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EFFECTS OF ANESTHETICS ON WATER PERMEABILITY AND LIPID METABOLISM IN *ACHOLEPLASMA LAIDLAWII* MEMBRANES

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The addition of tetracaine and diethyl ether to *Acholeplasma laidlawii* at concentrations commonly used for local anesthesia did not affect water permeability over the cell membrane, as measured by a pulsed magnetic field gradient NMR method. However, *A. laidlawii* changed its membrane lipid composition upon treatment with these anesthesics. Both tetracaine and diethyl ether addition resulted in a decrease in the molar ratio between the major membrane glucolipids, monoglucosyldiacylglycerol and diglucosyldiacylglycerol. The ratio between saturated and unsaturated acyl chains did not change. The results are in accordance with our proposal that *A. laidlawii* regulates its lipid composition in order to maintain optimal packing stability in the membrane (Wieslander, Å., Christiansson, A., Rilfors, L. and Lindblom, G. (1980) Biochemistry 19, 3650–3655). Introduction of anesthetics into the hybrophobic region of a bilayer is likely to affect the lipid packing. A membrane which contains lipids like monoglucosyldiacylglycerol, which forms a reversed hexagonal phase, will be destabilized unless the amounts of such lipids are reduced. The membrane concentration of anesthetics was estimated to one molecule per 12–15 lipid molecules. The fact that *A. laidlawii* regulates its lipid composition as a response to these concentrations, despite their negligible effect on water permeability, indicates a high sensitivity of this regulatory system.

Introduction

Every biological membrane contains a considerable number of different lipid molecules with different fatty acid compositions and polar head groups. The basis for this heterogeneity is only recently beginning to be understood. It is well known that the acyl chain composition of the lipids affects the physical properties of the bilayer [1] and the function of many membrane bound enzymes [2]. Recently the importance of the different polar head groups for the molecular packing in the bilayer [3–5] and for an optimal packing of proteins into the bilayer [6] has been proposed. Furthermore, lipids forming non-bilayer phases have been implicated in processes like membrane fusion and lipid flip-flop [7].

Acholeplasma laidlawii regulates its lipid composition as a response to changes in fatty acid composition, growth temperature and cholesterol [8–11]. The regulation involves not only changes in fatty acyl chain composition but furthermore an extensive regulation of polar head groups. We have recently proposed that this regulation is necessary for the cell to maintain optimal membrane stability and that the effects of environmental stimuli can be predicted on basis of their induced effects on the lipid packing in the bilayer [5]. This regulation keeps the balance between lipids forming lamellar and reversed hexagonal phase structures within certain limits, to maintain bilayer stability [4,5].

To further test the theory for regulation of lipid composition we have investigated whether the effects on lipid metabolism in *A. laidlawii* of membrane pertubing agents like anesthetics can be foreseen on basis of their disturbance of the bilayer. Anesthetics comprise a structurally very heterogenous group of chemicals whose potencies to induce anesthesia in nerve membranes are roughly correlated with their lipid

solubilities [12]. Reports have been presented on the ability of anesthetics to induce structural perturbations in both lipid bilayers and in proteins (for a review, see Ref. 13), though most theories hitherto seem to favour the action on lipids as the cause of anesthesia on nerve membranes. The introduction of small molecules into the bilayer is likely to affect its permeability for ions [14] and water [15]. We report here on the use of NMR to measure water diffusion over cell membranes with a pulsed magnetic field gradient method. The effect of anesthetics on water diffusion has been compared to the physiological regulation of lipid composition occurring upon addition of anesthetics. All investigations have been performed at clinically relevant concentrations of anesthetics, hopefully to gain some insight into the mechanism of nerve cell anesthesia at a molecular level, using *A. laidlawii* as a model system.

Materials and Methods

Organisms and growth conditions

Acholeplasma laidlawii A, strain EF22, was grown at 37°C in a lipid-depleted tryptose/bovine serum albumin medium as described previously [11]. The medium was supplemented with 75 μM of palmitic and 75 μM of oleic acid, added as sterile ethanolic solutions. To label the membrane lipids 10 μCi/l [1-^{14}C]oleic acid (56 mCi/mmol) and 30 μCi/l of [9,10-^{3}H]palmitic acid (57.9 mCi/mmol) (The Radiochemical Centre) were added. *Bacillus megaterium* Ft R32, a facultatively thermophilic strain, was grown in a tryptone-starch broth [16] at 50°C. This strain was used in experiments where the effect of anesthetics on growth of a common bacterium and the wall-less *Acholeplasma* was compared. The organisms were grown in side-arm flasks, containing different concentrations of anesthetics (see Table I). When volatile anesthetics were used, the flasks were closed with rubber stoppers, and a small volume of medium was contained in a big flask to assure accurate oxygen supply for the *Bacillus* cells. Growth was followed by measurement of absorbance at 540 nm.

To examine the effect of anesthetics on lipid metabolism, *A. laidlawii* was grown for 12 h in a medium without anesthetics. The culture was divided in two halves and one half was supplemented with anesthetics, the other remained unsupplemented. The drugs used were diethyl ether (final concentration 0.05 M) and tetracaine (final concentrations 0.22 mM). The drugs were added dissolved in fresh growth medium (approx. 4% of culture volume) and the unsupplemented culture received an equal volume of fresh medium. To prevent the evaporation of diethyl ether, this experiment was performed in rubber-stoppered flasks. After supplementation, incubation was carried on and further growth was measured by absorbance at 540 nm. Samples for lipid analysis were taken at intervals. Cells were harvested by centrifugation at 32 000 $\times g$ for 10 min at 5°C. The cells were washed and membranes prepared as in Ref. 9.

Lipid analysis

Membranes were extracted with chloroform/methanol (2 : 1, v/v) and the lipid extracts were purified as described earlier [9]. The individual polar lipid species were separated by thin-layer chromatography on silica gel H (Merck) plates (buffered with 1% w/v $Na_2B_4O_7 \cdot 10\ H_2O$) in chloroform/methanol/water (65 : 25 : 4, v/v). The lipids were visualized by exposure to iodine vapour and were scraped off the plates into Pasteur pipettes stoppered with glass wool. The lipids were eluted with chloroform/methanol (2 : 1, v/v) followed by methanol/formic acid (96 : 4, v/v) into scintillation vials. After evaporation of the solvent, scintillation cocktail (Omnifluor-toluene, New England Nuclear Co.) containing 6% (v/v) Biosolv (Beckman Instruments) was added and the samples were counted in a Nuclear Chicago (Mark II) liquid scintillation counter set for double label counting. Conversions from counts per minute (cpm) to distintegrations per minute (dpm) were obtained by the external standard ratio method, using calibration curves produced by quenching ready-for-use internal standards (LKB-Wallac, Finland) in scintillation cocktail of the same composition. Quantification of acyl chain and polar lipid composition in *A. laidlawii* by determination of radioactivity in the fatty acyl chains has been shown to agree well with GLC measurements under the conditions employed [8], since virtually no endogenous fatty acid synthesis is present [11].

Preparation of NMR samples

A. laidlawii was grown for 18 h at 37°C as described above and harvested by centrifugation

(32 000 × g, 10 min). The cells were washed in buffer (50 mM Tris, 130 mM NaCl, 2 mM $MgCl_2$, 5 mM $CaCl_2$, 1.2 mM Na_2HPO_4, 1 mM KCl, 0.1 mM sucrose, 0.1 mM glycerol, pH 7.4). This buffer permits no cell metabolism and has a stabilizing effect on the fragile *A. laidlawii* membrane [17]. After centrifugation the cells were resuspended in the same buffer with and without 2.23 mM tetracaine or 500 mM diethyl ether. The cell to drug ratio was the same as in the lipid metabolism study; see above. The suspensions were stirred very slowly for 30 min at room temperature and then centrifuged at 32 000 × g for 10 min. The obtained cell pellets were quantitatively transferred to graded NMR tubes, sealed with plastic stoppers and analyzed by NMR.

Determination of intra- and extracellular water

Cell pellets were prepared exactly as described above with the modification that the suspension buffer contained 0.16 μCi/ml [U-^{14}C]sucrose (610 mCi/mmol) and 0.4 μCi/ml [2-^{3}H]glycerol (500 mCi/mmol) (The Radiochemical Centre). After the last centrifugation, ^{14}C- and ^{3}H-activities were determined in the cell pellet and the supernatant (cf. Ref. 18). The amounts of labelled sucrose adsorbed to the cell surface was determined by adsorbing the cells on 0.22 μm Millipore filters and washing them with the appropriate buffer. The fraction of intracellular water (versus extracellular water) was found to be approx. 20% in the cell pellets intended for NMR analysis. This corresponds to roughly 1 μl of intracellular water per mg cell protein.

NMR diffusion measurements

All diffusion studies were done on a Bruker 322-s pulsed NMR-spectrometer operating at 61 mHz for protons. The spectrometer was equipped with a homebuilt pulsed magnetic field gradient unit. The water diffusion was measured in non-metabolizing cells with the pulsed magnetic field gradient method [19]. A 90°-τ-180° pulse sequence was used. Two magnetic field gradients, spaced Δ ms, of equal strength $\delta \cdot g$, where δ is the duration and g the size of the gradient, were applied on each side of the 180° radiofrequency pulse. A spin-echo was observed after a time interval of 2τ from the start of the pulse sequence. Diffusion of water molecules during the time Δ (the diffusion time) attenuated the spin-echo amplitude according to the equation:

$$E_g/E_0 = \exp(-(\gamma \cdot \delta \cdot g)^2 \cdot (\Delta - \delta/3) \cdot D)$$

where E_g and E_0 are the spin-echo amplitudes with and without magnetic field gradients, γ is the magnetogyric ratio for protons and D is the self-diffusion coefficient for the diffusing species.

In the case of restricted diffusion i.e. when the translational diffusion of the water molecule is limited in space due to barriers of some kind, the observed diffusion coefficient reaches an asymptotic value for diffusion times several times larger than the time it takes a molecule to diffuse the distance between the barriers.

In a heterogenous system like the *Acholeplasma* cells there are two fractions of mobile water, intra- and extracellular water. The spin-echo attenuation in such a system can be described by a superposition of two exponentials [20,21]. Assuming that the exchange rates for water molecules across the cell membrane are large compared to the transverse relaxation rates for the intra- and extracellular water the echo attenuation will depend on diffusion only [20,21].

The NMR signal of the cell sample is comprised of a fast decaying component due to extracellular water, that has its transverse relaxation rate greatly enhanced by the magnetic field gradient and a slow decaying component due to intracellular water (less enhanced relaxation rate) with a decay rate partly determined by the mean residence time τ_r of water molecules inside the cells. If the time it takes for a water molecule to transverse the cell interior is small compared to the mean residence time of a water molecule within the cell, τ_r is determined by the water diffusional permeability of the cell membranes. ($\tau_r \geq r^2/2D$, r = cell radius, D = self-diffusion coefficient for water molecules inside the cell [22].)

I. Variation of the magnetic field gradient. The magnitude of the magnetic field gradient was varied in discrete steps from 0.4 to 4 T/m while Δ was kept constant in each series. The following values were chosen for Δ: 13, 20, 26, 33, 39 ms and τ was 10, 15, 20, 25, 30 ms, respectively. δ was 1.5 ms in all measurements. The spin-echo amplitude was measured from an oscilloscope and $\ln(E_g/E_0)$ was plotted versus g^2. These measurements were carried out at the following temperatures: 5, 10.5, 16.5, 25, 37.7°C. The

effect of tetracaine on the water permeability was studied. Restricted diffusion was also tested by investigating lysed cells (Fig. 1). A reference of water free glycerol was used to calibrate the magnetic field gradient unit. The same settings on the spectrometer were used for the cell and the reference samples.

In a NMR experiment when magnetic field gradient pulses of sufficient large strength are applied the fast decaying component of the signal is much attenuated and the spin-echo amplitude reaches a limit due to the restricted motion of the intracellular water. For large $\delta \cdot g$ the signal amplitude can be described by the following expression [22].

$$E_g/E_0 = p_b \exp(-(2\tau/\tau_r) - (\gamma \cdot g \cdot \delta \cdot r)^2/5)$$

where p_b is the intracellular water fraction. $\ln(E_g/E_0)$ is plotted vs. g^2 and its intercept at $g = 0$ is determined. This intercept is a function of τ and thus τ_r can be determined.

II. Variation of the diffusion time. In this kind of experiment g was kept at a constant value while Δ, the diffusion time, was varied in discrete steps from 10 to 60 ms. $\ln(E_g/E_0)$ was plotted vs. Δ and δ was 1.5 ms.

Diffusion measurements on water (with small amount of Cu^{2+} added) were done in order to calibrate the magnitude of the magnetic field gradient unit. Self-diffusion coefficients for water at different temperatures were obtained from Ref. 23.

From these diffusion-studies we can determine a mean lifetime for water molecules inside the cells according to the method described by Andrasko [21]

$$\tau_r = \frac{D_a - D(1 + p_b/p_a)}{(\gamma \cdot \delta \cdot g)^2 D(D_a - D)}$$

where D_a is the self-diffusion coefficient for water at 25°C ($D_a = 2.4 \cdot 10^{-9}$ m$^2 \cdot$ s^{-1}), p_a and p_b are volume fractions of extra- and intracellular water, respectively. D is the observed diffusion coefficient obtained from the NMR measurements.

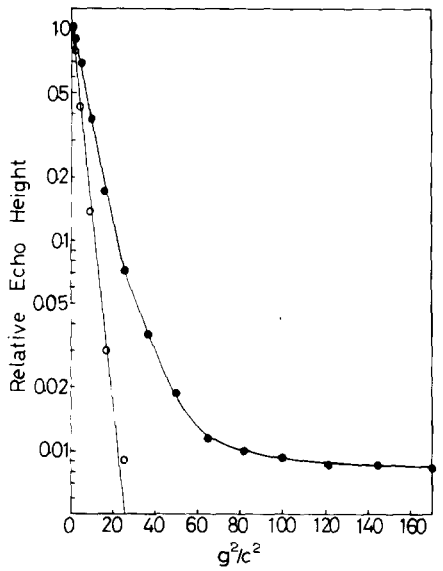

Fig. 1. Relative echo height vs. g^2/c^2 ($c = 0.37$ T/m) for lysed cells (o———o) and for intact cells (•———•) at 28°C. Δ was kept constant at 26 ms, δ was 1.5 ms and τ was 20 ms.

Results

Growth in the presence of anesthetics

In order to choose some suitable membrane-active drugs we examined the growth response of *A. laidlawii* in the presence of some anesthetics at different concentrations. Since these agents are lipophilic it might be expected that a change in growth would reflect a perturbation in the membrane (cf. Ref. 24). Fig. 2 illustrates the results of a typical experiment

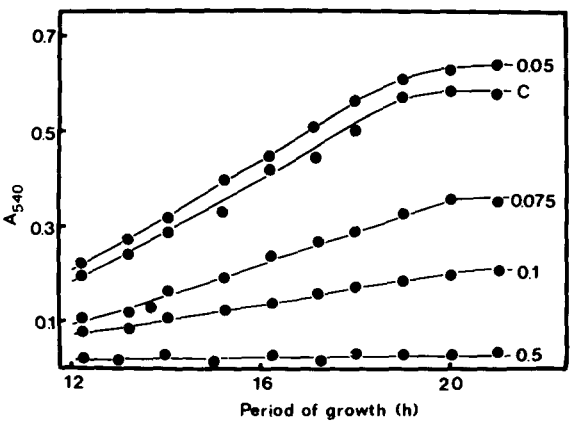

Fig. 2. Effect of diethyl ether on growth of *A. laidlawii.* Cells were grown at 37°C in a lipid-depleted tryptose-bovine serum albumin medium supplemented with 75 μM of palmitic and 75 μM of oleic acid. Different concentrations of diethyl ether (concn. in M) were added before inoculation. C, control culture without addition. Growth was estimated by absorbance measurements at 540 nm.

TABLE I

DIFFERENTIAL EFFECT OF MEMBRANE-ACTIVE DRUGS ON GROWTH OF *ACHOLEPLASMA LAIDLAWII* AND *BACILLUS MEGATERIUM* FtR 32

The drugs were added to the growth media (see Materials and Methods for composition) before inoculation. Growth temperatures were 37°C and 50°C, respectively, and growth was measured by absorbance at 540 nm. The growth rates are expressed in percentage of control cultures without drugs. Figures in parenthesis denote nerve-blocking concentrations of the drugs.

(A) Diethyl ether (0.05 M) *			(B) Ethanol (0.5 M) *		
Concn. (M)	Relative growth rate (%)		Concn. (M)	Relative growth rate (%)	
	A. laidlawii	*B. megaterium*		*A. laidlawii*	*B. megaterium*
0.05	108	100	0.25	–	93
0.075	44	–	0.50	0	68
0.10	31	100	0.63	0	48
0.25	–	92	0.75	0	0
0.50	0	0			
(C) Tetracaine (0.1–0.01 mM) **,***			(D) Procaine (4.6 mM) *		
Concn. (mM)	Relative growth rate (%)		Concn. (mM)	Relative growth rate (%)	
	A. laidlawii	*B. megaterium*		*A. laidlawii*	*B. megaterium*
0.11	97	100	1.9	62	100
0.22	65	–	3.8	5	100
0.25	–	85	19	0	100
0.50	–	62	46	0	100
0.66	2	–	115	–	84
0.75	–	0	230	–	34
			460	–	0

* Data taken from Seeman [12], obtained with frog sciatic nerve.
** Data taken from Seeman [12], obtained with squid giant axon (0.1 mM).
*** Data from Skou [37], obtained with frog sciatic nerve.

with diethyl ether. With increased concentrations of diethyl ether in the growth medium *A. laidlawii* changed its growth rate from slightly stimulated at 0.05 M diethyl ether to zero at 0.5 M, where the cells lysed. It should be noted that 0.05 M diethyl ether is the nerve blocking concentration for frog sciatic nerves [12]. Like all mycoplasmas, but in contrast to other bacteria, *A. laidlawii* lacks a cell wall [25]. Since the presence of a cell wall connected to the cytoplasmic membrane might stabilize the membrane structure, a comparison of the growth of *A. laidlawii* and a common bacterium in the presence of membrane active drugs was performed. Table I illustrates the differences in sensitivity of *A. laidlawii* and *B. megaterium* Ft R32 to diethyl ether, ethanol (general anesthetics), tetracaine and procaine (local anesthetics). It can be noted that both organisms were affected by diethyl ether, ethanol and tetracaine in the range of concentrations used for local anesthesia. However, the concentrations required to inhibit *B. megaterium* were always larger than those for *A. laidlawii.* Procaine inhibited growth of *A. laidlawii* below clinical concentrations, whereas *B. megaterium* remained growing until a 100-fold this concentration was applied. Detailed experiments were made with one general anesthetic, diethyl ether and with one local anesthetic, tetracaine.

Water diffusion

A comparison between the curves obtained in Fig. 1 strongly indicates restricted diffusion of water in intact cells. Almost free water diffusion occurred

TABLE II

THE MEAN RESIDENCE TIME FOR WATER MOLECULES INSIDE *ACHOLEPLASMA LAIDLAWII* CELLS AT DIFFERENT TEMPERATURES

t (°C)	5.0	10.5	16.5	25.0	37.7
τ_r (ms)	72	47	33	19	12

in the sample containing lysed cells. The water diffusion coefficients for this sample was found to be $4.1 \cdot 10^{-10}$ and $2.2 \cdot 10^{-10}$ $m^2 \cdot s^{-1}$ at 28°C and 18°C, respectively (cf. free water diffusion is $2.4 \cdot 10^{-9}$ $m^2 \cdot s^{-1}$ at 25°C [23]). The mean residence times τ_r for water molecules in intact cells obtained at different temperatures are summarized in Table II. As can be inferred from this table the permeability increases with increasing temperature. From the data in Table II an apparent activation energy of 36 kJ/mol was calculated. The permeability $p_d = r/3\tau_r$ may be calculated using the average value of the cell diameter equal to 0.6 μm. Our data are comparable to permeability values obtained for other systems [26,27]. The permeability may vary considerably depending on the lipid composition of a membrane [15].

The effect of tetracaine on the water permeability was also investigated. It was found that using the same batch of cells the mean residence time for water in cells supplemented with tetracaine ($\tau_r = 13 \pm 4$ ms) did not differ significantly from those without the drug ($\tau_r = 12 \pm 4$ ms). Similar results were obtained by using the method by Andrasko [21] on cells supplemented with diethyl ether.

Effects of anesthetics on lipid metabolism

To investigate the influence of anesthetics on water permeability cells were exposed to the drugs in a buffer. Due to the lack of nutrients in the buffer, the cells should not be able to compensate metabolically for the introduction of anesthetics into the membrane. Any disturbance created would be measurable without the masking influence of lipid compensating mechanisms. To examine the extent of metabolic compensation we employed a shift technique, adding an anesthetic to one half of an actively growing culture and leaving the other half unsupplemented.

After addition of anesthetics growth continued uninterrupted in both the diethyl ether culture and the tetracaine culture. There was a slight decrease in the growth rate of the diethyl ether culture compared to the control, whereas 0.22 mM tetracaine did not affect growth at all. Fig. 3 demonstrates the responses in lipid metabolism upon addition of anesthetics. During growth of the control cells there was a gradual decrease in the monoglucosyldiacylglycerol/diglucosyldiacylglycerol ratio due to an increased incorporation of oleic acid into the lipids (cf. Refs. 8 and 9). Immediately after the shift the glucolipid ratio decreased even further (Fig. 3A and B). This decrease was small compared to the magnitude of changes occurring upon temperature shift-down [9], but reproducible. Upon further growth the glucolipid ratio of the diethyl ether culture approached that of the control (Fig. 3A), whereas that of the tetracaine culture was larger than the control after 12 h of growth (Fig. 3B). The results obtained for the diethyl ether culture can probably be attributed to the difficulties to maintain the proper ether concentration after repeated samplings. Virtually no change occurred in the relative amount of ionic lipids of the diethyl ether culture when compared with the control (Fig. 3C). On the contrary, after addition of the positively charged tetracaine there was an increase of ionic lipids (negatively charged) (Fig. 3D). Analysis of total acyl chain composition revealed practically no differences (less than one per cent difference) between the anesthetic supplemented cultures and the controls.

Discussion

Previously we have proposed that variation in lipid composition is an important factor in maintaining optimal membrane stability in *A. laidlawii* [5]. The individual lipids in the bilayer can be visualized as building bricks with different geometries. Important factors determining the shape of the bricks are the volume of the hydrophobic part of the molecules, the hydrocarbon chain lengths and the optimal surface areas occupied by the polar head groups. These bricks will determine the shape of the aggregates formed [3]. Lipids with different molecular shapes may be accomodated in a bilayer as long as they can pack together to form a stable lamellar structure [4,5].

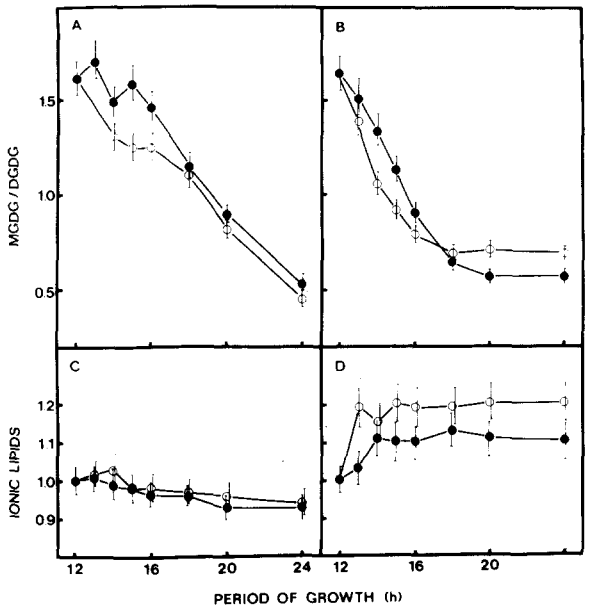

Fig. 3. Regulation of lipid composition in *A. laidlawii* upon addition of anesthetics. Cells were grown at 37°C for 12 h. At this time the culture was divided in two parts, one of which was supplemented with anesthetics (○———○) while the other remained unsupplemented (●———●). A and B: molar ratio monoglucosyldiacylglycerol/diglucosyldiacylglycerol (MGDG/DGDG). C and D: relative amount of ionic lipids. The percentage of ionic lipids in the membranes at 12 h was assigned the value of one. A and C: 0.05 M diethyl ether. B and D: 0.22 mM tetracaine. Lipid amounts were determined by liquid scintillation counting. Error bars denote 95% confidence limits determined by propagation of error.

The major lipids in *A. laidlawii* are monoglucosyldiacylglycerol and diglucosyldiacylglycerol. Monoglucosyldiacylglycerol is characterized by a small polar head group compared with the hydrophobic part, resulting in a reversed hexagonal (H_{II}) phase of the pure lipid in water, whereas diglucosyldiacylglycerol with a larger head group forms a lamellar phase [4,5,28]. One of the main lipid regulation mechanisms in *A. laidlawii* concerns the molar ratio monoglucosyldiacylglycerol/diglucosyldiacylglycerol. It has been found that in vitro mixtures of monoglucosyldiacylglycerol/diglucosyldiacylglycerol with ratios corresponding to those found in vivo form a lamellar phase, whereas larger ratios yield non-lamellar phases [29]. Another mechanism concerns the amount of ionic lipids (phosphatidylglycerol, glycerophosphorylmono- and diglucosyldiacylglycerol) in the membrane. Introduction of charges in a polar head group will change the optimal surface area and thus the packing properties of a lipid.

In the present investigation changes in acyl chain composition caused by addition of anesthetics were negligible and the results can therefore not be attributed to packing disturbances introduced by changes in acyl chain saturation. The lipophilic anesthetics, as shown for tetracaine [30,31], penetrate into the hydrocarbon region of the bilayer, and cause packing disturbances. This should lead to an increase in the hydrophobic bulkyness and a bilayer containing lipids with H_{II}-tendencies, e.g. monoglucosyldiacylglycerol, should be destabilized (cf. Ref. 32). Cholesterol, which also increases the hydrophobic volume of bilayers, was shown to induce H_{II} phase formation in in vitro mixtures of mono- and diglucosyldiacylglycerol [33]. *A. laidlawii* can therefore be expected to decrease the glucolipid ratio. Likewise, introduction of small molecules into the bilayer will dilute the charged lipids present, leading to a decrease in membrane surface charge [5,34]. We have shown previously that *A. laidlawii* regulates the amount of ionic lipids upon similar changes. Fig. 3A, B illustrates the regulation of the monoglucosyldiacylglycerol/diglucosyldiacylglycerol ratio upon addition of anesthetics. In both experiments the molar ratio decreased as predicted. No regulation of the amount of ionic lipids occurred in the diethyl ether culture (Fig. 3C). This can be explained by the fact that the diethyl ether concentration in the membrane was very low. Using membrane/buffer partition coefficients from Seeman [12] and Leo et al. [35] a rough estimate gives one anesthetic molecule per 12–15 lipids for both diethyl ether and tetracaine. This is equal to one molecule per approx. 800 Å^2 and the changes in surface charge introduced by the diethyl ether molecules may be small enough not to require compensation. Contrary to diethyl ether, the tetracaine shift resulted in a significant increase in the amount of ionic lipids (Fig. 3D). This increase seems to compensate for the amount of tetracaine molecules present in the membrane. Since the tertiary amine group of tetracaine is positively charged at the pH of the growth medium, its presence in the *Acholeplasma* membrane will lead to a charge neutralization when it interacts with the negatively charged ionic lipids. This will effectively reduce the optimal surface area per lipid molecule

and may lead to a bilayer destabilization. Thus the combined effects of tetracaine on the surface area and the hydrophobic volume may not be adequately compensated for merely by a decrease in the glucolipid ratio, but it also regulated by an increase in ionic lipids. A structurally related anesthetic, dibucaine, has been shown to induce formation of a H_{II} phase when interacting with diphosphatidylglycerol [36]. It can thus be concluded that the observed lipid metabolic regulations pertain nicely to the proposed theory. However no changes in water permeability was detected in non-metabolizing cells upon addition of anesthetics. This indicates that the disturbances introduced at clinical concentrations of anesthetics were too small if any to be measured. Similar conclusions have been reached for lipid membrane model systems using other physical methods, e.g. X-ray and neutron diffraction [38], NMR [39] and ESR [40]. However, although not always measurable, the introduction of anesthetics into a bilayer should lead to a concentration-dependent increase in the disorder or entropy of the system, as shown theoretically by Hill [41]. Since the cytoplasmic membrane is the only barrier to the outer milieu in *A. laidlawii*, it can be expected that a very sensitive lipid regulating systems should be at work to ensure optimal packing stability and functional intactness of the membrane. It is of interest to note that *B. megaterium* having a stabilizing cell wall outside the cytoplasmic membrane, was less sensitive to anesthetics than *A. laidlawii* (Table I).

The effects on lipid regulation observed in *A. laidlawii* upon addition of anesthetics may be relevant to the explanation of nerve cell anesthesia. The balance between lamellar and non-lamellar phases in mixtures of *A. laidlawii* glucolipids is very sensitive to changes in the hydrophobic region of the lipids [29,33], and is regulated upon addition of anesthetics, to maintain optimal stability of the lamellar phase. Very subtle changes can affect the phase structure of a lipid and thus its packing in a membrane (cf. 42). In a biological membrane the geometry of lipids are important for the correct embedding and probably function of membrane proteins [6]. In contrast to *A. laidlawii* nerve cells normally exist in a very constant environment and are not designed to rapidly change their lipid composition upon contact with e.g. anesthetics. The induced changes in the lamellar phase stability (or instability) might therefore be of crucial importance for the function of gating mechanisms regulating the transverse ion fluxes of the nerve impulse.

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