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CHRONIC EXPOSURE TO INHALED ANESTHETICS INCREASES CHOLESTEROL CONTENT IN *ACHOLEPLASMA LAIDLAWII*

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Acholeplasma laidlawii **cells were grown in cholesterol-enriched medium and exposed continuously to either air (control), 4.0 vol.% halothane in air at 1 atm pressure (4% atm halothane), or 80% cyclopropane in oxygen for 24 h at 37°C. Cells grown in the presence of 4% atm halothane or 80% cyclopropane had approximately twice as much membrane cholesterol content/mg protein as the control cells. Cells grown in an anesthetic environment also tended to have a higher membrane cholesterol/phospholipid molar ratio compared to control cells. Membranes isolated from halothane-exposed cells grown in a cholesterol-enriched medium were more ordered at 37°C (measurements were made with no anesthetic present) than membranes from control cells grown in an identically enriched medium. This difference in membrane physical state between control and anesthetic-exposed cells decreased as the temperature decreased, and disappeared at approx. 23°C. Continuous exposure of *A. laidlawii* to 4% atm halothane or 80% cyclopropane for 24 h did not markedly affect membrane fatty acid composition, either in cells grown on an unsupplemented medium or in cells grown in a medium enriched in myristic, palmitic or stearic acids. These results further support the hypothesis that an increased membrane cholesterol content may play a role in the tolerance or dependence that develops after chronic exposure to anesthetic agents.**

Introduction

Cellular systems may alter their membrane lipid composition when grown in the continuous presence of sedative-hypnotics and alcohol. However, the lipid changes observed depend upon the cell type and the drug examined. In *Escherichia coli*, short chain alcohols such as ethanol decrease the amounts of saturated fatty acids, whereas long chain alcohols such as hexanol increase the proportion of saturated fatty acids [1]. The reduced levels of saturated fatty acids in *E. coli* grown in the presence of ethanol result from a direct action of ethanol on the soluble enzymes of fatty acid synthesis [2,3]. Growth of *Tetrahymena* in the presence of ethanol decreases the hexadecanoic acids (16:1 and 16:2 and increases linoleic acid (18:2) [4]. Chinese hamster ovary cells increase their content of 16:0 and decrease that of 18:0 when grown in the presence of either ethanol or pentobarbitol [5]. The general anesthetic methoxyflurane causes a decrease in the fatty acid desaturase activity in *Tetrahymena*, and thereby decreases incorporation of [^{14}C]acetate into unsaturated fatty acids and increases incorporation into saturated fatty acids [6]. In certain cases, alterations in phospholipid head group composition also occur following long-term exposure to ethanol or barbiturates [4,5].

In mammals, chronic treatment with anesthetic agents can also alter the composition of neuronal membrane lipids. Again, however, these lipid altera-

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tions may depend upon the species and the preparation examined. The percentages of polyunsaturated fatty acids are reduced in a crude brain mitochondrial fraction of mice treated with vaporized ethanol [7,8]. In synaptic plasma membranes isolated from guinea-pigs chronically treated with ethanol, the proportion of monounsaturated fatty acids in choline and ethanolamine phosphoglycerides decreases and the proportion of polyunsaturated fatty acids in the ethanolamine phosphoglycerides increases [9]. Myelin membranes isolated from the brain stem of rats given ethanol for prolonged periods exhibit an increase in the proportions of 16:0, 18:0, and 18:1 and a decrease in 20:1, 20:4 and 22:6 compared to control animals [10]. Although fatty acid composition in the spinal cords of rat is not altered after chronic ingestion of alcohol, a change does occur in the phospholipid composition [11]. Phospholipid turnover in rats is increased following chronic ethanol treatement [12].

In addition, membrane cholesterol content may be altered after chronic drug exposure. Synaptic plasma membranes and red cell membranes of mice dependent upon alcohol exhibit increased cholesterol/protein and cholesterol/phospholipid molar ratios [13]. Brain microsomal membranes isolated from rats dependent upon alcohol also have an increased cholesterol content [4], but the cholesterol content of myelin is decreased [14,15]. Further, continuous exposure of mice to 50% nitrous oxide for 3 weeks increase the red cell membrane cholesterol/phospholipid ratio [16].

Such alterations in lipid compostition have been used to explain the development of tolerance (the requirement of a higher drug dose than normal to produce the same physiological effect) that may occur after continuous drug exposure. In addition, drug dependency may develop as evidenced by the appearance of withdrawal symptoms when the drug is discontinued [17,18]. Such dependency or tolerance may result from a modification of the lipid composition of certain critical membranes. It is possible that this modification returns the physical state of that membrane (in the presence of the drug) to its pre-affected value [7,13,19–23]. In particular, it has been suggested for synaptic membranes that an increase in the proportion of saturated fatty acids [7,24] or an increase in cholesterol content [13] induced by chronic exposure to anesthetic agents, may decrease the intrinsic fluidity of synaptic membranes and make them less susceptible to anesthetic perturbation.

However, the synaptic membrane lipid alterations seen in mammals [7,13,16] after long-term anesthetic exposure are usually very small in magnitude and border on the limits of detection. The apparent smallness may result from the heterogeneity of the synaptic membrane preparations, which dilutes any lipid alterations that might be seen in certain synaptic membrane regions specifically involved in tolerance development. Another difficulty is that larger alterations in lipid composition cannot be obtained by increasing the drug dose in these tolerance experiments, since an increase in dose would usually kill the animal. We therefore examined the influence of prolonged anesthetic exposure on the membrane lipid composition of *A. laidlawii*, a procaryote bound by a plasma membrane only and lacking a cell wall [25], but capable of adapting to a wide range of environments in part by modifying its membrane composition [26]. *A. laidlawii* does not require cholesterol for growth but is able to incorporate it into the membrane [27,57–59], a property that can be utilized to examine the role of membrane cholesterol in adapting to prolonged anesthetic exposure. Furthermore, considerable experimental work has been done using this membrane preparation [60–65].

Materials and Methods

Growth of cells. Acholeplasma laidlawii (strain B) was grown in a modified Razin medium [28] consisting (per l) 12 g bacto heart infusion broth/5 g bacto peptone/5 g bacto yeast extract/2.5 g NaCl/3.7 g Tris-HCl/2.5 g glucose/100 000 units penicillin G/1.5 g fatty acid-free bovine serum albumin adjusted to pH 8.2. Cholesterol was added to the medium from a stock solution of 10 mg cholesterol/ml ethanol to give a final concentration of 5 or 10 μg/ml. In some experiments, the medium was enriched with either myristic, palmitic or stearic acids which were added from a 10 mg/ml ethanol solution to give a final fatty acid composition of 34 mg/l medium. For these experiments, the amount of fatty acid-free bovine serum albumin increased to 4 g/l. Ethanol concentrations in the medium were usually below 0.05%.

Cells were grown in 125 ml flasks capped with rubber stroppers wrapped in aluminium foil. Two 18 gauge needles passed through the stoppers and served as inflow or outflow ports for air or the anesthetic gases. 3 ft. arterial lines were used to connect the flasks to the different gas mixtures. Gases passed through a bacterial filter from an anesthesia rebreathing circuit (Ohio Medical Products) before they entered the flasks.

75 ml medium were added to each of the flasks along with 0.75 ml of a growing cell suspension having an absorbance of approx. 0.6 at 640 nm. The cell suspensions were then continuously exposed either to a flow of air (control), halothane in air, or cyclopropane in oxygen for 24 h at 37°C with occasional agitation. Gas flow rates were regulated with Matheson 610 flowmeters and set at approx. 100 ml/min. Halothane in air was delivered from a premixed cylinder. Cyclopropane in oxygen was delivered by mixing the flows from separate oxygen and cyclopropane tanks. Anesthetic concentrations delivered to the cell suspension were checked by removing samples from the outflow port with a glass syringe. Halothane concentrations were measured by gas chromatography employing flame ionization detection with a 120 cm column packed with 10% SF-96 on chromosorb-WHP, 60/80 mesh (temperature = 50°C; nitrogen flow = 20 ml/min). Cyclopropane concentrations were measured with a Beckman LB-1 infrared analyzer.

Preparation of membranes and lipid analyses. Cells were harvested from each flask after 24 h of growth at 37°C. The cell suspension was centrifuged at 4500 $\times g$ for 20 min at 4°C. The supernatant was poured off and the cellular pellet was lysed and resuspended in 80 ml cold distilled water. This suspension was centrifuged at 25 000 $\times g$ for 20 min. The membrane pellet was washed once with cold distilled water.

Lipids were extracted from a portion of the membrane pellet (containing approx. 2 mg protein determined by the method of Lowry et al. [29]), once with 0.5 ml methanol and twice with 0.5 ml chloroform/methanol (1:1, v/v) solution. The lipid extracts were pooled, approx. 20 mg Sephadex G-25 was added, and the mixture was taken to dryness with a rotary evaporator. The dried mixture was immediately suspended in chloroform, and the Sephadex G-25 was removed by passage through a column packed with silane-treated glass wool. The column was washed with chloroform and the eluted mixture was dried under a stream of nitrogen and resuspended in 1.0 ml of chloroform. Aliquots of this suspension were then analyzed for fatty acids, cholesterol, and phosphate content after being evaporated to dryness. Fatty acid groups were transesterified to their methyl esters by a procedure that closely followed that of Morrison and Smith [30], except that BCl_3/CH_3OH instead of BF_3/CH_3OH was used. Fatty acid methyl esters were separated on a 6-ft. glass column placed with 10% SP-2330 cyanosilicone over a range of 160–200°C at a rate of 2°C/min. Peaks were identified by comparing retention times with fatty acid methyl ester standards. Inorganic phosphate was quantitated after digestion of the dried lipids with perchloric acid [31]. Cholesterol was quantitated by gas chromatography after derivitization with trimethylsilylimidazole, using 5 α-cholestane as the internal standard [16].

ESR measurements. For each sample, ml quantities of a 5-doxyl stearic acid solution in ethanol (0.708 mg/ml) were dried under nitrogen on the side of a 1.5 ml polypropylene centrifuge tube. The membrane suspension in 25 mM Hepes buffer, pH 7.4, was added to give a final concentration of 100 μg spin label/mg protein. The tube was then gently shaken at room temperature for 15 min. The sample was centrifuged (4°C) at 25 000 $\times g$ for 20 min and washed once with 25 mM Hepes buffer to obtain the labeled membrane pellet. The pellet was packed in a 100 μl glass capillary tube, and ESR spectra were recorded with a Varian E-3 ESR spectrometer interfaced to a PDP 11/10 computer at a power setting of 10 mW. Temperature was controlled and monitored with a digital thermometer. A 10-min equilibration period was allowed following each temperature change. Scan times were typically 10 min and taken at a gain of $5 \cdot 10^4$ with a 0.1-s filter time constant.

Chemicals. Reagents for the gas chromatographic analysis of the lipids were obtained from Supelco. All solvents employed in the lipid analyses were once distilled and saturated with inert gas. Low phosphate grade perchloric acid was from MCB. Halothane was donated by Ayerst. Cyclopropane was obtained from Matheson. Biochemicals were supplied by Sigma and salts were of analytical reagent grade.

Results

Exposure to inhalation anesthetics increases membrane cholesterol content

In the first series of experiments, cells were grown in a medium containing 5 μg cholesterol/ml, and continuously exposed to approx. 4% atm halothane, 80% cyclopropane, or air for 24 h at 37°C. Cells exposed to the inhalation anesthetics exhibited an increased cholesterol/protein ratio compared to control cells grown in an air environment (Table I). The cholesterol/protein ratio was doubled in the membranes of the cells exposed to halothane compared to the air control, and was approx. 50% greater in membranes isolated from cells exposed to cyclopropane. The molar ratio of cholesterol to lipid phosphorus also tended to be higher in the anesthetic-exposed cells compared to the control (Table I), but was not significantly different for the number of preparations examined. Cell growth was slightly inhibited by the anesthetics; absorbances at 640 nm were 0.580 ± 0.013 ($n = 4$) and 0.521 ± 0.009 ($n = 4$) for cultures exposed to halothane and cyclopropane, respectively, compared to a value of 0.688 ± 0.053 ($n = 4$) for the control in this experiment.

In a second series of experiments, we examined the effects of different halothane concentrations on cell growth in a medium containing 10 μg cholesterol/ml. Again, membranes isolated from cells exposed to 4% atm halothane for 24 h had a cholesterol/protein ratio that was approximately twice that of membranes isolated from the control cells (Table II). However, no significant increases in the cholesterol/protein ratio could be detected in cells exposed to 1.0 or 2.0% atm halothane for 24 h, although this ratio tended to be higher in the halothane-exposed compared to the control cells (Table II). Similarly, the molar ratio of cholesterol/phospholipid tended to be higher in the halothane-exposed cells (Table II). Cell growth in a medium containing 10 μg cholesterol/ml was inhibited by halothane; absorbances at nm for cells grown under 1.0, 2.0, and 4.0% atm halothane were 0.381 ± 0.049 ($n = 3$), 0.321 ± 0.055 ($n = 3$) and 0.282 ± 0.077 ($n = 5$), respectively, compared to a value of 0.542 ± 0.051 ($n = 11$) for the control cells in this experiment.

Exposure of cells to 80% cyclopropane for 24 h in a medium containing 10 μg cholesterol/ml also increased the membrane cholesterol content. The membrane μg cholesterol/mg protein ratio was 108.4 ± 10.3 ($n = 3$) in the cyclopropane-treated cells compared to 41.2 ± 5.9 ($n = 3$) in the air-exposed cells. These differences were significantly different ($P < 0.005$). Furthermore, a significant ($P < 0.05$) difference was found between the molar cholesterol/phospholipid ratios; this ratio was 1.63 ± 0.11 in the control cell membranes compared to 2.62 ± 0.29 in membranes isolated from the cyclopropane-treated cells. After 24 h exposure to cyclopropane, the absorbance of the cell culture at 640 nm

TABLE I

CHOLESTEROL/PROTEIN AND CHOLESTEROL/PHOSPHOLIPID RATIOS IN MEMBRANES ISOLATED FROM CELLS EXPOSED TO AN AIR, HALOTHANE, OR CYCLOPROPANE ENVIRONMENT

Cells were grown in a modified Razin medium containing 5 μg cholesterol/ml for 24 h. Mean values ± S.E. from four separate experiments.

Environment	mg cholesterol/ mg protein	μmol cholesterol/ μmol lipid P_i
Air	36.8 ± 4.2	0.859 ± 0.107
3.8% atm Halothane	73.4 ± 10.1	1.210 ± 0.098
83% Cyclopropane	57.0 ± 2.1	0.890 ± 0.074

TABLE II

MEMBRANE CHOLESTEROL/PROTEIN AND CHOLESTEROL/PHOSPHOLIPID RATIOS IN CELLS EXPOSED TO AIR AND DIFFERENT CONCENTRATIONS OF HALOTHANE

Cells were grown in a modified Razin medium containing 10 μg cholesterol/ml for 24 h. Mean values ± S.E.

Environment	μg cholesterol/ mg protein	μmol cholesterol/ μmol lipid P_i	N
Air	42.4 ± 12.4	1.47 ± 0.22	3
1.0% atm Halothane	46.7 ± 9.2	1.58 ± 0.05	3
Air	34.1 ± 4.2	1.39 ± 0.22	3
2.0% atm Halothane	44.6 ± 4.7	1.57 ± 0.05	3
Air	41.7 ± 6.9	1.46 ± 0.13	5
4.0% atm Halothane	92.4 ± 13.7	2.31 ± 0.41	5

was 0.159 ± 0.043 (n = 3) compared to 0.706 ± 0.020 (n = 3) in the control cultures.

Exposure to inhaled anesthetics does not alter membrane fatty acid composition

Continuous exposure of *A. laidlawii* to 4% atm halothane for 24 h had no marked effect on membrane fatty acid composition (Table III). No evidence for large anesthetic-induced changes in fatty acid composition could be detected either for cells grown on an unsupplemented medium or for cells grown in a medium enriched in myristic, palmitic, or stearic acids (Table III). Furthermore, no consistent alterations in the membrane fatty acid compositions could be detected after exposure to halothane when cholesterol (5 μg/ml) was added to the medium (data not presented). In addition, membranes isolated from cells grown in 80% cyclopropane for 24 h did not exhibit any gross alterations in fatty acid composition compared to the controls grown in air (data not presented).

ESR measurements

Membranes were labeled with 5-doxylstearic acid after being isolated from cells grown in a medium containing 5 μg cholesterol/ml and exposed either to 4% atm halothane or air for 24 h. When examined at temperatures near the growth temperature, and in the absence of anesthetics, the separation in G between the outermost peaks of the ESR spectra ($2T_{\parallel}$) was greater for membranes isolated from the halothane-exposed cells compared to the controls (Fig. 1). That is, membranes from the halothane-exposed cells were more ordered at this temperature. However, when membranes labeled with 5-doxylstearic acid were examined at lower temperatures, the difference in the

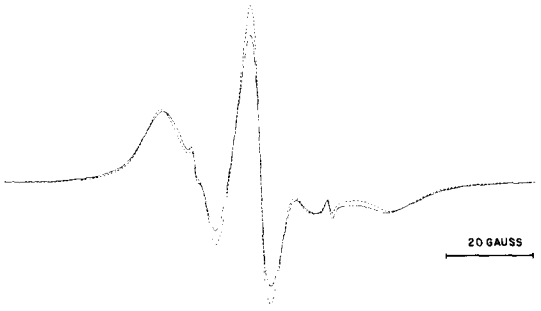

Fig. 1. ESR spectra of membranes isolated from *A. laidlawii* cells grown at either 4% atm halothane (broken line) or air (solid line) for 24 h in a medium containing 5 μg cholesterol/ml and labeled with 5-doxylstearic acid. The value of $2T_{\parallel}$ for the halothane exposed cells at 37°C was 52.6 G compared to 50.9 G in the air control.

TABLE III

MEMBRANE FATTY ACID COMPOSITIONS OF *A. LAIDLAWII* CELLS EXPOSED TO AIR AND 4.0% HALOTHANE

Cells were grown in the presence of 4.0% atm halothane or an air environment for 24 h. C, control; H, halothane-exposed.

Fatty acid [a]	Unsupplemented medium [b]		Supplemented with [c]					
	C	H	14 : 0		16 : 0		18 : 0	
			C	H	C	H	C	H
12 : 0	5.4 ± 0.2	1.9 ± 0.1	1.7	1.3	5.2	3.4	2.0	3.2
13 : 0	0.8 ± 0.1	0.2 ± 0.0						
14 : 0	26.9 ± 0.3	25.2 ± 0.6	62.1	61.3	16.5	15.9	12.0	13.7
15 : 0	1.5 ± 0.1	1.7 ± 0.1			0.1	0.4	0.4	
16 : 0	57.5 ± 0.2	58.3 ± 2.6	32.6	33.7	73.6	74.4	17.8	12.7
16 : 1	0.2 ± 0.1	1.4 ± 0.1	0.4	0.5	0.6	0.6	0.9	1.9
17 : 0	0.1 ± 0.1	1.0 ± 0.03					1.0	1.8
18 : 0	3.8 ± 0.5	2.8 ± 0.1	1.2	0.9	1.2	1.7	57.4	58.0
18 : 1	2.9 ± 0.2	5.6 ± 0.5	1.4	1.7	1.8	2.9	6.4	6.6
18 : 2	0.9 ± 0.1	1.6 ± 0.2	0.5	0.7	0.8	0.9	2.2	2.1

[a] Fatty acid composition expressed as percentage by weight of the listed fatty acids.
[b] Mean values ± S.E. from three experiments.
[c] Average value of duplicate experiments.

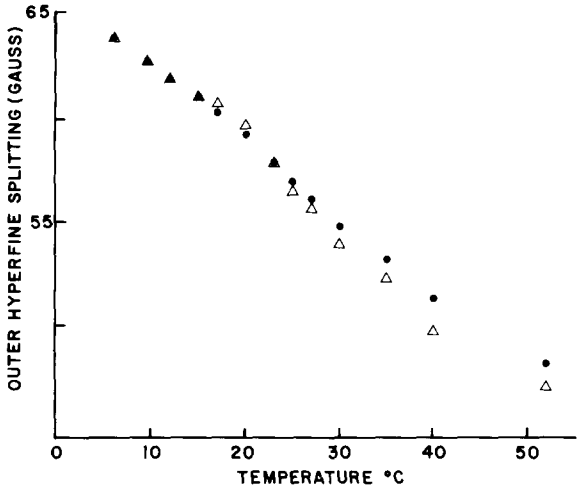

Fig. 2. The effect of temperature on the outer hyperfine splitting ($2T_{\parallel}$) of membranes labeled with 5-doxylstearic acid after being isolated from *A. laidlawii* cells grown at either 4% halothane (circles) or air (triangles) for 24 h in a medium containing 5 μg cholesterol/ml.

$2T_{\parallel}$ values between the halothane-exposed and control groups gradually decreased until at 23°C these values became identical (Fig. 2).

Discussion

The goal of these experiments was to test whether the organism *A. laidlawii* would respond to continuous anesthetic exposure by altering its membrane fatty acid composition or cholesterol content (relative to membrane protein or phospholipid). The advantages of using *A. laidlawii* are that its plasma membranes can be obtained in relatively large quantities in a purified form, and that it is able to withstand relatively high anesthetic concentrations for prolonged periods. The highest doses of halothane (4% atm) and cyclopropane (80%) used in these experiments are approx. 5–8-times the concentrations required to produce anesthesia in humans [32]. Only at these highest anesthetic concentrations could we detect marked increases in cholesterol content. The increases in cholesterol content for cells exposed to clinical doses (1 or 2% atm halothane) were not statistically significant for the number of experiments performed.

We found that cells continuously exposed to 4% atm halothane or 80% cyclopropane increased their membrane cholesterol content (Tables I and II) but did not alter their membrane fatty acid compositions (Table III). The increase in cholesterol content was mostly evidenced by an increase in the cholesterol/protein ratio, with relatively smaller increases in the cholesterol/phospholipid ratio. These increases in cholesterol content parallel the changes observed in synaptic plasma membranes and red cell membranes of mice made dependent on alcohol [13] or tolerant to nitrous oxide [16], but are larger in magnitude. The results from the alcohol experiments are relevant to the changes seen after chronic exposure to inhalation anesthetics, since a cross-tolerance exists between nitrous oxide and alcohol [33].

The cholesterol content in *A. laidlawii* membranes can also be altered by changing the growth temperature. Razin [34] reported that cholesterol/protein and cholesterol/phospholipid ratios are approx. 5-times greater when elaidate-enriched cells are grown at 37°C compared to 4°C *. Larger amounts of cho-

* It should be noted that in an earlier study by Razin et al. [35], it was reported that the amount of cholesterol in the membrane at equilibrium was unaffected by the membrane fatty acid composition [35]. This is seemingly in contradiction with the above conclusion that the incorporation of exogenous cholesterol is dependent on the physical state of the membrane, since alteration in membrane fatty acid composition can profoundly influence membrane fluidity. However, in those studies [34,35]. Tween 80 was added to the growth medium and it is not known to what extent Tween 80 was incorporated into the membrane under the different environmental conditions examined and to what degree this would alter the physical state of the membrane. We have also performed several experiments in which 0.01% Tween 80 and 20 μg/ml cholesterol were added to the growth medium, and have found an increased membrane cholesterol content after chronic anesthetic exposure; cells exposed to 4% halothane for 24 h had a membrane cholesterol/phospholipid molar ratio of 0.930 ± 0.055 compared to a value of 0.668 ± 0.048 for control cells exposed to air (±S.E., $n = 4$). However, the analysis of membrane fatty acid composition was complicated by the presence of Tween 80. When Tween 80 was carried through the derivitization and extraction procedures, its chromatogram exhibited approximately 20 peaks, many of which overlapped the peaks of the fatty acid methyl esters. We therefore could not accurately determine the membrane fatty acid composition when Tween 80 was added to the growth medium. This finding represents yet another reason why the use of Tween 80 as a carrier for cholesterol suffers from serious deficiencies [36].

lesterol were also incorporated into the membrane at 37°C compared to 4°C when *A. laidlawii* were grown on a oleate-enriched medium [34]. It was concluded from this study that the incorporation of exogenous cholesterol is enhanced when the membrane lipid bilayer is in the liquid-crystalline state and inhibited when the lipid bilayer is in the gel state. Thus, the continuous presence of inhalation anesthetics appears to have an effect similar to increased growth temperature on the incorporation of cholesterol into *A. laidlawii* membranes.

Indeed, both increases in temperature and the addition of inhalation anesthetics are known to increase the fluidity of other membrane preparations. Although inhalation agents cause a dose-related increase in the mobility of fatty acid chains in cholesterol-phospholipid bilayers [37–40], the magnitude of this fluidization depends upon the agent examined and upon the bilayer lipid composition [41–43]. In addition, the effect is small at clinical concentrations of anesthetics [43], and cannot even be detected when analyzed by X-ray or neutron diffraction [45,46]. In phospholipid-cholesterol (2 : 1) bilayers, the mean change in order parameter produced by anesthetizing levels of inhaled agents is similar to that produced by raising the temperature 0.32°C [47]. Inhaled agents also decrease the phase transition temperature of dipalmitoylphosphatidylcholine systems [48–50], but by less than 1°C at anesthetizing concentrations [51]. However, the mechanism(s) by which the presence of anesthetic agents or an increase in temperature allows for greater cholesterol incorporation into the membrane remains unknown.

Although we could not detect any changes in *A. laidlawii* membrane fatty acid composition after 24 h of exposure to high concentrations of halothane or cyclopropane, the possibility remains that longer exposure may alter fatty acid composition. In addition, other lipid components of *A. laidlawii* membranes were not measured and may have been altered after chronic anesthetic exposure. For example, the synthesis of monoglucosyl diacylglycerol is diminished by the presence of cholesterol but is stimulated by low temperature and saturated fatty acids [52]. The molar ratio of monoglycosyl diacylglycerol to diglucosyl diacylglycerol may vary over a wide range depending upon the nature of the exogenous fatty acid [53]. It remains to be determined whether or not continuous anesthetic exposure is able to alter the diacylglycerol composition.

The increase in membrane cholesterol content found after continuous exposure to high concentrations of halothane was paralleled by an increase in membrane order when measured with 5-doxylstearic acid near the growth temperature in the absence of the anesthetic (Fig. 1). In contrast, no differences in intrinsic fluidity could be detected in synaptosomal membranes of animals made tolerant to alcohol [20,54] or nitrous oxide [16] and their controls. The inability to detect differences in synaptosomal membrane order between anesthetic-adapted and control animals may arise from the smallness of the overall lipid changes observed in the heterogeneous synaptic membrane fraction following chronic anesthetic exposure.

The difference in membrane physical state between the control and the anesthetic-exposed cells disappeared as the temperature decreased (Fig. 2). This finding may be related to a dual role of cholesterol, which decreases the mobility of hydrocarbon chains of lipids above their phase transition temperature, and increases the mobility of lipids below their phase transition temperature [55]. A similar phenomenon has been found in comparing *A. laidlawii* membranes enriched in either palmitate or palmitate and cholesterol [56]. At a temperature of 40°C, membranes enriched in palmitate and cholesterol have a much higher order parameter than those enriched only in palmitate, and this difference in order parameter disappears as the membranes are cooled toward 20°C [56].

In conclusion, we have shown that *A. laidlawii* grown in a cholesterol-enriched medium and continuously exposed to high concentrations of inhaled anesthetics incorporate approximately twice the amount of cholesterol in their membrane (on a protein basis) compared to control cells grown in an air environment. This increase in membrane cholesterol content is accompanied by an increase in membrane order, as measured by 5-doxylstearic acid at the growth temperature of these cells. Such increases in membrane cholesterol content and membrane order might also occur in specific neuronal membrane regions of animals chronically exposed to anesthetics, and may play a role in the development of tolerance or dependence seen in these animals.

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