Title : INTERACTION OF LOCAL ANESTHETICS AND CELL MEMBRANE MODEL

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Introduction: Several hypotheses have been postulated for the molecular mechanism of local anesthetics (LAs) on the cell membrane. One of these is that LA makes the electrical potential at the membrane surface more positive and thus decreases excitability.[1] The purpose of this study is to clarify the mechanism of the electrical interaction between LA and the lecithin monolayer used as a cell membrane model.

Methods. Dipalmitoylphosphatidylcholine (DPPC) was dissolved in a solvent consisting of absolute ethanol and purified light petroleum ether (1:9 volume ratio). A trough of plexiglass 8 cm X 15.7 cm (125 cm^2) X 5 cm depth was constructed and a barrier bar was set from the top to 2 cm above the bottom to separate the rectangular surface into 50 cm^2 and 75 cm^2. Since the ratio of charged and uncharged forms of LA depends on the pH, a phosphate buffer solution was used to maintain the pH at 7.40. For each experiment, 375 ml of buffer solution was introduced into the trough. This volume and its buffering action maintained the pH at 7.40 consistently after the introduction of LA. DPPC was spread on the 50 cm^2 surface with a micropipette to form one molecule in 100 $Å^2$ or 60 $Å^2$. Surface potential was measured with an electrometer via an alpha emitter ionizing electrode on the DPPC monolayer and caromel reference electrode in the buffer solution. After the surface potential was stabilized, a calculated amount of LA in the 5 ml solution was introduced into the trough through the 75 cm^2 side by a syringe with a teflon catheter. Prior to each introduction of LA, 5 ml of buffer solution was withdrawn from the trough to keep the distance between the electrode and the surface of the lecithin monolayer constant. Injection of LA solution was performed quickly so that the LA would be distributed instantaneously throughout the 375 ml of buffer solution. Concentration of LA in the trough was increased stepwise from 0.5 to 2 milimoles for lidocaine and benzocaine, and from 0.125 to 0.5 milimoles for tetracaine when the one molecule of DPPC was spread in either 100 $Å^2$ or 60 $Å^2$. Surface potential was continuously recorded with a strip chart recorder as the concentration of each LA was increased. All experiments were performed at 22° C.

Results. When one molecule of DPPC occupied 100 $Å^2$, LAs in this study caused a change in surface potential. Tetracaine made the surface potential more positive as its concentration was increased. At 0.5 milimoles of tetracaine, surface potential increased by an average of 34 mV. Lidocaine also showed the dose related increase in the surface potential. Two milimoles of lidocaine increased the surface potential by an average of 31 mV. On the other hand, benzocaine decreased the surface potential. At a concentration of 2 milimoles, the surface potential was decreased by 64 mV. When one molecule of DPPC occupied 65 $Å^2$, all of the LAs in this study failed to change the surface potential.

Discussion. The hydrophilic polar group of the DPPC consists of a negatively charged phosphate group (negative P) and a positively charged trimethyl ammonium ion (positive N). When the DPPC forms a monolayer, the electrostatic dipole of the polar group is parallel to the membrane-water interface. Therefore, the positive N is near the negative P of the neighboring molecule and they attract each other creating the surface electrostatic interaction among DPPC molecules. When one molecule of DPPC occupies 100 $Å^2$ at 22°C, the electrostatic interaction among DPPC molecules is weaker than when it occupies 65 Å°. Therefore it is thought that LA molecules can penetrate and stay between the DPPC molecules resulting in the change of surface potential. At pH 7.40, more than 90% of the tetracaine and 70% of the lidocaine are positively charged at their hydrophilic end according to their pKa. Charged LA molecules are probably aligned in the monolayer with the hydrophobic benzene group between hydrocarbon chains of DPPC. The carbonyl group and positively charged hydrophilic end (positive E) are probably between hydrophilic groups of DPPC. By constructing the model structure of LA and DPPC molecules, one can visualize that the positive E of LA is near the negative P of DPPC and the carbonyl group is near the positive N of the other DPPC. Thus, the positive E of LA can neutralize electrostatically the negative P of DPPC. Carbonyl oxygen is negatively polarized and this weak negativity partially neutralizes the positive N of DPPC. Therefore, the electrostatic net effect of tetracaine and lidocaine on DPPC is that the positive N of DPPC becomes more predominant than the negative P of DPPC resulting in an increase in surface potential. Although benzocaine is electrically neutral at pH 7.40, it makes the surface potential less positive as opposed to the effect of tetracaine and lidocaine. It is speculated that the negative end of the carbonyl dipole in the benzocaine molecule partially neutralizes the positive N of DPPC, but the negative P of DPPC is not affected because benzocaine does not have a positively charged hydrophilic end. Therefore the negative P of DPPC becomes predominant, causing a decrease in the surface potential. When one molecule of DPPC occupies 65 $Å^2$ the surface electrostatic interaction among DPPC molecules is stronger than when one molecule occupies 100 $Å^2$. Therefore, it is thought that none of the LA molecules in this study penetrated into the monolayer and they failed to change the surface potential. In conclusion, local anesthetics changed the surface potential of the model cell membrane by perturbing the electrostatic interaction of phospholipid polar groups.

Reference

1. Strichartz G: Molecular mechanisms of nerve block by local anesthetics. ANESTHESIOLOGY 45:421-441, 1976