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Effects of Volatile Anesthetics on Light-induced Proton Uptake of Rhodopsin in Bovine Rod Outer Segment Disk Membrane

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The effects of volatile anesthetics upon the function of bovine rhodopsin were estimated from the measurements of light-induced proton uptake. The light-induced *p*H changes were measured at both 20° C and 37° C with suspensions to which volatile anesthetics were added in the liquid form. Each anesthetic depressed the light-induced proton uptake concentration-dependently. The anesthetic-induced depression was greater at 37° C than at 20° C. For each anesthetic the concentration needed to depress the proton uptake by 10% was roughly identical to that used clinically. Anesthetics also were added to the suspensions in the gaseous form with air. The light-induced proton uptake was decreased in proportion to the partial pressure of the anesthetic. The partial pressures of halothane and methoxyflurane that depressed the proton uptake by 10% at 37° C were 2.0×10^{-2} and 1.1×10^{-2} atm., respectively. From these facts it is suggested that volatile anesthetics affect the light-induced conformational changes of rhodopsin molecule during the metarhodopsin I to metarhodopsin II transition and cause inhibition of the light-induced proton uptake of rhodopsin in the rod outer segment disk membrane. (Key words: Anesthetics, volatile: diethyl ether; enflurane; halothane; methoxyflurane. Theories of anesthesia.)

ALTHOUGH the site of action of general anesthetics is unidentified at the molecular level, it is most likely to be in the excitable membrane within the central nervous system.[1] The lipid bilayer model has been most widely used for studies concerned with the molecular mechanism of general anesthesia, because a lipid bilayer is the basic element of biological membranes.[2] However, the differences between a simple lipid bilayer and an excitable cell membrane are too great to extrapolate the results of studies on lipid bilayers directly to the mechanism of anesthesia.

There have been a few reports about studies that employed excitable membranes as a model for study of the molecular mechanism of anesthesia. Young *et al.*[3] examined the action of anesthetics on a single synapse in acetylcholine receptor-rich membranes isolated from the electroplaque of Torpedo californica. They revealed that volatile anesthetics facilitated the desensitization of membrane-bound acetylcholine receptor *in vitro*. Cheng and Brunner[4,5] adapted gamma-aminobutylic acid (GABA) disposal by intact synaptosomes as a model system to simulate synapses in order to study molecular mechanisms of anesthesia.

In this study, a retinal rod outer segment (ROS) disk membrane was chosen as a model system, because the retina is regarded as a part of the central nervous system and is excitable. The visual pigment rhodopsin constitutes about 90% of the ROS disk membrane protein,[6] and the major portion of its mass is thought to penetrate into the phospholipid bilayer of the disk membrane.[7] Rhodopsin is directly involved in the early molecular events of vision.[8] When rhodopsin is exposed to light, it is bleached by a photochemical reaction.[8] During transition from metarhodopsin I to metarhodopsin II, the molecule changes its conformation on a large scale and the entropy increases.[9–11] The conformational change of rhodopsin is accompanied by changes in surface charge density[12,13] and pk of the ionizable amino acid groups.[14,15] These changes lead to light-induced proton uptake.[14,16,17]

In this study, the effects of volatile anesthetics on the function of rhodopsin protein in the ROS disk membrane were examined by measuring the light-induced proton uptake.

Materials and Methods

The bovine retinas were harvested from eye balls obtained from a meat processing plant. The ROS disk membranes were isolated from the bovine retinas using a linear sucrose density gradient technique according to the procedure described by Makino *et al.*[18] All procedures were carried out under dim red light at 4° C. Retinas were suspended in 36% sucrose solution containing 0.9% NaCl and 10 mM phosphate buffer (pH = 7.4), shaken vigorously, and centrifuged at 13,000 *g* for 45 min. The floating ROS disk membranes thus isolated were suspended in 100 mM KCl solution and centrifuged at 13,000 *g* for 45 min. The sediments were dispersed in 100 mM KCl solution and centrifuged again at 13,000 *g* for 45 min. to wash out phosphate buffer and sucrose. The washing was repeated three more times and the sediments were suspended in 100 mM KCl solution. The suspension contained 0.23 to 0.35 mg/ml of protein, as determined by the method of Lowry *et al.*[19] The *p*H of the suspensions was 6.25 ± 0.33 at 20° C. The *p*H was measured with a Radiometer® combination glass elec-

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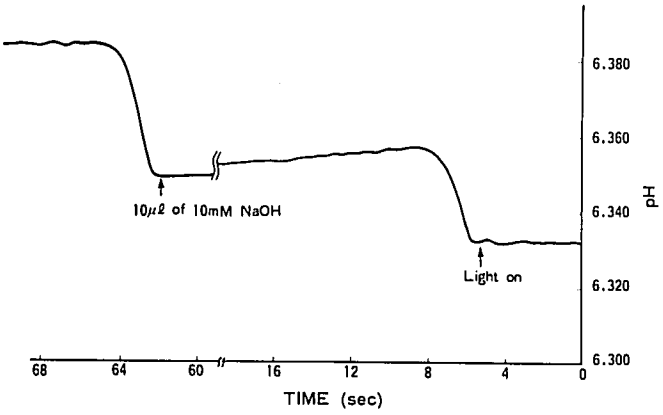

FIG. 1. Light-induced *p*H change and NaOH-induced *p*H change of the bovine ROS disk membrane suspension at 37° C. The two arrows indicate the start of illumination and the injection of 0.1 μmol NaOH, respectively. The extent of proton uptake was calculated by comparison between *p*H increases caused by illumination and by addition of NaOH.

trode GK2401C and a Radiometer Research® *p*H meter PMH84. An aliquot of 10 ml of the ROS disk membrane suspension was put into a cylindric Pyrex glass cell surrounded by a water jacket maintained at constant temperature by circulating water from a constant temperature water bath and equipped with a stirrer. The suspensions were stirred continuously until the *p*H became constant for 15–20 min before measurements. The disk membrane suspensions were illuminated by a slide projector located 20 cm from a reaction cell. The light was passed through a focusing lens and a water jacket surrounding the cell before entering the suspension. After illumination, NaOH 0.1 μmol was injected several times to calibrate the signal. The extent of proton uptake was calculated by comparison between *p*H increases caused by bleaching and by adding NaOH.

To examine the effects of volatile anesthetics on the function of rhodopsin in the ROS disk membrane, the light-induced *p*H changes were measured at 20° C and 37° C with the suspensions to which anethetics were added. Diethyl ether, enflurane, halothane, and methoxyflurane in the liquid form were added to the suspensions in stoppered glass test tubes. The suspensions were shaken vigorously and left in an icebox for 60 min. Each aliquot of 10 ml of the suspensions was put into a closed glass reaction cell having a volume of 65 cm^3. The suspensions were mixed continuously with a stirrer for 20 min before the *p*H measurements. Escape of a part of the anesthetic gas was inevitable during this procedure, and a large amount of the anesthetic was assumed to have moved into the gas phase from the suspension medium in the reaction cell during mixing. Therefore, the concentrations of anesthetic in the suspensions were determined after illumination measurements by a Shimadzu gas chromatograph GC-4CM equipped with a Shimadzu gas sampler. The addition of liquid anesthetics increased the *p*H of the suspensions slightly (maximum 0.1 *p*H unit). The *p*H increase was corrected by adding HCl before illumination.

Halothane and methoxyflurane also were added to the disk membrane suspensions in the gaseous form. Halothane and methoxyflurane were vaporized in specific vaporizers (Fluotec® and Pentec®, respectively) using air as a carrier. The anesthetic vapor was passed through the gas phase over the suspension in the reaction cell and equilibrated with the suspension by slow mixing with a stirrer for 20 min. The concentration of anesthetic in the solution was determined from the partial pressure of the anesthetic equilibrated with the suspension.

Results

Figure 1 shows the light-induced and NaOH-induced *p*H changes of the ROS disk membrane suspension with time. When the disk membrane suspension was illuminated continuously at 37° C, a rapid *p*H increase was followed by a slow decrease. The extent of proton uptake was calculated by comparison between the *p*H increases caused by illumination and by addition of NaOH.

Figure 2 shows the light-induced *p*H increases of the ROS disk membrane suspensions with or without halothane at 37° C. Halothane markedly depressed the light-induced proton uptake concentration-dependently. Figure 3 depicts the effects of diethyl ether, enflurane, halothane, and methoxyflurane, which were added in the liquid form, on the light-induced proton uptake of ROS disk membrane at 37° C (A) and 20° C (B). Each

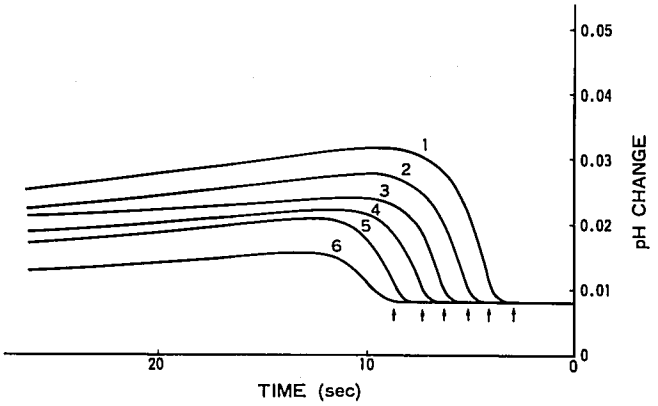

FIG. 2. Light-induced *p*H increase of the bovine ROS disk membrane suspensions with or without halothane at 37° C. Control (1), halothane, 0.46 mM (2), 1.07 mM (3), 1.75 mM (4), 2.67 mM (5), and 3.33 mM (6). Arrows indicate the time when illumination started. Ordinate represents the extent of *p*H change from the initial *p*H.

volatile anesthetic depressed the proton uptake concentration-dependently at both temperatures. The anesthetic-induced depression of the proton uptake was greater at 37° C than 20° C. As the gas/suspension partition coefficients of anesthetics were undetermined, the proper partial pressures could not be calculated. Using the gas/water partition coefficient as an approximation of the gas/suspension partition coefficient, the partial pressures of anesthetics needed to depress the proton uptake by 10% at 37° C were calculated to be 0.48×10^{-2}, 1.28×10^{-2}, 2.3×10^{-2}, and 4.3×10^{-2} atm. for methoxyflurane, halothane, enflurane, and diethyl ether, respectively. The order of depression in the proton uptake by these anesthetics roughly follows that of their anesthetic potencies.

Figure 4 depicts the inhibitory effects of gaseous halothane (A) and methoxyflurane (B) on the light-induced proton uptake at 37° C and 20° C. The per cent inhibition was statistically significant at and over each minimum concentration of anesthetics at 37° C and 20° C. The anesthetic-induced depression of the proton uptake was much greater at 37° C than at 20° C. Halothane at 3.0×10^{-2} atm. depressed the proton

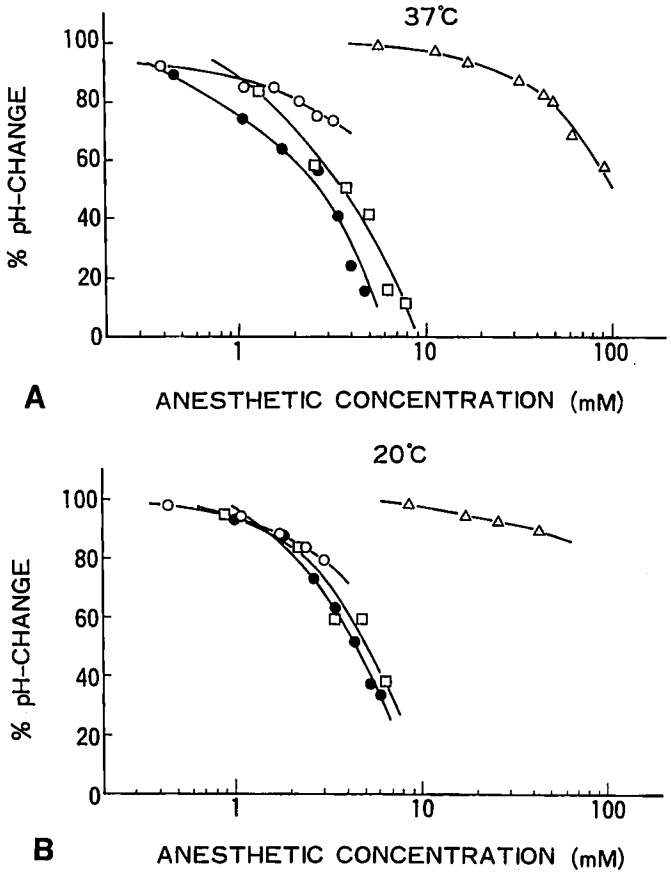

FIG. 3. Inhibitory effects of volatile anesthetics on the light-induced proton uptake of rhodopsin in the bovine ROS disk membrane at 37° C (*A*) and 20° C (*B*). Anesthetics—diethyl ether (△), enflurane (○), halothane (●) and methoxyflurane (□)—were added to the disk membrane suspensions in the liquid form. The concentrations of anesthetics are depicted on a logarithmic scale.

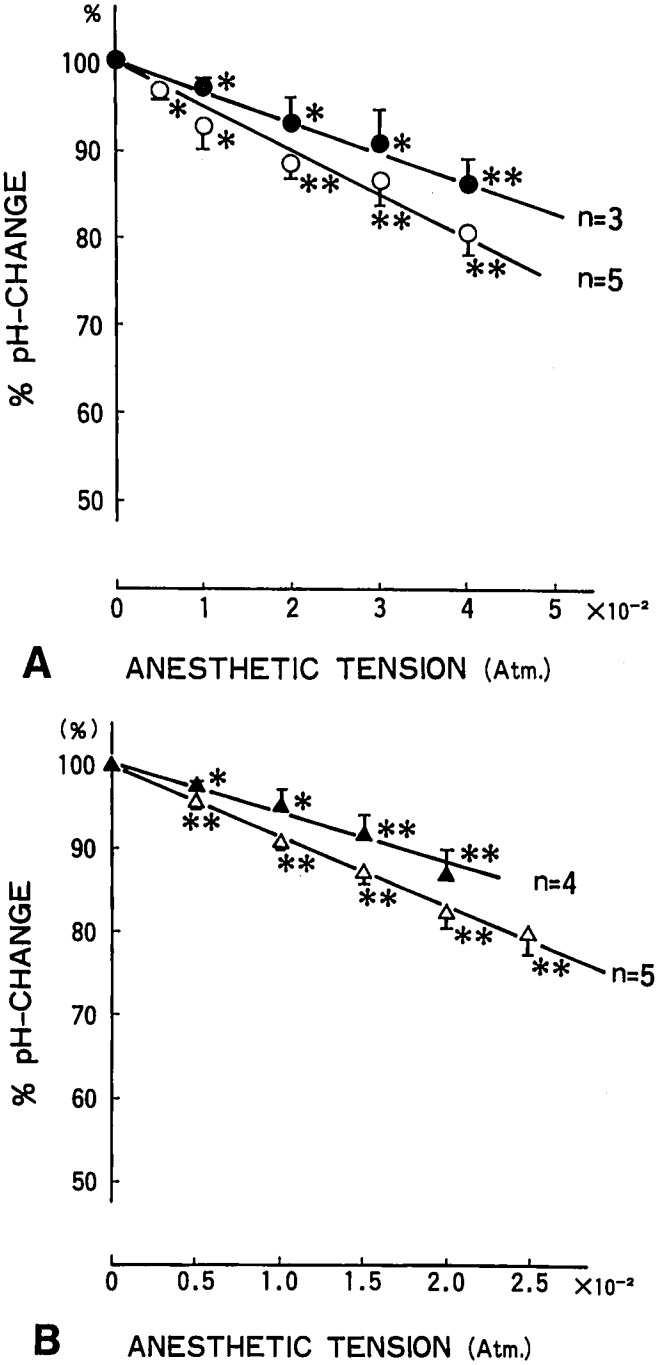

FIG. 4. Inhibition of the light-induced proton uptake of rhodopsin in the bovine ROS disk membrane by volatile anesthetics that were induced in the gaseous form. Figures *A* and *B* show effects of halothane at 37° C (○) and 20° C (●) and methoxyflurane at 37° C (△) and 20° C (▲), respectively. The values represent the means ± SEM. The per cent inhibition of light-induced proton uptake by both anesthetics is statistically significant (*$P < 0.05$, †$P < 0.01$).

uptake by about 15% at 37° C and by about 10% at 20° C. The partial pressures of halothane and methoxyflurane that depressed the proton uptake by 10% were 2.0×10^{-2} and 1.1×10^{-2} atm., respectively, at 37° C.

Discussion

The present study shows that volatile anesthetics decreased the light-induced proton uptake of rhodopsin concentration-dependently. Translating the molar concentrations of the anesthetics into partial pressures, the magnitudes of the decreases were in good agreement with MAC values.[20,21] This suggests that the molecular mechanism of the anesthetic-induced decrease in proton uptake closely is related to that of general anesthesia. Figure 3 shows that the molar concentrations at which diethyl ether decreases the proton uptake are extremely high, compared with those of the other anesthetics. This is due to the physical properties of diethyl ether, a higher water/gas partition coefficient and a lower oil/gas partition coefficient.

Possible mechanisms of the anesthetic-induced inhibition of light-induced proton uptake by rhodopsin in the ROS disk membrane may include the following: 1) Anesthetics affect directly the function and conformation of the rhodopsin molecule. Laasberg and Hedley-Whyte[22] demonstrated by optical rotatory dispersion measurement that halothane decreased reversibly helicity in the beta-chain of human hemoglobin. These data indicate that anesthetics directly affect conformation in the secondary structure of hemoglobin. Eyring *et al.*[23] concluded that volatile anesthetics induced a conformational change and volume increase in protein to explain the large entropy increase when anesthetic molecules were combined with an enzyme. We[24] have shown that a volatile anesthetic, diethyl ether, increased the partial molal volume of bovine serum albumin. 2) Anesthetics may indirectly influence rhodopsin by changing the fluidity of lipid components, especially lipid molecules surrounding rhodopsin, of the ROS disk membrane. Many investigators[25-28] reported that volatile anesthetics caused perturbation of the lipid moiety in both artificial and biologic membranes. As reported by Seeman and Roth,[29] anesthetics expand cell membranes at surgical concentrations. Furthermore, Fischer and Williams[30] demonstrated that different phospholipid species strongly affect the thermal stability of the rhodopsin molecule. With volatile anesthetics, the function and conformation of rhodopsin in the ROS disk membrane may be affected by change in the structure of the lipid moiety.

Volatile anesthetics affect directly and/or indirectly the metarhodopsin I to metarhodopsin II transition upon illumination and may inhibit excitation of the rhodopsin molecule. Raitta *et al.*[31] demonstrated that the amplitude of the a-wave of the electroretinogram (ERG) significantly diminished during general anesthesia. The a-wave of the ERG is thought to represent the receptors that include rod outer segment disk membranes. We[32] also have shown that volatile anesthetics decreased the amplitude of the a-wave of the ERG in rabbits. Similar inhibition by volatile anesthetics may occur in the excitable membranes in the central nervous system.

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