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A SPIN LABEL STUDY OF THE PERTURBATION EFFECT OF TERTIARY AMINE ANESTHETICS ON BRAIN LIPID LIPOSOMES AND SYNAPTOSOMES

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The membrane disordering efficiency of four local anesthetics, including lidocaine, tetracaine, dibucaine and heptacaine (piperidinoethyl ester of 2-heptyloxyphenylcarbamic acid) has been studied by spin-labeling methods. The disordering efficiency of the drugs in rat total brain lipid liposomes was quantitated with the initial slope value of the order parameter versus drug concentration curve, the so-called change-in-order parameter value. Using the positional isomers of *m*-doxyl stearic acids ($m = 5$, 12 and 16), it has been demonstrated that the tested drugs reveal quite different disordering efficiency. There is a clear tendency of increasing disordering efficiency towards the methyl terminal of the lipid acyl chains. By a comparison of order parameter versus drug concentration and temperature at three depths of rat brain total lipid liposomes and synaptosomes, it is shown that the 'fluidizing effect' of local anesthetics does not correspond to fluidization of membrane by temperature and that tetracaine and dibucaine do not have equal disordering efficiency as judged by their solubility in the membrane. The disordering efficiency of these drugs on the hydrocarbone core of a membrane qualitatively corresponds to their anesthetic potency. Similar results were obtained in liposomes and synaptosomes. It is assumed that there is a similar incorporation of the local anesthetics in the liposomes and in the lipid part of synaptosomes.

Introduction

It is generally accepted that most of the drugs causing anesthesia perturb the structure of the membrane; nevertheless, their mode of action is only partly understood. A common feature of different classes of anesthetics is that these compounds can disrupt the structure of functionally important membrane proteins either (i) specifically, by binding to a set of hydrophobic binding sites of proteins and displacing the annular lipid layer [1], or (ii) non-specifically, by fluidizing bulk lipid domains, an effect which in turn affects the packing of the annular lipid layer and may also induce protein conformational changes [2]. The local anesthesia is not abolished by cooling, which does not correspond with the theory of general fluidizing effects of local anesthetics in the membrane [3]. It is therefore of interest to study the temperature dependence of the molecular arrangement of membrane lipids in the presence of local anesthetics. Different probing techniques – while each operating on different time-scale – provide a deep insight into membrane-associated events. One of the available methods is spin labeling, which is useful due to its high sensitivity; it has been extensively used to study the effect of local anesthetics on the ordering of all brain lipid liposomes as detected by the cholestane spin label [4].

Abbreviation: *m*-DSA, stearic acid labeled with the dimethyloxazolidinyl (doxyl) group at the *m*th carbon of the stearic acid.

In the present work we have used spin-labeled stearic acids and have measured the perturbation effects of local anesthetics at three different depths in the total rat brain lipid membranes and in the synaptosomes. On the basis of these experiments, we have concluded that the 'fluidizing effect' of the tertiary amine local anesthetics lidocaine, tetracaine, dibucaine and heptacaine cannot be reversed by cooling, since anesthetic fluidization of these membranes is not equivalent to melting. It is further shown that the disordering effect of all the studied local anesthetics in the hydrocarbon core of the membranes investigated correlates with their potency to block the action potential on the nerve.

Methods

Materials

Doxylstearic acid (DSA) spin probes 2-(10-carboxydecyl)-2-hexyl-4,4-dimethyl-3-oxazolidinyloxyl (12-DSA), 2-(14-carboxytetradecyl)-2-ethyl-4,4-dimethyl-3-oxazolidinyloxyl (16-DSA), and 2-(3-carboxypropyl)-4,4-dimethyl-2-tridecyl-3-oxazolidinyloxyl (5-DSA) were purchased from Syva (Palo Alto, U.S.A.). The abbreviation *m*-DSA with $m = 5, 12, 18$ means the stearic acid spin-labeled with the doxyl (= dimethyloxazolidinyl group) at the *m*th carbon of stearic acid.

Lidocaine · HCl, tetracaine · HCl, dibucaine · HCl and heptacaine · HCl (piperidino ethyl ester of 2-heptyloxyphenylcarbamic acid) were provided by courtesy of Dr. L. Beneš (Institute of Experimental Pharmacology, Bratislava). Total lipids were extracted from rat brain according to the method of Folch et al. [5].

Preparation of liposomes

Procedure A. Total lipids and *m*-DSA spin probe were dissolved in chloroform/methanol at molar ratio of at least 100 : 1 and the solvent was evaporated in a stream of nitrogen. Residual traces of solvent were removed by evacuation. The total lipids were hydrated with 0.1 mol/l NaCl, 50 mmol/l Tris-HCl solution (pH 7.2) and multilamellar liposomes were prepared by 2-min sonication. Each sample contained 5% (w/v) lipids, approx. 65 mmol/l. Local anesthetics dissolved in the above-mentioned buffer were added to the liposomes and left to equilibrate overnight at 4°C.

Procedure B. Local anesthetics dissolved in the above-mentioned buffer were added to the dry lipids and samples were freezed-thawed (in solid CO_2) several times. After thawing, the samples were vigorously vortexed for 3–5 min and then left to equilibrate for 1–2 h at room temperature before measuring.

Preparation of synaptosomes

Procedure A. Synaptosomal fractions were separated from rat cerebral cortex after careful removal of the myelin-rich layer according to Hajós [6]. 6 μg of *m*-DSA spin probe was layered onto the bottom of plastic vials by evaporating the ethanol solution. 120 μl synaptosomal suspension in 0.32 mol/l sucrose, 10 mmol/l Tris-HCl solution (pH 7.4) were added and vigorously vortexed for 1 min. Local anesthetics were added as 0.32 mol/l sucrose solutions, vortexed for 1 min and left to equilibrate for 15 min.

Procedure B. A synaptosomal suspension with local anesthetics was filled into a plastic flat microcell, centrifuged for 5 min at $1000 \times g$, and left to equilibrate for 60 min at a given temperature. The final weight ration of synaptosomes to buffer was 1 : 30. The final pH of the samples has not been further adjusted.

ESR measurements

ESR spectra were recorded by a JEOL JES-PE-1X or Varian E-4 X-band spectrometer using the 100 kHz modulation technique. Typical instrumental settings were: 10 mW microvave power, modulation amplitude max 2 G, and a signal-to-noise ratio of at least 30. 100 μl sample suspension were filled into the flat microcell (Scanlon, U.S.A.), glass capillary or plastic flat microcell. The temperature was continously monitored by means of a digital thermometer with the thermocouple placed directly in the upper portion of the ESR cell.

Calculation of order parameter

To estimate the relative efficiency of local anesthetics in perturbing the membrane, the order parameter, *S*, was calculated from the ESR spectra of spin-labels *m*-DSA according to Gaffney [7] using the formula:

$$S = \frac{A'_{\parallel} - A'_{\perp}}{A_{33} - \frac{1}{2}(A_{11} + A_{22})} \cdot \frac{a}{a'} \qquad (1)$$

where $A'_{\parallel}$ and $A'_{\perp}$ were obtained from the outer and inner splitting, respectively, A_{ii} terms correspond to the diagonal elements of the hyperfine splitting tensor, $a = \frac{1}{3}\Sigma_i A_{ii}$, and $a' = \frac{1}{3}(A'_{\parallel} + 2A'_{\perp})$. The values of A_{ii} were taken from the literature [7].

Results

Perturbation effect of local anesthetics at different liposome membrane depths

The disordering efficiency of local anesthetics is best illustrated by change-in-order parameter vs. drug concentration plots according to Pang et al. [8]. As shown in Fig. 1, the order parameter, S, of spin label in total lipid liposomes is 0.532 ± 0.003 (at 30°C), when measuring at the C-12 acyl chain segment using the 12-DSA spin probe. The addition of increasing amounts of drug caused increasing disorder, i.e., decreasing S, with apparently fairly linear concentration dependence. On the basis of this feature, the drug disordering efficiency can be characterized with the slope value, $\Delta S/\Delta C$, where C is the concentration of local anesthetic, as given in Table I. For the sake of comparison, slope values at the C-5 and C-16 acyl chain segments are also given. It is clear from table that the perturbation effect at the C-5 segment increases in the order lidocaine < dibucaine < tetracaine < heptacaine, while at the C-12 and C-16 segments the order is lidocaine < tetracaine < dibucaine < heptacaine. Different preparation procedures affect only the numerical values of the order parameter, S, and not the order of efficiency of local anesthetics.

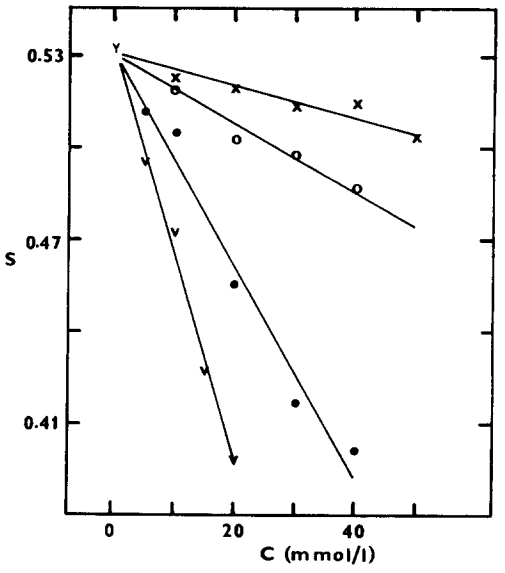

Fig. 1. Dependence of the order parameter, S, of the 12-DSA spin probe in total lipid liposomes on the local anesthetic concentrations. Liposomes were prepared by procedure A. The lipid/buffer ratio was 5% (w/v). Temperature, 30°C. Local anesthetics: ×, lidocaine·HCl; ○, tetracaine·HCl; ●, dibucaine·HCl; ∨, heptacaine·HCl; Y, control sample.

TABLE I

THE DISORDERING EFFICIENCY OF FOUR TERTIARY AMINE LOCAL ANESTHETICS AS QUANTITATED BY THE CHANGE-IN-ORDER PARAMETER SLOPE VALUES $\Delta S/\Delta C$

$\Delta S/\Delta C$ in synaptosomes was normalized for the 5-DSA spin probe setting its value for heptacaine $\Delta S/\Delta C = -2.63 \cdot 10^{-3}$ mM^{-1} (equal to $\Delta S/\Delta C$ in liposomes), and for the 16-DSA spin probe setting $\Delta S/\Delta C = -4.89 \cdot 10^{-3}$ mM^{-1} for dibucaine. Trend line analysis of $\Delta S/\Delta C$ curves gave $r^2 = 0.9$ or better, except for 16-DSA in synaptosomes. Abbreviations: Li, lidocaine·HCl; Te, tetracaine·HCl; Di, dibucaine·HCl; He, heptacaine·HCl. Samples were prepared by procedure A, except for synaptosomes with the 16-DSA spin probe, which were prepared by procedure B.

Samples	Spin probe	$\Delta S/\Delta C$ ($\times 10^3$) (mM^{-1})			
		Li	Te	Di	He
Liposomes	5-DSA	−0.148	−0.560	−0.283	−2.63
	12-DSA	−0.511	−1.13	−3.47	−6.68
	16-DSA	−0.342	−2.62	−4.89	−6.74
Synaptosomes	5-DSA	−0.180	−0.362	−0.231	−2.63
	16-DSA		−2.24	−4.89	

Disordering efficiency of local anesthetics in liposomes compared to temperature changes

To compare the perturbation effect caused by temperature and local anesthetics in total lipid liposomes, the changes in order parameter, S, versus concentrations of local anesthetics and temperatures 15–30°C at the three different depths of membrane have been studied. These relationships are fairly linear. Becuase of different $\Delta S/\Delta T$ gradients (where T is temperature) for the 5-, 12- and 16-DSA spin probes in the membrane, the parameter

$$P = \frac{\Delta S/\Delta C}{(\Delta S/\Delta T)_0} \qquad (2)$$

has been introduced. C is a concentration of local anesthetic in buffer, T is temperature and $(\Delta S/\Delta T)_0$ is the gradient of control sample. The parameter P expresses the temperature effect necessary to reach the same value of order parameter, S, in the control sample as in the sample with the fixed temperature, but with the local anesthetic. As is shown in Fig. 2, P increases for all local anesthetics with the depth of membrane. The values $\Delta S/\Delta C$ were taken from Table I and the values of $(\Delta S/\Delta T)_0$ for 5-, 12- and 16-DSA spin probes were -0.008, -0.01 and $-0.0032°C^{-1}$, respectively. The different values of parameter P at the three depths of membrane indicate that the effect of local anesthetics does not correspond to temperature changes, i.e., to the overall 'fluidization' of the membrane. It was possible to observe the ESR spectra of two spin probes, 5-DSA and 15-DSA, simultaneously incorporated in the same lipid dispersion. This approach was suitable in the temperature studies, because the effect in the two different membrane depths could be studied in the same sample, so that the possible differences due to history of sample were excluded. From the ESR spectrum, one can obtain $A'_{\|}$ for the 5-DSA spin probe and $A'_{\perp}$ for the 16-DSA spin probe, simultaneously. As shown in Fig. 3, dibucaine, (5 mmol/l) and tetracaine (10 mmol/l) have practically no effect on the $A'_{\|}$ of the 5-DSA spin probe, while the values of $A'_{\perp}$ for the 16-DSA spin probe are quite different for the anesthetics studied. Additionally therefore, different temperature effects were observed at the different membrane depths on the same membrane preparation. For example, the control sample must be heated by about 22°C and by 10°C to reach the same value of $A'_{\perp}$ as observed for sample with dibucaine (5 mmol/l) and with tetracaine (10 mmol/l), respectively. However, the change of $A'_{\|}$ with temperature was practically the same in all three samples studied. This means that the temperature change cannot compensate the perturbing effect of local anesthetics on the membrane.

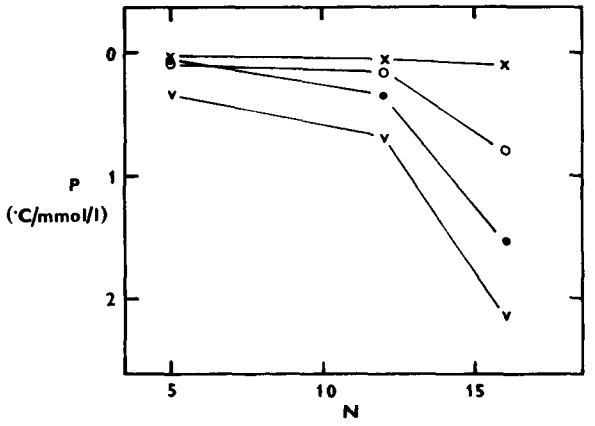

Fig. 2. Dependence of the parameter P on the carbon depth, N, of total lipid liposomes. Liposomes were prepared by procedure A. Lipid/buffer ratio was 5% (w/v). Temperature, 30°C. For symbols for local anesthetics, see Fig. 1.

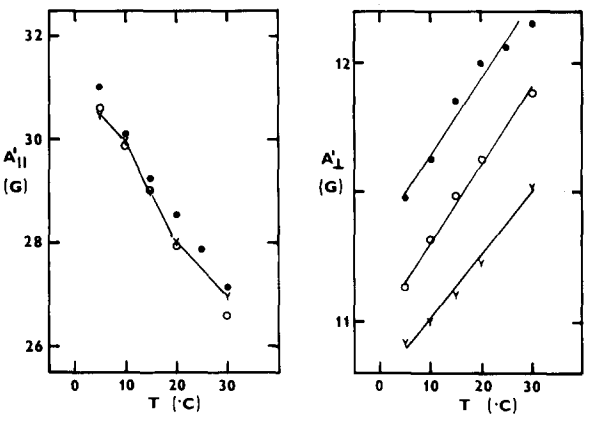

Fig. 3. Dependence of $A'_{\|}$ of the 5-DSA spin probe (left) and $A'_{\perp}$ of the 16-DSA spin probe (right) in total lipid liposomes on temperature (T) of control sample (Y), ●, sample with dibucaine·HCl (5 mmol/l) and ○, sample with tetracaine·HCl (10 mmol/l). Liposomes were prepared by procedure B. Volume ratio of buffer/lipid was 40.

Influence of the lipid concentration on the perturbation effect

We have observed that the change in S increases with the decrease in total lipid concentration in the sample at constant anesthetic concentration in the buffer added to the lipid. As seen from Fig. 4, with an increase in the water phase volume in the sample, the value of the order parameter, S, approaches within experimental error some limiting value. Comparing the limiting value of order parameter S with the temperature effect, it is seen that the perturbation caused by 5 mM dibucaine as detected by the 16-DSA spin probe is equivalent to the effect of the heating of the control sample without local anesthetic by 25–30°C.

Comparison of the perturbation effects in liposome and synaptosome membranes

As shown in Fig. 5, the addition of increasing amounts of drugs brought about a linearly increasing disorder in the synaptosomes as detected by the 5-DSA spin probe. Nevertheless, a comparison between the disordering efficiency in the synaptosomes and in liposomes is difficult to make, since the lipid-to-drug ratio cannot be given in the former case. This ambiguity in the dry lipid weight content of the synaptosomes will introduce an unknown scaling factor to our slope calculations, but should affect the slopes of different drugs

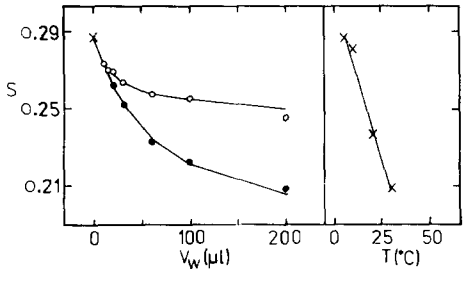

Fig. 4. Dependence of the order parameter, S, of the 16-DSA spin probe in total lipids on the amount of added buffer containing the constant 10 mM concentration of tetracaine·HCl (○) or 5 mM concentration of dibucaine·HCl (●). $V_L = 1$ µl. Temperature, 5°C (left). On the right, there is dependence of the order parameter S of the 16-DSA spin probe in total lipid liposomes on temperature (T) in the control sample (×) with buffer/lipid weight ratio 15. Liposomes were prepared by procedure 3.

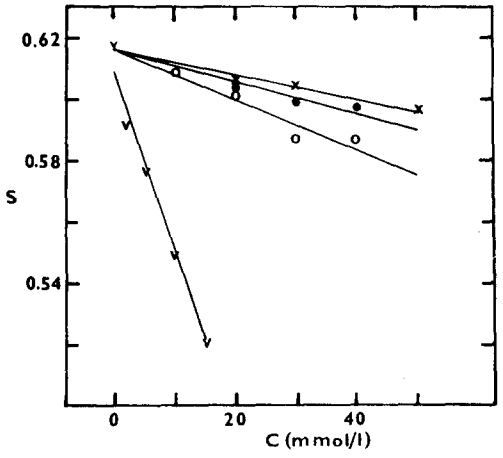

Fig. 5. Dependence of the order parameter, S, of 5-DSA spin probe in synaptosomes on the concentration of local anesthetics. Temperature, 36°C. Synaptosomes were prepared by procedure A. ×, lidocaine·HCl; ○, tetracaine·HCl; ●, dibucaine·HCl; ▽, heptacaine·HCl.

uniformly when using the same batch of synaptosome preparation. On the basis of this feature, we may normalize the slope values of Fig. 5 in such a way that the slope of heptacaine is arbitrarily set equal to that observed in the model experiments. As seen from Table I, and from the Fig. 9, all tertiary amine anesthetics tested display very similar disordering efficiencies in the synaptosomes as in the liposomes of extracted lipids.

It is seen from Fig. 6 that tetracaine is more efficient in decreasing the order parameter, S, of the 5-DSA spin probe in liposomes than is dibucaine (Fig. 6A), and dibucaine is more efficient in decreasing the order parameter, S, of the 16-DSA spin probe in liposomes than is tetracaine (Fig. 6B). Qualitatively similar results are seen from Fig. 7 for synaptosomes.

In Fig. 8, are presented the temperature dependences of the order parameter, S, in synaptosomes for the control sample, and samples with dibucaine (5 mmol/l) and tetracaine (5 mmol.l), as detected by the 5-DSA spin probe (Fig. 8A) and by the 16-DSA spin probe (Fig. 8B). It is seen that the gradient $\Delta S/\Delta T$ depends on the depth of the membrane. Taking this fact into account, we can evaluate the effect of local anesthetics in a temperature scale. For example, to reach the same value of order parameter, S, in the control sample as in

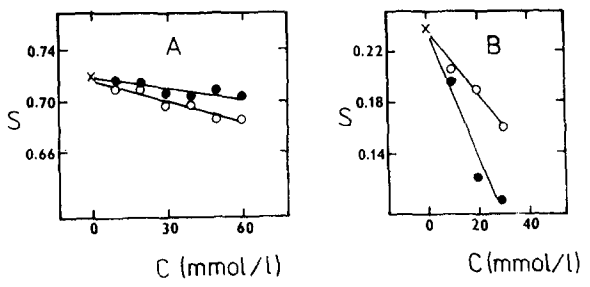

Fig. 6. Dependence of the order parameter S of the 5-DSA spin probe (A), temperature, 25°C, and the 16-DSA spin probe (B), temperature, 30°C, in total lipid liposomes on the local anesthetic concentrations. ●, dibucaine·HCl, ○, tetracaine·HCl. Liposomes were prepared by procedure A. Lipid/buffer ratio was 5% (w/v).

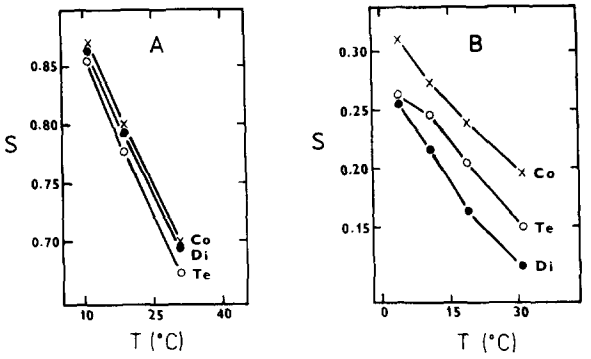

Fig. 8. Dependence of the order parameter, S, of the 5-DSA spin probe (A) and the 16-DSA spin probe (B) in synaptosomes on temperature (T), ×, control sample; ●, dibucaine·HCl (5 mmol/l); and ○, tetracaine·HCl (5 mmol/l).

the sample with dibucaine (5 mmol/l) it is necessary to heat the control sample by about 1°C in the case of the 5-DSA spin probe, but it is necessary to heat the control sample by about 15°C in the case of the 16-DSA spin probe. From this, it follows that the disordering effect of local anesthetics and temperature is different also in the membrane of synaptosomes. The extent of the observed effects was again dependent on the procedure of preparation of the synaptosomes, but the general trends were the same, independent of the preparation.

Discussion

The reversibility of anesthetic 'fluidization'

The key assumption of most of the approaches to anesthetic action is that the membrane acts as a solvent for the drugs, and since the partitioning of different drugs in the lipid and water phases differs considerably, their anesthetic potency will vary according to their lipid solubility. The primary effect of dissolved anesthetics is the 'fluidization' of the neuronal membranes, i.e., the lowering of its molecular ordering. A contraversial corollary of this hypothesis is that any effect which increases the molecular ordering, e.g., temperature or pressure should in principle reverse the anesthetic action. Although this was observed for ethanol, it failed for other drugs [1,3].

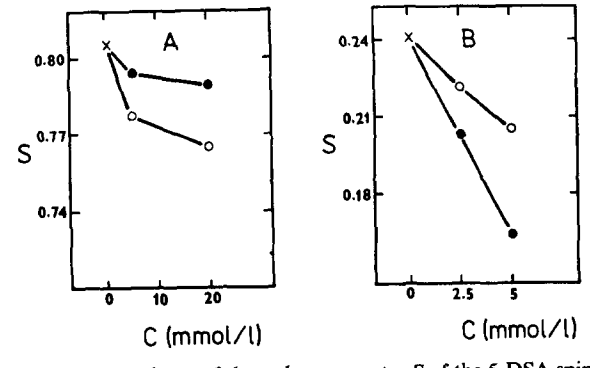

Fig. 7. Dependence of the order parameter S of the 5-DSA spin probe (A) and 16-DSA spin probe (B) in synaptosomes on the local anesthetic concentration, ●, dibucaine·HCl; ○, tetracaine·HCl. Temperature 19°C. Synaptosomes were prepared by procedure B.

We would like to emphasize that this model is essentially a phenomenological description and can thus hardly take into account the microscopic features of the bilayer. Namely, considering the shape and the size of amine anesthetics, it is evident that the fluidized bilayer can be described best as two-component system, whereby the drugs, owing to their amphiphatic nature, will be incorporated into the lipid array in an oriented fashion, disordering the membrane in dependence on their molecular structure. This is what we have found in the case of total lipid liposomes and synaptosomes.

The inequality of parameter P at the different depths of membranes clearly indicates that the effect of local anesthetics cannot be abolished by cooling in the case of liposomes. The same holds also for the membrane of synaptosomes, as clearly shown by the results demonstrated in Figs. 7 and 8.

Influence of lipid concentration on the extent of the perturbation effect

The dependence of order parameter, S, on the lipid concentration at a constant anesthetic concentration in the added buffer can be explained using the approach introduced by Ueda et al. [9] and Sikaris et al. [10]. Supposing that order parameter, S, of the *m*-DSA spin probe depends linearly on the local anesthetic concentration in the lipid phase, the change in S with the lipid/buffer ratio should depend on the partition coefficient, K_p, of the local anesthetic, which is defined as

$$K_p = \frac{n_L/V_L}{n_w/V_w} \tag{3}$$

where n_L and n_w are the numbers of local anesthetic molecules in lipid and water phases, respectively, V_L and V_w are the volumes of lipid and water phases, respectively, and density of both phases is 1.0 g/ml for simplicity. The change in the order parameter, S, as compared to the control sample without anesthetics can be quantified by $\Delta S = S_{Co} - S_v$, where S_{Co} is value of S for the control sample and S_v is value of S after addition of volume, V_w, of buffer containing k molecules per unit volume to the volume, V_L, of the dry lipid. It is easy to show that

$$\Delta S = \frac{\alpha \cdot k \cdot K_p \cdot V_w}{K_p \cdot V_L + V_w} \tag{4}$$

where α is constant. Fitting eq. 4 to the experimental data such as those shown in Fig. 4, we have obtained quite good agreement of the proposed theory with experimental results. In three experiments with dibucaine, the value of K_p was 62 ($r^2 = 0.996$), 35 ($r^2 = 0.975$) and 34 ($r^2 = 0.975$), in five experiments with tetracaine we have obtained the mean value $K_p = 23$ with mean quadratic deviation ± 9 and r^2 ranging from 0.973 to 0.986. It is clear that the accuracy of this approach is rather poor, because the changes in the order parameter, S, at low V_w/V_L ratios (under 10) are comparable to the error of ΔS estimation, while at a high V_w/V_L ratio (over 100) the noise in the spectra is increasing, again decreasing the accuracy of ΔS determination. Nevertheless, it is seen from the values given above that the partition coefficient of dibucaine is probably higher than that of tetracaine, so that the higher perturbation efficiency could be ascribed to the higher concentration of dibucaine in the lipid phase. Therefore, it was interesting to compare the perturbing effects of these anesthetics at the same anesthetic concentration in the lipid phase. This was done using the expression

$$R = \lim_{V_w \to \infty} \Delta S/K_p C \tag{5}$$

where C is the local anesthetic concentration in the added buffer. We have obtained $R = 0.24 \pm 0.03$ l/mol for tetracaine (the mean value and standard deviation from five experiments where C was 5–25 mM), and $R = 0.59 \pm 0.22$ l/mol for dibucaine (three experiments, $C = 2.5$ mM and 5.0 mM). The error of the estimation of R for dibucaine is high because of the limited number of experiments, but it is clearly seen that dibucaine is a more efficient perturbant than tetracaine at the same concentration in the lipid phase. However, it is important to notice that this conclusion was obtained with the 16-DSA spin probe. We are not using for the calculations of R the experimental data obtained with the 5-DSA spin probe, since it is known that this probe, with the bulky doxyl group located near the membrane polar region, disturbs the membrane significantly due to steric interactions [11].

Disordering efficiency of anesthetics

Local anesthetics can order, but also disorder, the membrane. The effect depends on the membrane composition as well as on the molecular structure and concentration of anesthetic. Butler et al. [12] and Neal et al. [4] have observed disordering of the membranes (using cholestane, 5-DSA and 12-DSA spin probes) formed from the total ox brain white matter lipids with the high concentration of cholesterol, but ordering was observed in membranes with cholesterol content 5%, both in the presence of 0.75–32 mM tetracaine. On the other hand, Rosenberg [13] has observed ordering (using the 5-DSA spin probe) of the mebrane of synaptosomes in the presence of lidocaine at a concentration of 0.1 mM, but disordering was observed at a concentration of 10 mM. One of the reviewers brought to our attention his/her observations with phosphatidylserine liposomes and

tetracaine – the effect of tetracaine was the increase of order at low concentrations and the decrease at high concentrations. He/she suggested that the intitial increase in order could be caused by the charge neutralization effects.

Which effect is more important from the biological point of view is unclear. Therefore, it is necessary to find correlations between the physical and biological effects of the anesthetics. In our experiments, we have observed both in liposomes as well as in synaptosomes only the disordering of the membranes at the higher concentrations indicated in the Fig. 1. Our results are thus in agreement with the observations on liposomes with high contents of cholesterol [12,4] as well as with observations on synaptosomes with higher concentrations of anesthetics [13]. Furthermore, we have found that the maximum disordering efficiency for liposomes and for synaptosomes is observed deep in the hydrocarbon core at C-16 acyl chain segments (Table I, Figs. 7 and 8). That is in agreement with the data of Boulanger et al. [14,15] for tetracaine in phosphatidylcholine membranes. They have found that the bulky benzenoid ring of tetracaine is located at least at the depth of the C-10 acyl chain segment. We have used data of Štolc and Beneš [17] together with the data obtained by Truanl and Takman [18] for the comparison of the relative potency of local anesthetics to block the action potential in isolated nerve with the perturbation effect of anesthetics on the liposome and synaptosome membranes observed in our experiments. In the Fig. 9, there is shown the correlation of the gradient $\Delta S/\Delta C$ for the three different depths of the membrane with the relative efficiency of local anesthetics to block action potential in the nerve. It is interesting that the biological effect correlates with the perturbation effect deeply in the hydrocarbon core of both the liposomal and synaptosomal membranes. Since the *m*-DSA spin probes mainly report the average membrane order, from the reasonably close similarity of disordering efficacies in the synaptosomes and liposomes of extracted lipids we concluded that the mode of action of this nonspecific lipid disordering displays close parallelism in the two systems. The greater propensity of local anesthetics to decrease order parameter S at the 16th carbon membrane depth than at the fifth one

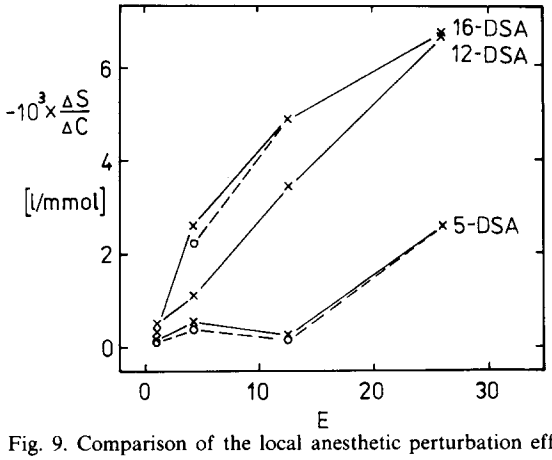

Fig. 9. Comparison of the local anesthetic perturbation effect with their relative potency to block action potential on the nerve, E. Full lines, total lipid liposomes; dotted lines, synaptosomes. Values of $\Delta S/\Delta C$ were taken from Table I. The relative potencies of local anesthetics were taken from literature [22,23]. Lidocaine·HCl, $E = 1.0$; tetracaine·HCl, $E = 4.2$; dibucaine·HCl, $E = 12.5$; heptacaine·HCl, $E = 26.0$.

in the liposomes and the synaptosomes supports the finding [14–16] that the polarizable part of the local anesthetic is located in the polar part of membrane, with the apolar tail penetrating into the hydrocarbon core parallel to the lipid acyl chains. It is possible that the main cause of the perturbation of the membrane structure is the creation of a free volume, ΔV, which is manifested in our experiment by the decrease of order parameter, S, at the 12th and 16th positions. This hypothesis is supported by our determination of parameter R, because R for dibucaine is greater than R for tetracaine. This can be explained by anesthetic-lipid interaction because of different shapes of dibucaine and tetracaine. Dibucaine produces a larger free volume in the C-16 depth of membrane than tetracaine. Because the stability of the membrane bilayer is dependent on the elastic energy $F \approx \Delta V^2$[19], the creation of free volume may induce a change in the overall structure of the membrane.

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