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STRUCTURAL ISOMERS OF TETRADECENOL DISCRIMINATE BETWEEN THE LIPID FLUIDITY AND PHASE TRANSITION THEORIES OF ANESTHESIA

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SUMMARY The long-chain unsaturated alcohols, cis- and trans-9,10-tetradecenol were found to be equipotent both as general anesthetics and local anesthetics. Their effects on the phase transition temperature of dipalmitoylphosphatidylcholine/water dispersions were measured spectroscopically by partitioning of the spin label 2,2,6,6-tetramethylpiperidine-1-oxyl. Also spectroscopic order parameters were obtained from spin labeled egg lecithin/cholesterol vesicles, in the absence and presence of the two alcohols. Whereas the egg lecithin/cholesterol membranes were fluidised by both alcohols to a similar extent, cis-tetradecenol lowered the transition temperature of dipalmitoylphosphatidylcholine, while the trans-isomer elevated the transition temperature. These results are thus inconsistent with the phase transition model of anesthetic action. The possible application of these alcohols to diagnose the origin of discontinuities in Arrhenius plots is discussed.

Contemporary theories of anesthesia suggest that anesthetic agents act either directly on those proteins in excitable membranes which are responsible for the ion fluxes associated with excitation, or on their surrounding lipids in such a way that the resulting perturbation is subsequently transmitted to the proteins (1,2). Experimental evidence in the last few years has led to an elaboration of the traditional lipid solubility theory of anesthesia (3), and two conflicting models have emerged. Firstly, spin label studies on aqueous lipid dispersions, or liposomes, with a large variety of anesthetics have shown that all the agents studied to date share the property of disordering PC[1]/cholesterol membranes (2,4,5), and also natural membranes (6)

[1]Abbreviations used: DPL, dipalmitoylphosphatidylcholine; TEMPO,2,2,6,6-tetramethylpiperidine-1-oxyl; PC, egg lecithin; 7,6-PC, 1-acyl-2 [8(4,4-dimethyloxazolidine-N-oxyl)] palmitoylphosphatidylcholine.

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provided that their cholesterol content exceeds about 30% (7,8). Such results support the so-called lipid fluidity model of anesthesia. The alternative model is based on the fact that pure lipids undergo a thermotropic phase transition from the gel to the liquid crystalline state. The idea that local anesthetics exert their effect by lowering the phase transition temperature of membrane lipids has been presented and tested with a wide variety of anesthetics by Lee (9) and Ueda et al. (10). A somewhat similar phase transition model was earlier postulated by Trudell (11,12) to account for general anesthesia. He suggested that general anesthetics lower the transition temperature of specific lipids in a mixed lipid membrane, thus preventing the possibility of lateral phase seperations within the membrane.

Both the fluidity and phase transition theories account for the phenomenon of pressure reversal of anesthesia (4,12,13), and both predict that the action of an anesthetic should be proportional to its membrane concentration to a first approximation. Thus, it is difficult to provide a critical test between the two types of theory unless one can find anesthetics whose action on the bilayers may not be proportional to their lipid solubility. We reasoned that because of their similar stereochemistry, the anesthetic trans-tetradecenol and the non-anesthetic tetradecanol might both raise the lipid phase transition temperature, whilst the corresponding cis-isomer, also an anesthetic, might lower it. The evidence reported here supports this reasoning.

MATERIALS AND METHODS

Cis- and trans-tetradecenol were purchased from Applied Science Labs., Inc., Pa., and DPL was obtained from Grand Island Biological Company, N.Y. TEMPO was synthesised by the method of Rozantsev (14), and 7,6-PC by the method of Hubbell and McConnell (15). TEMPO spectral partition coefficients (f) were calculated by the method of Shimshick and McConnell (16) and were determined on a Varian EM-500 electron spin resonance spectrometer. The microwave cavity was thermostated to ± 0.1°C. Samples of DPL (130mM) in phosphate buffer (0.1M NaCl, 10mM sodium phosphate) at pH 7.4, with 1mM TEMPO and appropriate alcohol were vortexed to homogeneity at 50°C, and placed in 1mm capillaries. Fifteen minutes were allowed for equilibration at each temperature. Order parameters were calculated by the method of Hubbell and

McConnell (15) using the spin label 7,6-PC (0.2mM) in sonicated vesicles containing PC and cholesterol (26mM; 1:1), and the appropriate alcohol, in 0.9% NaCl. Spectra were run at ambient temperature on a Varian E-109 spectrometer.

General anesthesia was determined by immersing groups of small tadpoles (ten per group) in 100ml of alcohol solution. Two concentrations of alcohol were employed, and at each concentration the loss of righting reflex was used as a criterion of anesthesia. Local anesthesia was determined in axon bundles dissected from the lobster walking leg. The bundles were arranged for conventional extracellular recording in a three-compartment lucite bath, using vaselined seals. Alcohols were applied in Ringer's solution to the central well, and the amplitude of the compound action potential was recorded as a function of time.

RESULTS

The general anesthetic potency of the tetradecenols was examined at 30μM and 60μM. At each concentration, the number of tadpoles anesthetised was determined after sufficient equilibration (up to 3 hrs.). The data was analysed according to the method of Waud (17), and within experimental error the two isomers were found to be equipotent full anesthetics with ED_{50}'s of 30μM (see Table 1) and their dose-response curves were similar in slope to the lower alcohols (Chang and Miller, unpublished results). Complete reversibility was effected by removing the tadpoles from the drug solutions and placing them in a large volume of distilled water overnight. The saturated alcohol n-tetradecanol was without anesthetic effect.

The alcohols were tested for local anesthetic activity on the lobster walking leg nerve. Both compounds produced complete axonal block at a concentration of 60μM, the time to half-block being identical in each case (see Table 1). Attempts to reverse the block by perfusion with drug-free Ringer's solution were only partially successful. This is not surprising since both compounds are sparingly water-soluble, but partition readily into lipid. In this context, reports on alkanes attest to the difficulty in obtaining good reversal (18). However, after complete block had been achieved, partial recovery (15%) was consistently obtained in six nerves by removing the axon bundles and allowing them to equilibrate over several hours in a large volume of drug-free Ringer's solution at $4^{o}C$. Control experiments (immediately after

Table 1. A comparison of the anesthetic potencies of cis- and trans-9,10-tetradecenol with their effects on lipid bilayer phase transitions and fluidity.

	9,10-Tetradecenol	
	Cis-isomer	Trans-isomer
Tadpole anesthesia, ED_{50} μM.	30 ± 6	30 ± 1.5
Lobster nerve, time to half block (mins.) at 60 μM.	13 ± 1 (n=4)	12 ± 3 (n=6)
Change in phase transition temperature of dipalmitoyl lecithin with 33 mole % of alcohol, (°C).	-7.0 ± 0.15	1 ± 0.18
Change in order parameter (S) of egg lecithin/cholesterol (1:1) with 11 mole % alcohol.	-0.028 ± 0.006	-0.022 ± 0.005

block) confirmed that recovery was not simply an artefact arising from the remaking of electrical seals.

The temperature-dependent partitioning of the spin label TEMPO (16) was used to determine the phase transition temperature of DPL. The tetradecenols were found to modify the transition temperature in a concentration-dependent manner. However, trans-tetradecenol elevated the transition temperature, whereas the cis-isomer lowered it (Figure 1). At 33 mole% incorporation, the difference between the two isomers was substantial (Table 1).

The order parameter (S) for PC/cholesterol (1:1) liposomes was found to be 0.686 ± 0.005 (S.D.). At ambient temperature (well above the phase transition temperature for this system), both alcohols (at 11 mole%) lowered the order parameter, that is, they both fluidised the lipid bilayer. Furthermore, within the experimental error, each alcohol induced the same increase in membrane fluidity (Table 1).

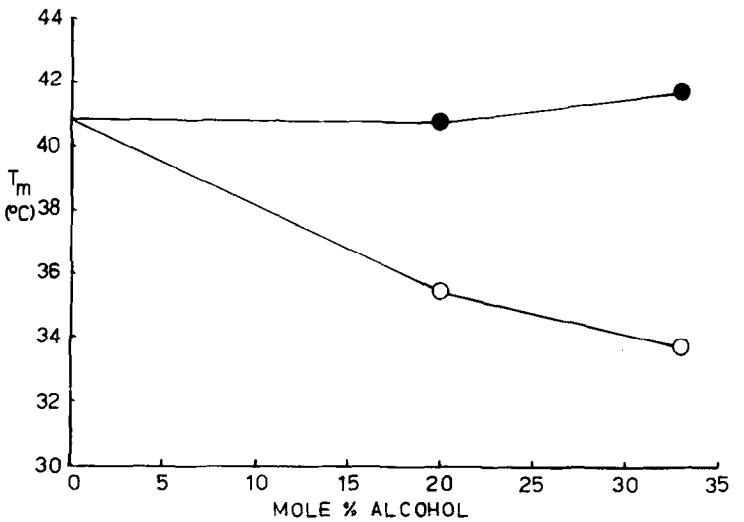

Figure 1. Plot of DPL phase transition temperature (T_m) versus alcohol concentration expressed as mole %, for cis- (open circles) and trans- (closed circles) 9,10-tetradecenol.

DISCUSSION

We have presented evidence in this report that the unsaturated long-chain alcohols, cis- and trans-tetradecenol are equipotent both as general and local anesthetics. Of the biophysical models which are currently invoked to explain anesthetic/membrane lipid interactions, our data are most consistent with the lipid fluidity model. Although we cannot unequivocally comment on the lateral phase separation model of Trudell (12), our evidence on the anesthetics, cis- and trans-tetradecenol, demonstrates that the idea that a simple depression in lipid phase transition temperature is a *sine qua non* for anesthetic action (9) is no longer universally true. It is possible that in the more complex lipid mixtures found in biomembranes, the situation would not be so clear-cut. However, these simple lipid models do successfully predict the cut-off (19,20, 21) in potency that is observed *in vivo* when alkanol chain length is increased beyond tridecanol (22). It thus seems probable both that the models are adequate to a good approximation and that there are no grounds for regarding long-chain alcohols as anomalous anesthetics.

Many biomembranes have been shown to exhibit thermotropic transitions, and in some cases a lipid transition has been demonstrated at temperatures at which breaks in Arrhenius plots of enzymatic activity occur (23). However, it is often technically difficult to unambiguously assign the origin of such discontinuities to lipids. In one such study, the effect of several short-chain alcohols in abolishing the discontinuity temperature for post-tetanic potentiation in Aplysia ganglia was used to suggest the involvement of lipids in the transition (24). One cannot, however, rule out the possibility that the alcohols might be acting directly on the protein. There are other reports that similar thermal discontinuities can occur in membrane ion-channel conductances (25,26). Demonstration of a differential effect of cis- and trans-isomeric alcohols in such cases could provide much stronger evidence for lipid involvement.

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