Title: ELECTRICAL CAPACITANCE OF LIPID MEMBRANES AND INHALATION ANESTHETICS. TWO MODES OF INTERACTION.

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Introduction: A few studies have been reported about the anesthetic effects upon the capacitance of planer bilayer lipid membranes. The two-molecular thickness of these membranes makes them appear black when observed by reflecting light because of the light interference; thus they are called black lipid membranes (BLM). These membranes are usually formed in a small pinhole about 1 mm diameter. Preparation of a giant size BLM (2 x 3 cm) with extended lifetime (24 hrs) has been reported (1). The present study demonstrates that inhalation anesthetics increase the capacitance of the giant BLM. The interaction occurs in two distinctively different steps.

Methods: Giant bilayers were prepared from cis-9-octadecenylamine on a teflon plate which had a window 2 x 3 cm. Film-forming solution was prepared by mixing octadecenylamine and decane 0.2:1 (v/v). A small volume (about 0.1 ml) of the film-forming solution was applied to the rim of the window of the teflon plate which separated a bath in two compartments. A 30 mM $NiCl_2$ solution was introduced into the bath from the bottom. The water level was raised gradually until it reached about 5 mm above the upper rim of the window. A rainbow-colored film formed over the window, which turned black in about 10 minutes. All reagents were the highest grade available, and water was purified by a Milli-Q system after distillation. Halothane, enflurane and chloroform were vaporized by an anesthesia machine and delivered to the aqueous phase by bubbling. The anesthetic concentrations in the gas and water were measured by gas chromatography (Shimadzu). The capacitance of the membrane was measured by a GenRad 1615-A capacitance bridge, a 1232-A tuned amplifier and a Tektronics memory oscilloscope.

Results & Discussion: The anesthetics increased the dc capacitance of the giant BLM. The increase with respect to the anesthetic concentration was nonlinear. According to the increase of the anesthetic concentrations, the capacitance increased asymptotically to a limiting value of 0.58 microF/cm^2. Further increase of the anesthetic concentration resulted in a sudden discontinuous increase of the capacitance. The anesthetic partial pressure at the breaking point was in the order of MAC values. The secondary increase was linear with respect to the anesthetic concentration until the destruction of the membrane at about 0.7 microF/cm^2. The contour of the primary increase followed the pattern of the Langmuir adsorption isotherm. The secondary increase is apparently caused by the mixing of the membrane molecules and the anesthetic molecules. The increase of the membrane capacitance indicates a decrease of the membrane thickness and/or an increase of the dielectric constant of the lipid core. The thickness of the BLM estimated from the capacitance was 3.4 nm without anesthetics. At anesthetic concentration at the break of the capacitance-anesthetic plot, the thickness was decreased to 3.2 nm. The decrement was 0.2 nm or about 6%. From the thickness of the membrane, the density and dielectric constant of the anesthetics, and the dielectric constant of the lipid core, the partition coefficient of the anesthetics was estimated between the BLM and the aqueous phase. At low anesthetic concentrations in the range of primary increase of the capacitance, the values were: halothane 3990, enflurane 2760, and chloroform 2660 . These values are in good agreement with previous reports using phospholipid vesicles. In this concentration range the anesthetic molecules are adsorbed to the surface of the membrane and do not penetrate into the lipid core. The partition coefficients of the anesthetics at high concentrations in the range of secondary increase of the capacitance were: halothane 5720, enflurane, 2700, and chloroform 1980. When expressed by the molarity partition coefficient, these values become: halothane 507, enflurane 220 and chloroform 176. These values are approximately double of olive oil/water partition coefficients. When one considers the condensation of the anesthetic molecules at the surface of the membrane, then it is not unreasonable to find larger partition coefficients of this magnitude than the bulk values for olive oil/water. Anesthetic molecules appear to mix with membrane molecules at these concentrations.

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References:

1. Yoshida T, Okuyama M, Itoh T: Bull Chem Soc Jpn 50:1399-1402, 1977.