pH-Dependent Effects of Local Anaesthetics in Perturbing Lipid Membranes

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Abstract. The effects of local anaesthetics lidocaine, benzocaine, carbisocaine and carbisocaine derivatives, KaQ-7 and Ka-O, in perturbing bovine brain lipid membranes or egg lecithin membranes were compared at pH 6.0; 7.0; and 8.0. The electron spin resonance method with stearic acid labeled at carbon position 16 as the spin probe was employed. The perturbation effects of lidocaine and Ka-O were found to increase with increasing pH of the sample, whereas the effect of carbisocaine decreased with increasing pH. The perturbation effects of benzocaine and KaQ-7 were independent of pH. The pH-dependent perturbation effects of the local anaesthetics tested on lipid membrane fairly corresponded with their pH-dependent potency to block nerve action potentials.

Key words: Local anaesthetics — Lipid membrane perturbation — ESR spectroscopy — pH dependence

Introduction

The potency of some local anaesthetics to block nerve action potentials depends on pH. Strobel and Bianchi (1970) observed that the local anaesthetic action of lidocaine on intact frog sciatic nerves was increased at pH 9.2 compared to that at pH 7.2, whereas the blocking potency of quaternary derivatives of lidocaine, QX-314 and QX-572 in squid giant axons was independent of pH (Frazier et al. 1970). The pH-dependence of the blocking potency of the lipophilic local anaesthetic carbisocaine and its hydrophilic derivative Ka-O and quaternary derivative KaQ-7 on rat sciatic nerve have been studied in our laboratory. The efficiency of carbisocaine has been found to decrease with increasing pH, whereas that of Ka-O to increase with increasing pH; the effect of KaQ—7 was independent of pH (Stankovičová et al. 1986)

The aim of the present work was to investigate the efficiency of local anaesthetics in perturbing lipid membrane at different pH, and to compare this efficiency with the potency of the local anaesthetics to block action potential on nerves.

Materials and Methods

Lidocaine. HCl was from Pfaltz and Bauer (USA). Benzocaine was from Medica (Czechoslovakia). Carbisocaine, Ka-O and KaQ-7 were synthesized by Dr. L. Beneš and Dr. H. Szöcsová (Inst. Exper. Pharmacol., Bratislava). The chemical formulae of the anaesthetics tested are shown in Fig. 1. The stearic acid spin label with a dimethyloxazolidinyl group at carbon 16, I(1,14), was purchased from SYVA (USA). All other chemicals were from commercial sources and were of analytical grade. Total lipids from bovine brain were isolated according to Folch et al. (1957), and yolk lecithin according to Singleton et al. (1965). Samples for ESR measurements were prepared as follows: Spin labeled stearic acid (spin probe lipid molar ratio of at least 1 : 100) was dissolved in chloroform methanol and the solvent was evaporated in a stream of nitrogen followed by evacuation. The lipids were hydrated with a buffer containing 100 mmol l NaCl, 20 mmol l TRIS and 20 mmol l PIPES . HCl at a given pH, and vortexed. The respective local anaesthetic in the buffer was added to the lipid suspension. In order to attain equilibration of the local anaesthetics in the lipid membrane the samples were sonicated in bath and subjected to freeze-thaw-vortex cycles for several times. The final lipid buffer ratio in the samples was 1 : 30 w w. The concentrations of local anaesthetics reported in the present paper refer to those in the aqueous phase immidiately after drug addition and not to final concentrations after equilibration with membranes. Fifty µl of the sample suspension was filled into a glass capillary and ESR spectra were recorded in an ERS-230 spectrometer.

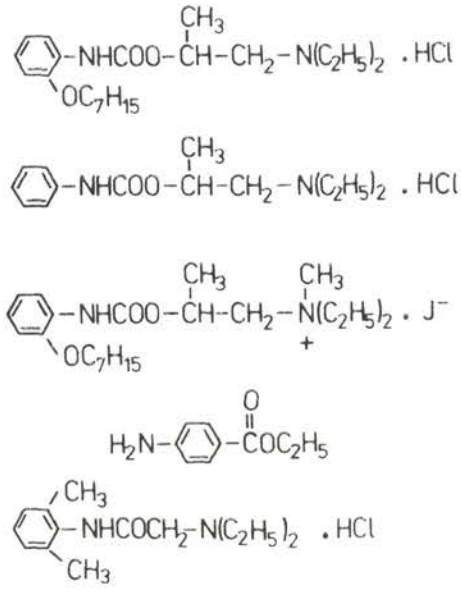

Fig. 1. Chemical formulae of local anaesthetics. From top to bottom: carbisocaine, Ka-O, KaQ-7, benzocaine and lidocaine.

To assess the relative efficiency of the local anaesthetics in perturbing the lipid membranes at different pH, the parameter $A_\perp$ was evaluated directly from the inner splitting of ESR spectra. The parameter $A_\perp$ is linearly proportional to the order parameter of the spin probe in the membrane (Gaffney 1976). The higher the $A_\perp$, the lower the order parameter, and the higher the disorder (fluidity) of the membrane. The values of $A_\perp$ were expressed in Tesla units. Since, in the samples containing lecithin the motion of the spin probe I(1,14) was not sufficiently anisotropic to allow resolution of the outer and inner hyperfine splittings, the rotational correlation time τ_B was calculated from the ESR spectra according to Lai and Cheng (1982). For relative comparison of the anaesthetic efficiency in perturbing membrane, the gradients $\Delta A_\perp/\Delta C$ were used (Ondriaš et al. 1983; Ondriaš et al. 1984). The higher the absolute value of the gradient, the higher the efficiency of the anaesthetic in perturbing lipid membrane.

Results

The perturbation effects of lidocaine and carbisocaine at the membrane carbon 16 level were pH-dependent, as revealed by the spin probe I(1,14) (Fig. 2). The parameter $A_\perp$ for control samples was higher at pH 6.0 than at pH 8.4. From a comparison of the gradients of $\Delta A_\perp/\Delta C$ at pH 8.4 and pH 6.0 it follows that lidocaine was 1.9 times more efficient in perturbing the membrane at pH 8.4 than at pH 6.0; carbisocaine acted in the opposite direction, being 9.4 times more efficient at pH 6.0 than at pH 8.4.

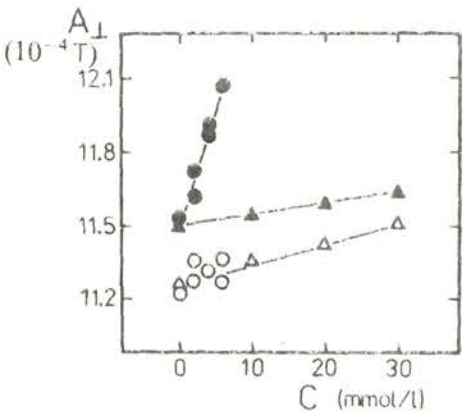

Fig. 2. The dependence of parameter $A_\perp$ of the spin probe on carbisocaine (circles) and lidocaine (triangles) concentrations (C) in total lipid membranes at pH 6.0 (full symbols) and pH 8.4 (open symbols). Temperature 23 °C.

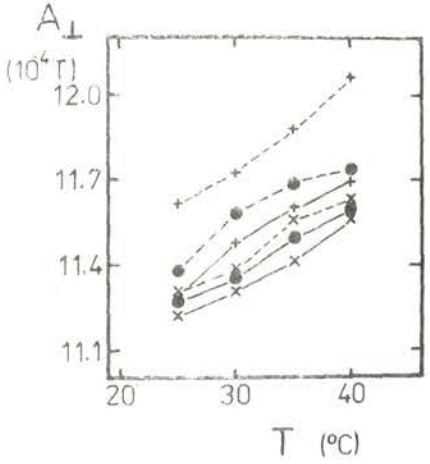

Fig. 3. The temperature dependence of parameter $A_\perp$ of the spin probe in total lipid membranes at pH 6.0 (+), 7.0 (●) and pH 8.0 (×). Full lines represent control samples and broken lines samples with carbisocaine (5 mmol/l).

The temperature dependence of the parameter $A_\perp$ of the spin probe in a control bovine lipid membrane and a membrane with carbisocaine are shown in Fig. 3. The parameter $A_\perp$ increased approximately linearly with the temperature in all

samples. In the control sample, $A_\perp$ increased with the decreasing pH. Carbisocaine shifted the parameter $A_\perp$ to higher values. To compare the effects of carbisocaine on membrane fluidity at different pH, the values of $A_\perp$ at 37 °C for carbisocaine and the control sample, calculated from the temperature dependence, were substracted at the given pH. The values of $\Delta A_\perp$ substracted for carbisocaine are given in Fig. 4. The parameter $\Delta A_\perp$ for 5 mmol/l and 10 mmol/l carbisocaine decreased with increasing pH. The parameters $\Delta A_\perp$ for Ka-O, KaQ-7 and benzocaine at different pH are shown in Fig. 5. The parameter $\Delta A_\perp$ increased with increasing pH for Ka-O, yet it did not significantly depend on pH for KaQ-7 or benzocaine.

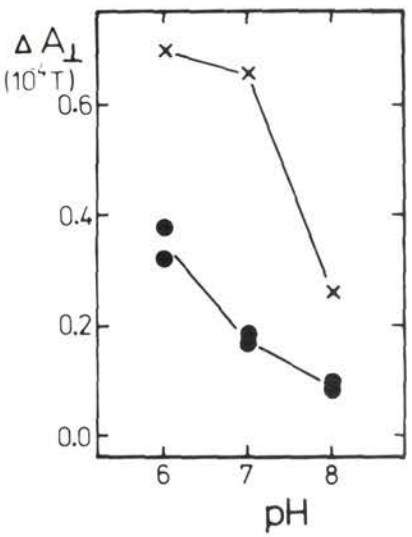

Fig. 4. pH-dependence of parameter $\Delta A_\perp$ of the spin probe in total lipid membranes for carbisocaine at 5 mmol/l (●) and 10 mmol/l (×). Temperature 37 °C.

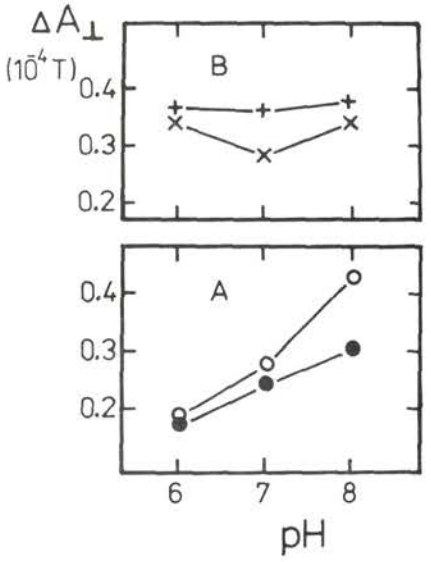

Fig. 5. pH-dependence of parameter $\Delta A_\perp$ of the spin probe in total lipid membranes containing local anaesthetics at concentrations: *A* — Ka-O, 40 mmol/l (●) and 80 mmol/l (○); *B* — KaQ-7, 5 mmol/l (+), and benzocaine 40 mmol/l (×). Temperature 37 °C.

To investigate the pH-dependent perturbation effect of carbisocaine on a simpler lipid membrane, the lecithin-buffer system was used. Since the motion of the spin probe I(1,14) was almost to be isotropic, the rotational correlation time τ_B was calculated from the ESR spectra. From the Debye equation the correlation time of the spin probe should depend linearly on η/T, where η is the viscosity and T is the temperature in °K. We therefore plotted the values of $\ln(T \,.\, \tau_B)$ versus temperature (Fig. 6) to estimate the fluidization effect of carbisocaine on the lecithin membrane. Fig. 6 shows that the value of $\ln(T \,.\, \tau_B)$

decreased with the increasing temperature for control samples and for samples with carbisocaine. The values for carbisocaine were lower than those for controls at each given pH. However, no significiant pH-dependent effect of carbisocaine could be observed.

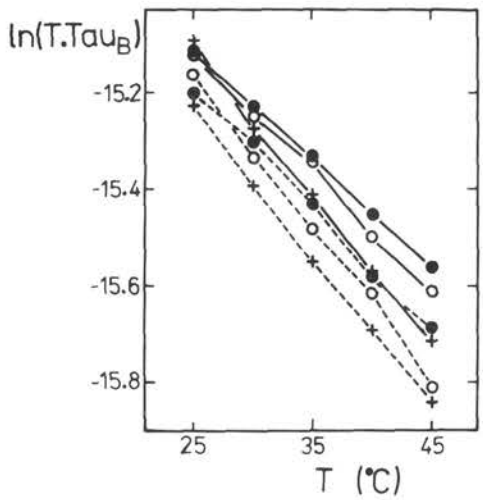

Fig. 6. The temperature dependence of parameter $\ln(T.\tau_B)$ of the spin probe in lecithin membranes at pH 6.0 (●) 7.0 (○) and 8.0 (+). Full lines represent control samples and broken lines samples with carbisocaine (10 mmol/l).

Discussion

We observed that the effect of some local anaesthetics in perturbing lipid membranes was pH-dependent. The perturbation effects of lidocaine and Ka-O (Figs 2, 5) increased with pH, corresponding to their pH-dependent anaesthetic potency. Strobel and Bianchi (1970) found that the local anaesthetic action of lidocaine on intact frog sciatic nerves was increased at pH 9.2 as compared to that at pH 7.2. Stankovičová et al. (1986) found the anaesthetic action of Ka-O on isolated rat sciatic nerve in vitro to increase with increasing pH. The EC_{50} values of Ka-O at pH 6.0, 7.2 and 8.4 were (in μmol/l) 230 ± 30; 24 ± 3; and 23 ± 3, respectively.

The perturbation effects of carbisocaine concentrations tested were pH-dependent in bovine brain lipid membranes, but they were pH independent in lecithin membranes. This may suggest that the pH-dependent effect would depend on the lipid composition of the membrane. The perturbation effect of carbisocaine in bovine brain lipid membranes decreased with increasing pH (Fig. 4) as corresponding to the pH-dependent potency of this anaesthetic to block action potential on isolated rat sciatic nerve in vitro, with EC_{50} values at pH 6.0, 7.2, and 8.4 pH of (in μmol/l) 0.8 ± 0.1; 7 ± 1; and 20 ± 2, respectively (Stankovičová et al. 1986).

The lipid membrane perturbing effects of benzocaine and KaQ-7 (Fig. 5) did not significantly depend on pH; this also is in agreement with their anaesthetic potency since Hille (1977) found that the effect of benzocaine on single myelinated fibers from frog sciatic nerve was pH-independent, and Stankovičová et al. (1986) found EC_{50} values of anaesthetic potency of KaQ-7 on isolated rat sciatic nerve in vitro of (in μmol/l) 25 ± 4; 44 ± 4; and 49 ± 3 at pH 6.0; 7.2; and 8.4, respectively.

The pK_a values of the tertiary amine local anaesthetics lidocaine, carbisocaine and Ka-O in solution are within a range of 7—9 (the pK_a values may be shifted in lipid membranes). Consequently, these anaesthetics are mostly positively charged at low pH while being mostly neutral at higher pH. Since the perturbation effects of the tertiary amine anaesthetics were pH-dependent, and the effects of the neutral anaesthetic benzocaine and the quaternary anaesthetic KaQ-7 were not significantly pH-dependent, the perturbation effects may be assumed to be associated with the ratio of neutral to positively charged anaesthetics in the membrane. The perturbation effect of carbisocaine decreased and that of Ka-O increased with increasing pH. Since the chemical formulations of the latter anaesthetics are similar, with carbisocaine being more lipophilic as compared to Ka-O, their opposite pH-dependent perturbation effects may be associated with their different lipophilicity (Štolc and Stankovičová 1986).

The correspondence between the pH-dependent propensities of the local anaesthetics to perturb lipid membranes and to block action potential on nerves suggests, that the pH-dependent anaesthetic potencies of these drugs may be mediated, in part at least, by their perturbation effects on membranes.

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