Effects of Local Anesthetics on Mechanical Characteristics of Lipid Bilayers and on the Ion Transport Dynamics

T. HIANIK[1], A. PALACKOVÁ[2] and J. PAVELKOVÁ[3]

1 *Department of Biophysics, Faculty of Mathematics and Physics, Comenius University, Mlynská dolina F1, 842 15 Bratislava, Czechoslovakia*
2 *Research Institute of Labour Security, Bratislava, Czechoslovakia*
3 *Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Czechoslovakia*

Abstract. Bilayer lipid membranes (BLM) of various composition were used to study the effects of local anesthetics (LA) carbisocaine and lidocaine on mechanical membrane characteristics and on the transport dynamics of ions across gramicidin D ionic channels. Carbisocaine concentrations of 20 μmol/l — 0.1 mmol/l caused a considerable decrease (by 15—40 %) in modulus of elasticity $E_\perp$ in direction perpendicular to membrane surface. The effect of lidocaine was approx. one order of magnitude weaker. LA-induced changes in $E_\perp$ were shown to depend on both the lipid composition of the membrane and the electrolyte pH. Neutral forms of LA induce marked changes in $E_\perp$. An analysis of current-voltage (I—V) characteristics of BLM modified by the channel forming agent gramicidin D revealed that carbisocaine significantly affects the superlinear segment of the I—V relationship; this suggests a strong effect on the transport dynamics of ions through the internal channel region. The results of the study suggest that the action of both carbisocaine and lidocaine may be non-specific. The effectivity of the non-specific action of LA is determined by the hydrophobic moiety of the local anesthetic molecule.

Key words: Local anesthetics — Lipid bilayers — Mechanical properties — Ion transport

Introduction

Studies into biophysical mechanisms of action of local anesthetics have left open the question concerning the relationship between the specific and non-specific action. It has been suggested that, in addition to specific interaction of anesthetics with membrane receptors, physical membrane characteristics may become altered due to some non-specific interactions. Both specific and non-specific

interactions of local anesthetics (LA) are usually characterized in terms of effective concentrations. E.g., sodium channels of brain synaptosomes are blocked at concentrations (0.1 mmol/l and below) that still do not induce any visible changes in the superficial or hydrophobic regions of lipid bilayers (Aksentsev et al. 1982). Higher LA concentrations (5 mmol/l and above) may induce considerable changes in the lipid bilayer characteristics: membrane microviscosity decreases and this may, in turn, induce aggregation of membrane proteins (Aksentsev 1983); the membrane ordering parameter, measured by the spin probe method (Butler et al. 1973; Neal et al. 1976), changes. The ordering of lipid bilayers has been shown to decrease with increasing LA concentrations; at constant LA concentration, the parameter has been shown to decrease with the increasing electrolyte pH. A correlation could be observed between the pK value of the given anesthetic and that of pH required to decrease membrane ordering (Butler et al. 1973). ^{2}H-NMR and UV-spectroscopy studies of the interactions of LA dibucaine and etidocaine with multilamellar liposomes of selectivity deuterated lipids showed that LA incorporation into the membrane is associated with an increase of the lipid bilayer surface and with conformational changes of the lipid head groups (Seelig et al. 1988). This observation suggests a non-specific interaction of LA with the membrane to occur at relatively low dibucaine concentrations (0.1 mmol/l). LA are known to affect lipid polymorphism: e.g., dibucaine and/or chlorpromazine induce lipid transition from lamellar to hexagonal H_{II} phase at an anesthetic: cardiolipin molar ratio 2 : 1 (Cullis and Verkleij 1979). Studies of LA effects on bilayer lipid membranes (BLM) have shown that pharmacological concentrations (1 mmol/l) of local anesthetic nurpecaine, cocaine, procaine (Ohki 1970), as well as carbisocaine (Ondriáš 1984) increase the conductivity of BLM prepared of various lipids in *n*-decane. McLaughlin (1975) reported that the tetracaine-induced increase in conductivity of BLM of phosphatidylethanolamine in *n*-decane is due to the transfer of the charged LA HAA^{+} complex (A^{+} and A are the charged and the natural LA form, respectively). LA induces changes in the microscopic membrane parameters. Ebihara et al. (1979) reported that benzylalcohol increased the thickness of *n*-alkanes-containing BLM, while membranes containing no solvent became attenuated. It is obvious from what has been said that local anesthetics may have different effect on model membranes of different composition. In addition to the known alterations in microscopic membrane parameters (microviscosity, the parameter of ordering, quadrupole splittings, conductivity), LA may induce changes in the lipid bilayer structure, which may become reflected in the macroscopic mechanical membrane properties. For instance, the conformational changes in lipid head groups, indicated by ^{2}H-NMR spectroscopy (Seelig et al. 1988), may by reflected in the ordering of the inner hydrophobic region of the membrane. Since LA considerably affect the

hydrophobic region of membranes, results of this interaction may be effectively detected by measuring the modulus of elasticity $E_\perp$ in direction perpendicular to the lipid bilayer surface (Passechnik and Hianik 1978). The parameter $E_\perp$ is very sensitive to structural changes of lipid bilayer such as those induced by adsorption of ions (Hianik et al. 1984), detergents (Passechnik and Hianik 1979) and proteins (Hianik et al. 1988). Moreover $E_\perp$ provides information on the general physical properties of the membrane; mechanical characteristics play a considerable role in the preservation of the shape of cells, e.g. erythrocytes.

Although ESR and NMR spectroscopy offer good tools for the study of LA interactions with membranes, the understanding of the mechanisms of LA action requires the knowledge of different independent parameters. Moreover, significant changes in ESR parameters occur at sufficiently high concentrations of LA (1 mmol/l and above) only (Ondriaš 1984; Frezatti et al. 1986). Even much more sensitive NMR technic has difficulties in assesion the effect of local anesthetics on relaxation time (Akber 1988).

The present work was aimed at studying the mechanical characteristics of BLM of various composition, under the action of LA lidocaine and carbisocaine. Lidocaine and carbisocaine differ from each other by their hydrophobic moieties. Carbisocaine has a large hydrophobic chain (Fig. 2*a*, 3*a*). The two LA studied allow an independent analysis of the role of the polar and/or hydrophobic moiety of the molecule in interaction with a membrane. In addition to $E_\perp$ measurements, also effects of lidocaine and carbisocaine on the transport dynamics of cations through gramicidin D induced ionic channels were studied by determining current-voltage characteristics. This approach allows a more comprehensive modelling of the effects of LA as agents capable of inhibiting the spreading of action potential in nerve by blocking sodium channels (Taylor 1959; Butterworth et al. 1988).

Materials and Methods

Bilayer lipid membranes were formed according to Mueller et al. (1962) from solutions of lipids in *n*-heptane, *n*-octane, *n*-decane (Fluka) or *n*-hexadecane (Merck), on a circular hole, approx. 0.5 mm in diameter, in the wall of a teflon cup. BLM were prepared of a mixture of egg phosphastidycholine (egg PC) (Plant of Chemical Preparations, Kharkov, USSR) with cholesterol (CH) (Fluka), w w ratio 4 : 1, in *n*-heptane or *n*-hexadecane (20 mg/ml); from a mixture of egg PC with cardiolipin (CL) (Plant of Chemical Preparations, Kharkov, USSR), w/w ratio 15 : 1, in *n*-heptane (20 mg ml); from total brain lipids (BL), isolated according to the method of Folsch (1957), in *n*-decane (20 mg ml); and from oxidized cholesterol (OCH), prepared according to the method of Tien et al. (1966), in *n*-octane (40 mg/ml). Also asolectin (AL) (Sigma) in *n*-heptane (40 mg ml) was used. As the electrolyte 0.1 mol/l NaCl in destilled water was used, with pH adjusted with 10 mmol l Tris HCl buffer (Fluka) and with 0.1 mol/l NaOH or HCl solution. The local anesthetics lidocaine (Pfaltz and Bauer, Inc.) and carbisocaine (1-methyl-2diethyl-aminoethylester of 2-*n*-heptyloxicarbanilic acid) (Institute of Experimental Pharmacology, Slovak Academy of Sciences, Bratislava) were used for

the experiments. The LA were dissolved in the same buffer as that used in membrane forming, in concentrations 1 mmol/l—0.1 mol/l. After membrane formation, LA were added to the electrolyte in one compartment of the teflon cup up to a final concentration between 10 μmol/l and 10 mmol/l. To balance hydrostatic pressure, electrolyte was added to the other compartment. Experiments were performed at 20 °C.

1. *Measurements of modulus of elasticity in direction perpendicular to the membrane surface, $E_\perp$.* BLM elasticity in direction perpendicular to the membrane surface is expressed in terms of the modulus of elasticity $E_\perp$ which characterizes the capacity of the membrane to attenuate upon the application of pressure p; this can be brought about by applying electric voltage U on the membrane. According to the method developed by Passechnik and Hianik (1977), alternating electric voltage $U = U_0 \sin 2\pi ft$ with a frequency f and amplitude U_0 will compress the membrane producing pressure $p = C_s U^2/2h$, where C_s is the specific capacity of the membrane, and h is the thickness of the hydrophobic region of the membrane. Membrane capacity depends on the electric voltage applied: $C = C_0(1 + \alpha U^2)$, where C_0 is membrane capacity at $U = 0$, and α is the coefficient of non-linearity. Due to nonlinearity of the capacity/voltage relationship, membrane current $i = \mathrm{d}(CU)/\mathrm{d}t$ will contain a component with frequency $3f$ and amplitude A_3, in addition to the component with frequency f and amplitude A_1. The parameter $E_\perp$ is then determined by $E_\perp = (3/4) \cdot (C_s U_0^2/h) \cdot (A_1/A_3)$. Thus, to obtain $E_\perp$, we need to measure but the amplitudes A_1 and A_3 of the membrane current harmonics, e.g. using standard electronic equipment and resonance amplifiers (cf. Passechnik and Hianik 1977). The amplitude of voltage applied on the membrane was $U_0 = 140$ mV. $E_\perp$ was calculated using the values of specific capacity C_s and membrane thickness h according to the solvent used. The respective values were: hexadecane C_{16}, $C_s = 0.6 \times 10^{-2}$ F/m^2, $h = 3.1$ nm according to the measurements for monoglycerides (Benz et al. 1975) and lecithins (Benz and Janko 1976); decane C_{10}, $C_s = 0.4 \times 10^{-2}$ F/m^2, $h = 5$ nm. Owing to negligible differences, values of C_s and h for C_{10} were used also for octane and heptane (C_8 and C_7, respectively). Differences in C_s and h due to the use of membranes of various composition were compensated for by measuring the parameter A_1 using a teflon cup with a large hole diameter $d \sim 1$ mm to maximally suppress the effect of the thick marginal region of the membrane Benz and Janko 1976. At the same time, dimensions of the "black area" representing the lipid bilayer were checked microscopically.

2. *Measurements of the coefficient of nonlinearity of membrane current-voltage characteristics* Current-voltage characteristics were determined by a new method described in detail by Flerov et al. (1981) and Passechnik et al. (1985). The method of direct measurement of current-voltage (I—V) characteristics of modified membranes is based on the recording of the third current harmonic generated at BLM, with nonlinear dependence of current i on voltage U. Only the principal relationships will be shown here. In first approximation, membrane I—V characteristic may be expressed as $i = gU(1 + \beta U^2)$, were g is the conductivity, and β is the coefficient of nonlinearity. If alternating voltage $U = U_0 \sin 2\pi ft$ with a sufficiently low frequency (to eliminate the capacitive current component) is applied to the membrane, the membrane current will contain another component with frequency $3f$ and an amplitude A_3 in addition to the current component with frequency f and amplitude A_1. The coefficient of nonlinearity is determined by $\beta = -4A_3(1 + rg)^3/E_0^2 A_1$, where r is the resistance of both the electrodes and the electrolyte, $g = A_1/(E_0 - rA_1)$, E_0 is the amplitude of alternating voltage applied to the system electrodes-electrolyte-membrane. The overall voltage amplitude E_0 is related to the amplitude of voltage decrement at the membrane, U_0, by $U_0 = E_0 - rA_1$. Hence, membrane I—V characteristics may be determined by measuring A_1, A_3, and resistance r. In our experiments, alternating voltage with amplitude $U_0 = 100$ mV and frequency $f = 40$ Hz was used. In measuring the coefficient of nonlinearity of I—V characteristics of modified membranes, the channel forming agent gramicidin D was first added to the electrolyte at both membrane sides. After equilibration of amplitudes A_1 and A_3, the local anesthetic carbisocaine (0.1 mmol l) or lidocaine (1 mmol l) was added to electrolyte.

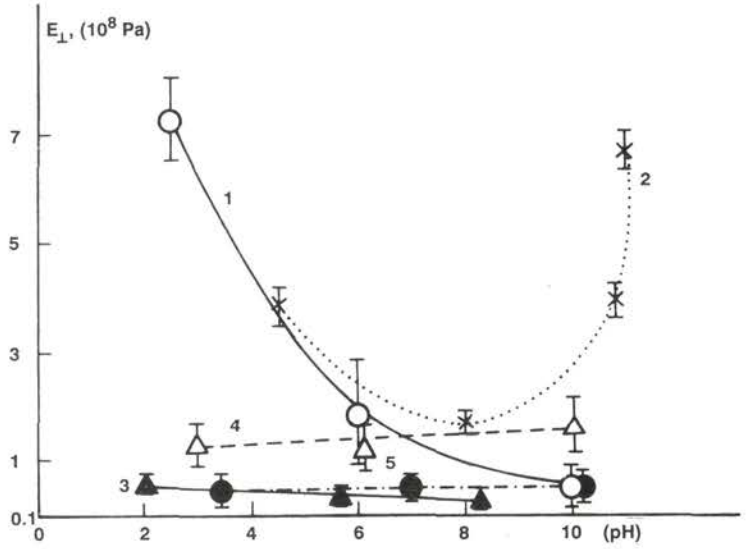

Fig. 1. The dependence of modulus of elasticity $E_\perp$ on electrolyte pH for BLM of various lipid composition: 1 — OCH in C_8; 2 — BL in C_{10}; 3 — AL in C_7; 4 — egg PC + CH in C_7; 5 — egg PC + CL in C_7.

Results and Discussion

First, let us discuss the problem of the effectivity of the local anesthetic action as depending on the LA molecule charge. The effect of local anesthetics depends on both the structure and ionization of the polar region. Depending on pH of the solution and on pK_a of the polar group of LA, the equilibrium shifts from natural (basic, B) to charged (protonised, BH^+) molecule (Pešák et al. 1980). For water solutions, the following relationship holds between the dissociation constant K_a, the concentrations of the basic [B] and the protonised [BH^+] forms, and proton concentration [H^+]: $K_a = [B].[H^+]/[BH^+]$. Both forms of local anesthetics, the basic and the protonised, are active upon LA acting on cell membrane, however, with different effectivities. Hence, we must know how LA induced changes in BLM parameters studied depend on electrolyte pH. The effectivity of LA action is also dependent on the concentration of the latter, and is determined by the parameter EC_{50}, i.e. the minimal anesthetic concentration required to reduce the action potential peak in isolated rat ischiadic nerve during 5 min in an electrolyte with pH 7.2—7.4 at a stimulation frequency of 30 pulses per s, by 50—60 %. For lidocaine, $EC_{50} = 0.56$ mmol/l, $pK_a = 7.97$; the respec-

tive values for carbisocaine are 10.3 μmol/l and 8.8 (Ondriáš 1984). Obviously the concentration dependence of membrane parameter changes is also an important aspect. In studying the LA action on lipid membranes it should be borne in mind that the mechanical membrane characteristics may also change in dependence on electrolyte pH (Hianik et al. 1984). This should be accounted for in analysing the results.

Fig. 1 shows the dependence of parameter $E_\perp$ on electrolyte (0.1 mol/l NaCl) pH for BLM of various lipid composition. It is obvious that for BLM of OCH in C_8, the parameter $E_\perp$ decreases monotonously with the increasing pH (curve 1); for membranes of BL in C_{10}, $E_\perp$ first decreases, reaching its minimum at pH 8, and then rapidly increases with the increasing pH (curve 2). The electrolyte pH-dependent changes in BLM $E_\perp$ may be due to condensation effects in the polar region of the lipid bilayer. Thus, in OCH BLM characterized by a negative surface charge (Hianik et al. 1984), dissociation of OH-groups in the polar region of the OCH molecule (Gibbsons et al. 1982) very likely results in gradual compensation of the negative charge with the increasing concentration of H^+ decrease in pH. As a result of this compensation, the electrostatic repulsing forces between the polar groups of the cholesterol molecules are reduced; consequently, a better ordering of both the polar and the inner non-polar region of the lipid bilayer occurs. This is also true for AL BLM (curve 3) which have a negative surface charge (Sokolov et al. 1980). BLM of BL are characterized by a mixture of various lipids with various pK values of the polar groups; this may be the major cause for the non-monotonous dependence of $E_\perp$ on electrolyte pH. The parameter $E_\perp$ for both BLM of a mixture of egg PC and CH (curve 4), and thosse of egg PC and CL (curve 5) is independent of electrolyte pH. Egg PC is a zwitterionic lipid with a zero summary charge at pH $6 \div 7$ (Ivkov and Berestovsky 1981). However, shifts towards lower or higher values result in the occurrence of a positive or negative summary charge respectively (Ermakov and Sokolov, personal communication). Moreover egg PC membranes contain also lipid peroxidation products carrying certain charges (Vladimirov and Archakov 1972). Thus the results obtained suggest that changes in surface charge of egg PC BLM remain without effect on the conformation of the hydrophobic regions of the membranes. Membranes containing CL carry a negative surface charge associated with two negative charges of the polar group of CL. However, probably due to both low CL concentrations and the presence of organic solvent (Hianik et al. 1984), the sensitivity of the experimental apparatus is insufficient to measure changes in $E_\perp$ in dependence on electrolyte pH for these membranes. It is obvious from the above that LA interactions with BLM can most adequately be studied using the latter two membrane types: those prepared of a mixture of egg PC with cholesterol, and those of egg PC and CL. Our studies of the action of carbisocaine and lidocaine

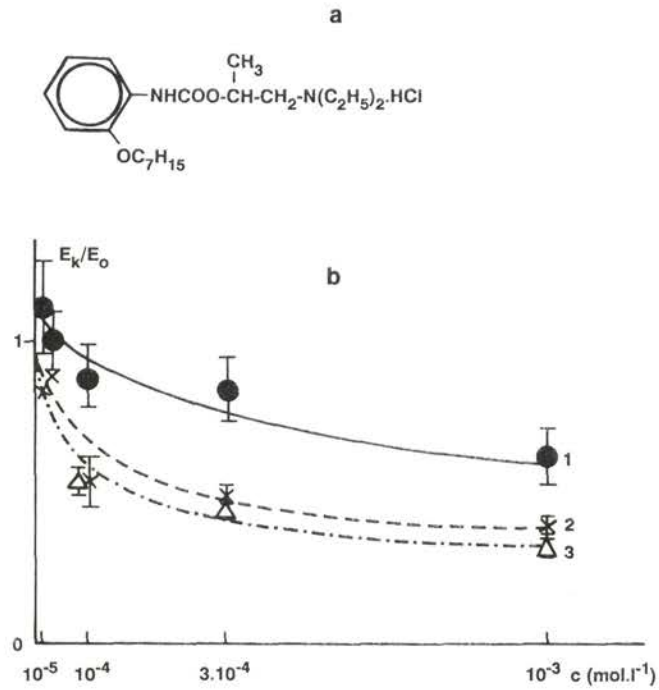

Fig. 2. *a*: Structural formula of carbisocaine. *b*: The dependence of the relative changes in modulus of elasticity E_k/E_0 for BLM of egg PC + CH in C_7, on carbisocaine concentration in the electrolyte (0.1 mol/l NaCl) for various pH of the water solution: 1 — pH 3.8, 2 — pH 7, 3 — pH 9.8.

were using membranes of the above composition.

Fig. 2*b* shows the dependence of the ratio of modulus of elasticity E_k, read after 10 min of the action of the respective LA concentration, to modulus of elasticity E_0 measured in absence of LA, on carbisocaine concentration for various electrolyte pH values. Relatively low LA concentrations (20 μmol/l—0.1 mmol/l) cause a rapid decrease in overall modulus of elasticity by 15—40 % as compared to the initial value of E_0. At LA concentration of 0.1 mmol/l, the changes in E_k/E_0 are slowlier, and they reach a steady state, with the initial value of E_0 decreasing by 63 % at 1 mmol/l. The results obtained indicate certain differences in the action between protonised and basic LA forms. Increasing concentrations of basic (natural LA) forms are associated with more pronounced changes in modulus of elasticity. These results agree with the report of Butler et al. (1973) who used the method of ESR spin probes and observed a decreasing ordering of lipid bilayers with the increasing concentrations of natural forms of other local anesthetics. The more pronounced activity of the basic forms of local anesthetics on change in modulus of elasticity $E_\perp$ may be due to the neutral

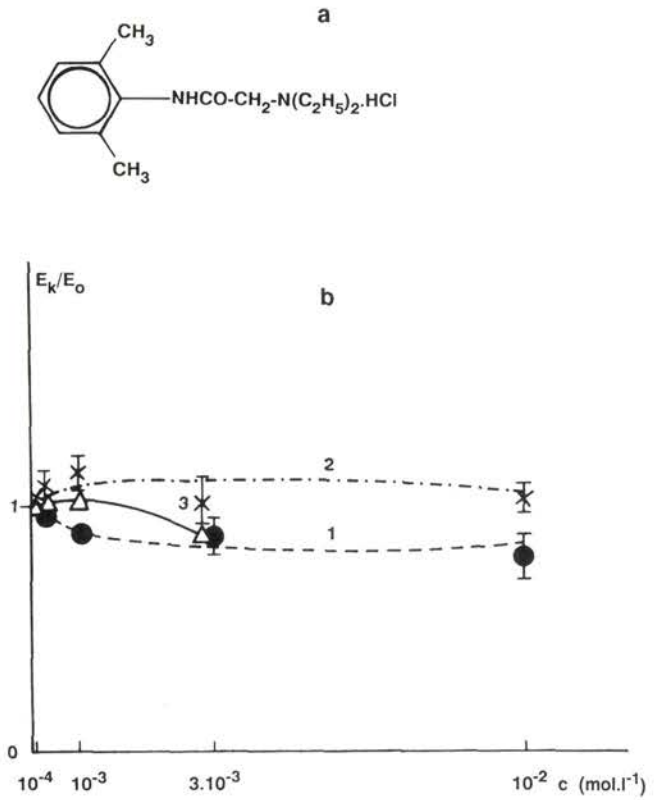

Fig. 3. *a*: Structural formula of lidocaine. *b*: The dependence of the relative changes in modulus of elasticity E_k E_0 for BLM of egg PC + CH in C_7, on lidocaine concentration in the electrolyte (0.1 mol/l NaCl) for various pH of the water solution: 1 — pH 3.8, 2 — pH 7, 3 — pH 9.8.

anesthetic forms being able to penetrate deeper into the hydophobic region of the membrane and thus to more strongly disturb the ordering of the membrane. Our results agree with those reported by Westman et al. (1982) and by Kelusky and Smith (1984) who study by ^{2}H NMR the binding of specially deuterated tetracaine and procaine. They could show that the depth of tetracaine penetration in egg PC depends mainly on the anesthetic charge. At pH 9.5, the uncharged tetracaine may sink lower into the bilayer, while at pH 5.5 the charged dimethylamino groups may prefer a position at the level of the trimethyl amonium group of egg PC. ESR-study of tetracaine interaction with egg PC vesicles (Frezatti et al. 1986) indicated a decrease of the lipid bilayer ordering at LA concentration of about 5.2 mmol/l with the increasing LA basicity.

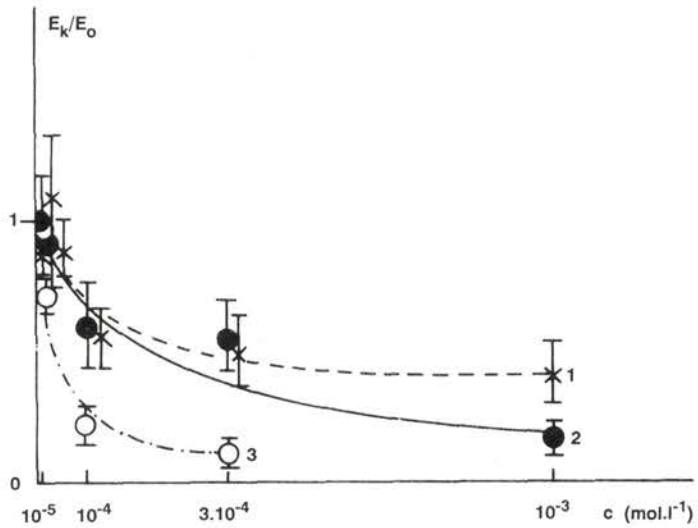

Fig. 4. The dependence of relative changes in modulus of elasticity E_k/E_0 for BLM of various composition prepared with various hydrocarbon solvents, on carbisocaine concentration in the electrolyte (0.1 mol/l NaCl), pH 7: 1 — egg PC + CH in C_7; 2 — egg PC + CH in C_{16}; 3 — egg PC + CL in C_7.

As compared to carbisocaine, even tenfold higher concentrations of lidocaine induce substantially smaller changes in E_k/E_0; the changes are independent of electrolyte pH (Fig. 3*b*). These observations are in agreement with the earlier results of Rosenberg et al. (1977) who have shown that lidocaine does not affect bilayer lipids. Lidocaine probably attacks non-lipid and charged radicals in the membrane (Sax and Pletcher 1969).

A comparison of the carbisocaine interaction with BLM with that of lidocaine clearly shows that the hydrophobic region of the local anesthetic molecule plays an important role in the non-specific interaction of the anesthetic with membranes.

The membrane solvent concentration has no significant effect on relative changes in modulus of elasticity induced by LA. This is clearly documented in Fig. 4 which shows the dependence of E_k/E_0 on carbisocaine concentration for BLM of egg PC and CH in C_7 (curve 1) and C_{16} (curve 2). It should be noted that due to shifts to the marginal regions of BLM (White 1975), C_{16} concentration in the membrane is substantially smaller. In contrast to solvent type, the membrane-LA interactions are largely dependent on the lipid composition of the membrane. Fig. 4 (curve 3) shows the dependence of E_k/E_0 on carbisocaine concentration in the electrolyte (pH 7) for BLM of egg PC + CL. As compared

to BLM of egg PC plus CH, changes in E_k/E_0 are much larger with the former. A reason for such differences can be the negative surface charge of egg PC + CL membranes and consequently a larger amount of LA adsorbed on such membranes as compared with egg PC + CH BLM (Ohki 1984). Our results agree with those obtained by Ondriáš (1984) who used the method of ESR spin probes to study lidocaine and carbisocaine effects on model bilayer membranes, and also observed changes in the parameter of ordering of both the polar and the hydrophobic region. However, the changes in parameter of ordering occurred at substantially higher LA concentrations than did those in modulus of elasticity: LA concentrations required to induce detectable changes in parameter of ordering of lipid bilayers (liposomes) were 5 mmol/l for carbisocaine and 50 mmol/l for lidocaine. The parameter of ordering of the lipid bilayer as measured using the probe 16 DSA, decreased by 10 % after carbisocaine and by 7 % after lidocaine. These results coincide well with the effectivity of the local anesthetic action. Carbisocaine is more effective in blocking action potential and in affecting mechanical characteristics of BLM that is lidocaine. Hence, the mechanical membrane characteristics are a quite sensitive indicator of the effectivity of the local anesthetic action.

The above results clearly document the significant effects of local anesthetics on the ordering of the hydrophobic region of lipid bilayers. Results of experiments with blocking Na^+ channels with the local anesthetic dicaine, obtained in brain synaptosomes (Aksentsev 1983) point to another possibility of modelling and testing effects of local anesthetics using BLM. Agents which induce the formation of ionic channels can be incorporated into BLM used to study the effects of LA on BLM characteristics. This approach was used to study dicaine, heptacaine and carbisocaine induced changes in conductivity of BLM modified with the channel forming agent gramicidin D (Ondriaš et al. 1986). LA concentrations of approx. 1 mmol/l were shown to increase the conductivity of non-modified lipid bilayers, and to reduce that of gramicidin D-modified BLM. Similar results were obtained with the patch clamp method. Brett et al. (1988) found that the LA isofurane causes the single acetylcholine receptor channels to "flicker" rapidly between open and close states. Nevertheless, the results obtained do not unambiguously explain the mechanism underlying the blocking of ionic channels by local anesthetics. There is no clear-cut answer to the question whether local anesthetics block ionic channels by their polar region thus preventing ions from entering the channel, or whether they affect ion transport through channels by altering the physical characteristics, and thus the structure of channels. Gramicidin D consists of two monomers, and may induce changes resulting in cleavage of dimers. Owing to this, our experiments were designed to investigate the effects of carbisocaine and lidocaine on another parameter of ionic channels which may provide significant information, namely the current-

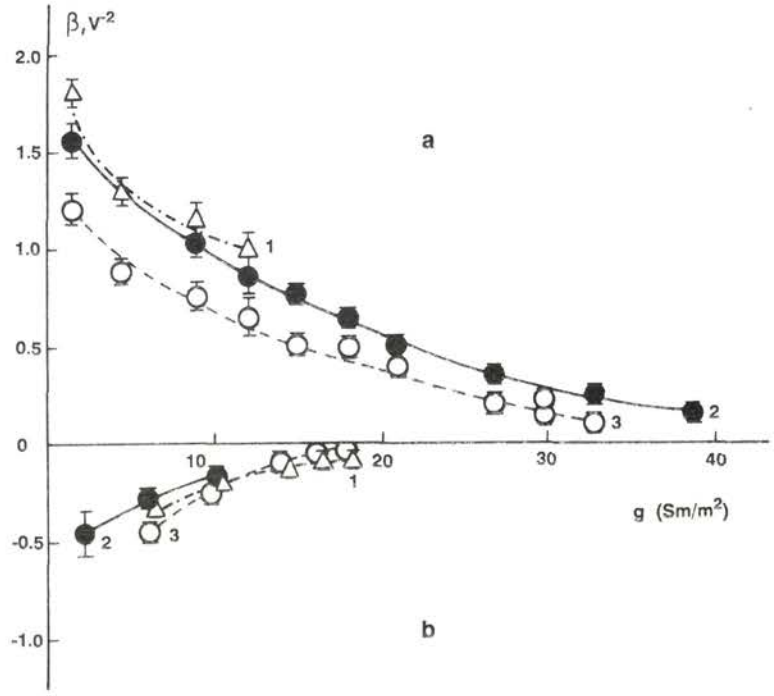

Fig. 5. The dependence of coefficient of nonlinearity of I—V characteristics of gramicidin D-modified membranes of egg PC + CH, on specific conductivity of the membrane, for various electrolyte (KCl) concentrations. *a*: c = 3 mol/l; *b*: c = 30 mmol/l, and on the presence of LA in the electrolyte: 1 — without LA; 2 — in the presence of 1 mmol/l lidocaine; 3 — in the presence of 0.1 mmol/l carbisocaine, pH 7.4. The concentration of gramicidin D in the electrolyte was 0.01 μmol/l.

voltage (I—V) characteristic. Changes in I—V characteristics were recorded for membranes prepared of a mixture of egg PC with CH (4 : 1, w/w) in *n*-heptane (20 mg/ml) modified with gramicidin D. In a previous report (Hianik et al. 1987) we could show that the shape of the I—V characteristic of gramicidin D-modified BLM, determined by the sign of the coefficient of nonlinearity β, is dependent on the electrolyte concentration. Gramicidin D-modified BLM prepared of a mixture of egg PC with cholesterol (4 : 1, w/w) have $\beta < 0$ at electrolyte (KCl) concentrations below 0.15 mol/l (I—V characteristic is sublinear, and ions are transported across the membrane as determined by the entrance area of the ionic channel); $\beta = 0$ at 0.15 mol/l (I—V characteristic is linear); and $\beta > 0$ at $c > 0.15$ mol/l (I—V characteristic is superlinear, and ion transport across the membrane is determined by the inner region of the ionic channel). Our experi-

ments were designed to study the effects of local anesthetics in two extreme conditions: at low KCl concentration ($c = 30$ mmol/l), when $\beta < 0$, and at high electrolyte concentration ($c = 3$ mol/l), when $\beta > 0$. Fig. 5 shows the dependence of parameter β on specific conductivity of modified BLM at physiological pH (7.4). The addition of carbisocaine (0.1 mmol/l) or lidocaine (1 mmol/l) to KCl electrolyte (3 mol/l) resulted in a gradual decrease in β as represented by three curves: 1 — BLM without LA; 2 — in the presence of lidocaine; 3 — in the presence of carbisocaine. The strongest effect (decrease in β by 32 %) was observed with carbisocaine. The addition of the local anesthetics to electrolyte with the low KCl concentration ($c = 30$ mmol/l) had different and varying effects. Parameter β decreased in the presence of lidocaine and increased with carbisocaine, with the relative change being smaller than observed with the high electrolyte concentration. Results obtained in approx. 10 membranes in each series were well reproducible as for the direction of change in β. The relatively large mean quadratic errors (Fig. 5) were due to differences in initial values of β of consecutively formed membranes of identical lipid composition. The results with testing LA effects on parameter β of gramicidin D-modified BLM suggest that LA predominantly interact with the structure of the inner channel region. The generalized restructuration of the lipid bilayer, as reflected in changes in the mechanical characteristics, thus has a significant influence on the operation of functional subunits of the membrane. The results, at least those obtained for LA with large hydrophobic moieties, support the concept of non-specific interaction of local anesthetics with lipid bilayers, mediated by alterations of the structural state of the latter.

Acknowledgement. The authors wish to express their gratitude to Dr. K. Ondriáš for generous gifts of local anesthetics and for stimulating discussions. We also thank Dr. A. Bajči for his assistance.

References

Akber S. F. (1988): Influence of anesthetics on relaxation times. Anesthesiology **63,** 290—291

Aksentsev S. L. (1983): Structural and functional specificity of biological membranes rebuilding. D. Sc. Thesis, Institute of Photobiology of Belorussian Acad. Sci., Minsk (in Russian)

Aksentsev S. L., Konev S. V., Okun I. M., Kulikov A. V. (1982): Blockage of sodium channels and transmitter uptake systems in synaptosomes by local anesthetics: lipid mediated or direct influence? Gen. Physiol. Biophys. **1,** 403—411

Benz B., Fröhlich O., Lauger P., Montal M. (1975): Electrical capacity of black lipid films and of lipid bilayers made from monolayers. Biochim. Biophys. Acta **394,** 323—334

Benz B., Janko K. (1976): Voltage-induced capacitance relaxation of lipid bilayer membranes. Effect of membrane composition. Biochim. Biophys. Acta **455,** 721—738

Brett R. S., Dilger J. P., Yland K. F. (1988): Isofurane causes "flickering" of the acetylcholine receptor channel: Observation using the patch clamp. Anesthesiology **69,** 161—176

Butler K. W., Schneider H., Smith I. C. P. (1973): The effects of local anesthetics on lipid multilayers. A spin probe study. Arch. Biochem. Biophys. **154,** 548—554

Butterworth J. F., Lief P. A., Strichartz G. R. (1988): The pH-dependent local anesthetic activity of diethylaminoethanol, a procaine metabolite. Anesthesiology **68,** 501—606

Cullis P. R., Verkleij A. J. (1979): Modulation of membrane structure by Ca^{2+} and dibucaine as detected by ^{31}P NMR. Biochim. Biophys. Acta **552,** 546—551

Ebihara L., Hall J. E., McDonald R. C., McIntosh T. J., Simon S. A. (1979): Effect of benzylalcohol on lipid bilayers, a comparison of bilayer systems. Biophys. J. **28,** 185—196

Flerov M. N., Passechnik V. I., Hianik T. (1981): Study of current-voltare characteristics of ionic channels by the transmembrane current harmonics. Biofizika **26,** 277—283 (in Russian)

Folsch J., Lees M., Stanley G. H. S. (1957): A simple method for the isolation and purification of total lipids from animals tissues. J. Biol. Chem. **226,** 497—509

Frezatti W. A., Toselli W. R., Schreirer S. (1986): Spin label study of local anesthetic-lipid membrane interactions. Phase separation of the uncharged form and bilayer micellization by the charged form of tetracaine. Biochim. Biophys. Acta **860,** 531—538

Gibbons G. F., Mitropoulos K. A., Myant N. B. (1982): Biochemistry of Cholesterol. Elsevier, Amsterdam

Hianik T., Miklovičová J., Bajči A., Chorvát D., Šajter V. (1984): Calcium and pH-induced changes in oxidized cholesterol bilayers. Stud. Biophys. **99,** 9—16

Hianik T., Bajči A., Laputková G., Pavelková J. (1987): Dependence of gramicidin D and valinomycin induced conductivity of lipid bilayer on cholesterol content. Biofizika, **32,** 458—461 (in Russian)

Hianik T., Kavečanský J., Zórad Š., Macho L. (1988): Insulin-induced changes in mechanical characteristics of lipid bilayers modified by liver plasma membrane fragments. Gen. Physiol. Biophys. **7,** 191—203

Ivkov V. G., Berestovsky G. N. (1981): Dynamic Structure of Lipid Bilayers. Nauka, Moscow (in Russian)

Kelusky E. C., Smith I. C. P. (1984): Anesthetic-membrane interaction: A ^2H nuclear magnetic resonance study of the binding of specially deuterated tetracaine and procaine to phosphatidylcholine. Can. J. Biochem. Cell Biol. **62,** 178—184

McLaughlin S. G. A. (1975): Local anesthetics and the electrical properties of phospholipid bilayer membranes. In: Molecular Mechanism of Anesthesia (Ed. B. R. Fink) pp. 193—220, Raven Press, New York

Mueller P., Rudin D. O., Tien H. Ti, Wecsott W. C. (1962): Reconstitution of cell membrane structure in vitro and its transformation into an excitable system. Nature **194,** 979—980

Neal M. J., Butler K. W., Polnaszek C. F., Smith I. C. P. (1976): The influence of anesthetics and cholesterol on the degree of molecular organization and mobility of ox-brain white matter. Mol. Pharmacol. **12,** 144—155

Ohki S. (1970): Effect of local anesthetics on phospholipid bilayers. Biochim. Biophys. Acta **219,** 18—27

Ohki S. (1984): Adsorption of local anesthetics on phospholipid membranes. Biochim. Biophys. Acta **777,** 56—66

Ondriáš K. (1984): Biophysical analysis of membrane effect of the local anesthetic. PhD. Thesis, Institute of Experimental Pharmacology, Slovak Acad. Sci., Bratislava (in Slovak)

Ondriáš K., Bérczi A., Mišik B., Štolc S. (1986): Influence of local anesthetics on electrical conductivity of planar lipid membrane doped with gramicidin. Stud. Biophys. **115,** 17—22

Passechnik V. I., Hianik T. (1977): Elastic properties of bilayer membranes in the direction perpendicular to the membrane plane. Kolloidn. Zh. **39,** 1180—1185 (in Russian)

Passechnik V. I., Hianik T. (1978): The estimate of viscoelastic properties of biological membranes from bilayer experiments. Biofizika **23,** 1005—1010 (in Russian)

Passechnik V. I., Hianik T. (1979): Modification of lipid bilayer elastic properties by different actions. Biofizika **24,** 460—466 (in Russian)

Passechnik V. I., Flerov M. N., Hianik T. (1985): The study of volt-ampere characteristic of ionic channels formed by gramicidin A. Gen. Physiol. Biophys. **4,** 35—54

Pešák M., Štolc S., Beneš L. (1980): Partition of local anesthetics in two phases in the form of the base and ionic pair. Českoslov. Farm. **39,** 272—275 (in Czech)

Rosenberg P. H., Janson S. E., Gripenberg J. (1977): Effect of halothane, thiopental, and lidocaine on fluidity of synaptic plasma membranes and artificial phospholipid membranes. Anesthesiology **46,** 322—326

Sax M., Pletcher J. (1969): Local anesthetics: Significance of hydrogen binding in mechanism of action. Science **166,** 1546—1548

Seelig A., Allegrini P. R., Seelig J. (1988): Partitioning of local anesthetics into membranes: surface charge effects monitored by the phospholipid head-group. Biochim. Biophys. Acta **939,** 267—276

Sokolov V. S., Chernyi V. V., Abidor I. G. (1980): Determination of the surface charge of bilayer lipid membranes. Dokl. Akad. Nauk USSR **251,** 236—239 (in Russian)

Taylor R. E. (1959): Effect of procaine on electrical properties of squid axon membrane. Amer. J. Physiol. **196,** 1071—1078

Tien H. Ti., Carbone S., Dawidowicz E. A. (1966): Formation of "black" lipid membranes by oxidation products of cholesterol. Nature **212,** 718—719

Vladimirov Yu. A., Archakov A. I. (1972): Lipid Peroxidation in Biological Membranes. Nauka, Moscow (in Russian)

Westman J., Boulanger Y., Ehrenberg A., Smith I. C. P. (1982): Change and pH dependent drug binding to model membranes. A ^{2}H-NMR and light absorption study. Biochim. Biophys. Acta **685,** 315—328

White S. H. (1975): Phase transitions in planar bilayer membranes. Biophys. J. **15,** 95—117

Final version accepted October 26, 1989