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 Štole 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

\[ D_m = \frac{\Delta S_m}{\Delta C} \]  

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

\[ P_m = \frac{\Delta S_m}{(\Delta S_m/\Delta T)_o} \]  

where \((\Delta S_m/\Delta T)_o\) 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
Perturbation of Synaptosomes by Local Anaesthetics

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 that 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 $S_1$ 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 $S_0$, 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 $(°C \cdot l/mmol)$ units.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$D_5$</th>
<th>$D_{12}$</th>
<th>$D_{16}$</th>
<th>$P_5$</th>
<th>$P_{12}$</th>
<th>$P_{16}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heptacaine</td>
<td>-8.5</td>
<td>-27</td>
<td>-36</td>
<td>1.2</td>
<td>2.2</td>
<td>10.6</td>
</tr>
<tr>
<td>IR-1</td>
<td>-0.4</td>
<td>-2.4</td>
<td>-2.5</td>
<td>0.05</td>
<td>0.2</td>
<td>0.7</td>
</tr>
</tbody>
</table>

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 °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 $-20 °C$) 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 $\text{Gd}^{3+}$ 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 dis ordering 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 $1.9 \times 10^{-5}$ mol/l and $6.9 \times 10^{-4}$ mol/l, respectively).

However, for definite conclusions concerning the correlations between the anaesthetic potency and its dis ordering 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 12th and 16th 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


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