

Perturbation Effect of Local Anaesthetics on Synaptosomes: Variation with Depth of the Spin Label Probe*

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Abstract. Stearic acids labeled with a doxyl group at the 5th, 12th and 16th carbon positions were used as the spin probes for an investigation of the perturbation effects of two local anaesthetics (the 2-piperidinoethylester of phenyl carbamic acid and the piperidinoethylester of 2-heptyloxyphenylcarbamic acid) on rat brain synaptosomes. For a quantitation of the perturbation effects we have studied the order parameter *S* of the spin probe, incorporated in the membrane, calculated from the ESR spectra as a function of temperature and concentration of local anaesthetics. The local anaesthetics had a different disordering efficiency at different depths of the membrane. It follows from our results that the perturbing effects of the anaesthetics increase relative to the depth of the membrane, and the extent of perturbation correlates with local anaesthetic activity.

Key words: Local anaesthetics — Synaptosomes — ESR spectroscopy — Drug-membrane interaction

Introduction

Local anaesthetics are drugs which block the propagation of action potentials on axonal membranes by affecting the permeability of Na⁺ channels (Taylor 1959). Anaesthetics also influence various membrane processes, such as membrane transport of Ca²⁺ ions (Nash-Adler et al. 1980), aggregation of cells (Feinstein et al. 1976), exocytosis (Poste and Allison 1973), and several enzymatic activities connected with lipid metabolism in the membrane (Horáková and Štolc 1982). Tertiary amine local anaesthetics also have antiarrhythmic effects (Beneš et al. 1979).

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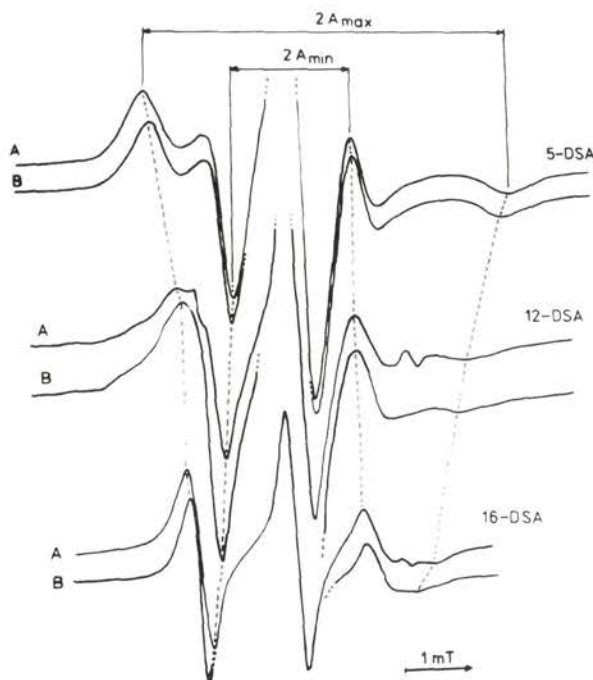


Fig. 2. ESR spectra of spin probes 5-DSA, 12-DSA and 16-DSA incorporated in the synaptosomal membranes. A — sample without anaesthetic. B — sample with 5 mmol/l, 2 mmol/l and 2 mmol/l heptacaine in 5-DSA 12-DSA and 16-DSA spectra, respectively, at room temperature.

perturb the membrane at a given depth. This efficiency was characterised using the parameter

$$D_m = \Delta S_m / \Delta C \quad (2)$$

where C is the anaesthetic concentration in the sample. Parameter D_m was introduced by Pang et al. (1980). To compare the efficiency with which anaesthetics perturb the membrane at different membrane depths, we have introduced the parameter P_m (Ondriaš et al. 1983) defined as

$$P_m = \frac{\Delta S_m / \Delta C}{(\Delta S_m / \Delta T)_0} \quad (3)$$

where $(\Delta S_m / \Delta T)_0$ is a temperature gradient of S_m in the control sample without anaesthetic. The parameter P allows to compare the anaesthetic disordering effect with that of temperature.

Results

Typical ESR spectra of spin probes 5-DSA, 12-DSA and 16-DSA incorporated in the synaptosomes are shown on Fig. 2, where spectrum A and B are a control

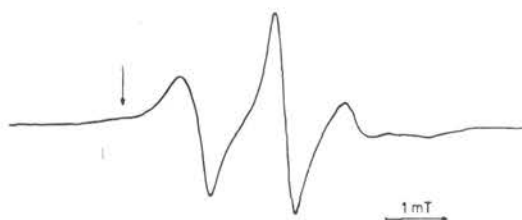


Fig. 3. ESR spectrum of spin probe 16-DSA in the synaptosomal membrane. Temperature 4 °C. Arrow indicates part of the immobilised spectra.

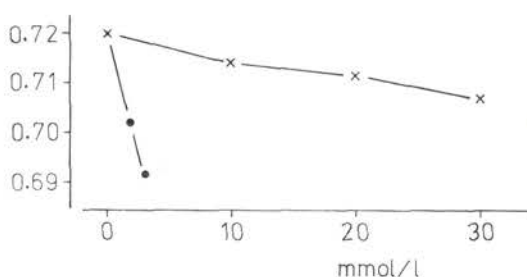


Fig. 4. Dependence of the parameter S_5 of 5-DSA spin probe in the synaptosomal membrane on local anaesthetic concentrations. Heptacaine (●), IR-1 (×). Temperature 25 °C.

sample and sample with the local anaesthetic heptacaine, respectively. On the spectra with the 16-DSA spin probe, we have seen superposition of strongly immobilised spin probe spectrum probably arising from the probe incorporated into the annular lipid region of the membrane proteins (marked by the arrow in Fig. 3) and the spectrum from the more mobile probe incorporated into the bulk fluid lipid region of the membrane. Such a superposition has been also observed with other membrane preparations, for example in the bovine rod outer segment membranes (Watts et al. 1979). Because of the poor signal: noise ratio of the immobilised spectrum in most of the samples, we have quantitatively evaluated only the mobile part of the spectra.

The values of the order parameter S_m calculated from the ESR spectra according to equation (1) in relation to different local anaesthetic concentrations are shown in Figs. 4–6. Fig. 4 shows the effect of heptacaine and IR-1 on the parameter S_5 as detected by 5-DSA spin probe. It is seen that local anaesthetics decrease the parameter S_5 in a concentration dependent manner, whereby heptacaine is several times more efficient than IR-1. Similar results are apparent in Fig. 5 and Fig. 6, where the parameter S_m was detected by 12-DSA and 16-DSA, respectively. To compare the efficiency of the anaesthetics to decrease S_m at one

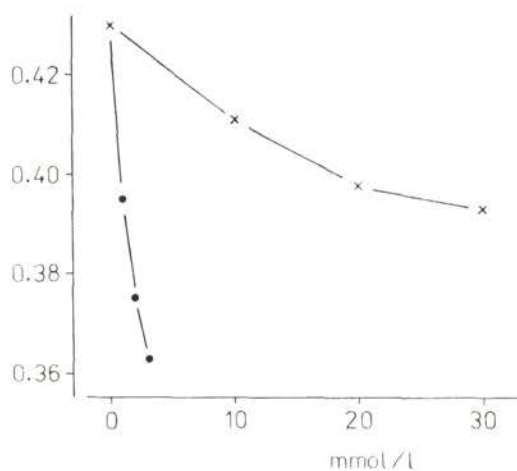


Fig. 5. Dependence of the parameter S_{12} of 12-DSA spin probe in the synaptosomal membrane on local anaesthetic concentrations. Heptacaine (●), IR-1 (×). Temperature 30 °C.

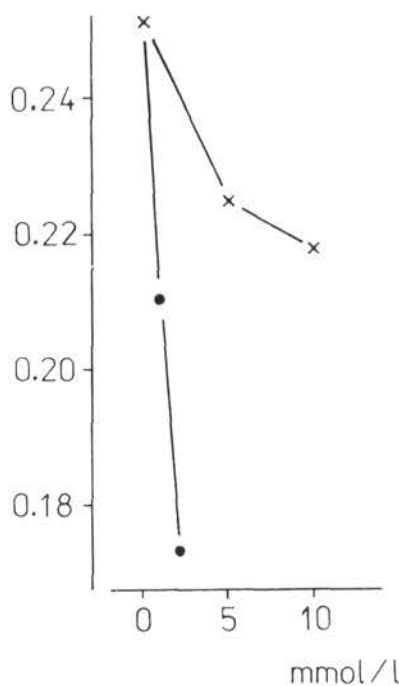


Fig. 6. Dependence of the parameter S_{16} of 16-DSA spin probe in the synaptosomal membrane on local anaesthetic concentrations. Heptacaine (●), IR-1 (×). Temperature 20 °C.

Table 1. Mean values (2–3 experiments) of efficiency parameters D_m and P_m of local anaesthetics heptacaine and IR-1 calculated from ESR spectra. D_m values are in (l/mmol) $\times 10^3$ units, P_m values are in ($^{\circ}\text{C} \cdot \text{l/mmol}$) units.

Sample	D_5	D_{12}	D_{16}	P_5	P_{12}	P_{16}
Heptacaine	-8.5	-27	-36	1.2	2.2	10.6
IR-1	-0.4	-2.4	-2.5	0.05	0.2	0.7

membrane depth the parameter D_m has been calculated according to equation (2). The D_m values are given in Table 1. Heptacaine is about 10–20 times more efficient in disordering the membrane than IR-1 at all three depths of the membrane as follows from a comparison of the D_m values in Table 1. The parameter S_m decreases approximately linearly with the increase in the temperature of the sample. The average gradients $(\Delta S_m/\Delta T)_0$ of the control samples (in the temperature range 25–30 $^{\circ}\text{C}$) were $(\Delta S_5/\Delta T)_0 = -0.008 \text{ K}^{-1}$, $(\Delta S_{12}/\Delta T)_0 = -0.011 \text{ K}^{-1}$, and $(\Delta S_{16}/\Delta T)_0 = -0.0034 \text{ K}^{-1}$. The gradient $(\Delta S_{16}/\Delta T)_0$ is approximately 2–3 times smaller than $(\Delta S_5/\Delta T)_0$ or $(\Delta S_{12}/\Delta T)_0$ ones. Taking into consideration this fact, we have compared the disordering effect of local anaesthetics at different depths of the membrane in the temperature scale, using the parameter P . The P_m values are given in Table 1. Comparison of the mean P values for heptacaine and IR-1 in the C_5 , C_{12} and C_{16} membrane depths shows that efficiency of local anaesthetics to disorder the membrane increases with the depth of the hydrocarbon core of the membrane and is more pronounced at the C_{16} carbon depth.

Discussion

As seen from the results summarised in Figs. 4–6, the values of the order parameters in control samples are decreasing in the order $S_5 > S_{12} > S_{16}$. At a fixed temperature (20 $^{\circ}\text{C}$), the average numerical values are $S_5 = 0.730$, $S_{12} = 0.530$ and $S_{16} = 0.241$. This trend is the same as observed by Harris et al. (1983) for rat synaptosomal membranes using different methods of preparation and handling (storage at -20 $^{\circ}\text{C}$) of synaptosomes. Such a decrease of order parameters in the hydrophobic core of the bilayer has been also observed on model phospholipid bilayers and various membrane preparations. Two different explanations have been offered for the gradient of order parameter in membranes. According to the first hypothesis, the acyl chains of lipids near the polar region of the membrane are cooperatively tilted with respect to the bilayer normal. This inclination of the chains near the polar region provides for an increased volume near the midplane of

the bilayer and, thus, decreased order. This explanation is mainly based on the results of ESR spectroscopy (McConnell 1976). Alternatively, a second explanation is based on Raman and NMR spectroscopy data, which indicate the average lipid chain direction coincident with the bilayer normal, and the presence of gauche isomers in the lipid chains (Gaber and Peticolas 1977; Seelig 1977). According to Dill and Flory (1980), the gradient of the order parameter is determined by the distribution of the chain ends in the hydrocarbon core of bilayers. Due to trans-gauche isomerisation, the chains can reverse direction and reenter the ordered layer which they emerged from, so that some of the chains terminate near the polar interface and the number of chains reaching deeper is diminished. Consequently, configurational freedom increases (i.e. order decreases) with depth. We prefer this explanation because of our experimental findings that the spin-lattice relaxation time, T_1 , of ^{13}C nuclei in terminal methyl groups of phosphatidylcholine acyl chains, determined by ^{13}C -NMR pulse techniques, is affected by the paramagnetic Gd^{3+} ions solvated in the water phase of water-lipid dispersion (Uhríková and Balgavý 1982). Let us explain our experimental observations using this second model of gradient of order parameter.

As shown in Figs. 4–6, and summarised in Table 1, local anaesthetics heptacaine and IR-1 decrease the order of the lipid part of the synaptosomal membrane. Rosenberg (1980) has observed ordering (using 5-DSA spin probe) of the synaptosomal membrane in the presence of lidocaine at a concentration of 0.1 mmol/l, but disordering was observed at a concentration of 10 mmol/l. In our study we have only observed the disordering effect, at all the local anaesthetic concentrations used — see Figs. 4–6. Furthermore, the absolute value of the D parameter is about 20 times higher for heptacaine, i.e. heptacaine is more efficient in disordering the membrane than IR-1. The biological anaesthetic potency is also higher in heptacaine as compared to IR-1 (Čižmárik et al. 1976). In our study, heptacaine was found to be 36 times more efficient than IR-1 in inhibiting the propagation of the action potential on the isolated rat sciatic nerve in vitro (EC_{50} for heptacaine and IR-1 were 1.9×10^{-5} mol/l and 6.9×10^{-4} mol/l, respectively).

However, for definite conclusions concerning the correlations between the anaesthetic potency and its disordering effect, the parameter D_m must be normalised using the partition coefficient between the water phase and the synaptosomal membrane, as reported for model phospholipid membranes in a previous study (Ondriaš et al. 1983).

The main conclusion of the present paper points to the relative values of the P_m parameter. The values of the P_m parameter increase in the order $P_5 < P_{12} < P_{16}$ for both local anaesthetics. These data indicate that both anaesthetics are incorporated into the lipid part of the membrane and affect the configurational freedom of the lipid chains. The greater propensity of anaesthetics to decrease the order parameter at the 16th carbon depth than at the 12th and the 5th carbon depths in

the synaptosomal membrane shows that anaesthetics penetrate into the membrane in a structure dependent manner. Disordering of the membrane which is dependent on the molecular structure of the anaesthetic is seen also from the ratios $P_5 : P_{12} : P_{16}$. When the experimental data are normalised so that the numerical values for P_5 are the same for both anaesthetics, we can deduce from the $P_5 : P_{12} : P_{16}$ ratios the extent of disordering of the membrane in terms of its depth and structure of the anaesthetics. The normalised numerical values for heptacaine are $P_5 : P_{12} : P_{16} = 1 : 2 : 9$, and for IR-1 $P_5 : P_{12} : P_{16} = 1 : 4 : 14$. It is clear from these data, that IR-1, despite of its lower absolute disordering efficiency, induces more configurational freedom for the acyl chains of lipids in the 12 th and 16 th carbon depth of the membrane than heptacaine. This is probably caused by the higher free volume created in the hydrophobic part of membrane after penetration into the bilayer in comparison with the heptacaine. In the case of heptacaine penetration into the membrane, the free volume is partly filled by heptyloxy chain. Because the free volume not only affects configurational freedom, but also the overall stability of the membrane (Derzhanski and Bivas 1979), its increase could eventually cause the local creation of nonlamellar structures in the synaptosomal membrane.

References

- Balgavý P., Ondriaš K. (1982): Effect of tetracaine and carbizocaine on the all brain lipid model membrane. In: Summer School "Liquid Crystals and Models of Biological Membranes". Smolenice, Czechoslovakia, September 6—10, 1982, Abstracts, p. 94
- Beneš L., Tichý M., Švec P., Kozlovský J., Štefek M. (1979): New basic carbanilates with local anesthetic and antiarrhythmic activity. *Europ. J. Med. Chim. Therap.* **14**, 283—284
- 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
- Čižmárik J., Borovanský A. (1975): Synthesis, u.v. and i.r. spectra of 2-piperidinoethyl esters of alkoxyphenylcarbamic acids. *Chem. Zvesti* **29**, 119—123
- Čižmárik J., Borovanský A., Švec P. (1976): Study of local anesthetics. LII. Piperidinoethyl esters of alkoxyphenylcarbamic acids. *Acta Fac. Pharm. Univ. Comenianae* **29**, 53—99
- Cullis P. R., Hornby A. P., Hope M. J. (1980): Effects of anesthetics on lipid polymorphism. In: *Molecular Mechanism of Anesthesia (Progress in Anesthesiology, Vol. 2)* (Ed. B. R. Fink), pp. 397—403, Raven Press, New York
- Derzhanski A., Bivas I. (1979): Influence of the lengths and conformation states of hydrocarbon chains on the stability of lyotropic phases. *Phys. Letters* **74A**, 372—374
- Dill K. A., Flory P. J. (1980): Interphases of chain molecules: Monolayers and lipid bilayer membranes. *Proc. Nat. Acad. Sci. USA* **77**, 3115—3119
- Feinstein M. B., Fiekers J., Fraser C. (1976): An analysis of the mechanism of local anesthetics inhibition of platelet aggregation and secretion. *J. Pharmacol. Exp. Ther.* **197**, 215—228
- Gaber B. P., Peticolas W. L. (1977): On the quantitative interpretation of biomembrane structure by Raman spectroscopy. *Biochim. Biophys. Acta* **465**, 260—274
- Gaffney B. J. (1976): Practical considerations for the calculation of order parameters for fatty acid or

- phospholipid spin labels in membranes. In: *Spin Labeling, Theory and Applications* (Ed. L. J. Berliner), pp. 564—571, Acad. Press, New York
- Hajós F. (1975): An improved method for the preparation of synaptosomal fractions in high purity. *Brain Res.* **93**, 485—489
- Harris J., Power T. J., Bieber A. L., Watts A. (1983): An electron-spin-resonance spin-label study of the interaction of purified mojave toxin with synaptosomal membranes from rat brain. *Europ. J. Biochem.* **131**, 559—565
- Hille B. (1977): Local anesthetics: Hydrophilic and hydrophobic pathways for the drug-receptor reaction. *J. Gen. Physiol.* **69**, 497—515
- Horáková L., Štolc S. (1982): Effect of some local anesthetics on phospholipid metabolism in the rat synaptosomal membranes. In: *Summer School "Liquid Crystals and Models of Biological Membranes"*. Abstracts. Smolenice, Czechoslovakia, Sept. 6—10, p. 97
- Jain M. K., Wu N. Y. M., Wray L. V. (1975): Drug induced phase change in bilayer as possible mode of action of membrane expanding drugs. *Nature* **225**, 494—495
- Lee A. G. (1976): Model for action of local anesthetic. *Nature* **262**, 545—548
- McConnell H. M. (1976): Molecular motion in biological membranes. In: *Spin Labeling, Theory and Applications* (Ed. L. J. Berliner), pp. 525—560, Acad. Press, New York
- Národa J., Balgavý P., Gawrisch K., Čizmárik J. (1983): Effect of local anesthetic heptacaine hydrochloride on the structured water in model phosphatidylcholine membrane: ^2D -NMR and ^{31}P -NMR study. *Gen. Physiol. Biophys.* **2**, 457—471
- Nash-Adler P., Louis C. F., Fudyma G., Katz A. M. (1980): The modification of unidirectional calcium fluxes by dibucaine in sarcoplasmic reticulum vesicles from rabbit fast skeletal muscle. *Mol. Pharmacol.* **17**, 61—65
- Ondriaš K., Balgavý P., Štolc S., Horváth L. I. (1983): A spin label study of the perturbation effect of tertiary amine anesthetics on brain lipid liposomes and synaptosomes. *Biochem. Biophys. Acta* **732**, 627—635
- Otero E. P., Otero E. A. (1975): Polimorfismo del sistema lecitina-agua en presencia de lidokaina — difracción de rayos X. Nueva fase laminar. *Anal. Quim.* **71**, 907—913
- Pang K. Y. Y., Braswell L. M., Chang L., Sommer T. J., Miller K. W. (1980): The perturbation of lipid bilayers by general anesthetics: A quantitative test of the disordered lipid hypothesis. *Mol. Pharmacol.* **18**, 84—90
- Poste G., Allison A. C. (1973): Membrane fusion. *Biochim. Biophys. Acta* **300**, 421—465
- Ráčanský V., Béderová E., Balgavý P. (1984): Decrease of gel-liquid crystal transition temperature in dipalmitoyl phosphatidylcholine model membrane in the presence of local anesthetics. *Stud. Biophys.* (in press)
- Richards C. D. (1980): In search of the mechanisms of anesthesia. *Trends Neurol. Sci.* **3**, 9—13
- Rosenberg P. H. (1980): Synaptosomal studies of fluidity caused by anesthetics. In: *"Molecular Mechanism of Anesthesia (Progress in Anesthesiology, Vol. 2),"* (Ed. B. R. Fink), pp. 325—334, Raven Press, New York
- Rosenberg P. H., Alila A. (1982): Hydrophobic membrane interaction of etidocaine, bupivacaine and 2-chloroprocaine. A spin and fluorescent probe study. *Naunyn-Schmied. Arch. Pharmacol.* **319**, 95—100
- Rosenberg P. H., Eibl H., Stier A. (1975): Biphasic effects of halothane on phospholipid and synaptic plasma membranes: A spin label study. *Mol. Pharmacol.* **11**, 879—882
- Seelig J. (1977): Deuterium magnetic resonance: Theory and application to lipid membranes. *Quart. Rev. Biophys.* **10**, 353—418
- Seeman P. (1972): The membrane actions of anesthetic and tranquilizers. *Pharmacol. Rev.* **24**, 583—655

- Štolc S., Beneš L. (1980): Effect of the carbanilates pentacaine (Pe) and heptacaine (He) on isolated motoric axons. *Čs. Fysiol.* **29**, 74 (in Slovak)
- Taylor R. E. (1959): Effect of procaine on electrical properties of squid axon membrane. *Am. J. Physiol.* **196**, 1071—1078
- Trudell J. R. (1977): A unitary theory of anesthesia based on lateral phase separations in nerve membranes. *Anesthesiology* **46**, 5—19
- Uhříková D., Balgavý P. (1982): ^{13}C -NMR spin-lattice relaxation times in egg yolk phosphatidylcholine model membranes. In: Proc. "Seminar on Molecular Mobility and Energy Transfer in Polymer Systems", (Ed. A. Havránek), High Tatras, Czechoslovakia, April 25 — May 1, pp. 220—221
- Watts A., Volotovskii I. D., Marsh D. (1979): Rhodopsin-lipid associations in bovine rod outer segment membranes. Identification of immobilized lipid by spin-labels. *Biochemistry* **18**, 5006—5013

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