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Effect of a local anaesthetic on hydrocarbon chain order in membranes

THE mode of action of anaesthetics has provoked considerable interest and speculation[1,2]. For tetrodotoxin[3], it is clear that direct interaction of the anaesthetic with the sodium channel blocks nerve conduction; in other cases changes in lipid bilayer structure, such as membrane expansion[2], fluidus expansion[1] or surface-charge modulation[4], have been invoked. Recently, a mechanism involving an increase in lipid bilayer thickness has been suggested from results obtained using black film techniques[5–7]. We have investigated the hydrocarbon chain length of a lecithin bilayer in the presence and absence of the anaesthetic benzyl alcohol[8,9] using high-field deuterium nuclear magnetic resonance (NMR) spectroscopy of specifically deuterated lipids[10–16], to determine the changes in bilayer structure that occur on addition of the anaesthetic alcohol. We report here that at concentrations used for local anaesthesia there is no change in membrane thickness. By comparison, cholesterol (at 0.3 mol fraction) causes an ~0.46 nm increase in membrane thickness. We therefore suggest that the amount of solvent (tetradecane) in black lipid membranes, and hence their thickness, may be influenced by the presence of benzyl alcohol and cholesterol[5].

Figure 1*a* shows the effect of benzyl alcohol on the deuterium quadrupole splitting of a dimyristoyl lecithin labelled as $^2H_2$ in the sixth position of the 2-chain. As the concentration of benzyl alcohol increases, the quadrupole splitting and therefore the order parameter of the labelled methylene segment[13] decreases. This suggests a decrease in order of the 2-chain; cholesterol causes an increase[10,11,15,16]. Determination of the change in order parameter at each segment in the hydrocarbon chain permits evaluation of the total change in hydrocarbon chain length[10,13,17]. We determined the quadrupole splittings, and the order parameters, for a range of dimyristoylphosphatidylcholines labelled (as C^2H_2 or C^2H_3) at one of positions C-2, C-3, C-4, C-6, C-8, C-10, C-12, or C-14, in the 2-chain, at a total lipid–anaesthetic mol ratio of 1:3. In Fig. 1*b* representative spectra illustrate the effect of benzyl alcohol on the deuterium quadrupole splittings of a series of ^2H-labelled phosphatidylcholines, and Fig. 1*c* shows the effects produced by benzyl alcohol and cholesterol on these splittings. Benzyl alcohol and cholesterol have opposite effects on the order of the phosphatidylcholine acyl chains. Benzyl alcohol (above the gel–liquid crystal phase transition temperature of the phospholipid) causes a decrease in hydrocarbon chain quadrupole splitting or order parameter; cholesterol (above the gel–liquid crystal phase transition temperature of the phospholipid) causes an increase in hydrocarbon chain quadrupole splitting, or order parameters. Qualitatively, therefore, benzyl alcohol decreases and cholesterol increases hydrocarbon chain order.

At anaesthetic concentration, however, there is no effect of benzyl alcohol on membrane thickness. Local anaesthetic concentrations of benzyl alcohol are 4 mM for rat phrenic nerve and 12 mM for frog sciatic nerve[9]. At 40 °C the partition coefficient of benzyl alcohol between multi-bilayers of dimyristoyl

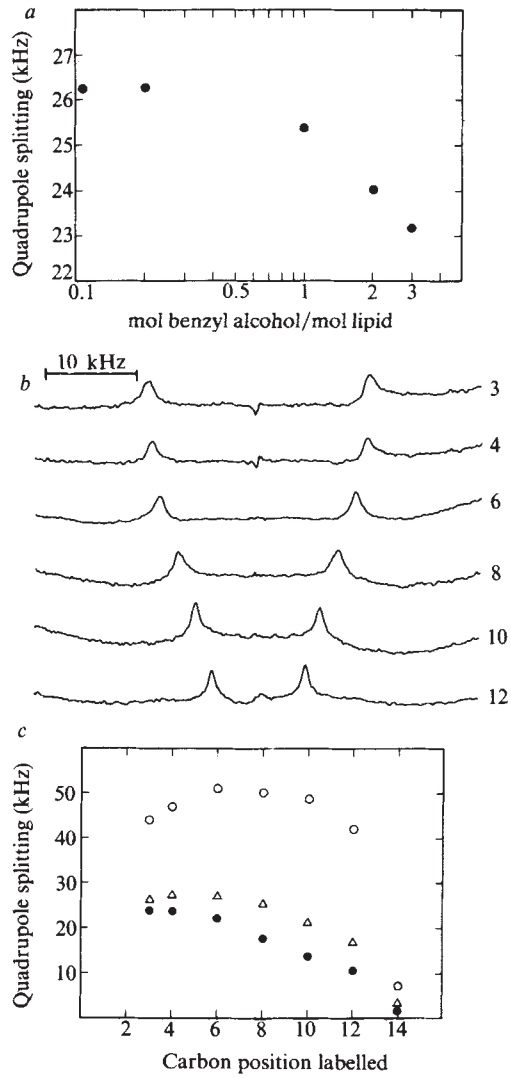

Fig. 1 Effects of benzyl alcohol, and of cholesterol, on deuterium NMR quadrupole splittings of specifically ^2H-labelled dimyristoylphosphatidylcholines. *a*, Graph of the quadrupole splitting of 2-(6′, 6′-d_2)dimyristoylphosphatidylcholine (10 mg) dispersed in excess deuterium-depleted water (250 μl) containing benzyl alcohol at 38° C, as a function of the benzyl alcohol–lipid mol ratio. The pure lipid quadrupole splitting value is 26.2 ± 0.5 kHz. *b*, Representative deuterium Fourier transform NMR spectra of dimyristoylphosphatidylcholines, labelled in the 2-chain as C^2H_2 at the positions indicated. The samples contained a benzyl alcohol–lipid mol ratio of 3:1; spectra were recorded at 38 °C. *c*, Graph of quadrupole splittings of specifically deuterated dimyristoylphosphatidylcholines as a function of position labelled in the hydrocarbon chain, in the presence and absence of benzyl alcohol (3:1 benzyl alcohol–lipid mol ratio) or cholesterol (30 mol % cholesterol). Pure lipid and lipid–benzyl alcohol data obtained at 38 °C, lipid–cholesterol data obtained at 30 °C. Deuterium NMR spectra were obtained using the Fourier transform method at 5.2T (corresponding to a deuterium resonance frequency of 34 MHz) on a homebuilt instrument[10]. The synthesis of dimyristoylphosphatidylcholines specifically deuterated in the 2-chain are described elsewhere[10]. Benzyl alcohol was added as an aqueous solution to dry phospholipid and the mixtures homogenised at about 40 °C. △, Dimyristoylphosphatidylcholine alone; ○, in the presence of cholesterol; ●, in the presence of benzyl alcohol.

0028-0836/79/0277—0669$01.00

lecithin and water is 13.9 according to Katz and Diamond[18]. At local anaesthetic levels, the benzyl alcohol–dimyristoyl lecithin mol ratio is 0.04–0.11. As may be seen from Fig. 1*a* there is no decrease in the electric quadrupole splitting from the pure lipid value of 26.2±0.5 kHz in this range of benzyl alcohol concentration, a result consistent with spin-label studies[9], although on a different timescale and without the possible complications of a perturbing probe effect.

At higher benzyl alcohol–dimyristoyl lecithin mol ratios there is a decrease in quadrupole splitting (or order parameter) with increasing concentration of benzyl alcohol, which agrees with spin-label studies[9] (Fig. 1). From these results it is relatively straightforward to determine quantitatively the projections of each C–C segment onto the bilayer normal[10,13,17], and so determine the effective length of the hydrocarbon chain, or the total membrane thickness[10,13,17,19]. Calculated chain lengths are rather insensitive to chain tilt. For example, for pure dimyristoylphosphatidylcholine at 60 °C, the distance from C-2 to C-14 is 10.2(6) Å assuming no chain tilt, or 10.5(2) Å assuming a gaussian distribution of chain orientations with a probable tilt angle of 25° (refs 10, 17). Similar average chain lengths are obtained using flat or lorentzian distribution functions (E.O. and R. Jacobs, unpublished). Consequently, we obtain from the NMR data a model-insensitive value for the average chain length, or membrane thickness. These lengths are in excellent agreement with those determined using high-resolution neutron diffraction (D. Worcester, M. Meadows, D. Rice and E.O., unpublished). For example, 23 °C lecithin–cholesterol data give a total hydrocarbon region thickness of about 30–31 Å, and neutron diffraction measurements[10] indicate a thickness of about 33 Å (Table III of ref. 10). From the data in Fig. 1*b* and *c* we can calculate the thickness of the dimyristoylphosphatidylcholine membrane at 38° C and obtain values of about 2.5(0) nm for the hydrocarbon region thickness in the absence of benzyl alcohol and about 2.3(6) nm in the presence of benzyl alcohol. The fluidisation of the bilayer caused by benzyl alcohol[8,9] decreases the membrane thickness. Cholesterol (30 mol%) causes an increase in hydrocarbon chain order, corresponding to about a 0.46-nm increase in membrane thickness at 23 °C (ref. 10).

Previous workers[5,6] have found, using black lipid membrane capacitance and conductance measurements, a 1.2-nm increase in membrane thickness in the presence of benzyl alcohol, and a decrease in hydrocarbon region thickness in the presence of cholesterol. Although the fatty acid composition of the lecithin used in the black lipid membrane studies differed slightly from ours, neutron beam and X-ray diffraction measurements on the egg-lecithin–cholesterol system indicate no significant decrease in membrane thickness on addition of cholesterol[21–23]. Similarly, other ^{2}H NMR and electron spin resonance spin-label studies of egg lecithin and egg-lecithin–cholesterol systems indicate an ordering of the hydrocarbon chains by cholesterol[15,24,25], which must correspond to an increase in projected chain length. In the absence of hydrocarbon solvents, such as tetradecane, an increase in projected chain length must correspond to an increase in membrane thickness, as neutron and X-ray data rule out ≥0.2 nm-chain interdigitation.

We therefore suggest that the previous black lipid film results[5,6] may be attributable to the inclusion of variable concentrations of solvent tetradecane in the lipid membrane, the exact amount being a function of the amount of cholesterol or benzyl alcohol present.

Deuterium nuclear magnetic resonance results suggest that the direct effect of benzyl alcohol on membrane bilayer thickness is negligible at the anaesthetic levels commonly used in clinical studies. At much higher levels (corresponding to an ~30–80-fold overload) there are small (≈0.1 nm) decreases in membrane thickness which are consistent with recent X-ray and neutron-beam studies of the effects of gaseous general anaesthetics on lipid bilayer thickness[26]. Taken together, our results cast considerable doubt on the importance of membrane thickness changes as a factor in the mechanism of action of many anaesthetics.

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X-ray diffraction patterns from molecular arrangements with 38-nm periodicities around muscle thin filaments

A SERIES of meridional and near-meridional reflections have been observed in X-ray diffraction patterns (ref. 1 and Fig. 1*a*) from the crab *Plagusia* leg muscle in the living, resting state, and have been attributed to troponin molecules which lie in pairs on thin filaments at 38-nm intervals. In the pattern (ref. 1 and Fig. 1*b*) obtained from the same muscle in rigor, the corresponding series of reflections was observed to have stronger intensities and higher order reflections. The reflections were interpreted as arising from both troponin and the cross-bridges. Electron micrograph[2] showed that cross-bridges occurred in symmetrical pairs around the thin filaments with a repeating distance of 38 nm in which one or a few closely spaced pairs may be included. Recently, reflections indexed as orders of 76 nm in X-ray diffraction patterns from insect flight muscle in rigor[3] and those from scallop striated muscle in rigor[4] have also been interpreted in terms of the cross-bridges attached in pairs to thin filaments with a repeating distance of 38 nm. We report here a method that enables us to analyse diffractions generated by molecular arrangements of this type. These arrangements are described by a set of identical helices, and the systematic modulation of intensities, indexed as orders of 76 nm, is interpreted in terms of displacement between these helices.

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