Anesthesiology
46:322–326, 1977

Effects of Halothane, Thiopental, and Lidocaine on Fluidity of Synaptic Plasma Membranes and Artificial Phospholipid Membranes

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The effects of halothane, thiopental, and lidocaine were studied with spin-labeling methods in synaptic plasma membranes (order parameter) and artificial phospholipid membranes (lateral diffusion). Halothane had a biphasic action, low concentrations (0.64 mM) ordering and high concentrations (2.9 mM) fluidizing both types of membranes. A biphasic effect in phospholipid membranes was also seen with thiopental, 0.1 mM ordering and 10 mM fluidizing, whereas in synaptic plasma membranes both low and high concentrations caused an increased order in the lipid bilayer region. At high thiopental concentrations, a considerable number of molecules may have reacted with membrane proteins or accumulated in the highly fluidic hydrophobic interior region of the membrane without affecting the rotational movement of the labeled fatty acid. Lidocaine alone, or together with calcium chloride, at various concentrations to 10 mM had no significant effect, and a fluidizing effect of 1 mM calcium chloride was possibly a result of interaction of calcium chloride with the label. The results indicate that the three lipid-soluble anesthetics interact differently with the lipid part of membranes. Lidocaine did not seem to affect bilayer lipids, while thiopental and halothane in phospholipid vesicles and halothane alone in synaptic membranes caused a dose-dependent biphasic effect. (Key words: Theories of anesthesia; Membrane, effect of anesthetics; Anesthetics, volatile, halothane; Anesthetics, intravenous, thiopental; Anesthetics, local, lidocaine.)

ANESTHETICS include a variety of small lipid-soluble molecules that show good correlation between anesthetic potency and lipid solubility. Modern hypothesis of anesthesia suggest that they act at a hydrophobic site in nerve membranes by physical mechanisms. However, the molecular mechanisms of the actions of different anesthetic drugs have remained largely unknown or controversial. Inhalation anesthetics, and other anesthetics as well, seem to expand biological membranes,[1] possibly by filling up a critical fraction of the total volume in the membrane molecular lattice.[2] Concentrations of halothane and enflurane used in clinical anesthesia have an ordering effect on the hydrophobic part of synaptic plasma membranes and artificial phospholipid membranes,[3,4] while higher concentrations of inhalation anesthetics have a disordering or fluidizing effect.[4,5] Less is known about the molecular mechanism of barbiturates. Pentobarbital (ED_{50} concentration 7 mM) has been shown to increase the order in multibilayer membranes from ox brain white matter,[6] whereas pentobarbital at 16 mM fluidizes lecithin membranes, which contain phosphatidic acid and cholesterol.[7]

Depending on the lipid composition, phospholipid membranes have been both stabilized[6,8] and perturbed[9] by local anesthetics, which possibly compete with calcium in their action on nerve cell membranes.[10,11] Calcium, on the other hand, is considered to have a stabilizing effect on phospholipid and biological membranes.[12,13]

As it was possible earlier to demonstrate a biphasic effect of volatile anesthetics on fluidity in phospholipid and synaptic plasma membranes,[3,4] we now have used spin-label techniques to study the effects of various concentrations of volatile, intravenous, and local anesthetics on the order parameter S[14] in synaptic plasma membranes and the rate of lateral diffusion[15,16] in phospholipid membranes.

Material and Methods

The spin-label technique applied to problems of biological membranes gives information about the molecular architecture and the dynamic behavior of the membrane. Motion characteristics of fatty acid spin labels incorporated into biological membranes or lipid vesicle membranes provide a measure of the fluidity of the lipid hydrocarbon chains.[14]

Synaptic plasma membranes were isolated from brain tissue of adult Sprague-Dawley rats according to the sucrose gradient centrifugation method of Jones and Matus.[17] Electron microscopy was used to check the purity of the fractions. Protein was assayed according to the method of Lowry *et al.*[18] The spin labels, N-oxyl-4′,4-dimethyloxazolidine derivatives of stearic acid, the doxyl group positioned at C-5 [I(12,3)] or C-12 [I(5,10)] (Syva Inc., Palo Alto, California), coated on small glass beads, were incorporated in the synaptic membranes during gentle shaking of the membrane suspensions in 0.32 M sucrose (60 μg label/300 μl sus-

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Received from the Department of Anesthesiology, Helsinki University Central Hospital, 00290 Helsinki 29, Finland, and Department of Anatomy, University of Helsinki, Siltavuorenpenger 20, 00170 Helsinki 17, Finland. Accepted for publication January 4, 1977. Supported by grants from the Finska Läkaresällskapet, the Alexander von Humboldt-Stiftung and the Finnish Medical Research Council.

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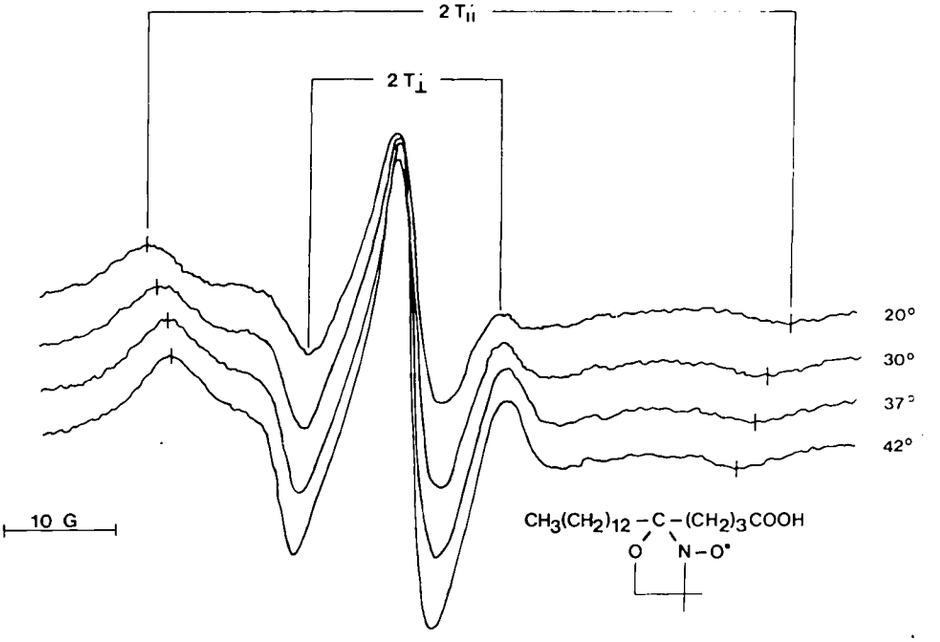

FIG. 1. First-derivative spectra of stearic acid spin label I(12,3) in synaptic plasma membranes. The order parameter S was derived from the formula

$$S = \frac{T_{\parallel}' - T_{\perp}'}{T_{zz} - T_{xx}}$$

where $T_{\parallel}' - T_{\perp}'$ was measured from the spectra as indicated in the figure, and $T_{zz} - T_{xx} = 26.1$ G (Gauss).[14]

pension) at 37 C for 20 minutes. The order parameter S[14] was calculated as indicated in the legend of figure 1; the lower the S parameter, the more fluid the membranes.

1,2 - Dipalmitoyl - *rac* - glycerol - 3 - phosphoryl - choline (DPL) and 1,2-dimyristoyl-*rac*-glycerol-3-phosphorylcholine (DML) (Sigma Chemical Company, St. Louis, Missouri) were used without further purification. Spin labels and phospholipids in chloroform solutions were mixed at molar ratios 0.05, 0.1 and 0.2, and after evaporation the mixtures were pumped under vacuum to remove chloroform. Dispersions (5 mg/ml) were prepared by cosonication for 5 minutes under nitrogen in 0.01 M Tris–HCl buffer, *p*H 7.2, which contained 0.1 M KCl. The sonication temperatures, as well as the electron spin resonance (ESR) recording temperatures, were 45 C for DPL (transition temperature 41 C) and 25 C for DML (transition temperature 41 C) and 25 C for DML (transition temperature 23 C). According to the interpretation of Sackmann and Träuble,[15,16] who investigated the same lipid system in detail, the width of the central peak in the ESR spectra was used as a measure of the rate of lateral diffusion. As the broadened ESR peaks were clearly resolved without disturbing background noise, the peak widths were extracted as exactly as possible by visual examination.

Drug preparations included a saturated calcium-free water solution of freshly distilled halothane (Leiras OY, Turku, Finland), thiopental sodium (Leiras OY, Turku, Finland), lidocaine hydrochloride (Astra, Södertälje, Sweden), and calcium chloride. The halothane concentrations in the capillary tubes, used for measurements, were checked gas-chromatographically after the ESR recordings.

The ESR spectra were obtained on a Varian E 4 spectrometer operating in the X-band region, and equipped with a temperature-regulation accessory.

The significances of changes from control values of the order parameter S and in the rates of lateral diffusion were evaluated using the matched-pair t-test.

Results

Synaptic Plasma Membranes

A high degree of order was seen when spin label I(12,3) was incorporated into synaptic plasma membranes (fig. 1). As indicated in the figure, the outer hyperfine splitting ($2T_{\parallel}'$) decreased with increasing temperature. Halothane at 0.64 mM increased the order parameter S in the synaptic membranes in a temperature range from 20 to 42 C (fig. 2*a*). On the other hand, at a higher concentration (2.9 mM), halothane decreased the order. The difference from control values showed statistical significance with 0.64 mM halothane at 37 C ($P < 0.01$) and 42 C ($P < 0.005$) and with 2.9 mM halothane at 30 C ($p < 0.05$), 37 C ($P < 0.01$) and 42 C ($P < 0.005$). At each temperature the differences between the means were rather small, but in each of the six experiments all values obtained with 0.64 mM halothane were higher and those with 2.9 mM halothane lower than the respective control values.

Thiopental at 0.1 and 10 mM (fig. 2*b*), and at 1.5 mM increased the order parameter in the whole temperature range observed. As with halothane, the changes were small in all experiments. Statistically significant changes were seen with 0.1

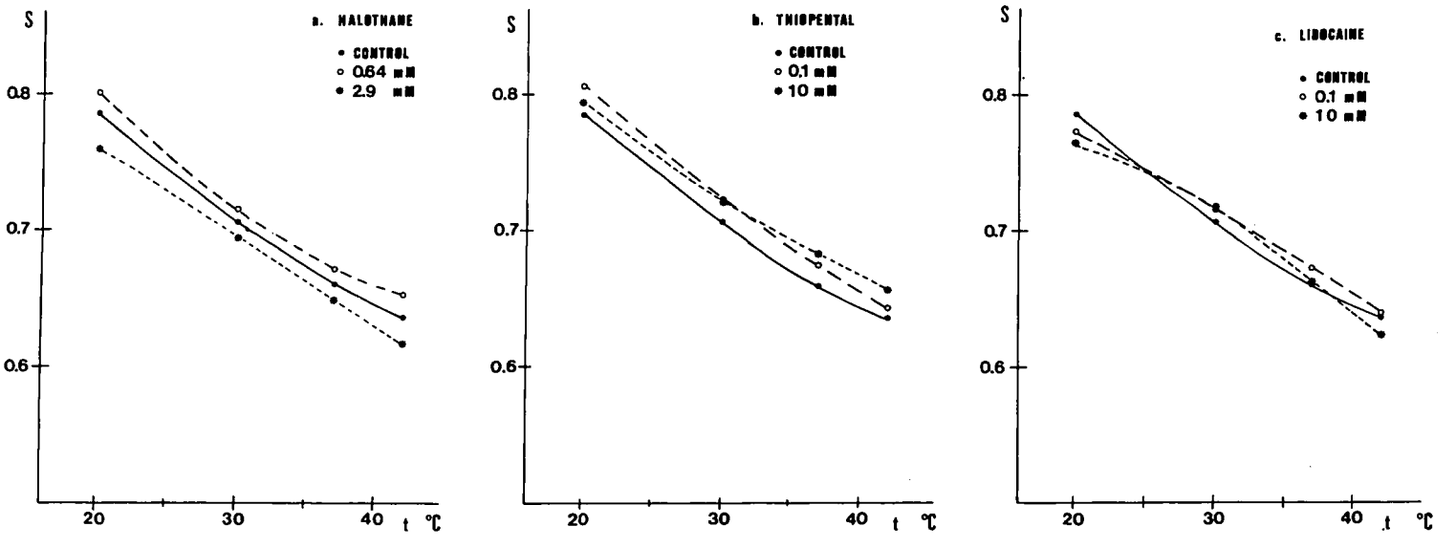

FIG. 2. Effects of halothane (*a*), thiopental (*b*) and lidocaine (*c*) on the temperature dependence of the order parameter S (means of six preparations) of spin label I(12,3) incorporated into synaptic plasma membranes of rat brain. The standard errors have not been indicated in the graphs because in most cases vertical bars would have been covered by the mean value symbols. Protein concentration 1–2 mg/ml.

mM thiopental at 37 C ($P < 0.05$) and with 10 mM thiopental at 37 C ($P < 0.01$) and 42 C ($P < 0.05$).

Lidocaine at 0.1 and 10 mM (fig. 2*c*), and at 1 mM caused small and variable changes in the order parameter. Calcium chloride at 1 mM caused at 30 and 37 C ($P < 0.05$) a consistent increase in the order parameter S compared with the control. There were no further changes when lidocaine, 1 mM, was added together with calcium chloride.

With spin label I(5,10) a temperature-dependent decrease in the order parameter S from about 0.58 (20 C) to about 0.40 (42 C) was recorded in synaptic plasma membranes, showing that the interior of the lipid bilayer exists in a highly fluid state. When this spin label was used, none of the drugs at the above-mentioned concentrations affected the order parameter.

Artificial Phospholipid Membranes

The ESR spectra of high concentrations of spin label I(5,10) in DML and DPL vesicles showed line broadening and dependence on the label-to-lipid ratio typical for spin exchange (table 1). The more there was spin exchange interaction between the labels, due to labeled molecules colliding with each other, the broader were the spectral peaks.[15] A clear "stabilization," *i.e.*, a consistent inhibition of the rate of lateral diffusion, was observed in DPL vesicles with 0.64 mM halothane (table 1). In DML vesicles the line width decreased only at a label-to-lipid ratio of 0.2. Promotion of lateral diffusion with 2.9 mM halothane was small or nil in the different preparations.

The low dose of thiopental (0.1 mM) consistently decreased the line widths in both DPL and DML vesicles at label-to-lipid ratios of 0.05 and 0.1. At ratio 0.2 there was no change from the control values. The high dose (10 mM) increased the line widths in both lipids at all ratios except 0.1 in DPL vesicles. When thiopental, 10 mM, was added, a small but distinct isotropic spectrum appeared superimposed on the typical broad-peaked anisotropic spectrum. On standing, a loose sedimentation was visible in these glass capillary tubes. The changes in the halothane and thiopental experiments were not statistically significant.

Low (0.1 mM) or high (10 mM) doses of lidocaine caused no consistent change. An unexpected increase in line width was seen at a label-to-lipid ratio of 0.05 (every twentieth molecule a label) in DPL vesicles after treatment with calcium chloride at 1 or 10 mM, as well as after the addition of 1 mM lidocaine and 1 mM calcium chloride together (table 1). At a ratio of 0.2, calcium chloride decreased the line width, but lidocaine at 1 mM was without further effect.

Discussion

Although anesthesia ultimately may be produced by a blocking of sodium fluxes through nerve cell membranes,[19] the present results indicate that halothane, thiopental, and lidocaine differ in their interactions with the lipid region of cellular membranes. According to the "critical volume hypothesis of anesthesia,"[2] small anesthetic molecules stabilize membranes by occupying free space within the

membranes, whereas an excess number of molecules would cause disorder. Results with halothane fit well with this scheme, low concentrations ordering and high concentrations fluidizing both synaptic plasma and artificial lipid membranes. These observations are in agreement with earlier results.[3] The partitioning of halothane in these membrane preparations is not known, but assuming a partition coefficient about the same as that of halothane in octanol/water (64.5 w/w),[20] the lower concentration used in the present study is probably at the "clinical" level (0.5–1 mM in blood).[21] At different temperatures the partitioning of halothane between lipid and water may not change significantly, since the solubilities of halothane in, *e.g.*, brain and water change at almost equal rates with temperature.[22] The temperature dependence of the partitioning of thiopental or lidocaine seems not to be known.

Like halothane, thiopental also had a biphasic action on artificial model membranes, while the higher concentrations of thiopental failed to fluidize biological membranes. This could be due to excess barbiturate molecules accumulating at a membrane site where the rotational movement of the spin label is not affected. Indeed, by using the spin label I(5,10), which has the paramagnetic nitroxide attached near the apolar end of the fatty acid chain, it was shown that the hydrophobic region of the phospholipid bilayer in synaptic membranes is in a very fluid state. Thus, a large number of small lipid-soluble molecules may gather in this region without a detectable change in the order parameter. In biological membranes, excess barbiturate molecules may further bind to membrane proteins and in this way escape detection by the present spin-label technique. Thiopental at high concentrations may, furthermore, have interacted with the spin-label binding in phospholipid vesicles, since an additional small isotropic spectral component appeared in these cases. The low dose of thiopental (0.1 mM) used in this study seems to be comparable to "clinical" levels (0.1–0.2 mM in serum).[23]

Local anesthetics have been regarded as powerful membrane "stabilizers,"[24] but lidocaine had little effect on our preparations. The absence of significant lidocaine effects on the neutral artificial membranes was not surprising, because local anesthetics seem to exert their action in a charged form.[25] The lack of effect on biological membranes may indicate that local anesthetics primarily affect non-lipid constituents of membranes.

The lack of distinct stabilizing effects of calcium chloride on biological membranes is difficult to explain. Thus, the nature of the suggested interaction between calcium and local anesthetics[10] remains unclear. Strong evidence against a direct interaction between calcium and local anesthetics has lately been presented by Strichartz.[26] The fluidizing effect of calcium chloride on DPL vesicles might have been a technical artifact; binding of calcium to ionized carboxylic groups of the labels might have approached neighboring labels and thus increased the rate of spin exchange interaction.

We conclude that the anesthetic agents used, halothane, thiopental, and lidocaine, affect cellular membranes in different manners. Both halothane and thiopental exert their actions primarily on the lipid region of the membranes, halothane according to the critical-volume hypothesis of anesthesia by which low concentrations have an ordering effect and high concentrations fluidize the membranes, while an excess of thiopental deviated from this scheme in being apparently inactivated in a highly fluidic region of the membranes. Lidocaine, on the other hand, appears to attack primarily non-lipid and charged radicals in the membranes.

TABLE 1. Effects of Different Concentrations of Halothane, Thiopental, Lidocaine, and Calcium Chloride on Central Line Width (Gauss) of ESR Spectra from Stearic Acid Spin Label I(5,10) Incorporated in Different Molar Ratios into DML and DPL Vesicles (Means of Three Preparations). In the DML Experiments the Temperature was 25 C and in the DPL Experiments, 45 C. Phospholipid Concentration 5 mg/ml

Label/Lipid	Control	Halothane (mM)		Thiopental (mM)		Lidocaine (mM)		Calcium Chloride (mM)		Calcium Chloride 1 mM + Lidocaine 1 mM
		0.64	2.90	0.10	10	0.10	10	1	10	
I(5,10)/DML										
0.05	3.37	3.37	3.50	3.25	3.63	3.50	3.43			
0.10	5.50	5.50	5.63	5.38	5.75	5.75	5.75			
0.20	6.75	6.25	6.75	6.75	7.00	6.75	6.50			
I(5,10)/DPL										
0.05	4.00	3.88	4.00	3.88	4.25	4.19	3.85	4.60	4.52	4.78
0.10	6.00	5.50	6.00	5.75	6.00	6.00	5.64	6.00	5.76	5.56
0.20	7.25	7.00	7.37	7.25	7.37	7.19	7.37	7.03	7.19	7.03

The authors gratefully acknowledge valuable technical assistance by Mrs. Marja Nykänen and Mrs. Armi Savolainen.

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