INTERACTION OF NEUTRAL AND CHARGED LOCAL ANESTHETIC WITH PURPLE MEMBRANE

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The circular dichroic spectra of a purple membrane (PM) in the presence of tetracaine (TTC) showed that neutral TTC lowered the exciton band intensity with a blue shift, whereas charged TTC resulted only in a lowering of the band intensity without the blue shift. This suggests that there is a difference in the mode of action on the PM between the two forms of TTC.

KEY WORDS local anesthetic; purple membrane; circular dichroism; exciton band

At physiological pH, clinically used local anesthetics exist in both the neutral and charged forms because their pK_a values are between 7.5 and 9. Narahashi et al.[1] proposed that local anesthetics penetrate the nerve cell membrane in the neutral form and inhibit the nerve action by binding to the effector sites from the inside of the cell membrane in its positively charged form. This theory suggests that there are at least two separate modes of action among the neutral and charged forms that show local anesthetic action. Agin et al.[2] and Hersh,[3] on the other hand, suggested that neutral and charged local anesthetics may interact with living and model systems in an identical mechanism.

A possible way to get an insight into the anesthetic action at the molecular level is to use model membranes which allow more specific testing of the contributions of protein and lipid in the local anesthetic-membrane interaction. The purple membrane (PM) of *Halobacterium halobium* containing protein incorporated into the lipid bilayer is an appropriate model system for investigating the interaction of local anesthetics on excitable membranes. Seventy-five percent of the isolated dry PM consists of a single protein, bacteriorhodopsin (bR), and 25 % is lipids. [4] Here we report the effects of the neutral and charged local anesthetics, tetracaine (TTC), on the conformation of bR and on the two-dimensional aggregates of bR in the PM using circular dichroism (CD) spectrophotometry.

PMs were obtained from the strain S-9 of *Halobacterium halobium* as already described.[5] After sucrose-gradient centrifugation, pellet fractionation was replaced by repeated washings, each followed by centrifugation, and then stored at 4 °C. Before use, the PM fragments were suspended in 10 mM phosphate buffer at the desired pH. TTC hydrochloride was obtained from Sigma. The size distribution of the PM fragments was determined using a Nicomp 370 particle sizer (Particle Sizing Systems Products, Santa Barbara, CA). The CD was recorded using a JASC J-600 spectrometer (Tokyo) equipped with a data processor under a constant nitrogen flush at 22 ± 0.5 °C. The instruments were calibrated with d-10-camphorsulfonic acid.

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In the visible CD spectra of the PM suspension, the exciton coupling effects between the retinal chromophores of the neighboring bR molecules in the PM can be used to monitor the state of the close packing and the restricted mobility of the protein molecules, and to distinguish between the monomeric and aggregated bR .[6] ^Figures 1 and 2 show the visible CD spectra of bR in the PM with various concentrations of TTC at pH 8.4 and 6.8, respectively. The sample preparations were incubated with various concentrations of TTC for 5 h in the light-adapted state with stirring at 23 °C. No change in the CD spectra of the native PM in aqueous solutions in the range of pH 6 to 9 was observed. The TTC aqueous solution at pH 8.4, which corresponds to the pK_a value, consists of a 50/50 % mixture of neutral and charged species. The addition of TTC at pH 8.4 diminished the bilobed band intensities, and is closely linked to the aggregated form of bR, with the band shift to shorter wavelengths, 475 nm, and at 5.0 mM TTC, the exciton bands almost completely disappeared (Fig. 1). On the other hand, the charged TTC at pH 6.8 resulted only in a lowering of the bilobed band intensities without the blue shift, and at 15 mM TTC, the exciton band disappeared (Fig. 2). The concentration of the charged TTC required to induce the disappearance of the exciton band of bR is about 3 times that the 50 % mixture of neutral and charged TTC at pH 8.4, due to a difference in partitioning of the neutral and charged TTC between the PM and the buffer solution.[7] TTC is an amphiphilic amine compound. Therefore, the neutral TTC would be expected to show extensive binding to the PM, either to lipid hydrophobic moieties or to hydrophobic sites on the bRs, whereas the charged TTC at pH 6.8 would primarily bind to the polar head of the lipids in the PM, which is made up of almost all negatively charged lipids.[8] and to negatively charged amino acid residues in the loop region of bR exposed to the aqueous phase.[9] The binding of TTC with two forms is considered to cause the perturbation of the lipid bilayer in the PM[10] and a loosening of the bR-bR interactions in the aggregate. We also

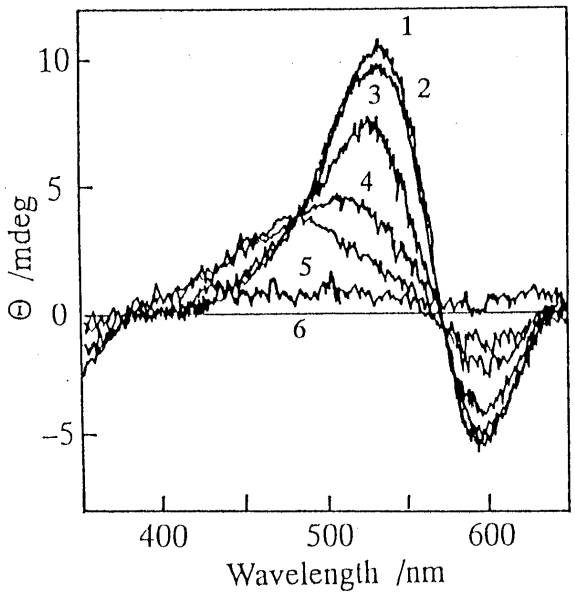

Fig.1. The Effect of a 50 %(M/M) Mixture of Neutral and Charged Tetracaine at pH 8.4 on the CD Spectra of Purple Membranes. 1, 0; 2, 0.5; 3, 1.0; 4, 2.0; 5, 3.0; 6, 5.0 mM.

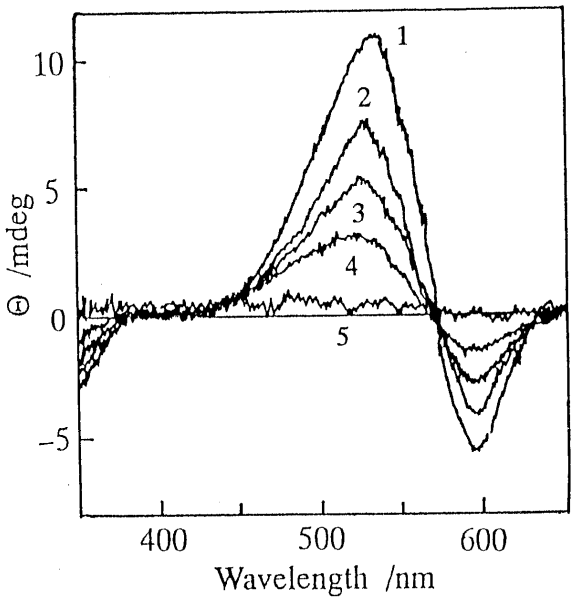

Fig.2. The Effect of Charged Tetracaine at pH 6.8 on the CD Spectra of Purple Membranes. 1, 0; 2, 3.0; 3, 5.0; 4, 10.0; 5, 15.0 mM.

found that the PM fragments with a diameter of about 550 nm in the native state became smaller with the concentration of TTC (neutral and charged forms); e.g., at 10 mM charged TTC, its mean diameter was about 350 nm. The results suggest that the addition of TTC leads to a more fluid state for the PM and increases the protein mobility in the membrane. Therefore, the lowering of the bilobed band intensities in Figs. 1 and 2 reflects the anesthetic-induced breaking of the two-dimensional aggregates of bR in the PM.

It is noteworthy that the neutral TTC shifts the exciton band of bR to shorter wavelengths and changes the purple color (Fig. 1), but the charged TTC does not cause a shift (Fig. 2). The purple color of this protein is due to the retinal covalently binding the protein and is influenced by the intramolecular interactions of the retinal with the surrounding amino acid residues.[11] Taking into account the consensus that the hydrophobic bilayer of the biological membrane is permeable to neutral local anesthetics,[12] neutral TTC also penetrates the PM and interacts with hydrophobic amino acid residues distributed around the transmembrane α-helices of bR, leading to conformational deformation of bR molecules, together with the breaking of the two-dimensional aggregates of bR in the PM. The blue shift of the CD spectra of bR in the presence of neutral TTC is explained by the conformational deformation of bR which is linked to the changes in the purple color of bR. Furthermore, we found that the effects of charged (pH7.0) and neutral dibucaine (pH8.7) on the CD coupling band of bR in the PM were similar in change in band intensities and band shift to those of neutral and charged TTC (data not shown). The CD spectroscopic studies suggest that the neutral and charged TTC interact with both the protein and lipid in the PM in a different way and break the aggregates of the bR molecules.

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