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Biphasic Effects of Halothane on Phospholipid and Synaptic Plasma Membranes: a Spin Label Study

PER H. ROSENBERG,[1] HANSJÖRG EIBL, AND ANTON STIER

Max-Planck Institute of Biophysical Chemistry, D-3400 Göttingen, German Federal Republic

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SUMMARY

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Halothane at 0.16 and 0.32 mM increased, while at 9.3 mM it decreased, the order parameter in palmitoyllauroyllecithin vesicles containing stearic acid spin label I(12,3). A corresponding effect was seen in rat brain synaptic plasma membranes. In dipalmitoyllecithin vesicles the lateral diffusion of stearic acid spin label I(5,10) was inhibited by halothane at 0.32–1.60 mM and promoted by it at 9.3 mM. Halothane in these three membrane systems exerted a biphasic effect, low concentrations ordering and high concentrations disordering the lipid structure of the membranes.

INTRODUCTION

The lipid portion of the nerve cell membranes was long ago considered to be the primary site of action of inhalation anesthetics (1, 2). Model studies on erythrocyte membranes have shown that inhalation anesthetics exert a biphasic effect in the sense that low concentrations inhibit hypotonic hemolysis and high concentrations enhance hemolysis (3). With the application of spin label techniques to a less complex model, volatile anesthetics have been observed to cause perturbation (fluidization) of phospholipid vesicle membranes (4).

The aim of this study was to investigate the effect of halothane on different parameters characterizing the fluidity in different kinds of membranes and to show a possible biphasic pattern of action of the anesthetic on the membranes.

1-Palmitoyl-2-lauroyl-*sn*-glycerol-3-phosphorylcholine (16-12-lecithin) was synthesized starting from 1-palmitoyl-2-lauroyl-*sn*-glycerol, into which the phosphorylcholine group was introduced according to Eibl and co-workers (5). The product showed only a single spot on thin-layer chromatography in different solvent systems. The purity of the compound was found to be 95% with respect to the fatty acid distribution between positions 1 and 2 in the glycerol molecule as checked by gas chromatography (6) of the lysolecithin resulting after phospholipase A_2 treatment. DPL[2] was purchased from Sigma Chemical Company and used without further purification. The *N*-oxyl-4′,4-dimethyloxazolidine derivatives of stearic acid, the doxyl group positioned at C-5[I(12,3)] (see formula in Fig. 2) and C-12 [I(5,10)], respectively, were products of Syva, Inc., Palo Alto. 17β-Hydroxy-4′,4′-dimethylspiro)5α-androstan-3,2′-oxazolidine)-3′-yloxyl (androstane spin label) was synthesized according to Keana and co-workers (7) (see formula in Fig. 2).

Dispersions of the phospholipids (5

[1] Humboldt Foundation grant fellow. Permanent address, Department of Anesthesiology, Helsinki University Central Hospital, 00290 Helsinki 29, Finland.

[2] The abbreviation used is: DPL, 1,2-dipalmitoyl-*rac*-glycerol-3-phosphorylcholine.

mg/ml) and spin labels were prepared by low-power cosonication for 5 min in Tris-HCl buffer containing 0.1 M KCl, pH 7.2, under N_2 with a Branson Sonifier to produce a clear dispersion in the case of 16-12-lecithin and a slightly opalescent dispersion in the case of DPL.

Rat brain synaptic plasma membranes were isolated by the gradient centrifugation method of Jones and Matus (8). The fractions were assayed for acetylcholinesterase (EC 3.1.1.7) activity (9), cytochrome *c* oxidase (EC 1.9.3.1) activity (10), 2',3'-cyclic nucleotide 3'-phosphohydrolase activity (11), and protein concentration (12). About 80% of the particles in the synaptosomal fraction were considered to be of synaptic origin (8).

The fatty acid and androstane spin labels were incorporated into the synaptic plasma membranes by equilibration during gentle shaking with spin label-coated glass beads [100 μg of spin label per milliliter of membrane suspension in 28.5% (w/w) sucrose] at 37° for 30 min.

Different amounts of saturated aqueous solutions of freshly distilled halothane were incubated with the phospholipid vesicles or the synaptic plasma membranes in sealed capillary glass tubes at room temperature. The lipid/water partition coefficients for halothane in these preparations are not known, and thus the concentrations of halothane incubated with the membranes are expressed as millimoles per liter of the membrane suspensions.

ESR spectra were recorded on a Varian E9 spectrometer equipped with a temperature regulation accessory.

The order parameter S, calculated from the spectra (13) and plotted against temperature, showed in the case of spin label I(12,3) and 16-12-lecithin vesicles an increase when 0.16 mM or 0.32 mM halothane was added, and a decrease in a narrower temperature range in the presence of 9.3 mM or 18.6 mM halothane (Fig. 1). A smooth decrease in the order parameter with increasing temperature indicates a lack of phase transition between 10° and 50° for this phospholipid.

In synaptic plasma membranes a high degree of order was observed during incorporation of the spin label I(12,3) (Fig. 2). The outer hyperfine splitting ($2T'_{\parallel}$) decreased from about 60 G (10°) to about 52 G (40°). With spin label I(5,10) the hyperfine splitting varied from about 55 G (10°) to about 44 G (40°), and with androstane spin label (Fig. 2), from about 45 G (10°) to about 36 G (40°). These findings are characteristic for spin labels undergoing rapid anisotropic rotation in a highly ordered lipid matrix of membranes (13), and are in accordance with results of spin label studies on other biological membranes (see ref. 14).

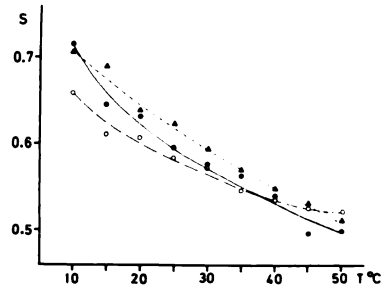

FIG. 1. *Effect of halothane on temperature dependence of order parameter S in 16-12-lecithin vesicles containing stearic acid spin label I(12,3)*

$$S = \frac{T'_{\parallel} - T'_{\perp}}{T_{zz} - T_{xx}}$$

where $T'_{\parallel} - T'_{\perp}$ was measured from the ESR spectra as indicated in Fig. 2, and $T_{zz} - T_{xx} = 26.1$ G (13). The dispersions of spin label (label-to-lipid molar ratio = 0.02) and lipid (5 mg/ml) were prepared by cosonication in 0.01 M Tris-HCl (pH 7.2) containing 0.1 M KCl. Halothane was incubated with the dispersions in sealed capillary glass tubes at room temperature. ▲, halothane, 0.32 mM; ●, no halothane; ○, halothane, 18.6 mM. The figure shows a representative temperature plot of one of the five preparations examined.

The order parameter in the temperature range from 15° to 35° from spectra of spin label I(12,3) incorporated into synaptic plasma membranes increased in the presence of halothane at 0.32 mM ($p < 0.005$) and 0.64 mM and decreased slightly at concentrations above 2.95 mM (Fig. 3).

The ESR spectra of high concentrations of spin label I(5,10) incorporated into DPL vesicles showed line broadening and dependence on the label-to-lipid ratio typical for spin exchange (15, 16). In the DPL vesi-

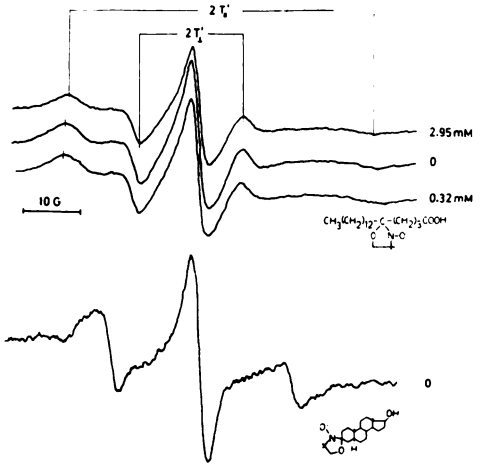

FIG. 2. *First derivative ESR spectra (35°) of spin label I(12,3) (upper three spectra) and androstane spin label (lower spectrum) incorporated into synaptic plasma membranes of rat brain*

The spin labels were incorporated into the membranes (protein concentration, 8–10 mg/ml) by incubation with spin label-coated glass beads at 37° for 30 min. The effect of halothane, the millimolar concentrations of which are indicated on the right side of the figure, on the outer hyperfine splitting ($2T'_0$) is clearly visible.

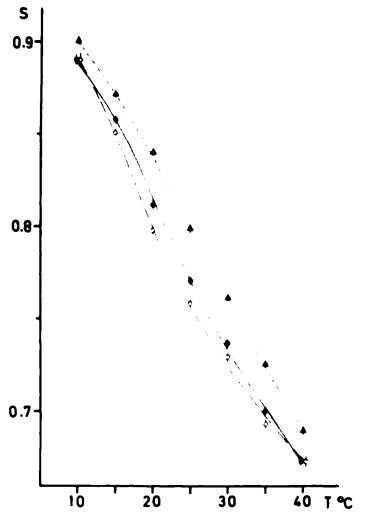

FIG. 3. *Effect of halothane on temperature dependence of order parameter S (means ± standard errors) of spin label I(12,3) incorporated into synaptic plasma membranes of rat brain*

The order parameter S is defined in the legend to Fig. 1. ▲, halothane, 0.32 mM; ●, no halothane; ○, halothane, 2.95 mM. The effect of halothane in six different preparations was statistically significant at temperatures from 15° to 40° (20–25°, $p < 0.001$; 15° and 40°, $0.001 < p < 0.005$). The effect of halothane at 2.95 mM was statistically significant only at 20° ($0.005 < p < 0.01$), but in none of the six preparations at temperatures between 15° and 35° was the S value higher than the respective control value.

cles halothane at low concentrations (0.32–1.60 mM) caused narrowing, whereas 9.3 mM and higher concentrations broadened the spectral lines in the whole range of label-to-lipid ratios (Table 1). If one accepts the interpretation of Sackmann and Träuble (15, 16), who investigated the same model system in depth, it may be concluded that halothane has a biphasic effect on the rate of lateral diffusion, low concentrations inhibiting and high concentrations promoting the diffusion.

Our results indicate a biphasic effect of halothane on two parameters characterizing mobility of labels in the lipid structure of membranes. This biphasicity was observed in three different membrane systems. The effect of "high" halothane concentrations is in accordance with fluidization of the membrane lipids as recently suggested by Trudell and co-workers (4), whose lowest halothane concentration studied lies in the range of our "high" concentrations. The effect of "low" halothane concentrations in our study may be interpreted in terms of the "critical volume theory of anesthesia" of Mullins (17), who suggested that the membrane stabilization of anesthesia would be due to filling of free space in the lattice of membrane structures by small anesthetic molecules. The occurrence of free space seems to be evident in the 16-12-lectin membrane preparation, which we chose because of the 4-carbon difference in the fatty acid chains, but in the case of DPL vesicles free space may also be available because of a difference in packing density of the outer and inner lamellae of the bilayer membranes (18). Despite the unknown structure of the synaptic plasma membranes it may also be reasonable to assume the occurrence of conformational "holes" within these membranes (17).

On the assumption of a partition coefficient of 64.5 [octanol/water (w/w), ref. 19], the calculated equilibrium concentrations

TABLE 1

Effect of different halothane concentrations on central line width of ESR spectra (45°) from stearic acid spin label I(5,10) incorporated in different concentrations into DPL vesicles

The spin label-containing lipid dispersions (5 mg/ml) were prepared as described in the legend to Fig. 1, and halothane was incubated with the dispersions in sealed glass tubes at room temperature.

Label-to-lipid molar ratio	Central line width at various halothane concentrations					
	0 mM	0.32 mM	0.64 mM	1.60 mM	2.95 mM	9.30 mM
	MHz	*MHz*	*MHz*	*MHz*	*MHz*	*MHz*
0.05	8.78	8.57	8.43	8.43	8.57	8.78
0.10	10.90	10.17	9.85	10.40	10.90	11.22
0.13	15.47	15.10	14.72	15.10	15.10	17.20
0.20	17.55	17.20	17.20	16.83	17.55	17.90

of halothane in the water phase of our systems would be about 20% lower than the values given here. The "low" concentrations of halothane which we found to decrease membrane fluidity are thus in the range of the equilibrium concentrations known to produce clinical anesthesia (20). It is interesting that in the synaptic plasma membrane preparation the ordering effect of these low concentrations is more pronounced than the fluidizing effect of the higher concentrations of the anesthetic we used.

For the complex biological phenomenon of anesthesia, however, dual effects of the anesthetics—ordering the disordering of the lipid structure of the membranes—may contribute on the molecular level.

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