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Effect of anesthetics on the self-sustained oscillation in an artificial membrane induced by repetitive conformational change of DOPH molecules between hydrophilic and hydrophobic phases

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Abstract
Purpose. The mechanism of anesthesia was approached from a study of an artificial excitable membrane that well reproduced the active electrical properties of the nerve membrane.
Methods. Self-sustained oscillations of the membrane potential in a model membrane in which dioleyl phosphate (DOPH) was infiltrated into the pores of a millipore filter were utilized to investigate the effect of volatile anesthetic agents on the repetitive conformational change of DOPH molecules between hydrophilic multibilayers and hydrophobic oil droplets, while this process was coupled with diffusion of K^+ across the membrane placed between KCl aqueous solutions.
Results. The period of the self-sustained oscillations increased due to the addition of volatile anesthetics to the aqueous solutions, and there were critical values of concentrations of volatile anesthetics above which the self-sustained oscillations disappeared.
Conclusion. The volatile anesthetic agents affected the hydrophobic oil droplets of the DOPH molecules and impeded their repetitive conformational change between the hydrophilic and hydrophobic phases, just as local anesthetics had been reported to do.

Key words: DOPH model membrane, Self-sustained oscillation, Volatile anesthetics

Introduction

The mechanism of anesthesia is still unknown, and many hypotheses have been proposed. Since Meyer and Overton [1] revealed the correlation between anesthetic potency and oil solubility of anesthetics, it has been widely believed that anesthetics act by disturbing the structure or dynamic properties of the lipid bilayer of cell membranes. However, those simple lipid hypotheses were inconsistent with the evidence that enzymes without lipid bilayers were inactivated by anesthetics, and were then refined to postulate that specialized domains such as the hydrophobic center in membranes are sensitive to anesthetics [2–7]. Since the actual hydrophobic center in nerve membranes has not yet been found, it would be necessary to investigate the effect of anesthetics on some hydrophobic center in an artificial model membrane that can reproduce the electrical excitability of the nerve membrane.

Investigations of the electrophysiological properties of the protoplasmic droplets of Nitella and squid giant axons have suggested that the excitation mechanism of the actual nerve membrane can be explained by a transition of membrane structure. To make this finding more concrete, it has been shown that the excitation phenomena observed in the actual nerve membrane can be well simulated by a model membrane system in which a transformation of molecular conformation in the membrane takes place with variation in environmental conditions or with external stimulation [8]. It was reported that a model membrane in which a lipid analogue (dioleyl phosphate DOPH) was infiltrated into pores of a millipore filter manifested a self-sustained oscillation of the membrane potential under a direct electrical current and pressure difference across the membrane, depending on the concentration gradient of a salt such as KCl [9,10].

The mechanism of oscillation was clarified on the basis of the phase transition of the DOPH molecules and ionic flow across the membrane. A repetitive conformational change of DOPH molecules between hydrophilic multibilayers and hydrophobic oil droplets in the pore is coupled with diffusion of K^+ across the membrane placed between KCl aqueous solutions [11,12].

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The effects of three local anesthetics (tetracaine, procaine, and lidocaine) and of *n*-alkanols on the self-sustained oscillation of the membrane potential were investigated [13]. The local anesthetics and *n*-alkanols for *n* less than 12 were shown to depress the self-sustained oscillation of the membrane potential [14]. This fact has been understood to support the reliability of the DOPH-impregnated filter as a model of the actual membrane system [14].

We studied the response of the self-sustained oscillation of the model membrane impregnated with DOPH to four volatile anesthetics: halothane, enflurane, isoflurane, and sevoflurane.

Materials and methods

The anesthetic agents we used were halothane (Fluothane, Takeda Yakuhin Kogyo & Imperial Chemical Industries, Osaka, Japan), enflurane (Ethrane, Abbott Laboratories, Osaka, Japan), isoflurane (Forane, Abbott Laboratories, Osaka, Japan), and sevoflurane (Sevofrane, Maruishi Seiyaku, Tokyo, Japan). Cellulose ester filter paper, 5.0-μm pore size (Type SMWP, Nihon Millipore, Tokyo, Japan) was used to prepare model membrane filters by immersion in DOPH lipid. DOPH was synthesized by hydrolysis of the reaction product of oleyl alcohol and phosphorus oxychloride [15,16]. A DOPH-impregnated model membrane filter was prepared by immersing a filter in a solution of DOPH in benzene for 1 min and then drying it in air. The quantity of impregnated DOPH was adjusted to about 10 mg·cm^{-2} by regulating the concentration of the DOPH-benzene solution. The model membrane filter was preconditioned in 100 mM KCl aqueous solution for more than 12 h, and then in 1 mM KCl aqueous solution for a few hours before experiments. The DOPH-impregnated model membrane filter will be called a DOPH-membrane filter.

We designed an experimental device to investigate the effect of the anesthetic molecules on the hydrophobic part of the DOPH molecule by observing the change in periods of self-sustained oscillations of electrical potential due to anesthetics. The device is shown schematically in Fig. 1.

The DOPH-membrane filter was placed in the circular window between two closed vinyl chloride cells under a KCl gradient with a pressure difference and electrical current. The area of the circular window was about 20 mm^2. One cell was filled with 100 mM and the other with 5 mM KCl aqueous solution. The volume of each cell was about 30 cm^3. In the cell with the more dilute KCl aqueous solution, a small stirrer was used to dissolve the volatile anesthetic agents in the solution. The pressure in the cell with 100 mM KCl concentration was kept 30 cm H_2O higher than that in the other cell. Electrical current was applied from a constant current/voltage source (Metronix Model 6912, Tokyo, Japan). The membrane electrical potential was monitored by an electrometer (Nihon Kohden Dual-Beam Memory Oscilloscope VC-10, Tokyo, Japan) with Ag/AgCl electrodes, and recorded by an X-recorder (Pantos Model U228, Tokyo, Japan) as well as a digital storage FFT frequency analyzer (Canopus ADF-16 and Wave Master II on PC, Kobe, Japan).

The filter papers were impregnated with DOPH to produce a membrane resistance of hundreds of megaohms per square centimeter. A constant electrical current between 0.1 and 1 μA was applied to induce a membrane potential of 3 to 8 V. Then, a stable and rhythmic oscillation of electrical potential occurred after the start of the application of the constant electrical current. This oscillation has been called a self-sustained oscillation [9–12].

Undiluted drugs of each anesthetic were prepared. After a self-sustained oscillation of electrical potential across a DOPH-membrane filter had been obtained, each of the four anesthetic agents was injected into the closed cell with 5 mM KCl concentration, and the change in amplitude and period of the self-sustained oscillation was recorded with respect to the amount of the anesthetic agent injected. A fixed amount of each anesthetic was injected repeatedly at 3-min intervals. Experiments were performed at 25 ± 2°C so as to maintain standard conditions for the self-sustained oscilla-

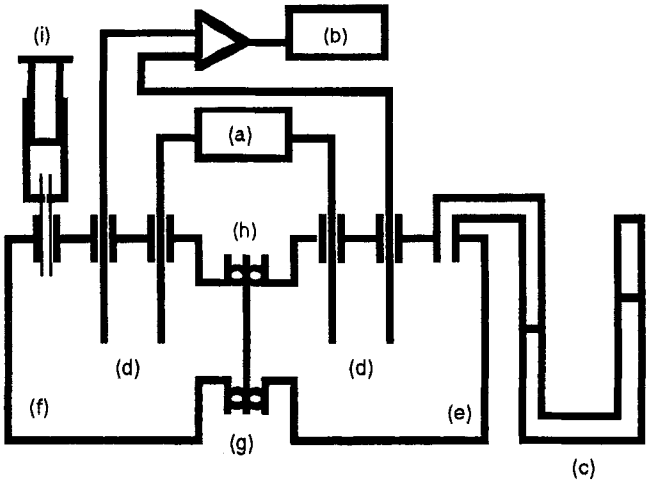

Fig. 1. Schematic diagram of the experimental setup. (*a*) Current source (Metronix Model 6912, Tokyo, Japan), (*b*) pen recorder (Pantos Model U228, Tokyo, Japan), (*c*) pressure tank, (*d*) Ag/AgCl electrode, (*e*) 100 mM KCl aqueous solution, (*f*) 5 mM KCl aqueous solution, (*g*) silicon rubber gasket, (*h*) membrane filter impregnated with lipid, and (*i*) injector (microsyringe)

tion of electrical potential across a DOPH-membrane filter [9–11]. No essential change could be found when preliminary experiments were performed at body temperature (about 36°C), and therefore we performed experiments at 25 ± 2°C so that we could easily maintain stable conditions.

DOPH was in the hydrophobic phase of oil droplets at these temperatures if the KCl concentration was less than the critical concentration (approximately 30 mM), and in the hydrophilic phase of micelles or bilayer (including multilayer) leaflets if the KCl concentration was higher than the critical concentration [12]. Therefore, the interface between the DOPH-membrane filter and the 5 mM KCl aqueous solution formed the hydrophobic phase, and that between the DOPH-membrane filter and the 100 mM KCl aqueous solution formed the hydrophilic phase. Under these conditions, volatile anesthetics injected into the closed cell with 5 mM KCl aqueous solution could affect principally the hydrophobic oil droplets of the DOPH molecules, but not the hydrophilic phase.

As a control for the experiment with volatile anesthetics, we performed the same experiment with nonanesthetic agents such as HCl, NaCl, and quinine. Recording of the electrical potential was continued until the self-sustained oscillation disappeared. The final anesthetic concentration of the 5 mM KCl solution in the closed cell was measured by liquid chromatography, and the part-way anesthetic concentration was calculated from the final one. The experiments were performed 10 times for each anesthetic agent.

Results

A representative oscillation curve of a self-sustained oscillation of electrical potential across a DOPH-membrane filter is shown in Fig. 2. The amplitude and frequency of the self-sustained oscillation were approximately 0.2 V and 0.5 Hz, respectively.

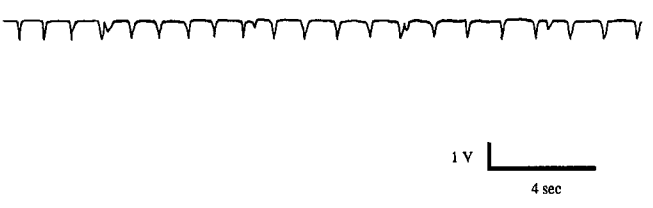

Fig. 2. Representative oscillation curve of electrical potential across a DOPH-membrane filter. The amplitude and frequency of the self-sustained oscillation are approximately 0.2 V and 0.5 Hz, respectively

Halothane

The concentration range of halothane was from 0 to 1.0×10^{-3} M. The period of the self-sustained oscillation of the DOPH-membrane filter increased after injection of halothane into the closed cell with 5 mM KCl concentration, whereas no changes in amplitude were observed. A representative result is shown in Fig. 3 as a sequence of three-dimensional return maps of the period of the self-sustained oscillation. The period was kept almost constant until the halothane concentration reached a threshold value $(5.0 \pm 1.1) \times 10^{-4}$ M, and began to increase as the halothane concentration was increased. There was a critical value $(1.0 \pm 0.2) \times 10^{-3}$ M of halothane concentration above which the self-sustained oscillation disappeared.

Enflurane

The concentration range of enflurane was from 0 to 5.0×10^{-4} M. The period of the self-sustained oscillation of the DOPH-membrane filter also increased after injection of enflurane into the closed cell with 5 mM KCl concentration, whereas no changes in amplitude were observed. A representative result is shown by a sequence of three-dimensional return maps of the period of the self-sustained oscillation in Fig. 4. The period began to increase as the enflurane concentration was increased; there was no threshold value. There was a critical value $(5.0 \pm 1.0) \times 10^{-4}$ M of enflurane concentration above which the self-sustained oscillation disappeared.

Isoflurane

The concentration range of isoflurane was from 0 to 8.0×10^{-4} M. The period of the self-sustained oscillation of the DOPH membrane filter also increased after injection of isoflurane into the closed cell with 5 mM KCl concentration, as can be seen from a representative result shown by a sequence of three-dimensional return maps of the period of the self-sustained oscillation in Fig. 5. No changes in amplitude were observed. The period began to increase but fluctuated considerably until the isoflurane concentration reached $(5.0 \pm 1.2) \times 10^{-4}$ M. The period increased as the isoflurane concentration was increased further. The self-sustained oscillation disappeared above a critical value $(8.0 \pm 1.2) \times 10^{-4}$ M of isoflurane concentration.

Sevoflurane

The concentration range of sevoflurane was from 0 to 5.0×10^{-4} M. The period of the self-sustained oscillation of the DOPH-membrane filter was kept almost constant

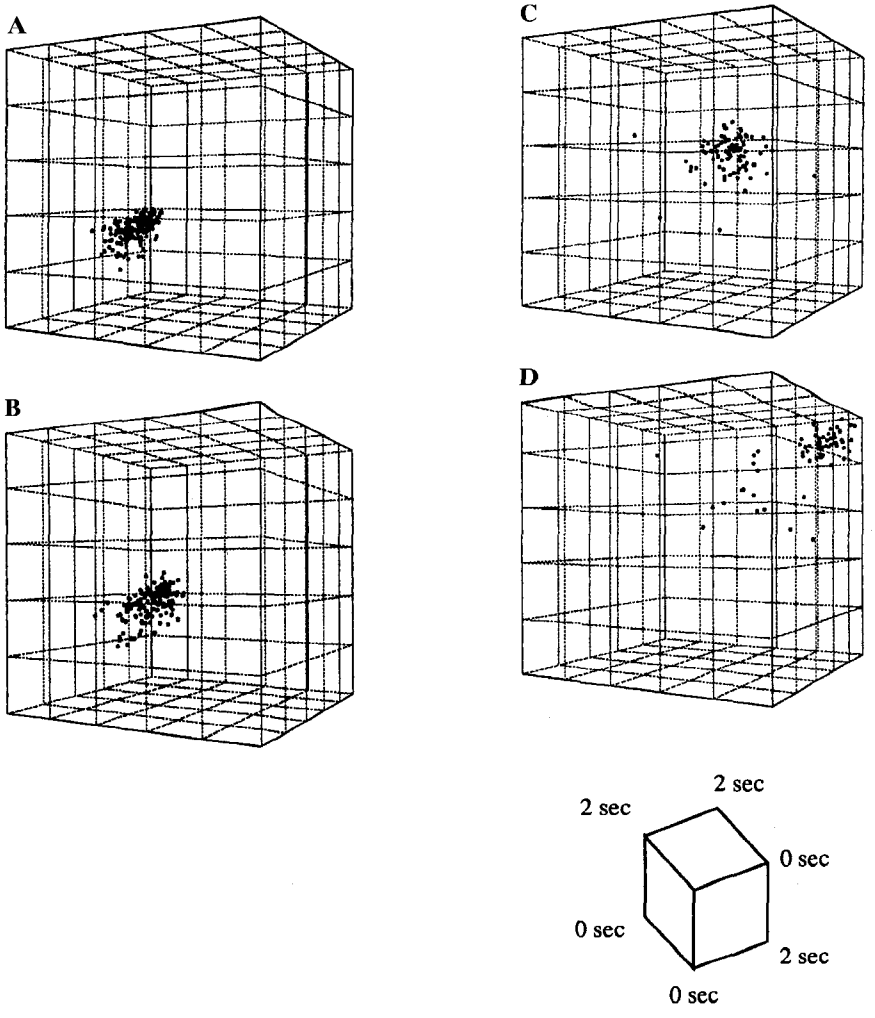

Fig. 3. Relationship between oscillation period and halothane concentration is shown by a sequence of three-dimensional return maps of the period of the self-sustained oscillation. The temporal change of the period $T_1, T_2, T_3, \ldots, T_n$ is given by three-dimensional plots of the series of points (T_1, T_2, T_3), (T_2, T_3, T_4), $(T_3, T_4, T_5), \ldots, (T_{n-2}, T_{n-1}, T_n)$. The halothane concentrations were (**A**) 0 M, (**B**) $(5.0 \pm 1.1) \times 10^{-4}$ M, (**C**) $(6.5 \pm 1.1) \times 10^{-4}$ M, and (**D**) $(1.0 \pm 0.2) \times 10^{-3}$ M. Time scales in the three axes are the same for **A–D**, and numerical values of the grid lines are indicated schematically; 0 to 2 s for each of the three axes

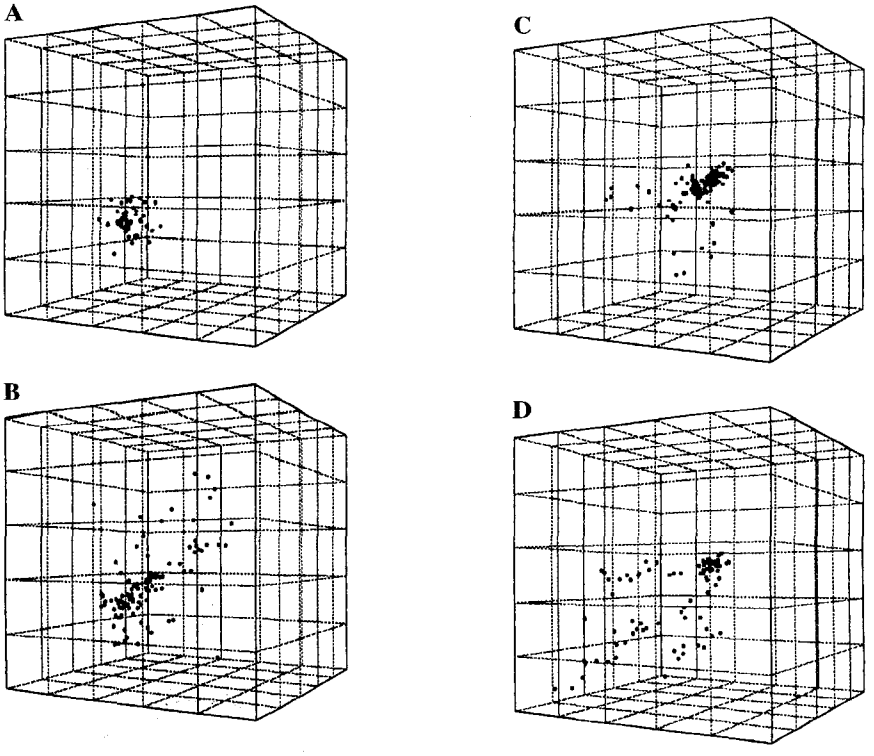

Fig. 4. Relationship between oscillation period and enflurane concentration is shown by a sequence of three-dimensional return maps of the period of the self-sustained oscillation. Time scales in the three axes are the same as in Fig. 3, and numerical values of the grid lines are not indicated. The enflurane concentrations were (**A**) 0 M, (**B**) $(1.5 \pm 0.3) \times 10^{-4}$ M, (**C**) $(3.0 \pm 0.5) \times 10^{-4}$ M, and (**D**) $(5.0 \pm 1.0) \times 10^{-4}$ M

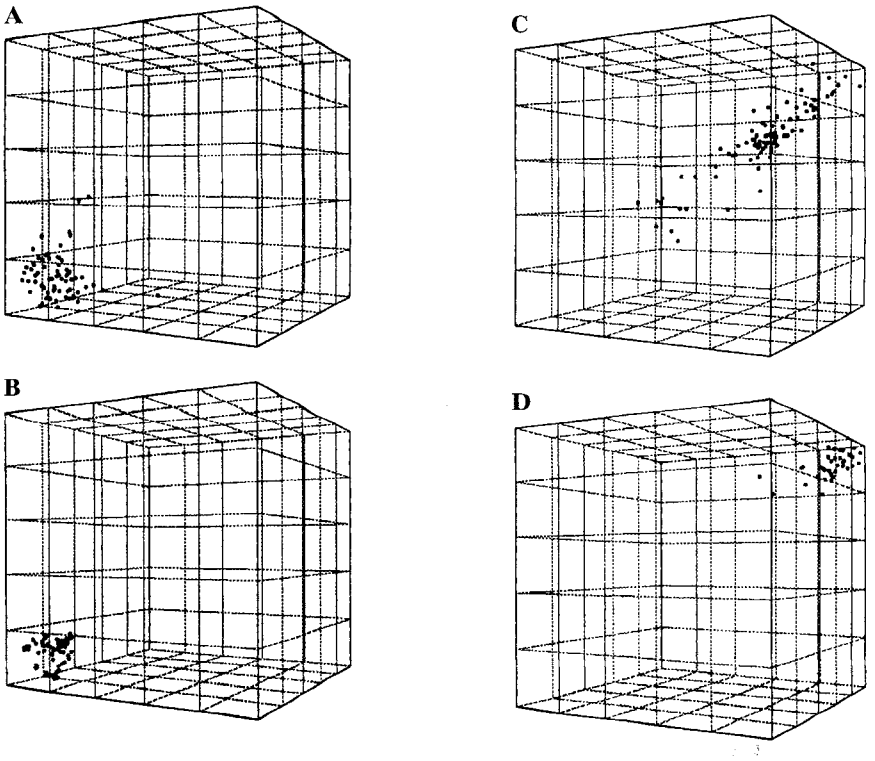

Fig. 5. Relationship between oscillation period and isoflurane concentration is shown by a sequence of three-dimensional return maps of the period of the self-sustained oscillation. Time scales in the three axes are the same as in Fig. 3, and numerical values of the grid lines are not indicated. The isoflurane concentrations were (**A**) 0M, (**B**) $(1.5 \pm 0.3) \times 10^{-4}$M, (**C**) $(5.0 \pm 1.2) \times 10^{-4}$M, and (**D**) $(8.0 \pm 1.2) \times 10^{-4}$M

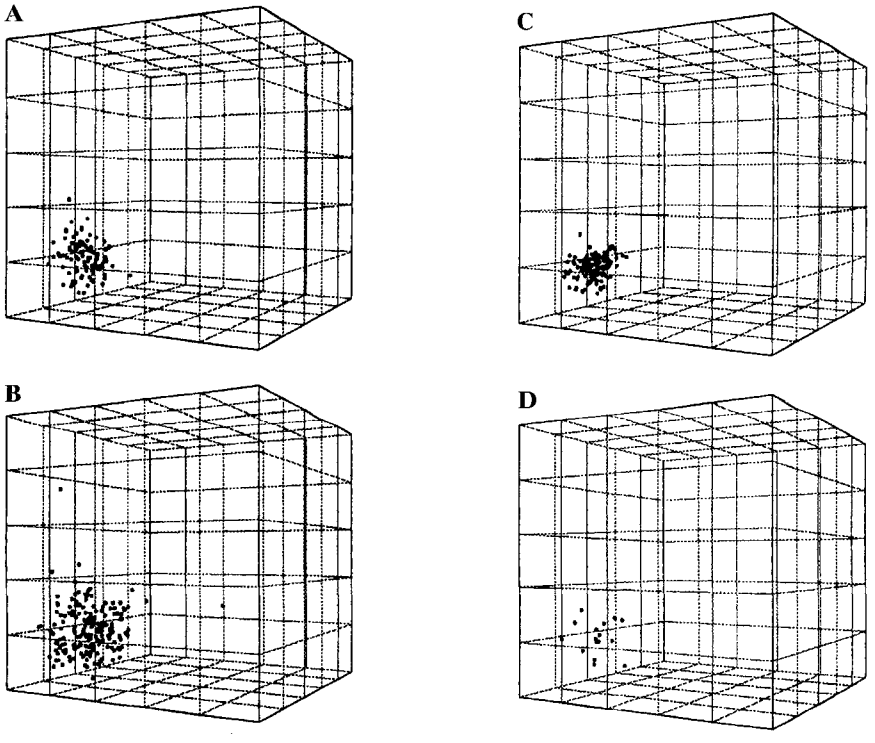

Fig. 6. Relationship between oscillation period and sevoflurane concentration is shown by a sequence of three-dimensional return maps of the period of the self-sustained oscillation. Time scales in the three axes are the same as in Fig. 3, and numerical values of the grid lines are not indicated. The sevoflurane concentrations were (**A**) 0M, (**B**) $(1.5 \pm 0.3) \times 10^{-4}$M, (**C**) $(3.0 \pm 0.5) \times 10^{-4}$M, and (**D**) $(5.0 \pm 1.3) \times 10^{-4}$M

until the sevoflurane concentration reached $(3.0 \pm 0.5) \times 10^{-4}$M after injection of sevoflurane into the closed cell with 5mM KCl concentration. The period did not begin to increase as the sevoflurane concentration was increased further, but there was a critical value $(5.0 \pm 1.3) \times 10^{-4}$M of the sevoflurane concentration above which the self-sustained oscillation disappeared. A typical result is shown by a sequence of three-dimensional return maps of the period of the self-sustained oscillation in Fig. 6.

Control group

The period of the self-sustained oscillation of the DOPH membrane filter fluctuated or increased as each nonanesthetic concentration was increased. There was no critical value of the nonanesthetic concentration above which the self-sustained oscillation disappeared, at least over a reasonable range of concentrations.

This result implies that we can use the critical value of the anesthetic concentration above which the self-sustained oscillation disappeared as a characteristic variable in our experimental study. For the purpose of verifying this implication, we estimated the statistical correlation coefficient between the critical values of oscillation disappearance and the anesthetic concentrations of the volatile anesthetic agents, obtaining a high value (0.824).

Discussion

The modeling of anesthesia is indispensable for the investigation of the mechanism of anesthesia. After the finding of bacterial and firefly luciferase (membrane free) enzymes as important model anesthetic systems [2–4], interest was focused on the hydrophobic regions in proteins, rather than lipids, as the sites of anesthetic action [5]. There, anesthetic agents were shown to compete with aldehyde for binding within the hydrophobic active center of the bacterial luciferase enzyme [1,6]. Anesthetic agents are hydrophobic molecules and have a tendency to couple with the hydrophobic parts of other molecules. The brain sites of anesthetic action were therefore searched for within the hydrophobic regions of neural proteins, such as ion channels, receptors, enzymes, and gap junctions, although they have not yet been found.

We have used the DOPH-membrane filter with pores impregnated with DOPH as a model membrane system manifesting an excitability similar to that of the actual cell membrane. This model membrane system with microscopic pores choked up with DOPH could simulate the activity of membranes with hydrophobic active centers, for example, by generating a temporal change in ion density and current in terms of repetitive conformational changes between the hydrophilic and hydrophobic phases of the DOPH molecules in the pore. Although the existence of such a mechanism in real membranes has not yet been directly observed, it would be plausible, considering the fact that many aspects of the electrostatic and electrodynamic activity of the DOPH-membrane filter are similar to those of the neural membrane [11,12].

One of the electrodynamic activities of the present model membrane system, that is, the self-sustained oscillation of electrical potential across the membrane driven by the repetitive conformational change between the hydrophilic and hydrophobic phases of the DOPH molecules, has been used to study the effects of the four volatile anesthetic agents on the simulated mechanism of activity of the excitable membrane. The period of the self-sustained oscillations increased after the addition of halothane, enflurane, and isoflurane to the aqueous solutions, and there were critical values of concentrations of the volatile anesthetics, above which the self-sustained oscillations disappeared. The period did not begin to increase as the sevoflurane concentration increased, but there was a critical value of the sevoflurane concentration above which the self-sustained oscillation disappeared. This difference between sevoflurane and the other three volatile anesthetics may be explained by the fact that the value of the gas-water partition coefficient of sevoflurane is much lower than those of the other three volatile anesthetics. Since the partition coefficient of sevoflurane is low, the KCl aqueous solution behaves like a nonlinear penetration gate, and a sudden rather than a gradual penetration takes part, resulting in the disappearance of the self-sustained oscillation without a phase of gradual change of the period of oscillation.

The breakdown of the self-sustained oscillations resulting from the addition of volatile anesthetics to the aqueous solutions would be associated with the inhibition of dynamic membrane activity in animals. In view of the fact that the anesthetic agent was injected into the 5 mM KCl solution adjacent to the hydrophobic phase of the DOPH-membrane filter, it can be suggested that the volatile anesthetic agents affected the hydrophobic phase of the DOPH molecules and impeded their repetitive conformational change between the hydrophilic and hydrophobic phases.

Like the functional neural protein conformational changes (channel opening, receptor transduction, etc.), the repetitive conformational change of the DOPH molecule in the pore depends on the mechanism of electron mobility within the hydrophobic part of the DOPH molecule, and anesthetics may act by inhibiting that mobility. The present experimental study may support the validity of a new unitary mechanism for anesthesia in terms of a common mechanism of electron mobility within hydrophobic regions [7].

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