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The influence of general, volatile anesthetics on the dynamic properties of model membranes

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The microscopic mechanism of general anesthesia is still not known but it might be located in the membrane region of nerve cells. In the present article microwave experiments on model membrane systems are discussed. At these particular frequencies a significant decrease of the systems' complex dielectric function is measured when it is exposed to volatile anesthetics. This effect is reversible as well as being identical for chemically quite different anesthetics. The corresponding anesthetic concentrations in the samples are relevant for medical anesthesia. The underlying microscopic mechanism is a decrease in the Debye relaxation frequency (and correspondingly an increase in viscosity) of those water molecules which are localized at the membranes' surfaces. General anesthesia might be a consequence of such a viscosity change at hydrophilic surfaces.

Introduction

Inhaling volatile anesthetics is one of the most frequently applied medical procedures for inducing general anesthesia [1]. Apparently changes in the properties of the nerve cell membrane region are responsible for anesthesia, since this is the place of information processing in the body [2,3]. It is generally assumed that, in contrast to most other drugs, volatile anesthetics only work by their presence (solution) in a biological system without any metabolism or other specific biochemical processes. This idea is mainly supported by the chemical diversity of anesthetics (most striking is the noble gas xenon) [1–3] and the pressure reversal (decrease of anesthetic effects if the organism is exposed to a higher hydrostatic pressure) [1–4]. In this sense, anesthesia is the result of a general physical change of biological membranes that have been doped with anesthetics. Although there have been numerous theoretical and experimental efforts to correlate anesthesia with changes in the membrane region, the most common dealing with the membrane lipid bilayer matrix [5–8], water [9,10] and proteins [11,12], the data available until now neither elucidate the site of action nor point to the microscopic mechanism of general anesthetics within membrane regions.

In the present work a new experimental approach is described with which dynamical changes of lipid bilayer membranes can be continuously recorded as a function of time. Thus the influence of anesthetic gases on these model membrane systems can be monitored from the very beginning of the absorption. The lipid L-α-dimyristoylphosphatidylcholin (DMPC) mixed with water was used, since it belongs to the relatively well known class of phosphatidylcholines which form bilayer membranes in the presence of water.

One of the primary ideas of this work was to induce anesthesia-related changes most quickly by applying the anesthetic gas in the highest, saturated concentration. Thus changes will occur in a very short time giving best measurement resolution and low influence of measurement system drift which enables the detection of minor alterations, too.

First, the dynamics of water in terms of dielectric relaxation are briefly recapitulated. Then a preparation technique for membrane/water layers is explained which yields a preferential macroscopic layer orientation. Thus anisotropic dielectric measurements are possible, facilitating a unique interpretation of the measured alterations in effective dielectric data. Furthermore, gravimetric absorption measurements were performed to determine the total amount of anesthetic molecules in the samples during the microwave experiments.

The continuously recorded microwave data show an immediate decrease in both parts of the dielectric function of the DMPC/H_2O system, as soon as an exposure

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to an anesthetic gas is started. This effect is reversible and identical for chemically quite different anesthetics. Although very high anesthetic concentrations in the gas were used, the time dependence of the gravimetric and dielectric data together give evidence that the observed decreases in dielectric function are caused by very low, medically relevant anesthetic concentrations in the samples.

The relaxation of bound water

Below 40 weight% H_2O, the DMPC/water system consists of periodically stacked bilayer membranes. They are separated by water layers of certain thickness which depends on the water content [13]. This water is of fundamental importance, necessary for forming the bilayer structure and determining its phase behavior. It is known to have properties different from the bulk water [14–16].

The motivation for the present work evolved from several dielectric experiments which were performed on isotropic DMPC/water systems. A reasonable Debye relaxation analysis was possible since the water content of the samples, the temperature and the measuring frequencies were varied. Thus the relaxation frequencies of the so-called bound water molecules [15], of the phosphatidylcholine headgroups [17] and of the alkyl chains [18] could be separated from each other and were determined; at microwave frequencies the water dominates the dielectric dispersion of the samples if the water content is above 15 weight%.

However, the inner geometry of isotropic membrane systems, i.e., an alignment or curvature of the membrane stacks, is not exactly known and was not considered in these dielectric relaxation studies. In other words, the depolarization factors of dielectric boundaries in the samples are unknown. They have a different influence on the real and imaginary part of the dielectric function and can induce errors in the calculation of the Debye frequencies. Therefore, anisotropic samples were prepared for the present experiment which have a known orientation of the membrane layers. The results of [15,17,18] were definitely proved by this procedure, too.

The Debye frequency $f_D = 1/(2\pi\tau_D)$ corresponds to the mean relaxation time τ_D within which an electric dipole changes its equilibrium position above a potential barrier ΔG [19]. The complex Debye equation describes the contribution to the complex dielectric function $\epsilon = \epsilon_1 - i\epsilon_2$. Separated in the real- and imaginary part it is given by:

$$\epsilon_1 = \epsilon_\infty + \frac{\epsilon_S - \epsilon_\infty}{1 + (f_M/f_D)^2}$$

$$\epsilon_2 = \frac{(\epsilon_S - \epsilon_\infty)(f_M/f_D)}{1 + (f_M/f_D)^2}$$

with

$$f_D = \text{const} \times \exp\left(-\frac{\Delta G}{k_B T}\right)$$

(ϵ_S and ϵ_∞ are the real, low and high frequency limits of the dielectric function with respect to f_D; f_M is the measuring frequency, k_B Boltzmann's constant, T the absolute temperature). The Debye frequency of water is of special interest here. It is a dynamic measure of the rotational relaxation motion of a H_2O molecule due to several changes in the hydrogen bond pattern of water [20–23] and is a time measure for the redistribution of water molecules in the water lattice. ΔG is the free enthalpy barrier for this rate process. Thus, the Debye frequency is also a measure of diffusion and behaves inversely proportional to the viscosity [19]. The rapid molecular time scale with its large energy and density fluctuations is averaged out at microwave frequencies [23,24], but these complicated processes are the presupposition that a position change of a water molecule in the hydrogen bond network can take place. Therefore an anisotropy in the water relaxation behavior in the vicinity to hydrophilic surfaces will hardly be measurable: the rapid molecular time scale will be highly anisotropic due to the different hydrogen bond strengths at the surface; however, the sum of molecular alterations necessary for a rotational relaxation of a water molecule involves its whole surrounding and will average the anisotropies out.

The Debye frequency of water molecules becomes smaller when their distance to the surface of the DMPC bilayers is reduced, as is shown in Fig. 1 for isotropic samples having different water content [15]. Consider-

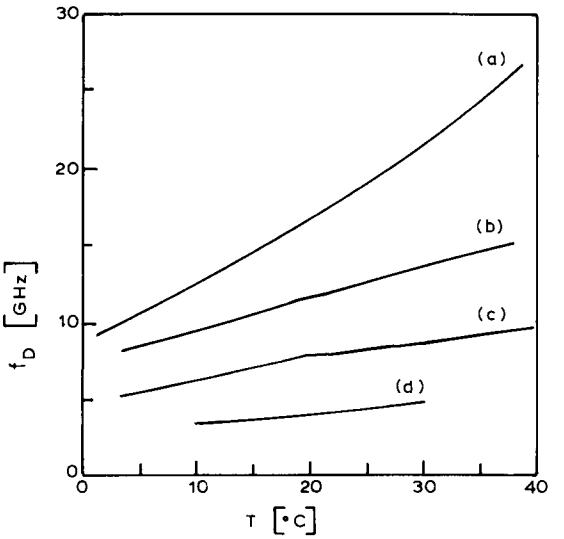

Fig. 1. Temperature-dependent Debye-frequency f_D of free (bulk) water (a), and of the most loosely bound water in isotropically oriented DMPC/H_2O samples having (b) 46, (c) 35 and (d) 12 weight% water content [15].

ing the thermally activated Debye frequency behavior, the reorientation barrier ΔG continuously becomes higher, in agreement with the continuously stronger binding as found by Parsegian et al. [14].

Preparation

DMPC was a gift from Nattermann Phospholipid GmbH, Köln. According to a quantitative element analysis and to a thin-layer chromatography, it proved to be better purified than most commercially available products. It was mixed with about 40 weight% H_2O from an ELGA UHQ purifier; a vessel made from the Teflon derivative PFA (DuPont) was used. The vessel was tempered at 80 °C for two days; on the first day a 15min centrifugation at $5000 \times g$ was applied twice to remove the air bubbles. By squeezing the material between squares of 12 μm PFA-foils, a multi-layered stack of 1.1 mm thickness was prepared that consisted of 10 material layers between 11 foils. Appropiately cut out segments were filled in two PFA sample-containers (inner dimensions $10 \times 1.1 \times 17.4$ mm, maximum sample weight about 200 mg). The alignment of the foils to the containers' cross section as well as to the electric field vector $\vec{E}$ of the microwave is sketched in Fig. 2. In the following, the prefixes $\perp$ for perpendicular and $\parallel$ for parallel alignment in relation to $\vec{E}$ will be used. The containers were sealed with 37.5 μm, two-layered Kapton/PFA foils (DuPont). The actual water content was determined by a coulometric Karl-Fischer-titration (Mitsubishi moisture meter MCI CA05) to be in the range (38 ± 3) weight% H_2O, which corresponds to 23 H_2O-molecules per DMPC-molecule.

The orientation of the membrane layers in the PFA-containers was checked at room temperature. Small-angle X-ray diffraction with a line focus and a camera of the Kratky-type was used. The periodicity of the membrane/water layers forms a diffraction grating for the X-rays, if the plane of the incident line focus is aligned parallel to the plane of the layers. Having perfect perpendicular orientation, no diffraction pattern should occur. The ratios of the integral first-order peak intensities belonging to the layer periodicity were larger by 5 : 1, mostly 10 : 1, for the parallel compared to the perpendicular orientations. This was checked at several positions and proved, that the membrane/water layers preferentially orient themselves parallel to the boundaries of the PFA foils.

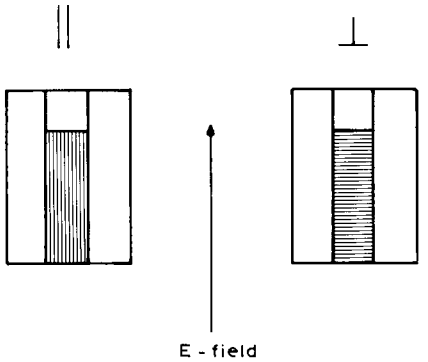

Fig. 2. Schematic cross section of both PFA sample containers; the middle sections show the alignment of the PFA foils in relation to the electric field vector of the microwave.

Method

The sample is placed in a rectangular X-band waveguide as shown in Fig. 3. This arrangement represents a partially filled waveguide structure and is part of a microwave bridge. The rectangular waveguide is embedded in a temperature controlled copper block of 30 cm length (temperature stability ± 0.03 K at the sample location). The microwave bridge including the temperature control and computer data acquisition system was described recently [25] as well as the evaluation of the sample's complex dielectric function from the H_{10}-mode wave vector [26]. The latter in turn is calculated from the measured phase and amplitude of the transmitted wave via a transmission line evaluation. The differences between the analytically exact L^3-method [26] and the approximative transmission line evaluation [26,27] is negligible if the dielectric response is as low as with the present samples. A measuring frequency f_M of 9.105 GHz was used during all microwave experiments.

The evaluated dielectric function comprises the response of the sample layers and the PFA foils, i.e., the middle sections of Fig. 2. By an extension of the transverse resonance principle [28] it was shown [27] that the anisotropic values of the interesting DMPC/H_2O stacks can be calculated analytically exact. This was performed

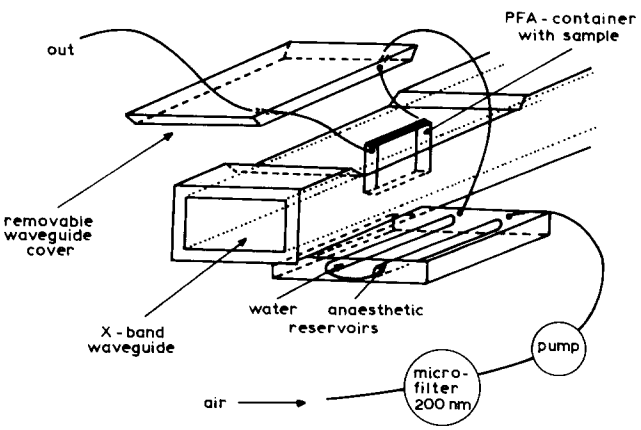

Fig. 3. Sample location in waveguide. Gas flow is maintained with a pump and is led above the liquid surfaces in the anesthetic and in the water reservoirs. The anesthetic reservoir can be filled and emptied through a second teflon capillary (not shown). The whole arrangement is embedded in a box for thermal isolation; the inner part of the sample waveguide section is thermally decoupled by insertion of Kapton foils which avoids convection.

for both the ⊥- and the ||-samples. However, the influence of the 'air gap' above the sample in the container, as sketched in Fig. 2, is not considered in these calculations. It cannot be handled analytically at all. Therefore, first the completely filled containers were measured without gas flow (see next chapter), thus gaining reference values. After this, 1 mm of the sample substance was removed from the top and teflon capillaries (600 μm o.d., 300 μm i.d.) were fixed in the containers' walls. Constant gas flows between 1 and 2 ml/min were used.

Reference Measurements

Fig. 4 illustrates the value of dielectric microwave experiments. According to the maximum in $\epsilon_2(T)$, the measuring frequency f_M lies in a range of dominating Debye relaxation processes which were shown to belong to the spectrum of bound water relaxation [15]. The following points facilitate the interpretation of the measurements described in the next paragraph:

(1) The anisotropy seen between the ⊥- and ||-sample by X-ray diffraction expresses itself also in the different values of the dielectric function shown in Fig. 4. The ⊥-sample can be thought of as a series capacitance formed by the water and the membrane layers. Such an arrangement yields a lower effective dielectric function than the corresponding one of the ||-sample. This simple picture is valid because of the small layer thicknesses in comparison with the measuring wavelength; it should not be applied to the dielectric response separation of the PFA foils from the intermediate membrane stacks [27]. In spite of their different parameters both samples nearly show equal behavior with respect to Debye relaxation analysis: the ratios of changes in the imaginary and the real part due to temperature changes, $[\Delta\epsilon_2(T)]/[\Delta\epsilon_1(T)]$, and the temperatures of the maxima in $\epsilon_2(T)$ are similar. Considering the error in the determination of the dielectric function ($\leq 20\%$ for the absolute values of both the real and imaginary part under the present conditions [27]) and the tolerance in the determination of water content, the water relaxation analysis is identical for both samples. This reveals the following consequence:

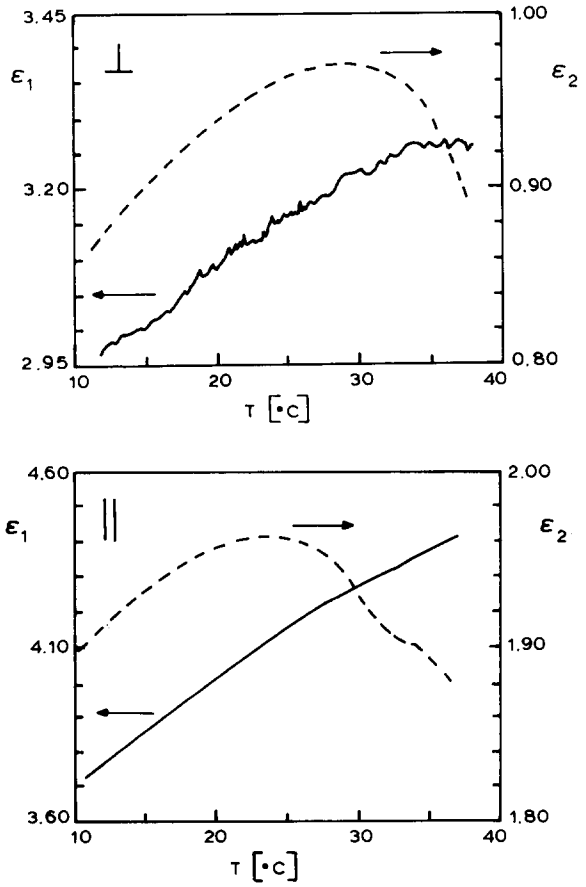

Fig. 4. Temperature-dependent dielectric function of DMPC/H_2O, reference measurements having completely filled PFA containers; above: ⊥-sample, below: ||-sample; solid lines: $\epsilon_1(T)$, dashed lines: $\epsilon_2(T)$. Measuring frequency: $f_M = 9.105$ GHz.

The inner structures of both samples represent two extremes with respect to an isotropic layer orientation and cause different values of the dielectric functions. However, the Debye relaxation analysis yields identical values for the dynamical water properties in both, at least in the framework of the achieved measurement accuracy. Contrary to many other heterogeneous systems, the inner structure of these DMPC/H_2O samples is irrelevant for the given analysis of water relaxation, i.e., first, that the Debye frequency of water molecules decreases in direction to the membrane surface from the bulk water value to values below 1 GHz and second, that this decrease is quasi-continuous. Changes in this behavior can be simply analyzed considering the values $\Delta\epsilon_1$ and $\Delta\epsilon_2$.

(2) The temperature dependent dielectric functions are determined by the most loosely bound water molecules in the samples, i.e., molecules having their Debye frequency next to that of bulk water and being most distant to the membrane surface [15,16]. Approximating this by a single Debye relaxation process allows the evaluation of $f_D(T)$, as shown in Fig. 1. Consistent with the conclusion of point 1.), both ⊥- and ||-samples indeed have slopes and values similar to the isotropic sample (c) of Fig. 1 with 35 weight% H_2O. The relaxation behavior of the strongest bound water molecules (the first two per DMPC molecule) was not observable by microwaves at all accessible temperatures, i.e., their Debye frequency is much lower in any case [15,27]. All other bound water molecules lie in the range between these two extremes. This continuous variation was observed in several experiments [14–16]. In Fig. 1, slight variations of f_D can be seen at the gel-fluid phase transition of the DMPC membranes at about 22°C. This has no relevance for the present work, since the

anesthetic measurements were always performed at constant temperatures above the transition, i.e., in the fluid phase which is supposed to be the biologically relevant one [29].

(3) A decrease in f_D always lowers the contribution of a relaxation process to ϵ_1 and lowers or raises it to ϵ_2. If changes in $\epsilon_{1,2}$ are both negative and the condition $(-\Delta\epsilon_2)/(-\Delta\epsilon_1) > 1$ is fulfilled, the initial f_D of a single relaxation process already must have been lower than f_M. If the measured, integral values of the spectrum of water relaxators show the same behavior, this conclusion proves right also for the changing segment of the spectrum. It becomes evident by calculations simulating the measured integral changes $\Delta\epsilon_{1,2}$: if some water molecules with $f_D > f_M$ would also change their properties significantly, a correspondingly larger amount with $f_D < f_M$ had to compensate it, since the former give larger contributions to the measured integral values. Such a large amount is not available having only 23 H_2O molecules per lipid molecule at all.

Measurements with anesthetic gases

The gas path through teflon capillaries is illustrated in Fig. 3. Above the water surface in the reservoir the gas is saturated with water vapour at the respective temperature of the sample; thus the sample's water content remains constant. After filling the anesthetic

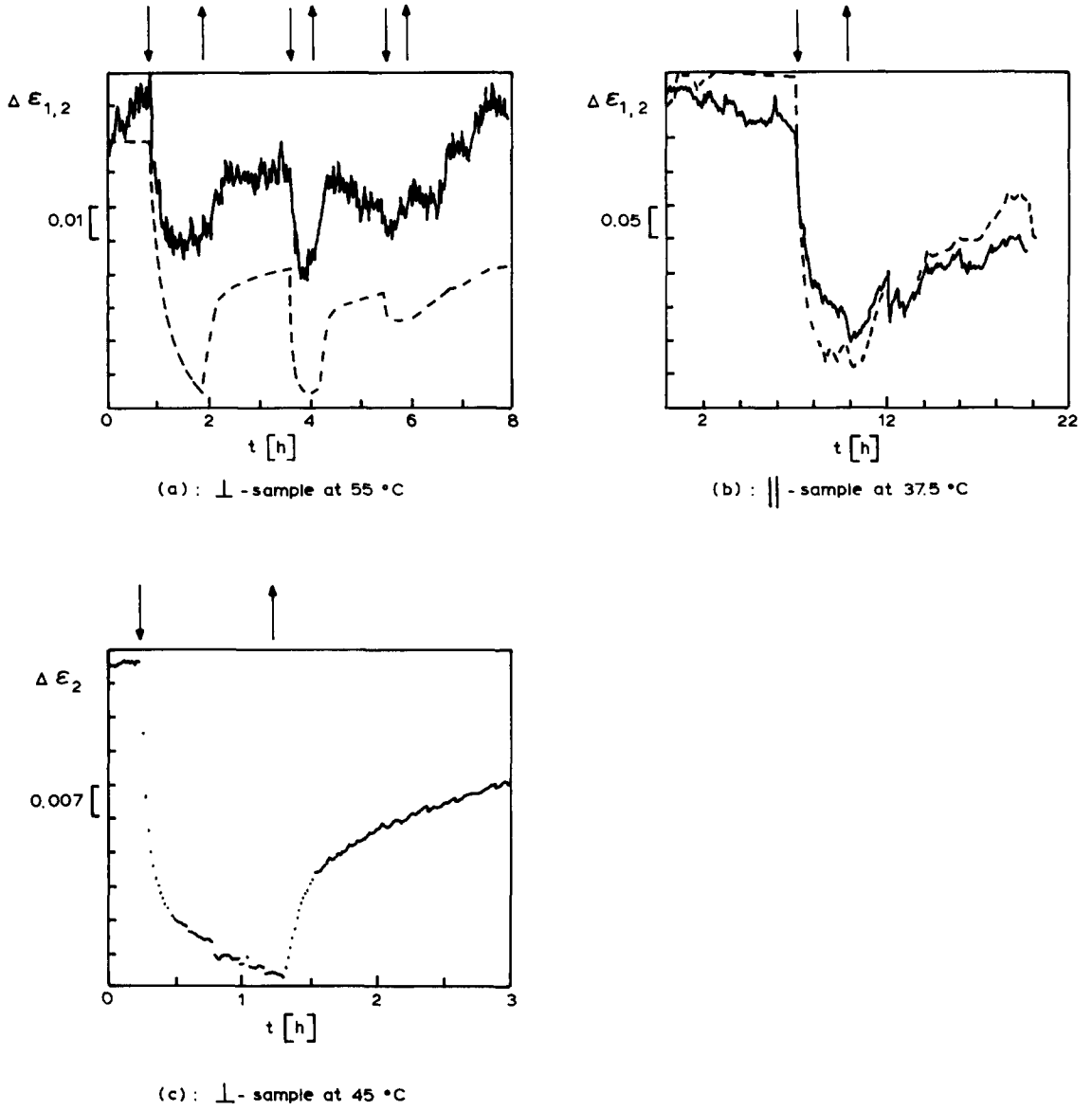

Fig. 5. Time-dependent dielectric function of DMPC/H_2O (relative scales) after filling (arrow down) or emptying (arrow up) the anesthetic reservoir. In (a, b) the solid lines represent the ϵ_1-function, the dashed ones those for ϵ_2. In (a) trichloroethylene was applied first, then chloroform and methoxyfluran; in (b) *n*-hexane was used. In (c) all ϵ_2-measuring points (1-min intervals) are displayed under the influence of chloroform. Measuring frequency: $f_M = 9.105$ GHz.

reservoir the gas is additionally saturated with the anesthetic vapour. In Fig. 5 examples of the influence of anesthetic gases on the dielectric function are displayed. Several points must be considered, before the negative steps can be interpreted without ambiguity:

(1) No data were available on the speed of absorption and the dynamics of the distribution process of the anesthetic molecules in the present samples. It will be different from real biological systems, since there is only diffusion and no macro- and microcirculation, i.e., no convection. The uptake and distribution of anesthetic molecules depends not only on thermodynamic conditions but also on measurement geometries, absolute gas flow and, presumably also important, on the diffusion processes into the water and in the water phase and from the water into the lipid phase. The diffusion behavior will strongly depend e.g. on the macroscopic alignment of the membranes, since this produces less structural defects.

Therefore, additional gravimetric absorption measurements were performed using both samples, the same measurement conditions at a constant room temperature of 28°C and the anesthetics trichloroethylene, chloroform and methoxyflurane. The samples were placed on a balance (Sartorius R160P), in all cases the measurable deviations after 40 min of anesthetic gas flow were not resolvable and were definitely below 0.25 mg. Thus, the concentration lies below a maximal limit of 1 anesthetic molecule per 50 DMPC or about 1000 H_2O molecules which, of course, must be proved to be an upper concentration limit over the whole sample volume for the observed effects (see next point). This would correspond to the physiological range estimated for medical anesthesia and would be some orders of magnitude below the equilibrium gas/lipid partition coefficients [2,6,30]. Equilibrium with the saturated gas is reached after weeks if it is reached at all; the samples should swell macroscopically which was never observed after the first hours.

More recently, high resolution absorption experiments elucidated differences in uptake (Enders, A., unpublished data): in the same time interval only 1/7 methoxyfluran molecules were absorbed compared, e.g., to those of chloroform. This explains the weak effects measured, though it is the most potent anesthetic in medical use. The last two drops of Fig. 5a are not part of the following Table I. There might be interference since the initial values were not reached before applying the next anesthetic. In Fig. 5b the measurement was finished after 20h; then the sample was heated to 70°C to accelerate the desorption of n-hexane thus saving measuring time.

(2) Now the dielectric response has to be analyzed after starting an exposure to an anesthetic gas. The molecular changes leading to the observed decrease in the dielectric function must extend, must be completed and thus must be equal over the whole sample volume when the decrease in the dielectric function comes to a saturation, as measured. This is a simple fact, since dielectric measurements are integral over the whole sample volume. Consequently, the molecular places (binding sites), where the dielectric changes are caused due to the presence of anesthetic molecules, must also be saturated in the whole sample; this also shows that for these special sites the thermodynamic equilibrium with the gas phase is readily established. At the latest after two hours the measured decreases in dielectric function saturate and roughly 2/3 of the decrease happens within the first ten minutes. The upper limit of total anesthetic content in the sample is known at this point of time due to the absorption experiments. Therefore, the corresponding average concentration in the sample is an upper limit for the saturation concentration at these special binding sites and is in the range of very low, i.e., physiologically relevant concentrations. Of course, the same changes in dielectric function should occur using lower partial pressure of the anesthetics and equilibrating for a longer time but it is a difficult task achieving such stable measurement conditions.

TABLE I

Changes in dielectric function under the influence of anesthetics

T (°C)	$-\Delta\epsilon_1$	$-\Delta\epsilon_2$	Sample	Anesthetic
70.0	0.04	0.06	⊥	trichloroethylene
55.0	0.05	0.07	⊥	trichloroethylene
45.0	0.03	0.05	⊥	trichloroethylene
45.0	0.03	0.06	⊥	chloroform
45.0	0.02	0.02	⊥	methoxyfluran
37.5	0.04	0.09	⊥	trichloroethylene
37.5	0.04	0.10	⊥	chloroform
27.0	0.03	0.08	⊥	ether
37.5	0.10	0.20	∥	trichloroethylene
37.5	0.06	0.12	∥	carbon tetrachloride
37.5	0.08	0.20	∥	chloroform
37.5	0.22	0.37	∥	n-hexane

(3) Neutron and X-ray diffraction experiments show that structural changes of model membranes must be very small and cannot be detected at these low anesthetic concentrations [30], hence the observed dielectric changes cannot be explained by static molecular alterations due to changes in the electron distribution.

(4) A change in the samples' water content under the influence of anesthetics could influence the absorption as well as the microwave results. This was excluded by a continuous water titration of the capillary gas flow behind the sample. The capillary outlet was placed directly in the electrolytic cell of the MCI CA05-titrator during the gravimetric absorption measurements, the water titration accuracy being about 0.1 μg/s (resolution of 0.01 μg/s). No significant changes were detected.

According to these arguments, only a change in water relaxation properties can be responsible for the dielectric behavior. In Table I all measurements are listed giving the integral $\epsilon_{1,2}$ changes of the first 40 min application of the anesthetics. According to point (3) of the preceding paragraph, the relation $(-\Delta\epsilon_2)/(-\Delta\epsilon_1) > 1$ ensures that in all cases water molecules with $f_D < f_M$ lower their Debye frequencies further. Since f_M is lower than the Debye frequencies of the most loosely bound water molecules (which may be seen from Figs. 1, 2) only a more strongly bound part of the whole water in the sample can be involved. This proves that the observed changes take place directly at the membrane surface. Their radius of influence into the surrounding water phase is shorter than the present water layer thickness constituted by 23 H_2O molecules per DMPC molecule. On the other hand, the measured changes amount to 5 to 10% of the total sample response in ϵ_2 which points to drastically reduced Debye frequencies of the involved water molecules.

A more quantitative interpretation was performed by simulation calculations. Changes in the dielectric function of individual water molecules were integrated from those directly bound to the membrane surface up to a certain distance in the water layer. The initial Debye frequencies were extrapolated from Fig. 1 at the respective measurement temperatures, their changes were estimated as being constant over the changing part of the spectrum. The simulations yielded limits which may be summarized as follows:

In the range of the first seven to fifteen H_2O molecules per DMPC molecule the water Debye frequency is lowered by a factor of about three under the influence of general, volatile anesthetics. This corresponds to a three-fold slowing down of all diffusion-related processes in this area, as explained in the second paragraph: the Debye frequency is an integrated value for the time scale of those molecular alterations in the hydrogen bond structure of water which lead to a redistribution of molecules. If this behavior is compared with the bulk water properties, it can be said that the viscosity would be increased three-fold.

Fig. 5c demonstrates also that the effects take place at low concentrations: already within the first minutes after the application of chloroform there is a large drop in the dielectric response. However, the increase after the exposure is much slower, the initial values being reached after about 8 h. This behavior may be explained by a two-stage-absorption of anesthetic molecules: At first they quickly diffuse through the water layers and are adsorbed in low concentrations at the hydrophilic surface of the membrane, giving rise to the observed effects. When the concentration is higher, this site becomes saturated and the molecules penetrate into the hydrophobic membrane core that has much larger absorption capability; however, the latter process does not affect the dielectric function. After terminating the exposure the release from the core determines the concentration at the hydrophilic site and thus the slow increase of the dielectric function. This agrees with a two-stage absorption model developed from low frequency capacitance measurements [31]. Another recent experiment, though withdrawn with regard to conclusions on saturation, shows two distinct binding environments of the anesthetic halothan in the brain, too [32,33].

Conclusions

The experimental results given here prove that there is a low-dose absorption mechanism for volatile anesthetics in membrane systems, which has a general influence on the membrane's hydrophilic properties. The measurements have been made in the fluid phase where the membrane's hydrophilic head groups are disordered [13]. Nevertheless, there is a subtle alteration due to an ab- or adsorption of at most 1 anesthetic on 50 DMPC molecules which generally alters the boundary conditions for the range of the first 7 to 15 H_2O molecules bound to the DMPC head group. Though it is not yet clear which physical processes are involved, the observed effect on the dynamics is drastic: the transport velocities of this water region slow down by about a factor of three, thus, e.g., detaining all diffusion-related processes at and through the membrane surface. In contrast to the known water theories on anesthesia ('clathrate-, iceberg-models') [9,10] there is a general change in the membrane's hydrophilic properties due to a low-dose doping; anesthesia is not caused by the direct influence of the anesthetic molecule on only its surrounding water.

One point is worth mentioning which concerns the correlations found between anesthetic potency and the solubility of anesthetics in various media, the oil/gas partition coefficient yielding the best correlation [2,6]. At first this correlation seems to show that the site of anesthesia action is located in an oil-like phase, i.e., in the hydrophobic membrane core. However, this correlation is merely a relation between an attractive force (which is responsible for the solution behavior) and the anesthetic potency and says nothing about partitioning on the microscopic, membrane scale. The attractive force in an unpolar liquid is established by van der Waals forces, so they are responsible for the attraction of anesthetic molecules. But, however, van der Waals forces are always present between molecules, and the above mentioned correlation could as well be established by the van der Waals attraction (which causes adsorption) of the anesthetic molecules on the membrane surfaces. Of course, this hypothesis has to be verified by measurement. Nevertheless, it could bridge the gap between an anesthesia mechanism effective in a

hydrophilic environment like the one reported here and the good correlations found between anesthetic potency and the solubilities in unpolar media.

Similarly, the reported influence of anesthetics on bound water is presumably effective at other hydrophilic surfaces (e.g., of proteins) which would explain the variety of biological observations. It will be interesting to see which of the numerous potential mechanisms actually cause anesthesia; in addition to the direct effect on molecular transport, more complicated effects are thinkable, too, e.g., a different time behavior between the active and passive conformational states of proteins. Anyway, the present mechanism might be the molecular basis.

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