth, S. H. Physical mechanisms of anesthesia. *Annu. Rev. Pharmacol. Toxicol.* **19**:159–178 (1979).
2. Brawley, P., and J. C. Duffield. The pharmacology of hallucinogens. *Pharmacol. Rev.* **24**:31–66 (1972).
3. Kupfermann, I. Modulatory actions of neurotransmitters. *Annu. Rev. Neurosci.* **2**:447–465 (1979).
4. Boarder, M. R. The mode of action of indolamine and related hallucinogens. *Essays Neurochem. Neuropharmacol.* **2**:21–48 (1977).
5. Bach, D., A. Raz, and R. Goldman. The effect of hashish compounds on phospholipid phase transition. *Biochim. Biophys. Acta* **436**:889–894 (1976).
6. Lee, A. G. Interactions between anesthetics and lipid mixtures: normal alcohols. *Biochemistry* **15**:2448–2454 (1976).
7. Lee, A. G. Model for action of local anaesthetics. *Nature (Lond.)* **262**:545–548 (1976).
8. Ueda, I., C. Tashiro, and K. Arakawa. Depression of phase-transition temperature in a model cell membrane by local anesthetics. *Anesthesiology* **46**:327–332 (1977).
9. Vanderkooi, J. M., R. Landesberg, H. Selik, II, and C. C. McDonald. Interaction of general anesthetics with phospholipid vesicles and biological membranes. *Biochim. Biophys. Acta* **464**:1–16 (1977).
10. Myers, R. D. Psychopharmacology of alcohol. *Annu. Rev. Pharmacol. Toxicol.* **18**:125–144 (1978).
11. Chin, J. H., and D. B. Goldstein. Effects of low concentrations of ethanol on the fluidity of spin-labeled erythrocyte and brain membranes. *Mol. Pharmacol.* **13**:435–441 (1977).
12. Chin, J. H., D. B. Goldstein, and L. M. Parsons. Fluidity and lipid composition of mouse biomembranes during adaptation to ethanol. *Alcoholism Clin. Exp. Res.* **3**:47–49 (1979).
13. Rawan, R. G. Toxic effects of ethanol: possible role of acetaldehyde, tetrahydroisoquinolines and tetrahydro-β-carbolines. *Toxicol. Appl. Pharmacol.* **34**:2–27 (1975).
14. Abramovitch, R. A., and I. D. Spenser. The carbolines. *Adv. Heterocyclic Chem.* **3**:79–207 (1964).
15. Usdin, E., and D. H. Efron. Psychotropic drugs and related compounds. Publication No. HSM 72-9074, Department of Health, Education and Welfare, Washington, D. C., 1115–1118 (1972).
16. Sandler, M., S. B. Carter, K. R. Hunter, and G. M. Stern. Tetrahydroisoquinoline alkaloids *in vivo* metabolites of L-dopa in man. *Nature (Lond.)* **241**:439–443 (1973).
17. Collins, M. A., W. P. Nijm, G. F. Borge, G. Teas, and G. Goldfarb. Dopamine-related tetrahydroisoquinolines: significant urinary excretion by alcoholics after alcohol consumption. *Science (Wash. D. C.)* **206**:1184–1186 (1979).
18. McIsaac, W. M. Formation of 1-methyl-6-methoxy-1,2,3,4-tetrahydro-2-carboline under physiological conditions. *Biochim. Biophys. Acta* **52**:607–609 (1961).
19. Airaksinen, M. M., and W. M. McIsaac. Indolealkylamines and behavior. *Annu. Med. Exp. Fenn.* **46**:367–381 (1968).
20. Holman, R., G. Elliot, E. Seagraves, J. R. DoAmaral, J. Vernicos-Daniellis, K. Kellar, and J. Barchas. On the role of acetaldehyde in the actions of ethanol, in *Satellite Symposium: 6th International Congress on Pharmacology* (K. O. Lindroos and C. L. P. Erikson, eds.). The Finnish Foundation for Alcohol Studies, Helsinki, 207–219 (1975).

21. Peura, P., I. Kari, and M. M. Airaksinen. Identification by selective ion monitoring of 1-methyl-tetrahydro-β-carboline in human platelets and plasma after ethanol intake. *Biomed. Mass Spectrom.* **7**:553–555 (1980).
22. Whaley, W. M., and T. R. Govindachari. The Pictet-Spengler synthesis of tetrahydroisoquinolines and related compounds, in *Organic Reactions* (R. Adams, ed.), Vol. VI. John Wiley & Sons, New York, Chap. 3 (1951).
23. Peura, P., and E. Nousiainen. Syntheses and spectral data of some 1-alkyl-substituted 1,2,3,4-tetrahydro-β-carbolines. *Acta Pharm. Fenn.* **90**:175–178 (1981).
24. Gregoriadis, G. Enzyme entrapment in liposomes. *Methods Enzymol.* **44**:218–227 (1976).
25. Brunner, J., P. Skraval, and H. Hauser. Single bilayer vesicles prepared without sonication: physico-chemical properties. *Biochim. Biophys. Acta* **455**:322–331 (1976).
26. Radda, G. K., and J. Vanderkooi. Can fluorescent probes tell us anything about membranes? *Biochim. Biophys. Acta* **265**:509–549 (1972).
27. Jacobson, K., and D. Papahadjopoulos. Effect of a phase transition on the binding of 1,8-ANS to phospholipid membranes. *Biophys. J.* **16**:549–560 (1976).
28. Eling, T. E., and R. P. DiAugustine. Role of phospholipids in the binding and metabolism of drugs by hepatic microsomes. *Biochem. J.* **123**:539–549 (1971).
29. Ritchie, J. M. The aliphatic alcohols, in *The Pharmacological Basis of Therapeutics* (A. Goodman, L. S. Goodman, and A. Gilman, eds.), Ed. 6. Macmillan, New York, 376–385 (1980).
30. Goldstein, D. B., and V. W. Arnold. Drinking patterns as predictors of alcohol withdrawal reactions in DBA/2J mice. *J. Pharmacol. Exp. Ther.* **199**:408–411 (1976).
31. Chin, J. H., and D. B. Goldstein. Drug tolerance in biomembranes: a spin label study of the effects of ethanol. *Science* (*Wash. D. C.*) **196**:684–685 (1977).
32. Chin, J. H., L. M. Parsons, and D. B. Goldstein. Increased cholesterol content of erythrocyte and brain membranes in ethanol-tolerant mice. *Biochim. Biophys. Acta* **513**:358–363 (1978).
33. Littleton, J. M., and G. John. Synaptosomal membrane lipids of mice during continuous exposure to ethanol. *J. Pharm. Pharmacol.* **29**:579–580 (1977).
34. Paterson, S. J., K. W. Butler, P. Huang, J. Labelle, I. C. P. Smith, and H. Schneider. The effects of alcohols on lipid bilayers: a spin label study. *Biochim. Biophys. Acta* **266**:597–602 (1972).
35. Kenyhercz, T. M., and P. T. Kissinger. High-performance liquid chromatographic assay of isoquinoline alkaloid formation from reaction of biogenic amines and aldehydes. *J. Pharm. Sci.* **67**:112–113 (1979).

Send reprint requests to: Dr. Pekka Peura, Department of Pharmacy, University of Kuopio, P.O.B. 138, 70101 Kuopio 10, Finland.