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Local anesthetics and pressure: a comparison of dibucaine binding to lipid monolayers and bilayers

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The binding of the local anesthetic dibucaine to monolayers composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine was studied with a Langmuir trough at pH 5.5 (22°C, 0.1 M NaCl). At this pH value only the charged form of the local anesthetic exists in solution. Charged dibucaine was found to be surface active and to penetrate into the lipid monolayer, with the hydrophobic part of the molecule being accommodated between the fatty acyl chains of the lipid. The dibucaine intercalation could be quantitated by measuring the expansion of the film area, ΔA, at constant surface pressure, π. At a given surface pressure, ΔA increased with increasing dibucaine in the buffer phase. On the other hand, keeping the dibucaine concentration constant, the area increase, ΔA, was strongly dependent on the surface pressure. The area increase, ΔA, was large at low surface pressure and decreased with increasing surface pressure. A plot of the relative change in surface area, $\Delta A/A$, versus the surface pressure yielded straight lines in the pressure range of 25–36 mN/m for five different concentrations. The $\Delta A/A$ vs. π isotherms intersected at $\pi = 39.5 \pm 1$ mN/m with $\Delta A = 0$, indicating that charged dibucaine apparently can no longer penetrate into the monolayer film. By making judicial assumptions about the area requirement of dibucaine the monolayer expansion curves could be transformed into true binding isotherms. Dibucaine binding isotherms were constructed for different monolayer pressures and were compared to a bilayer binding isotherm measured under similar conditions with ultraviolet spectroscopy. The best agreement between monolayer and bilayer binding data was obtained for a monolayer held at a pressure of 30.7 to 32.5 mN/m, which can thus be considered as the bilayer-monolayer equivalence pressure. It is further suggested from this analogy that the binding of dibucaine does not change the internal pressure in the bilayer phase, at least not in the concentration range of physiological interest (0–2 mM dibucaine) but induces a lateral expansion. At higher molar ratios of cationic dibucaine to lipid, x_b, in the monolayer ($x_b > 0.20$) the area increase is larger than would be expected from the molecular dimensions of dibucaine. This is probably due to charge repulsion effects, which at still higher molar ratios ($x_b > 0.6$) lead to a micellisation. The pressure dependence of the intercalation of cationic dibucaine into lipid membranes may also be of relevance for the phenomenon of pressure reversal in anesthesia.

Abbreviation: POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine.

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Introduction

General and local anesthetics intercalate readily into the inner hydrophobic core of membranes thereby altering the packing of lipid molecules

and perhaps also of membrane proteins. In general, anesthetics have been shown to increase the fluidity of lipid bilayers [1], to decrease the order parameter of the hydrocarbon chains [2,3], to depress the temperature of the gel to liquid crystalline phase transition [4,5], and to expand the surface area of monolayer films [6] and of erythrocyte membranes [6,7]. Although these experiments refer to different physical parameters, the molecular origin of the observed changes is probably identical in all cases, namely a loosening of the lipid packing under the influence of intercalating anesthetics. In the concentration range of physiological interest these effects are, however,small. Most of these experiments were performed on pure lipid membranes. The rationale for using these simple model systems derives from the experimental evidence that the anesthetic potency correlates approximately with the oil-water partition coefficient of the anesthetics [8].

In the present study we are interested in another membrane parameter, namely, the internal pressure of membranes. Little is known about the effect of anesthetics on the internal pressure of membranes. On the other hand, it is well documented that an externally applied hydrostatic pressure reverses general [9] as well as local anesthesia [10,11]. This leads to a number of questions: (1) How large is the internal membrane pressure in a lipid bilayer? (2) Does the internal membrane pressure change in the presence of increasing concentration of a local anesthetic? (3) Does the concentration of anesthetics in the membrane change when an external pressure is applied?

In contrast to the parameters mentioned above, pressure in bilayers is ill defined and can not be measured directly. Hence, depending on the theoretical or experimental approach different authors have arrived at quite different operational definitions for the pressure in a lipid bilayer and, consequently, have reported quite a large range of numerical values. In our opinion, the most conclusive approach to study this problem is to compare lipid bilayers with lipid monolayers. On this basis different authors with different experimental techniques determined monolayer-bilayer equivalence pressures in the range of 25–35 mN/m [12,14]. In the past, most binding studies of anesthetics to monolayers have, however, been performed at much lower pressures.

Only a few authors discuss the effect of anesthetics on the internal membrane pressure. It has been suggested that local anesthetics achieve conduction block by increasing the surface pressure of the nerve membrane, thereby closing the pores through which ions move [15,16]. This interpretation was based on monolayer experiments performed at constant area. The pressure increase upon anesthetic incorporation was measured. Thereby it was observed that the effectiveness of anesthetics in increasing the surface pressure of the lipid monolayers was proportional to their relative anesthetic potency. Electrical capacitance measurements on black lipid membranes in the presence of *n*-octanol have also been interpreted in the sense of a pressure increase in bilayer membranes [17]. The concept of a pressure increase induced by anesthetics is, however, consistent with neither a laterally expanding fluidized membrane nor with the phenomenon of pressure reversal.

For the present study, dibucaine, a local anesthetic with a high relative anesthetic potency, has been choosen. Dibucaine, like most local anesthetics, is a tertiary amine, and at neutral pH exists in an equilibrium between a neutral and a charged form. The uncharged form partitions readily into the inner hydrophobic core of lipid bilayers. In contrast, the charged form is thought to act specifically on the membrane proteins and to block, for example, the sodium channels in squid axon [8]. However, the chemical structure of the charged form of most local anesthetics resembles closely that of hydrophobic ions which are known to bind quite strongly to pure lipid membranes [19]. Hence the modification of the membrane surface by the binding of the charged form of a local anesthetic, e.g., via the membrane surface potential, could constitute an additional mechanism by which the electrophysiological properties of membrane-bound proteins could be regulated [20].

While monolayer experiments were usually performed at constant monolayer area, observing the pressure increase upon incorporation of the compound of interest, the present experiments were recorded in a different mode. By means of an

electronic feed-back system the surface pressure of the film was kept at a predefined value and the intercalation of dibucaine was monitored by the increase of the surface area. Comprehensive monolayer binding isotherms were measured for dibucaine in the concentration range of 0.5–4 mM (pH 5.5, 0.1 M NaCl, 22° C). The POPC monolayer pressures were varied in the range of 25–36 mN/m. In order to compare the behavior of monolayers and bilayers the binding of dibucaine to POPC bilayer liposomes was measured by ultraviolet spectroscopy. From the identity of monolayer and bilayer binding isotherms under certain conditions a monolayer equivalence pressure could be established. Furthermore, from the pressure dependence of dibucaine binding it was obvious that at high surface pressures the intercalation of dibucaine into POPC monolayers was no longer possible. This phenomenon may also occur in lipid bilayers and may bear on the theory of pressure reversal in anesthesia.

Materials and Methods

Materials

Dibucaine-HCl was purchased from Sigma, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) was obtained from Avanti Polar Lipids (Alabama). Water used for buffers and solutions was double ion-exchanged and glass distilled. If not otherwise cited the buffer used was 50 mM K_2HPO_4-KH_2PO_4 (pH 5.5)/0.1 M NaCl. pH was specifically chosen as pH 5.5 in order to study exclusively the charged form of dibucaine. The concentration range of dibucaine solutions measured was 0.5–4 mM. Representative commercial solutions of dibucaine in clinical use for spinal anesthesia are in the range of 1.7–10 mM. The equilibrium effective concentration of dibucaine is however smaller.

Monolayer experiments

A round Teflon trough designed by Fromherz [21] was used, with a total area of 362 cm^2 divided into eight compartments (Type RMC 2-T, Mayer Feintechnik, Göttingen, F.R.G.). The surface pressure was measured by the Wilhelmy method, using plates cut from filter paper (Whatman, No. 1). Before each measurement, the trough and the filter paper were thoroughly cleaned with methanol and distilled water. For binding studies two segments of the trough were filled with 20 ml of buffer solution each. Known amounts of POPC dissolved in hexane/ethanol (9:1, v/v) were released onto the water surface using a Hamilton syringe. A monolayer with a pressure of around 15 mN/m formed within a few seconds and was left to attain equilibrium. The monolayer was then compressed by a barrier to the desired lateral pressure. The area covered by the final POPC monolayer was between 53 and 56 cm^2, thus being slightly larger than the area of one segment. A 1 M dibucaine solution was then injected into the buffer subphase of each compartment with a Hamilton syringe to attain dibucaine concentrations in the range of $5 \cdot 10^{-4}$–$4 \cdot 10^{-3}$ M. The buffer phases in each segment were stirred by tiny magnets to assure a homogeneous distribution of dibucaine. The instrument was used in the constant pressure mode and the increase in surface area was recorded as a function of time. All measurements were done at room temperature (21 ± 1° C).

Ultraviolet absorption spectroscopy

The binding of dibucaine to POPC bilayers was studied by ultraviolet spectroscopy. The extinction coefficient of dibucaine at 325 nm and pH 5.5 is 4144 cm^{-1} (Allegrini, P., Seelig, A., Seelig, J., unpublished results). Bilayer liposomes were prepared by suspending an accurately weighed amount of POPC (approx. 20 mg) in 400 µl of buffer containing a known amount of dibucaine from a stock solution. Equilibrium was attained by vortexing and freeze-thawing the suspension several times. The samples were then centrifuged at 30000 × *g* for 40 min at 22° C. The supernatant was removed and the concentration of dibucaine in the supernatant (c_{eq}) was measured by ultraviolet absorption spectroscopy. From the difference of the concentration of the starting solution (c_o) and the concentration of the supernatant (c_{eq}) the molar amount of dibucaine bound per mol of POPC was determined (x_b). Measurements were performed with a Uvikon 860 spectrophotometer.

Results

Surface activity of charged and non-charged dibucaine

The chemical structure of dibucaine is shown in Fig. 1. The tertiary amino group has a pK value of around 8.5 [22]. At pH 5.5 dibucaine occurs only in its charged form in aqueous solution, whereas at pH 7.4 both charged dibucaine (93%) and non-charged dibucaine (7%) are present. We have investigated the surface-active properties at both pH values by injecting corresponding dibucaine solutions into the compartments of the Langmuir trough with a clean air-water interface, choosing a final dibucaine concentration of 1 mM. The charged as well as the neutral form were found to be surface active. The equilibrium surface pressure at pH 5.5 was determined to be 6.1 mN/m but increased to 15.5 mN/m at pH 7.4 (22° C, 0.1 M NaCl, 50 mM potassium phosphate).

The variation of the surface pressure with dibucaine concentration at pH 5.5 is shown in Fig. 2. The ionic strength also has an important influence on surface pressure. Higher salt concentration increases the surface pressure at both pH 5.5 and pH 7.4.

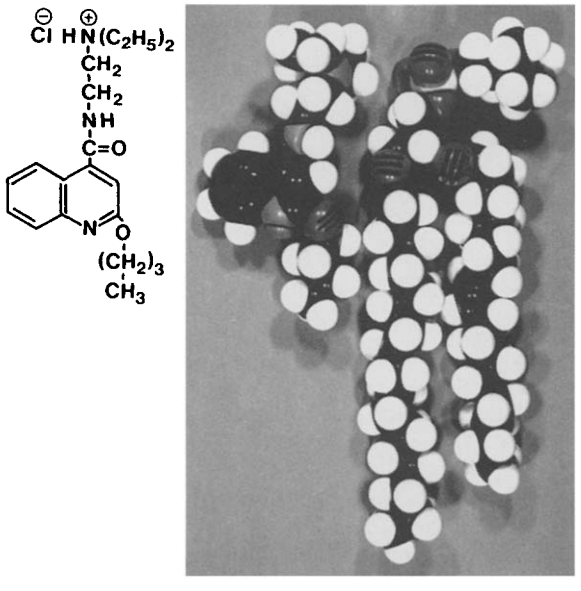

Fig. 1. Chemical structure of dibucaine hydrochloride and CPK models of dibucaine and POPC.

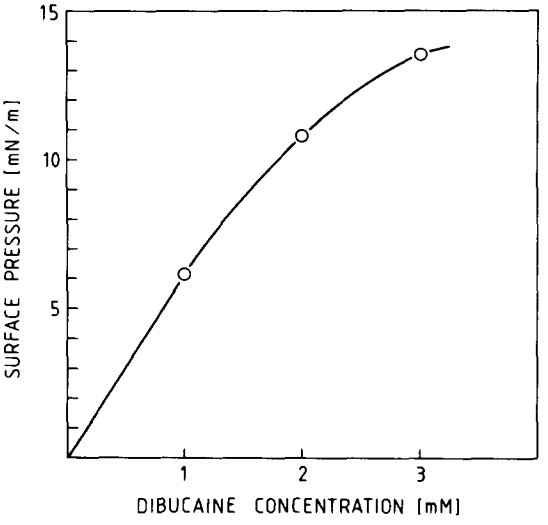

Fig. 2. Surface pressure of cationic dibucaine as a function of dibucaine concentration. (50 mM potassium phosphate (pH 5.5)/0.1 M NaCl).

Binding to and penetration of cationic dibucaine into POPC monolayers

The binding of water-soluble substrates to a lipid bilayer may follow different mechanisms. In the case of non-charged and partially hydrophobic molecules such as the primary aliphatic alcohols or benzylalcohol the driving forces are essentially hydrophobic interactions and the molecules will penetrate into the hydrophobic core of the lipid bilayer. In contrast, metal ions remain on the outside of the membrane surface and interact purely electrostatically with the lipid polar groups [23,24]. In the uncharged form, tertiary amine anesthetics belong to the first group of compounds. In the charged form, however, they combine features of hydrophobic molecules and metal ions. As a first step in eludicating the binding mechanism, we have studied therefore the penetration power of charged dibucaine using the monolayer approach.

Monolayers were formed by spreading a solution of 1-palmitoyl-2-oleoyl-*sn*-glycerol-3-phosphocholine in hexane/ethanol (9 : 1, v/v) onto the buffer phase at pH 5.5. The dibucaine concentration in the subphase was adjusted by injecting small amounts of a highly concentrated dibucaine stock solution. The surface pressure was kept constant at a preset value by an electronic control

system and the expansion of the lipid film due to incorporation of dibucaine was recorded. A typical time-course for the change in surface area A is shown in Fig. 3 for a dibucaine concentration of 1 mM. One observes an initial rapid increase of the monolayer area which levels off with time, yielding a stable equilibrium situation after about 50 min. The penetration kinetics depends on a number of experimental parameters such as the stirring rate in the subphase; however, the end values of the monolayer expansion were found to be very reproducible for a given monolayer pressure and dibucaine concentration. Fig. 4 summarizes the relative increase in monolayer area, $(\Delta A/A)$, as a function of the preset monolayer pressure for five different dibucaine concentrations. For each surface pressure value a new POPC monolayer was prepared. Inspection of Fig. 4 allows the following conclusions. (1) The penetration power of the charged form of dibucaine as measured by the relative area increase is a linear function of the monolayer pressure in the range of 25–36 mN/m. The monolayer expansion is large at low surface pressures and small at high pressures. Hence, the intercalation of the local anesthetic between the monolayer lipids becomes increasingly difficult with increasing packing density of the lipids. (2) Keeping the monolayer pressure constant, the relative area increase is dependent on the dibucaine concentration in the subphase. Increasing dibucaine concentrations result in increasingly larger changes in the surface area. Plots of $\Delta A/A$ vs. log c yield straight lines within the limit of experimental accuracy (not shown). (3) The straight lines in

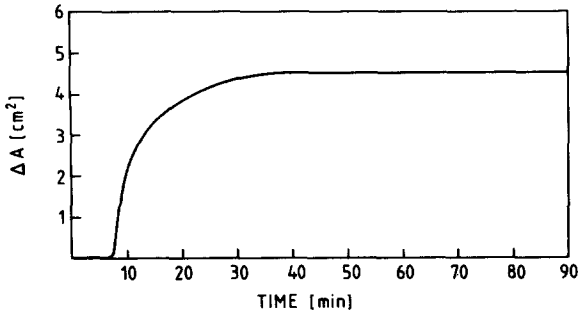

Fig. 3. Area increase (ΔA) due to intercalation of cationic dibucaine (1 mM) into a POPC monolayer.

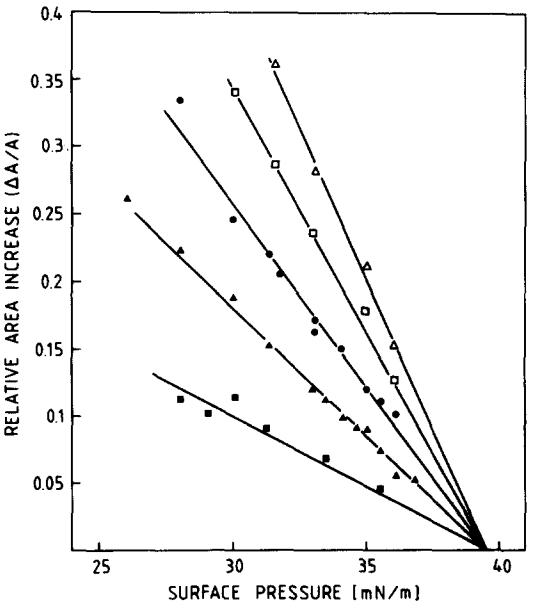

Fig. 4. Relative area increase $(\Delta A/A)$ as a function of the surface pressure at five dibucaine concentrations: ■, 0.5 mM; ▲, 1 mM; ●, 2 mM; □, 3 mM; △, 4 mM: (50 mM potassium phosphate (pH 5.5)/0.1 M NaCl).

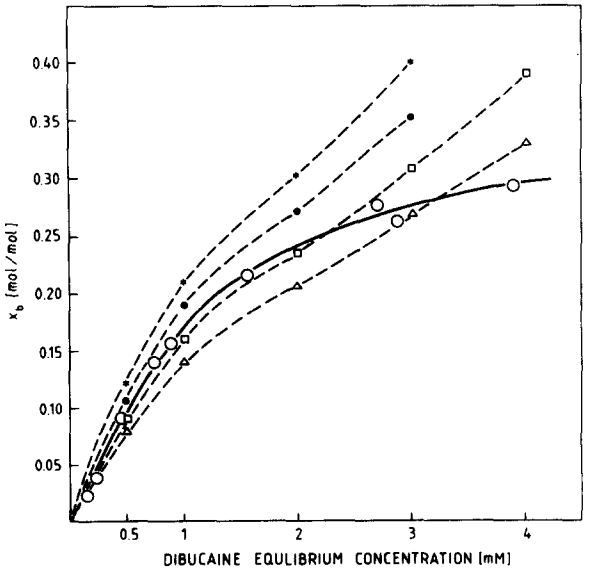

Fig. 5. x_b (moles dibucaine/mol POPC) as a function of dibucaine equilibrium concentration, as determined by ultraviolet spectroscopy for bilayer liposomes (○) and as determined for lipid monolayers at four different surface pressures. △, 33 mN/m; □, 32 mN/m; ●, 31 mN/m; *, 30 mN/m.

Fig. 4 extrapolate to a limiting surface pressure of 39.5 ± 1 mN/m at which pressure the change in surface area is zero. This is also seen in pressure-area isotherms which are reversible and which, after an incorporation time of 50 min, no longer show hysteresis. When dibucaine is injected at pressures around 39 mN/m there is still, however, a small area increase which might be due merely to a disruption of the highly packed film by the injection needle, making subsequent dibucaine intercalation easier.

Binding of cationic dibucanine to POPC bilayers

POPC bilayer dispersions (coarse liposomes) were formed in the same buffer and in the same dibucaine concentration range as used for the monolayer experiments. The amount of membrane-bound dibucaine was determined by ultraviolet absorption difference spectroscopy as described in Materials and Methods. Fig. 5 shows the variation of the molar amount of dibucaine bound per mol of POPC with the dibucaine equilibrium concentration (solid line). Qualitatively, the binding isotherm has the appearance of a Langmuir adsorption isotherm. Even at rather low equilibrium concentrations, a considerable binding of dibucaine to POPC is noted. The dashed lines in Fig. 5 correspond to binding isotherms calculated from monolayer expansion curves as discussed below.

Discussion

The monolayer experiments in the absence of phospholipids quite clearly demonstrate that both forms of dibucaine are surface active, the non-charged form exhibiting a stronger surface activity than the charged form. This is paralleled by the hydrophobicity of the two species, since the water solubility is low for the neutral form and increases for the charged form.

When the charged form of dibucaine is injected underneath a phospholipid monolayer a film expansion is observed under most conditions. This area increase argues against a surface adsorption of charged dibucaine to the phospholipid polar groups; rather, it suggests an intercalation of the hydrophobic part of the molecules between the fatty acyl chains, while the tertiary ammonium group resides in the lipid-water interface [2].

The amount of incorporated dibucaine depends critically on the monolayer pressure. A quantitative description of the change in surface area can be given as follows. Starting with a pure POPC monolayer with a constant number, n_L, of lipid molecules the surface area, A, is also a function of the monolayer pressure. If n_D molecules of dibucaine penetrate into the monolayer the total surface area, A_T, is given by

$$A_T = n_D A_D + n_L A_L \tag{1}$$

where A_D denotes the surface area of dibucaine. The increase in the surface area, A, relative to the area of the pure lipid monolayer can thus be calculated according to

$$\frac{\Delta A}{A} = \frac{\Delta A}{n_L A_L} = \frac{A_T - n_L A_L}{n_L A_L} = \frac{n_D}{n_L} \cdot \frac{A_D}{A_L} = x_b \frac{A_D}{A_L} \tag{2}$$

x_b may be interpreted as the molar amount of dibucaine bound per mol of POPC.

It should be noted that the surface area of POPC is not constant but rather depends on the applied surface pressure. The pressure area isotherms of this system are well documented and the area, A_L, for a given temperature and pressure can be determined from the published data [25]. At high surface pressure (37 mN/m) a minimum lipid area of $A_L = 62$ Å^2 has been determined.

Unfortunately, no comparable information is available for dibucaine. From inspection of molecular models (cf. Fig. 1B) a minimum cross sectional area of $A_D = 55$ Å^2 can be estimated, but the pressure dependence of A_D is unknown. We consider two different models. In the first, the surface area A_D is assumed to be constant with $A_D = 55$ Å^2 at all surface pressures. This behavior parallels that of cholesterol, which is known to form a stable condensed film with approximately constant surface area [23]. Eqn. 2 then takes the form

$$x_b = (\Delta A/A)(A_L/55) \tag{3}$$

where A_L is measured in Å^2 and taken from POPC pressure-area diagrams. In the pressure range of interest, i.e., 25–37 mN/m, A_L varies from 68 Å^2 to 62 Å^2.

In the second model, the surface area of dibucaine varies with surface pressure and is assumed to have the same functional dependence on the surface pressure as observed for POPC monolayers. The area ratio A_L/A_D is thus constant for the whole pressure range of 25–37 mN/m and may be calculated from the limiting areas at high surface pressure as $A_L/A_D = 62/55$. In this model the amount of bound dibucaine is calculated according to

$$x_b = \left(\frac{\Delta A}{A}\right)\left(\frac{62}{55}\right) \quad (4)$$

In the pressure range studied here the differences between the two models are only small. In the following we will discuss the second model in more detail. Eqn. 4 demonstrates that the change in relative surface area is linearly related to the amount of bound dibucaine. Hence, from the measured increase in the relative surface area it is possible to obtain an estimate of the amount of charged dibucaine bound to the POPC monolayer. As the amount of bound dibucaine depends critically on the monolayer surface pressure, the question arises at which pressure can the binding of dibucaine to monolayers be correlated to the binding of the same molecule to bilayers? We make the assumption that for a given dibucaine buffer concentration the same amount of local anesthetic should be bound to the bilayer as to the monolayer. This postulate leads to the operational definition of a monolayer-bilayer equivalence pressure π_{equiv}. In order to determine this equivalence pressure we have compared in Fig. 5 the bilayer binding isotherm with various binding isotherms for monolayers at various surface pressures. Almost superimposable binding isotherms for monolayer and bilayer are obtained at a monolayer pressure of 31.7 mN/m which is hence identical to the monolayer-bilayer equivalence pressure.

The concept of a monolayer-bilayer equivalence pressure has earlier been approached by different methods. By comparing the action of phospholipases on erythrocytes and lipid monolayers it has been suggested that the packing of the phospholipids in the outer layer of the human erythrocytes is comparable to a lipid film with a lateral pressure between 31 and 35 mN/m. The outer layer of erythrocytes is composed of choline-containing phospholipids; the lipid film was formed from POPC [12]. A comparative study of the phase transitions of phospholipid bilayers and monolayers yielded an equivalence pressure of 30 mN/m [13]. A closely related quantity, the equilibrium surface pressure for the bilayer-monolayer transition in a system of dioleoylphosphatidylcholine vesicles in equilibrium with a POPC monolayer, has been found to be about 25 mN/m [14]. Our result obtianed for POPC by yet another method is in good agreement with these studies.

As indicated by Eqn. 2 the binding isotherm derived from monolayer experiments depends on a judicious estimate of the surface area of the local anesthetic. For the above calculation a value of 55 Å^2 was used. If a smaller (larger) surface area of 50 Å^2 (62 Å^2) is assumed, an equivalence pressure of 32.5 mN/m (30.7 mN/m) results for the optimum binding isotherm. Hence, in the concentration range of up to 2 mM dibucaine, a change in the surface area by more than 20% induces a variation in the equivalence pressure of less than 10%.

Inspection of Fig. 5 further reveals that the equivalence pressure is not influenced by the concentration of cationic dibucaine at least up to a dibucaine concentration of 2 mM. However, the parallel behaviour of monolayer and bilayer isotherms breaks down at concentrations higher than 2 mM. This can be explained as an effect of the large amount of charged dibucaine bound at these higher concentrations. As may be estimated from Fig. 5 an equilibrium concentration of about 2 mM leads to a value of x_b of approximately 0.25, which means that one dibucaine occupies four to five lipids. From CPK models of dibucaine and POPC it can be seen that at least four lipids are required to completely surround one dibucaine molecule. If in this situation further binding of dibucaine occurs, it is obvious that some dibucaine molecules must come into direct contact. The strong electrostatic repulsion should drastically modify the monolayer properties and would explain the seemingly higher increase of x_b with concentration in the monolayer isotherm compared to the bilayer isotherm. Similar electrostatic effects have been observed for long-chain amines which form much more expanded films when spread on acidic subphases, where they become

positively charged, than on neutral subphases [26].

The determination of x_b from $\Delta A/A$ is therefore only possible so long as no charge repulsion effects come into play. At x_b values over 0.2 to 0.25, A_D can no longer be regarded as constant but increases with increasing concentration of dibucaine in the monolayer. This variable area requirement shall be called A'_D. As an example we calculate A'_D at a $c_{eq} = 4$ mM the highest of the measured concentrations according to Eqn. 5

$$\frac{\Delta A}{A} = \frac{A'_D}{A_L} \cdot x_b \tag{5}$$

and get a value of $A_D = 72$ Å^2 which is considerably larger than $A_D = 55$ Å^2. It should be recognized that not only monolayers but also bilayers are affected in a comparable way by high charge densities. This is most obvious at high equilibrium concentrations and high x_b values ($x_b = 0.6$–0.7), where charge repulsion forces become so strong as to induce a transition from a bilayer to a micellar arrangement (Allegrini, P., Seelig, A., Seelig, J., unpublished results).

An interesting feature is the reversibility of dibucaine intercalation with increasing monolayer pressure. The large area increase $\Delta A/A$ (cf. Fig. 4) at low monolayer pressure can be reversed by increasing this pressure. Hence, for a given dibucaine concentration in the subphase, it is in principle possible in a single monolayer experiment to establish reversibly all $\Delta A/A$ values given by the corresponding straight line in the area expansion versus pressure diagram of Fig. 4. Area A has to be corrected, however, for the compressibility of the pure lipid.

Of physiological relevance is the observation that at monolayer pressures of about 39.5 ± 1 mN/m the charged dibucaine is completely pushed out of the monolayer. This result may provide insight into one aspect of the molecular mechanism of pressure reversal of local anesthesia. It is known for general anesthetics [9] and has also been found for local anesthetics [10,11] that the physiological effects of these molecules can be antagonized by applying pressure of the order of 100–150 atm. However, negative reports have also appeared. For example, pressure fails to reverse the effects of general anesthesia on fresh water shrimps [27,28]. Local anesthesia induced by drugs intercalating into the hydrophobic core of the membrane is generally antagonized by pressure. However, the effect of tetrodotoxin, which specifically blocks sodium channels, is not reversed by pressure [10,11].

The molecular mechanisms leading to reversal of anesthesia by pressure are largely unknown. Different factors, such as the effect of pressure on membrane bound prpoteins [29] or on the reordering of the hydrocarbon chains [30] have been implicated. It has also been the general contention that the anesthetic molecules are not expelled from the membrane when hydrostatic pressure is applied. However, recent studies on the partitioning of inhalation anesthetics between water and liquid crystalline bilayer domains have demonstrated that the partition coefficient decreases with pressure, leaving less anesthetic in the pressurized bilayers [31]. This result on bilayer model membranes and non-polar general anesthetics parallels the present finding obtained with monolayers and a charged local anesthetic. In both cases a pressure increase decreases the concentration of anesthetics in the membrane.

This correlation can be carried further by making the following quantitative comparison. Experimentally it is known that pressure reversal of anesthesia requires pressures of about 100 atm. Studies on bilayer model membranes composed of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine have shown that the application of a pressure of 100 atm increases the gel-to-liquid phase transition by about 2°C [32]. In monolayers the corresponding analogue would be the liquid expanded-to-condensed transition, which is also temperature and pressure dependent. Extensive data on this transition have been reported for 1,2-dihexadecyl-*sn*-glycero-3-phosphocholine which is chemically closely related to 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine with the exception of ether instead of ester linkages of the aliphatic chains [33], Fig. 2). These studies demonstrate that a change in the temperature by 2°C (from 35°C to 37°C) shifts the corresponding critical surface pressure by 7 mN/m (from 32 to 39 mN/m). Under the effect of a hydrostatic pressure of 100 atm, the lateral pressure in a lipid bilayer would thus be shifted from the natural value of 32 mN/m to

value of 39 mN/m at which charged dibucaine can no longer intercalate.

In summary, the following conclusions can been drawn. (1) A comparison of the binding of cationic dibucaine to POPC monolayers and bilayers leads to a monolayer-bilayer equivalence pressure 30.7–32.5 mN/m. (2) The internal pressure in a bilayer does not change upon dibucaine intercalation, at least not in the concentration range of physiological interest (0–2 mM). (3) At ratios of cationic dibucaine to POPC larger than 0.2, charge repulsion effects lead to an increase in the area requirement of cationic dibucaine in monolayers. The area requirement is increasing with increasing charge concentration. Comparable effects might occur in bilayers and might explain the micellisation of membranes containing large amounts of dibucaine. (4) The intercalation of cationic dibucaine into POPC monolayers is linearly dependent on the surface pressure in the range of 25–36 mN/m. At 39.5 ± 1 mN/m, dibucaine is extruded from monolayers and bilayers. This effect is reversible and could be one reason for pressure reversal of dibucaine anesthesia.

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