Biochimica et Biophysica Acta, 734 (1983) 319–328
Elsevier

BBA 71847

THE EFFECT OF ANAESTHETICS ON PROTEIN CONFORMATION IN MEMBRANES AS STUDIED BY THE SPIN-LABELLING TECHNIQUE

P. GRÓF * and J. BELÁGYI

Central Laboratory, Medical University, Pécs (Hungary)

(Received June 28th, 1983)

Key words: Anesthetic-membrane interaction; Lipid-protein interaction; Protein conformation; Spin label; (Frog sciatic nerve)

The effect of general and local anaesthetics was studied on the membrane components (proteins and lipids) of frog sciatic nerve using the spin-labelling technique. A rapid and effective method was developed to incorporate fatty acid labels into the lipid regions and to attach the maleimide spin labels to the thiol sites of the proteins of the membrane. On the basis of the rotational correlation time of the attached maleimide spin labels, at least three different protein domains were identified. One part of the labels has a preferred orientation with respect to the axis of the nerve fibre. The effect of halothane and of local anaesthetics such as lidocaine and tetracaine, which influence primarily the lipid regions of the membrane, is efficiently transferred to the spin-labelled membrane proteins via strong lipid-protein interaction. The results support the concept that the architecture and the physiological activity of the membrane-bound proteins are sensitive to changes in the physical state of membrane lipids.

Introduction

Anaesthetics are clinically useful drugs which exert their effects on membrane of the nerve by blocking conductance changes. In general, the mechanism of anaesthesia has been explained in terms of conductance changes in the nerve membranes, but the molecular events producing these alterations are not yet known exactly.

It has been suggested that the anaesthetics act at the hydrophobic site of the nerve membrane by certain physical mechanisms. It was found that anaesthetics expanded biological membranes [1] and the anaesthesia could be reversed by application of pressure [2–4]. It was also reported that the pressure effect on anaesthetics is not unambiguous [5,6].

In the last decade, spectroscopic techniques utilizing the spin-labelling method have been used to study the structural and motional characteristics of biological membranes [7–10]. Using these techniques, several important details about the inherent dynamical features of the membranes and model systems have been reported [11–13].

It has recently been suggested that local anaesthetics interact strongly with the polar headgroups of the phospholipid chains and produce an increase in the fluidity of the membranes [14–18]. The various drug molecules can trigger their effect by direct binding to specific sites of proteins or by perturbing the lipid regions necessary for the stability of protein conformation [19]. The perturbation produced by drugs in the lipid region of the membrane may translocate to the proteins and, as a result of this interaction, the tertiary structure of proteins may alter [20]. However, no evidence is as yet available to prove that it is on the nerve's membrane proteins that the local

* Permanent address: Biophysical Institute, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary.

anaesthetics primarily act, but an anaesthetic perturbation of protein structure was suggested [6].

In the present investigation, we report covalent labelling of proteins in nerve membrane with maleimide spin labels. The labels are rigidly bound to a protein domain which rotates with a correlation time of about 100 ns. The EPR spectra provide evidence that the effect of general and local anaesthetics is translocated from the lipid region to the protein segments bearing the spin labels. Measuring the rate of motion of spin probes, we compare the effect of halothane on the lipid and protein components of the membrane. A strong correlation between the protein conformational state and the physical state of the lipid region was observed.

Materials and Methods

Preparation and spin-labelling of membranes and membrane proteins

The frogs used in the experiments were of the species *Rana esculenta*. The nerves (n. ischiadicus) were always freshly prepared and desheathed before the experiments and were kept in refrigerator at 4°C in Ringer's solution for 1 h before spin-labelling. The composition of the Ringer's solution was 115.0 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$ and 2.4 mM $NaHCO_3$ in 1000 ml of bidistilled water (pH 7.0).

In earlier experiments, an exchange reaction, between bovine serum albumin-bound fatty acid and the membrane was used for incorporation of fatty acids into the lipid region of the nerve membrane [21,22]. In order to achieve an effective and rapid incorporation of the probe molecules into the lipid region of the membrane or for labelling the membrane proteins, the following procedure was used. First, 100–200 μg of the spin label was dissolved in 10–20 μl halothane, then using a TP 18 Ultra-Turrax (Jahnke-Kunkel) homogenizer, it was thoroughly mixed with 1.2 Ringer's solution at 4°C. The final concentration of spin label was $(3–7) \cdot 10^{-4}$ M. The nerves were immersed in the spin-labelling solution, either for 2–3 min in the case of fatty acid and for 12 min to label the membrane proteins. During the reaction, the solution was carefully shaken at room temperature to achieve a high degree of labelling. The spin labels used in the experiments were the fatty acid label from Syva (Palo Alto, CA; 2-(3-carboxypropyl)-4,4-dimethyl-2-tridecyl-3-oxazolidinyloxyl) or, for membrane proteins, the maleimide label.

It is known that the maleimide labels react very rapidly with thiol groups on different immobilizing sites. Therefore, the EPR spectra exhibit the spectra of labels arising from both weakly and strongly immobilizing sites [23]. But pretreating the frog nerves with *N*-ethylmaleimide at 1 mM concentration for 15 min before spin labelling (with or without halothane), it is possible to obtain an EPR spectrum which reflects the spin labels attached only to strongly immobilizing sites. After incubation, the nerves were transferred to a large volume of Ringer's solution to remove the labels from the water space of the nerve by stirring gently. Finally, the physiological state of the nerves was controlled in equipment consisting of an oscillograph and an impulse-generator. An irreversible blocking of action potential occurred between 10 and 15 min using –SH-directed reagents, but in the case of fatty acid labels, the effect of halothane could still be restored by washing the nerves for 30 min in Ringer's solution (Fig. 1).

EPR experiment

The spin-labelled nerves were usually measured in Zeiss flat cells (Carl Zeiss, G.D.R.) at room temperature. In other cases, the nerve was placed in a small capillary tube, which was transferred into the usual EPR sample tube. EPR spectra were taken by an ERS 220 spectrometer and temperature controller (Center of Scientific Instruments, G.D.R.) in the temperature range 0–40°C. Temperature was measured by a thermocouple and digital thermometer with an accuracy of 0.2°C. The thermocouple was mounted in the capillary tube. The stability of the temperature regulation was better than 0.5°C during a scan.

For conventional EPR measurement (first harmonic absorption in-phase), 100 kHz field modulation (0.02 mT amplitude) and 20 mW microwave were used. Second harmonic absorption out-of-phase (v_2') spectra were recorded with 50 kHz field modulation and detection at 100 kHz out-of-phase. The out-of-phase setting was achieved by shifting the phase of the modulation field at low microwave power, usually 1 mW, to

obtain a minimum signal [24]. The saturation-transfer spectra were taken at 80 mW microwave power, which corresponds to a microwave field amplitude of 0.026 mT. Field modulation amplitude was 0.05 mT and field calibration was made by nuclear magnetic resonance magnetometer (MJ-11OR Radiopan, Poland).

Results

Fatty acid labels in frog nerve membrane

The conventional first harmonic absorption in-phase EPR spectra of frog nerve membrane incorporated within the spin-labelled fatty acid are demonstrated in Fig. 2, at room temperature in two orientations: the long axis of the nerve was parallel ($H \parallel k$) and perpendicular ($H \perp k$) to the applied magnetic field. The experimental spectra belong to the sample, the action potential of which was imaged in Fig. 1. The spectra show clearly the preferred orientation of the labels with cylindrical symmetry. The motion of the labels in the lipid region can be characterized with a rotational correlation time of about $\tau_2 \approx 10^{-8}$ s estimated from the spectral parameters. Previous experiments had indicated that the fatty acid spin probes are oriented with their long molecular axis approximately parallel to the membrane and they rotate rapidly around this axis [7,8,25].

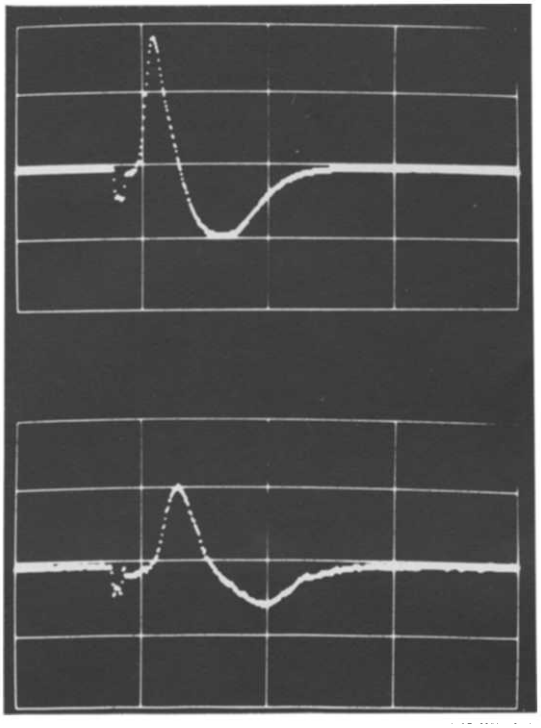

Fig. 1. Action potentials of n, ischiadicus from frog before (upper curve) and after (lower curve) incorporation of 192 μg fatty acid labels with 10 μl halothane in 1.2 ml of Ringer's solution into the lipid region of the membrane. Incubation time was 3 min. After labelling, the nerve was washed in a large excess of Ringer's solution for 30 min. Conditions for stimulation: $I = 50$ μA and $t = 0.1$ ms. Time base: 5 μs $\times 1024$; 16 single potentials were averaged for presentation.

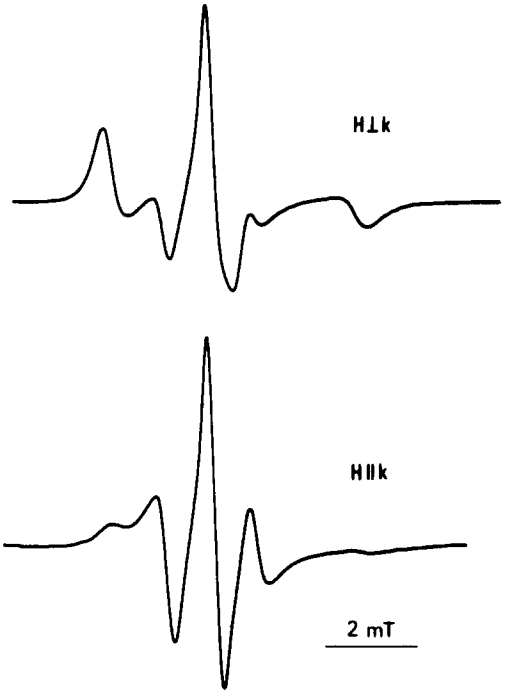

Fig. 2. EPR spectra of fatty acid spin labels incorporated into the lipid region of the nerve membrane from frog. The long axis of the nerve was oriented perpendicular ($H \perp k$) and parallel ($H \parallel k$) to the static magnetic field. The nerve was the same as in Fig. 1.

EPR spectra of spin-labelled membrane proteins

The conventional and saturation-transfer EPR spectra of membrane proteins labelled with the maleimide spin label are shown in Fig. 3. The nerves were pretreated before spin-labelling with *N*-ethylmaleimide for 10 min in order to block the fast-reacting groups in the membrane. Without

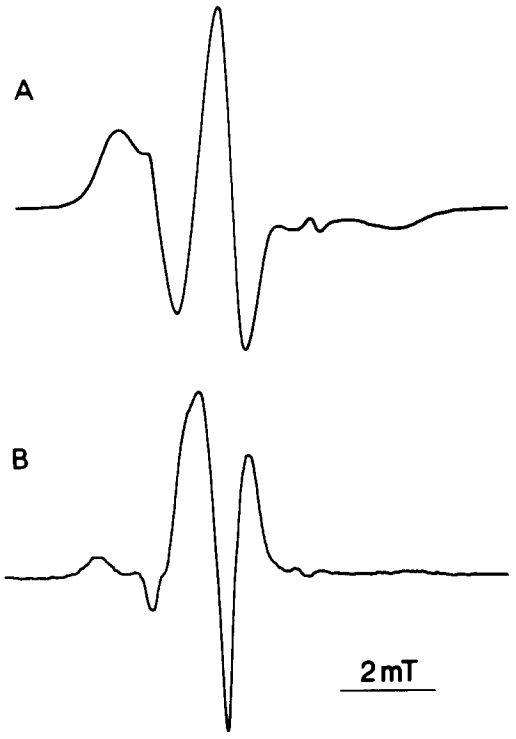

Fig. 3. EPR spectra of frog sciatic nerve labelled with the maleimide spin label. (A) First harmonic absorption in-phase EPR spectrum; (B) second harmonic absorption out-of-phase EPR spectrum. The nerve was pretreated with 1 mM *N*-ethylmaleimide for 10 min in 1.2 ml of Ringer's solution in the presence of 10 μl halothane, spin-labelled in 1.2 ml of Ringer's solution with $7 \cdot 10^{-4}$ M maleimide spin-label and 10 μl halothane. Reaction time was 12 min.

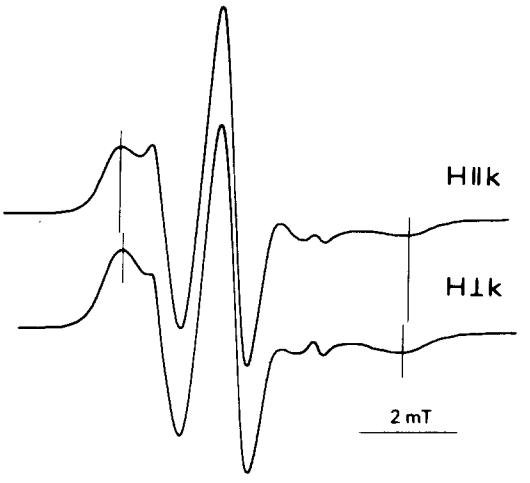

Fig. 4. Orientation dependence of the EPR spectra of spin-labelled membrane protein with respect to the long axis of the nerve. The nerve was treated with 1 mM *N*-ethylmaleimide for 15 min before spin-labelling in the presence of 10 μl/ml halothane in the bathing solution.

pretreatment with *N*-ethylmaleimide, the EPR spectrum of the nerve can be characterized as a superposition of the EPR spectra arising from radicals attached to both strongly and weakly immobilizing sites. The amount of spin labels bound to weakly immobilizing sites was estimated to be about 10% of the total absorption. The basic parameters measured in the experiments are the $A'_{\|}$ and $A'_{\perp}$ components of the motionally averaged nitrogen hyperfine tensor. The values were estimated from the EPR spectra, they are $A'_{\|} = 2.949$ mT and $A'_{\perp} = 1.076$ mT.

Orientation-dependence of the EPR spectra

It was found that the spectra of the nerves labelled with the maleimide spin labels after treatment with *N*-ethylmaleimide exhibit a dependence on orientation. The anisotropic distribution of spin labels with respect to the fibre axis of the nerves provides evidence that the labels are located on the same sites in a protein or a protein domain (Fig. 4).

Rotational motion of the spin labeles

In order to obtain the apparent rotational correlation time of the protein-bound spin label, the method of McCalley and co-workers [26] was performed using sucrose solutions of different concentration to vary viscosity. The values of viscosity were taken from the work of Othmer and Silvis [27]. The nerves were incubated for 1 h in Ringer's solution containing different amounts of sucrose and subsequently spectra were recorded. Failing an adequate method for determination of the microviscosity in the environment of the labelled membrane proteins, it was supposed that the viscosity of the membrane is the same as the viscosity of the bathing solution. In order to avoid artefacts due to specific interactions between the protein and sucrose, we have compared the physical situation of the probe molecule using glycerol, too, to enhance viscosity. The best-fit parameters for both sets of measurements were practically the same; therefore a single best-fit parameter was

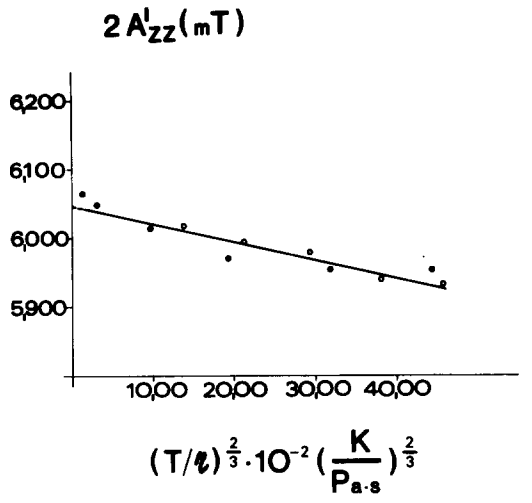

Fig. 5. Variation of $2A'_{zz}$ against $(T/\eta)^{2/3}$ for determination of the rotational correlation time of maleimide spin-labelled membrane proteins (○, sucrose; ●, glycerol). The nerve was pretreated and spin-labelled as described in Fig. 3.

used (Fig. 5). The extrapolation procedure resulted in an $2A'_{zz}$ value of 6.510 ± 0.03 and 6.010 ± 0.03 mT for spin-labelled membrane proteins. The smaller value was obtained for samples pretreated with *N*-ethylmaleimide before spin labelling. The rotational correlation times were calculated according to Goldman and co-workers [28]; they are 35 ns and 62 ns, respectively, at 23°C and at an apparent viscosity, η, of 1 mPa · s. The rotational correlation times for the weakly immobilized spin labels was calculated to be about 1 ns. From the plot of $1/\tau_2$ against T/η following Kuznetsov and co-workers [29], it can be derived that the labels are rigidly bound to the protein moieties in both cases, and therefore the apparent rotational correlation times reflect the motion of the entire protein or a large domain of it.

For samples spin-labelled after pretreatment with *N*-ethylmaleimide, the rotational correlation time is 0.12 μs, estimated from the C'/C parameter of the ST-EPR spectrum using the results of Thomas and co-workers [24]. The difference between the two values of τ_2 estimated from the conventional and saturation transfer EPR spectra can be accounted for the smaller sensitivity of ST-EPR parameters in the submicrosecond time range and/or anisotropic rotational motion of the protein.

For samples labelled with the maleimide spin labels without pretreatment with *N*-ethylmaleimide the presence of strong signals from weakly immobilized labeles prevented the direct estimation of the rotational correlation time of the strongly immobilized labels from the ST-EPR spectrum.

Temperature dependence of the EPR spectra

Spectra were recorded over the temperature range from 0 to 40°C (Fig. 6). The temperature stability of the labelled region provided different characteristics. The plot against temperature above 10°C shows a greater slope for nerves which had been pretreated with *N*-ethylmaleimide, then those labelled with the spin label without pretreatment. The results can be interpreted by the assumption that the labels are localized on protein domains in the interior of the membrane, where the tight molecular packing of the lipids is already reduced. An increase in temperature leads to a more drastic change in the fluidity in this region than on the surface of the membrane. This supports the fact that the protein conformation depends on the

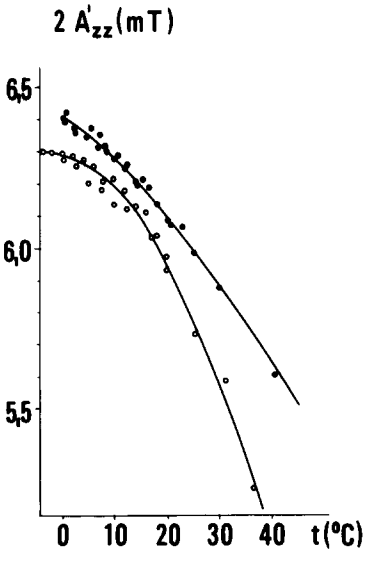

Fig. 6. Temperature dependence of the $2A'_{zz}$ parameter of spin-labelled frog nerves: ●, Frog nerves were labelled with $5 \cdot 10^{-4}$ M maleimide spin labels for 15 min in Ringer's solution in the presence of 10 μl/ml halothane. ○, Frog nerves were treated with 1 mM *N*-ethylmaleimide in the presence of 10 μl/ml of halothane before spin-labelling.

physical state of the lipid or water environment in a very sensitive manner. Over the temperature range recorded, no phase-transition was observed. On the other hand, it is interesting to note that a phase transition was observed at 38°C in the lipid region of the frog nerve using fatty acid labels [29]. It is possible that the heat denaturation of the protein alters the interaction between the hydrocarbon chains and the protein neighbourhood of the thiol sites [30].

Effect of general anaesthetics on nerve membrane

The influence of general anaesthetics (halothane, *n*-butanol and benzyl alcohol) on the mobility of the attached labels are demonstrated on Figs. 7 and 8. The nerves were kept in Ringer's solution containing halothane for 15 min at room temperature and label mobility was subsequently measured. In general, the measurements reveal that at a low concentration of general anaesthetics there are only minor and reversible changes in the EPR spectral parameters $2A'_{zz}$. But at higher concentration, $2A'_{zz}$ alters very rapidly and the spectral change exhibits a strong dependence on concentration (Table I). Above a critical concentration of anesthetics, irreversible changes in the protein conformation could be detected. Removing the halothane by washing the nerves overnight in Ringer's solution, a higher $2A'_{zz}$ (6.207 mT) was observed, which is greater than the value obtained for the rigid limit with the sucrose extrapolation. This indicates a change in the polarity as a consequence of irreversible structural alteration.

From the experimental findings, we can state that (1) the different labels reflect different dependence on anaesthetic concentration (see Table I); (2) the spin labels in the perturbed membrane are always more mobilized than the labels in the untreated membranes (Fig. 7). The first statement can be explained by the fact that the halothane which penetrates deeply into the membrane is not very effective at the surface of the membrane where the nitroxide groups of the fatty acid labels are localized. It is known that general anaesthetics fluidize the membrane by disordering the mem-

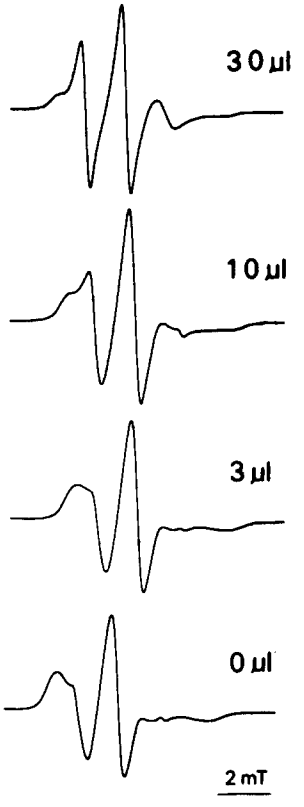

Fig. 7. Effect of halothane on the spin-labelled sites of membrane protein. The nerves were pretreated and spin-labelled as described in Fig. 3, and then were transferred for 15 min into Ringer's solution containing increasing amounts of halothane (per ml) as indicated on the figure. All spectra were taken at room temperature.

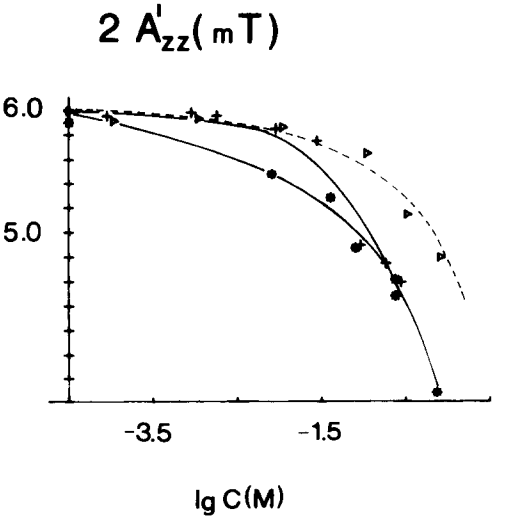

Fig. 8. Variation of $2A'_{zz}$ vs. drug concentration: +, halothane; ▽, *n*-butanol; * benzyl alcohol. The nerves were pretreated and spin-labelled as in Fig. 3. Incubation with the given drug concentration lasted for 15 min.

TABLE I

EFFECT OF HALOTHANE ON THE LIPID AND PROTEIN COMPONENTS OF THE FROG NERVE MEMBRANE

190 μg of the fatty acid label was disolved in 10 μl of halothane, mixed in 1.2 ml Ringer's solution. Incubation time was 3 min. After 30 min washing in a large excess of Ringer's solution, the nerves were immersed in Ringer's solutions containing the given amount of halothane, for 15 min at room temperature. Using covalent labels, the nerves were pretreated with 1 mM *N*-ethylmaleimide for 10 min and labelled in 1.2 ml Ringer's solution with $7 \cdot 10^{-4}$ M maleimide spin-label for 12 min in the presence of 10 μl of halothane. Incubation with the given amount of halothane lasted for 15 min. The pH was always 7.0. At the EPR measurements the long axis of the nerve was oriented perpendicular to the static magnetic field.

Halothane concentration in Ringer's solution (μl/ml)	$\Delta 2A'_{zz}$ (mT) [a]	
	Fatty acid label	Maleimide label
0.2	– [b]	0.04
1	– [b]	0.11
3	– [b]	0.19
10	0.13	1.17
30	0.23	1.37 [c]
50	0.27	–
80	0.29	– [d]
100	0.39	–

[a] $\Delta 2A'_{zz}$ is the decrease in the separation of the extrema after addition of halothane.

[b] No significant effect was observed within the limits of error (±0.025 mT).

[c] Irreversible changes in the EPR spectra were observed after removing the halothane by washing the nerves overnight in Ringer's solution.

[d] The presence of strong signals from weakly immobilized labels prevented the estimation of the separation between the outermost peaks.

brane components. Therefore, the increased mobility may indicate that a certain minimum rigidity of the lipid is essential for maintaining the tertiary structure of the membrane protein.

Influence of local anaesthetics on protein conformation

Three different local anaesthetics were studied and the results are given in Fig. 9 and Table II. It was already shown that the local anaesthetics interact strongly with the polar groups of the phospholipids at the membrane surface and in the interior of the membrane [15]. The procaine molecules are oriented horizontally to the surface, and the interaction is driven by ion-ion or ion-dipole attractive forces [16]. The increase in mobility obtained in our experiments reflects that the perturbations known to occur within the lipid region of the membrane are not attenuated in the immediate environment of the local anaesthetic molecules, but that they extend to the protein segment bearing the spin labels and they induce the loosening of the protein structure. The interaction is reversible and the effect on mobility correlate with the pharmacological potency of the local anaesthetics and depend on the concentration.

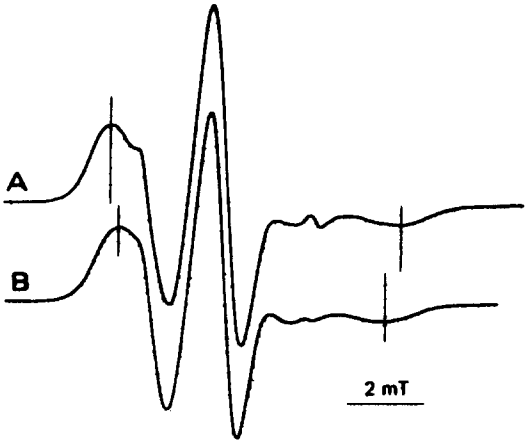

Fig. 9. Effect of tetracaine on the environment of the spin-labelled sites in membrane protein. (A) EPR spectrum of the nerve. Reaction conditions were the same as in Fig. 3. (B) EPR spectrum of the same nerve after treatment with 16.6 mM tetracaine·HCl for 15 min in Ringer's solution at room temperature (pH 7.0).

TABLE II

EFFECT OF LOCAL ANAESTHETICS ON THE EPR SPECTRAL PARAMETER OF THE MALEIMIDE SPIN-LABELLED NERVE FROM FROG

The nerves were pretreated and spin-labelled as in Table I. The nerves were incubated for 15 min in the solution of given concentration of the local anaesthetics at room temperature and subsequently the EPR spectra were measured.

	C (mM)	$\Delta A'_{zz}$ (mT) [a]
Procaine·HCl	3.6	no significant effect
	36.0	
Lidocaine·HCl	2.5	0.034 ± 0.025
	5.1	0.071 ± 0.025
	13.2	0.143 ± 0.025
	28.3	0.172 ± 0.025
	42.3	0.184 ± 0.025
	85.0	0.185 ± 0.025
Tetracaine·HCl	1.6	0.034 ± 0.025
	3.3	0.051 ± 0.025
	16.6	0.371 ± 0.025
	33.2	0.486 ± 0.025

[a] $\Delta 2A'_{zz}$ is the difference in the separation of the extrema evoked by addition of local anaesthetics.

Discussion

Excitability and spin-labelling of frog nerve

Anaesthetics exert their effect on membrane by blocking conductance changes which are thought to be essential to the nerve impulse transmission. The irreversible effect on excitability achieved with *N*-ethylmaleimide (and thiol-directed spin labels) also speaks in favour of the involvement of proteins playing an important role in the transient gating of ions occurring during excitation [32]. Our preliminary experiments on the bioelectric properties of frog sciatic nerves indicate a correlation between the disappearance of the electric signal associated with the nerve impulse and the appearance of a component in the EPR spectrum, which is characteristic for labels on weakly immobilizing sites. The EPR spectrum of the nerves, the excitability of which was blocked with the maleimide spin label, consists of a small strongly immobilized components ($2A'_{zz} = 6.207$ mT) with a strong mobile component superimposed. The rotational correlation time for these mobile labels is about 1 ns and the environment of the labels reflects a water-like polarity.

It can be concluded that the maleimide labels attached to a reactive site of the membrane proteins probably perturb the structure of the proteins and therefore,they lost the ability to contribute to the excitability.

Rotational motion of the labelled protein and the polarity of the labelled sites

In order to avoid the spectral component from a mobile population of labelled sites localized on the surface of the membrane, we pretreated the nerves with *N*-ethylmaleimide prior to spin labelling with maleimide analogue in the presence of a small amount of halothane. This procedure allows to identify a protein or a protein domain that rotates in the submicrosecond time range. Conventional and ST-EPR, used for the determination of the rotational correlation time of the labels gave $\tau_2 = 62$ ns, and 120 ns, respectively. From previous studies, it is known that the maleimide labels are attached to both strongly and weakly immobilizing sites adjacent to the polar regions of membrane [23,33]. The labels on weakly immobilizing sites in our case reflect a polar environment. The polarity of the components bound to strongly immobilizing sites, however, reflect an environment of hydrophobic polarity. It is interesting to note that an ascorbic acid solution caused an immediate disappearance of the EPR signal, but the unpaired electron is not accessible to paramagnetic ions such as nickel or manganese because no line broadening was observed after washing the labelled nerves in Ringer's solution containing 100 mM $NiCl_2$ or 1 mM $MnCl_2$.

The mechanism of anaesthetic action

Recently, two classes of structural perturbation were supposed to interpret the mechanism of anaesthetic action: (a) perturbation of protein structure through direct interaction with the anaesthetics; (b) perturbation of lipid structure which leads to perturbation of the protein structure via lipid-protein interaction [19].

Experiments reported in this study do not provide evidence against either of the hypotheses. A given concentration of general anaesthetics have smaller effect on the packing of the lipid molecules

near to their headgroups, but influence greatly the interior of lipid regions and the annular lipid around the protein molecules. The interaction between the anaesthetics and lipids leads to certain alteration in the protein dynamics in the neighbourhood of the labelled sites. This particular change in dynamics is manifested as mobilization of labels in the EPR spectra.

The experiments presented above, however, cannot exclude the possibility of a direct interaction between the local anaesthetic molecules and proteins which are responsible for nerve conduction. The binding of a local anaesthetic molecule to a specific site may lead to a new conformational state which is not favourable for the maintenance of nerve impulse transmission.

It should be noted that the accurate distribution of covalently attached labels in the protein system of the membrane is not known. The labelling procedures used in our experiments allow us to bind the spin labels to -SH groups which differ mainly in reactivity, polarity and mobility. Thus, the blocking of the -SH groups with maleimide derivatives does not result in a biologically site-specific labelling, but the reaction is mainly controlled by the physical and chemical properties of the environments of the thiol groups. Therefore, the anaesthetic-induced changes observed in the EPR spectra cannot be related entirely to conformational changes of specific proteins, such as those of sodium channels, which are responsible for nerve conduction. In this way, the EPR spectral reflect the superposition of structural perturbations from different domains of different proteins.

The negative result obtained with procaine can be explained by the fact that procaine molecules are preferentially oriented horizontally to the surface of the membrane and they are distributed mainly in the water region; therefore, the interaction is driven by electrostatic forces [16]. However, the tetracaine molecules are oriented parallel to the hydrocarbon chains and the molecules are located in the lipid bilayer [34]. This means that the tetracaine molecules may interact strongly with the hydrophobic interior of the membrane.

Calcium ions influence significantly the degree of molecular organization of lipid region [35,36]. It was suggested that the anionic groups of the phospholipid molecules might serve as binding sites for local anaesthetics and calcium ions. The calcium ions are able to exert a stabilizing effect by interaction with the anionic headgroups of the phospholipid molecules, whereas the local anaesthetic molecules fluidize the membrane by disordering the packing of the membrane components. The electrostatic nature of the interaction supposed earlier favours the well-known antagonism between local anaesthetics and calcium ions.

The Ca^{2+} concentration (18 mM) used in our study for perturbation of the membrane produced only a small increase in the separation of the extrema ($\Delta 2A'_{zz} \sim 0.1$ mT) which reflects a further immobilization in the environment of the attached labels. But in the presence of both tetracaine and Ca^{2+}, we observed only the effect of tetracaine. A possible explanation for this phenomenon is that the labels are localized in the interior of the membrane proteins and Ca^{2+} have smaller effect on the hydrocarbon core than the tetracaine.

Although the experiments presented above do not define the precise molecular events of local anaesthetic action, they nevertheless clearly demonstrate that some of the membrane proteins, or segments of them, are very sensitive to the presence of the anaesthetic molecules. All of our observations call attention of the fact that most, if not all, of the perturbations on the membrane lipids could affect the dynamic state of the membrane proteins. In order to obtain more insight into the nature of the molecular changes in the architecture of the membranes, it is planned to investigate protein-containing model systems with the help of the presented techniques.

Acknowledgements

The work is part of the research project 'Mechanism of Regulation in Life Processes', and was supported by a grant of the Hungarian Ministry of Health. The authors thank Dr. K. Hideg for supplying the maleimide spin label.

References

1 Seemann, P. and Roth, S. (1972) Biochim. Biophys. Acta 255, 171–177
2 Trudell, J.R., Hubbell, W.L. and Cohen, E.N. (1972) Ann. N.Y. Acad. Sci. 195, 530–537

3 Trudell, J.R., Hubbell, W.L. and Cohen, E.N. (1973) Biochim. Biophys. Acta 291, 321–327
4 Trudell, J.R., Hubbell, W.L. and Cohen, E.N. (1973) Biochim. Biophys. Acta 291, 328–334
5 Boggs, J.M., Roth, S.H., Yoong, T., Wong, E. and Hsia, J.C. (1976) Mol. Pharmacol. 12, 136–143
6 Boggs, J.M., Yoong, T. and Hsia, J.C. (1976) Mol. Pharmacol. 12, 127–136
7 McConnell, H.M. and McFarland, B.J. (1970) Q. Rev. Biophys. 3, 91–136
8 Jost, P.C., Waggoner, A.S. and Griffith, O.H. (1971) in Structure and Functions of Biological Membranes (Rothfield, L.I., ed.), p. 83–144, Academic Press, New York
9 Smith, I.C.P. and Butler, K.W. (1976) in Spin Labeling, Theory and Applications, (Berliner, L.J., ed.), pp. 411–451, Academic Press, New York
10 Schreier, S., Polnaszek, C.F. and Smith, I.C.P. (1978) Biochim. Biophys. Acta 515, 395–436
11 McConnell, H.M. (1976) in Spin Labelling, Theory and Applications (Berliner, L.J., ed.), pp. 525–560, Academic Press, New York
12 Griffith, O.H. and Jost, P.C. (1976) Spin Labelling, Theor and Applications (Berliner, L.J., ed.), pp. 433–523, Academic Press, New York
13 Marsh, D. (1981) in Electron Spin Resonance: Spin Labels in Membrane Spectroscopy, (Grell, E., ed.), pp. 51–142, Springer-Verlag, Berlin
14 Hubbell, W.L., Metcalfe, J.C., Metcalfe, S.M. and McConnell, H.M. (1970) Biochim. Biophys. Acta 219, 415–427
15 Ohki, S. (1970) Biochim. Biophys. Acta 219, 18–27
16 Papahadjopoulos, D. (1972) Biochim. Biophys. Acta 265, 169–186
17 Papahadjopoulos, D., Jacobson, K., Poste, G. and Shepard, G. (1975) Biochim. Biophys. Acta 394, 504–519
18 Giotta, G.J., Cargiulo, R.J. and Wang, H.H. (1973) J. Membrane Biol. 13, 233–244
19 Richards, C.D., Martin, K., Gregory, S., Keightley, C.A., Hesketh, T.R., Smith, G.A., Warren, G.B. and Metcalfe, J.C. (1978) Nature 276, 773–779
20 Papahadjopoulos, D., Moscarello, M., Eylar, E.M. and Isac, T. (1975) Biochim. Biophys. Acta 401, 317–335
21 Hubbell, W.L. and McConnell, H.M. (1969) Proc. Natl. Acad. Sci. U.S.A. 64, 20–27
22 Landsberger, F.R., Lenard, J., Paxton, J. and Compans, R.W. (1971) Proc. Natl. Acad. Sci. U.S.A. 68, 2579–2583
23 Jost, P., Brooks, U.J. and Griffith, O.H. (1973) J. Mol. Biol. 76, 313–318
24 Thomas, D.D., Dalton, L.R. and Hyde, J.S. (1976) J. Chem. Phys. 65, 3006–3024.
25 Libertini, L.J., Waggoner, A.S., Jost, P.C. and Griffith, O.H. (1969) Proc. Natl. Acad. Sci. U.S.A. 64, 13–19
26 McCalley, R.C., Shimshick, E.J. and McConnell, H.M. (1972) Chem. Phys. Lett. 13, 115–119
27 Othmer, D.F. and Silvis, S.J. (1948) Sugar 43, 32–33
28 Goldman, S.A., Bruno, G.V. and Freed, J.H. (1972) J. Phys. Chem. 76, 1858–1860
29 Kuznetsov, A.N., Ebert, B., Lassmann, G. and Shapiro, A.B. (1975) Biochim. Biophys. Acta 379, 139–146
30 Shummer, U., Hegner, D., Schnepel, G.H. and Wellhöner, H.H. (1975) Biochim. Biophys. Acta 394, 93–101
31 Eletr, S. and Inesi, G. (1972) Biochim. Biophys. Acta 290, 178–185
32 Huneens-Cox, F., Fernandez, H.L. and Smith, B.H. (1966) Biophys. J. 6, 675–689
33 Giotta, G.J. and Wang, H.H. (1973) Biochim. Biophys. Acta 298, 986–994
34 Johansson, L.B.-A. and Lindblom, S. (1981) Biophys. J. 36, 775–791
35 Papahadjopoulos, D. (1970) Biochim. Biophys. Acta 211, 467–477
36 Neal, M.J., Butler, K.W., Polnaszek, C.F. and Smith, I.C.P. (1976) Mol. Pharmacol. 12, 144–155