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Influence of Inhalation Anesthetics on Ion Transport across a Planar Bilayer Lipid Membrane

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Ion transport from one aqueous phase (W1) to another (W2) across a planar bilayer lipid membrane (BLM) in the presence of inhalation anesthetics was electrochemically investigated. In the absence of inhalation anesthetics in the BLM system, no ion transport current flowed between W1 and W2 across the BLM. When inhalation anesthetics such as halothane, chloroform, diethyl ether and trichloroethylene were added to the two aqueous phases or the BLM, the ion transport current quite clearly appeared. When the ratio of the concentration of KCl or NaCl in W1 to that in W2 was varied, the zero current potential across the BLM was shifted. By considering the magnitude of the potential shift, we concluded that the ion transport current can be predominantly ascribed to the transport of Cl^- across the BLM. Since the dielectric constants of these anesthetics are larger than that of the inner hydrophobic domain of the BLM, the concentration of hydrophilic electrolyte ions in the BLM increases with the increase in the dielectric constant of the inner hydrophobic domain caused by addition of these anesthetics. These situations lead to an increase in the ion permeability coefficient.

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Introduction

The ion transport across a nerve cell membrane is closely related to neurotransmission.[1,2] Since lipid bilayers serve as large energy barriers to the membrane transport of small inorganic ions such as K^+, Na^+ and Cl^-,[3] ionic channels located in the synapse play an important role in the ion transport across the nerve cell membrane. The effect of local anesthetics such as lidocaine and procaine is generally explained in terms of the sodium channel blocking of nerve axons.[4,5] Although there are some published works which mention similar channel blocking mechanism for inhalation anesthetics,[6-8] such a mechanism is hard to accept because the specific binding sites are unknown.[9] It has frequently been proposed that the structural change of channel proteins, which is caused by the phase separation of lipid bilayers, the increase of membrane fluidity and the increase of membrane volume, causes the invalidation of the channel function.[9–12] Nowadays, there exists no single explanation of the function on the inhalation anesthetics. On the other hand, variations in the conductance and the dielectric constant of lipid bilayers induced by addition of inhalation anesthetics have also been reported.[13,14] However, the relation between such variations and the transmembrane ion movement remains to be elucidated.

The ion transport between two aqueous phases (W1 and W2) separated by a bilayer lipid membrane (BLM), which is considered as one of the simple biomembrane models, has been investigated in order to analyze the features of the ion transport across biomembranes.[15,16] In particular, a planar BLM system has been utilized, since the ionic composition in aqueous phases and in lipid components in the BLM can easily be regulated.

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In the present work, the facilitated transport of small hydrophilic ions such as K^+, Na^+ and Cl^- across the BLM on the addition of inhalation anesthetics was electrochemically investigated in order to understand the anesthesia mechanism.

Experimental

Reagents and chemicals

Lecithin (PC, Wako Pure Chemical Ind., Ltd.) and cholesterol (Ch, Kanto Chemical Co. Inc.) were used to form the BLM. BLM-forming solution was prepared by dissolving a mixture of about 10 mg of PC and about 5 mg of Ch in 1 mL of *n*-decane (Wako Pure Chemical Ind., Ltd.). Chloroform (Chl, Wako Pure Chemical Ind., Ltd.), diethyl ether (DE, Wako Pure Chemical Ind., Ltd.), halothane (Hal, Sigma-Aldrich Co.) and trichloroethylene (TE, Sigma-Aldrich Co.) were used as inhalation anesthetics. In order to form anesthetics-containing BLM, we prepared the BLM-forming solution by dissolving 10 mg of PC and 5 mg of Ch in 1 mL of a x:$(1 - x)$ mixture of the anesthetic and *n*-decane. All other reagents were of reagent grade and were used without further purification.

Apparatus

The electrochemical cell used for electrochemical measurements with the BLM system was the same as that used in the previous works.[15,16] The BLM was constructed as a black lipid membrane by brushing the BLM-forming solution on a 1.0-mm diameter aperture created on the tetrafluoroethylene resin sheet of the electrochemical cell. The formation of the BLM was confirmed by microscopic observations and capacitance measurements.[15,17]

The electrochemical cell system employed in the present study is indicated by Eq. (1).

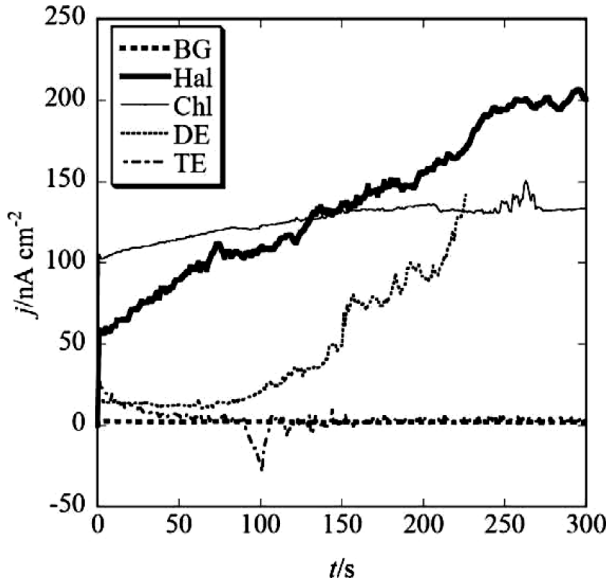
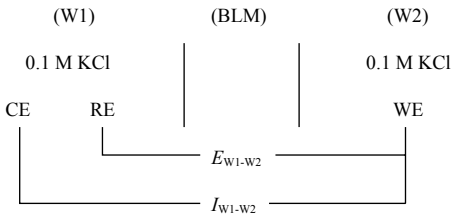


Fig. 1 Time-courses of the current densities at 0.05 V of the applied potential through the BLMs in the absence or presence of inhalation anesthetics in W1 and W2.



(1)

The cell has two aqueous compartments separated by a tetrafluoroethylene resin sheet of a thickness of 0.2 mm, the two compartments, W1 and W2, were filled with 15 mL of aqueous solution. The cell was then placed in a Faraday cage in order to decrease the background noise during the electrochemical measurements. Electrochemical measurements were performed on an automatic polarization system (Hokuto Denko Co., HSV-100). The potential difference, $E_{W1\text{-}W2}$, was applied between two Ag|AgCl electrodes (RE and WE) and the current, $I_{W1\text{-}W2}$, between WE and a Pt wire electrode (CE) was recorded. The current density, $j_{W1\text{-}W2}$, was obtained by dividing $I_{W1\text{-}W2}$ by the area of the BLM. All voltammograms were measured at a scan rate of 10 mV s^{-1} and at 25 ± 1°C, unless otherwise mentioned.

Results and Discussion

Facilitated ion transport across the planar BLM by the addition of inhalation anesthesics

Figure 1 shows the time-courses of the current densities observed at 50 mV of the membrane potential, $E_{W1\text{-}W2}$, between W1 and W2 across the BLM, where W1 and W2 contained 0.1 M KCl while the BLM contained inhalation anesthetics such as halothane (Hal), chloroform (Chl), diethylether (DE) and trichloroethylene (TE). In the absence of these inhalation anesthetics, no current due to the transfer of any ions was observed. This means that the BLM serves as a barrier to the permeation of hydrophilic ions such as K^+, Cl^-, *etc*. When 0.1 M KCl solutions saturated with Hal, Chl, DE or TE were used as W1 and W2, the ion transport currents were distinctly observed. The point in time just after the formation of the BLM was regarded as the starting point ($t = 0$). Although the electrochemical cell was not a closed system, the concentration of Hal, Chl, DE or TE in 0.1 M KCl containing excess anesthetics was almost constant. Except for TE, the current densities increased with time. Since these anesthetics were distributed from aqueous phases to the BLM, the concentrations of these anesthetics in the BLM could be increased gradually. It caused the increase in the relative permittivity of the inside of the BLM. In case of TE, the current density decreased gradually. The solubility of TE in water is less than that of other inhalation anesthetics and the partition coefficient of TE between aqueous and octanol is higher than that of other inhalation anesthetics, as summarized in Table 1. Under the initial condition, the ion transport current was observed, since a small amount of TE was distributed from both W1 and W2 to the BLM. As time went on, most of TE seemed to be moved to the residual *n*-decane phase which was dispersed into W1 and W2. On the other hand, the current densities were also measured at 50 mV of $E_{W1\text{-}W2}$ in

Table 1 Dielectric constants, ε, solubilities in water (g per 100 g of water), partition coefficients between octanol and water[18-20]

	Dielectric constant, ε	Solubility in water	Partition coefficient (log P)
Halothane	4.66[a]	0.45	2.30
Chloroform	4.8069	0.80	1.97
Diethyl ether	4.2666	6.3	0.89
Trichloroethylene	3.390	0.128	2.53
n-Decane	1.9853	0.0000015	6.25
BLM	2.09[b]		

Data except those noted *a* and *b* are from Ref. 18.
a. Ref. 19.
b. Ref. 20.

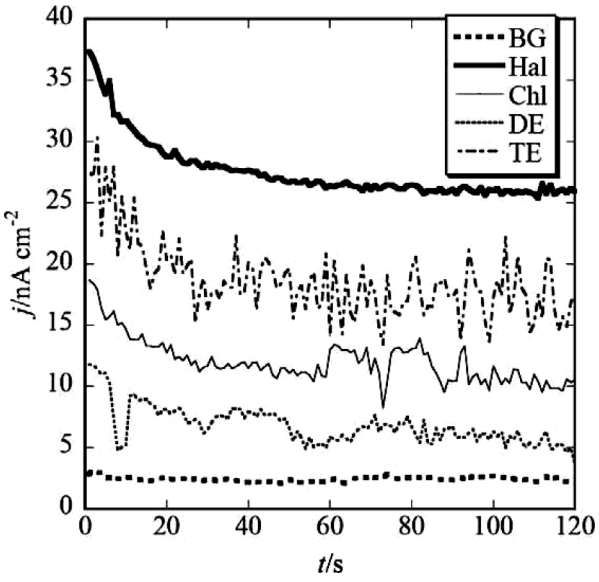


Fig. 2 Time-courses of the current densities at 0.05 V of the applied potential through the BLMs in the absence or presence of inhalation anesthetics in the *n*-decane solutions to form the BLMs.

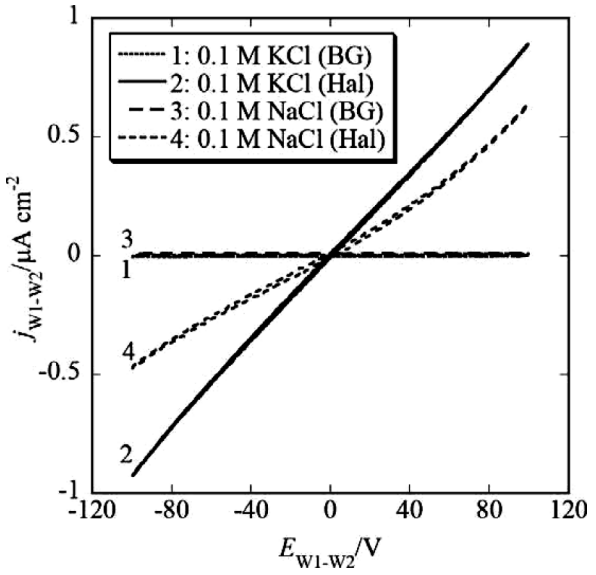


Fig. 3 Cyclic voltammograms for the ion transfer across the BLM between W1 and W2 containing 0.1 M KCl (curves 1 and 2) or 0.1 M NaCl (curves 3 and 4). Curves 2 and 4 were obtained by use of the aqueous solutions saturated with Hal. Potential scanning rate of $E_{W1\text{-}W2}$: 10 mV s^{-1}.

the presence of Hal, Chl, DE or TE in the BLMs. In this case, 0.3:0.7 mixtures of the anesthetic and *n*-decane were used as solvents to prepare the BLM-forming solutions. The time-courses of the current densities at $E_{W1\text{-}W2} = 50$ mV are shown in Fig. 2. The current densities in the presence of Hal, Chl, DE and TE were 3 to 10 times larger than the current density in the absence of anesthetics just after the BLMs were formed. In all cases, the current densities decreased with time. This may be attributed to the decrease of the anesthetics in the BLMs, since the anesthetics were leached from the BLMs to aqueous phases to reach the distribution equilibria. Since it was difficult to control the concentration of these anesthetics in the BLM, the reproducibility of the current densities was poor. Shibata *et al*. reported the current fluctuation of the ion transport across lipid bilayers in the presence of local anesthetics such as tetracaine and benzocaine,[21] in which the formation of pores in the lipid membrane by the interaction with the local anesthetics was proposed. It seems, however, that the ion transport should not be ascribed to the formation of pores in the present case, because the ion transport current flowed continuously.

Voltammetric interpretation of the ion transport across the planar BLM in the presence of halothane

In order to decrease the fluctuation of the concentration of the inhalation anesthetics in the BLM, the relation between the applied $E_{W1\text{-}W2}$ and the current density, $j_{W1\text{-}W2}$, was investigated using a cell system, of which all phases contained Hal. The aqueous solution used in this procedure was saturated with Hal, and a mixture of Hal and *n*-decane was used as a solvent to prepare the BLM-forming solution. When the Hal/*n*-decane ratio of the mixed solvent was larger than 0.15, the current densities across the BLM at a given $E_{W1\text{-}W2}$ became reproducible to a level of about ±20%. Figure 3 shows cyclic voltammograms for the ion transfer between W1 and W2 containing 0.1 M KCl in the absence and presence of Hal at 10 mV s^{-1} in the potential region between −100 and +100 mV. No Faradaic current was observed in the absence of Hal in the cell system. In the presence of Hal in all phases, however, the voltammogram became steady state and was a symmetrical rotated-sigmoidal curve about the origin (0 V, 0 A), as shown by curve 2 in Fig. 3. The current density was almost proportional to $E_{W1\text{-}W2}$ in the potential range from +70 to −70 mV. When NaCl was used instead of KCl, a similar characteristic voltammogram was obtained, as shown by curve 3 in Fig. 3. The current densities at $E_{W1\text{-}W2} = 10$, 25 and 50 mV in the case of 0.1 M KCl were 140 ± 30, 300 ± 50 and 550 ± 100 nA cm^{-2}, respectively. On the other hand, those in the case of 0.1 M NaCl were 61 ± 6, 140 ± 20 and 300 ± 30 nA cm^{-2}, respectively. The current densities at the same $E_{W1\text{-}W2}$ were about 50% less than those observed in the case of KCl. It is well known that the ion transfer current density has been described by Goldman–Hodgkin–Katz equation, when the mass transfer within the BLM is the rate-determining step.[16,22] Therefore, the current density is expressed by Eq. (2):

$$j_{W1\text{-}W2} = \frac{c_W P z^2 F^2}{RT} E_{W1\text{-}W2} \quad (2)$$

where c_W, P, z, R, T and F denote the concentration of a salt MX in W1 and W2, the permeation coefficient, the charge number of the ions, gas constant, temperature and the Faraday constant. The current density is directly proportional to the permeation coefficient (P), which is a function of the distribution coefficient of the electrolyte ions between W and the BLM (β), the diffusion coefficient of the ion (D), and the thickness of the BLM (d), and is given by Eq. (3):

$$P = \beta D/d \quad (3)$$

Here, β can be related to the standard Gibbs transfer free energies of M^+ and X^- from W to BLM ($\Delta G^o_{tr,M^+}$ and $\Delta G^o_{tr,X^-}$):[16,23]

$$\ln \beta = -\frac{\Delta G^o_{tr,M^+} + \Delta G^o_{tr,X^-}}{2RT} \quad (4)$$

When the same potential difference was applied as $E_{W1\text{-}W2}$, the ratio of the current density, $j_{W1\text{-}W2}$(NaCl), in the case of 0.1 M NaCl to that, $j_{W1\text{-}W2}$(KCl), in the case of 0.1 M KCl is represented by Eq. (5):

$$\frac{j_{W1\text{-}W2}(\mathrm{NaCl})}{j_{W1\text{-}W2}(\mathrm{KCl})} = \frac{P_{\mathrm{NaCl}}}{P_{\mathrm{KCl}}} \quad (5)$$

where P_{NaCl} is the sum of permeation coefficient of Na^+, P_{Na^+}, and that of Cl^-, P_{Cl^-}, and P_{KCl} is the sum of the permeation coefficient of K^+, P_{K^+}, and P_{Cl^-}. Equation (5) can be rewritten as Eq. (6) by assuming the fixed thickness of the BLM:

$$\frac{j_{W1\text{-}W2}(\mathrm{NaCl})}{j_{W1\text{-}W2}(\mathrm{KCl})} = \frac{\beta_{\mathrm{NaCl}}(D_{Na^+} + D_{Cl^-})}{\beta_{\mathrm{KCl}}(D_{K^+} + D_{Cl^-})} \quad (6)$$

in which β_{NaCl} and β_{KCl} are the distribution coefficients of NaCl and KCl between W and the BLM and D_{K^+}, D_{Na^+} and D_{Cl^-} are the diffusion coefficients of K^+, Na^+ and Cl^- within the BLM. Based on the Stokes–Einstein relation,[24] $(D_{Na^+} + D_{Cl^-})/(D_{K^+} + D_{Cl^-})$ is estimated to be 0.83 by use of Shannon's ionic radii (Na^+, 0.116 nm; K^+, 0.152 nm; Cl^-, 0.181 nm).[25] Therefore, Eq. (6) can be converted to Eq. (7):

$$\frac{\beta_{\mathrm{NaCl}}}{\beta_{\mathrm{KCl}}} = 0.43 \quad (7)$$

If one takes into account the relation between Eqs. (3) and (7), the following equation can be producted:

$$\exp\frac{\Delta G^{o}_{tr,K^+}-\Delta G^{o}_{tr,Na^+}}{2RT}=0.43 \quad (8)$$

Since ΔG^{o}_{tr} values, however, cannot be evaluated exactly, we may utilize the estimated values of ΔG^{o}_{tr} of K^+, Na^+ and Cl^- ($\Delta G^{o}_{tr,K^+}$, $\Delta G^{o}_{tr,Na^+}$ and $\Delta G^{o}_{tr,Cl^-}$) based on the concept proposed by Parsegian.[26,27]

$$\Delta G^{o}_{tr}=\frac{N_A e^2}{8\pi\varepsilon_0 a}\left(\frac{1}{\varepsilon_M}-\frac{1}{\varepsilon_W}\right)-\frac{N_A e^2}{4\pi\varepsilon_0\varepsilon_M d}\ln\left(\frac{2\varepsilon_W}{\varepsilon_W+\varepsilon_M}\right) \quad (9)$$

Here, N_A is the Avogadro constant, e the elementary electric charge, ε_0 the permittivity of free space, ε_W the dielectric constant of water, ε_M the dielectric constant of the hydrocarbon layers within the BLM, a the ionic radius of the transport ion, and d the thickness of the BLM. When Eq. (9) is substituted in Eq. (8), Eq. (10) is obtained.

$$\left(\frac{1}{a_{K^+}}-\frac{1}{a_{Na^+}}\right)\left(\frac{1}{\varepsilon_M}-\frac{1}{\varepsilon_W}\right)=\frac{16\pi\varepsilon_0 RT}{N_A e^2}\ln 0.43 \quad (10)$$

From Eq. (10), a value of 7.4 is derived as ε_M. This value seems to be too high, but it is clear that the ion permeability across the BLM depends on the dielectric constant of the inner layer of the BLM.[27] Although it is still larger than the dielectric constant of Hal (4.66), it is certain that the increase of the dielectric constant of the hydrocarbon layers within the BLM brings about the increase of the concentration of electrolyte ions in the BLM.

Figure 4 indicates cyclic voltammograms for the ion transport between W1 and W2, of which ionic compositions were asymmetrical with respect to KCl, across the BLM in the presence of Hal in all phases. In the absence of Hal, $j_{W1\text{-}W2}$ was very small. In the presence of Hal in all phases, however, a large $j_{W1\text{-}W2}$ was observed. When the concentration of KCl in W1 was identical to that in W2 (1 or 10 mM), the voltammogram was in a steady state and the waveform was symmetrical about the origin. When the voltammogram was measured between W1 containing 10 mM KCl and W2 containing 1 mM KCl, the waveform changed and became asymmetrical in shape. It is thought that the influence of the transport of K^+ on that of Cl^- is negligibly small, because the permeability of Cl^- is much higher than that of K^+.[14] The zero current membrane potential, $E_{W1\text{-}W2}$, $j = 0$ ($E_{W1\text{-}W2}$ at $j_{W1\text{-}W2} = 0$ μA cm^{-2}) was also shifted to the negative direction by about –55 mV. The authors have already proposed that the theoretical value of $E_{W1\text{-}W2,j=0}$ is represented by Eq. (11) in a cell system with an asymmetrical ionic composition.[16]

$$E_{W1\text{-}W2,\,j=0}=-\frac{RT}{F}\ln\frac{D_{M^+}C_{ratio}+D_{X^-}}{D_{M^+}+D_{X^-}C_{ratio}} \quad (11)$$

where C_{ratio} is the ratio of the concentration of KCl in W2 to that in W1. The experimental membrane potential, $E_{W1\text{-}W2,exp}$, was compared with the theoretical membrane potential, $E_{W1\text{-}W2,theo}$, calculated in the case of $C_{ratio} = 0.10$. Since the $E_{W1\text{-}W2,exp}$ value at $C_{ratio} = 0.10$ was -57 ± 1 mV, the ratio of D_{K^+} to D_{Cl^-} was estimated at 0.089. This indicates that D_{Cl^-} was about ten times larger than D_{K^+}. Yoshida *et al*. reported the similar influence of the addition of Hal on the ion transport across the planar lipid bilayer prepared from oleylamine.[14] In addition, they also reported that the relative permittivity within the BLM was changed from 2.10 to 2.46 on the addition of Hal. This result

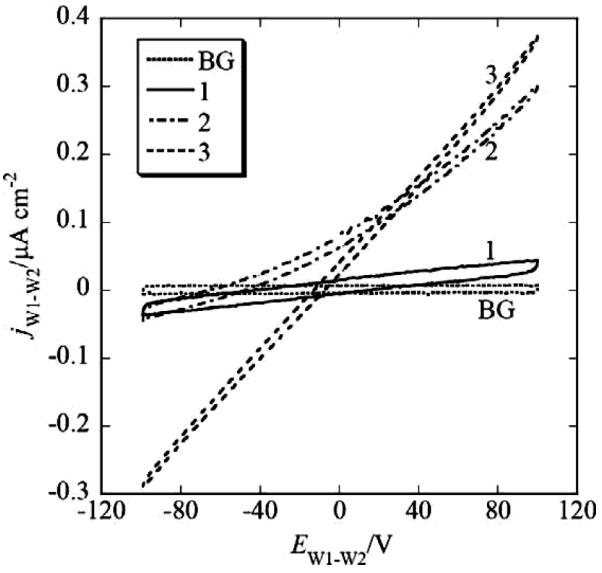


Fig. 4 Cyclic voltammograms for the ion transfer across the BLM in the presence of Hal between W1 and W2 saturated with Hal. Curve 1, 1 mM KCl in W1 and W2; curve 2, 10 mM KCl in W1 and 1 mM KCl in W2; curve 3, 10 mM KCl in W1 and W2. Potential scanning rate of $E_{W1\text{-}W2}$: 10 mV s^{-1}.

may be interpreted by our proposal on the facilitated ion transport across the BLM by the addition of inhalation anesthetics. However, they considered that the counter cation was not transported across the BLM. Similar phenomena on the permeability of several ion species across lipid bilayers in the presence of 1-chlorodecane were noted by Dilger *et al*.[27] These authors proposed the ion transport mechanism including two processes; i) the distribution process and ii) the ion transport process. In the distribution process, K^+ (or Na^+) is distributed with Cl^- from W1 or W2 to the BLM by the addition of inhalation anesthetics. The ion concentration in the BLM increases with an increase in relative permittivity in the BLM. In the ion transport process, Cl^- and K^+ (or Na^+) were simultaneously transported across the BLM depending on the applied membrane potential. In this case, K^+ (or Na^+) and Cl^- are transported across the BLM in the opposite directions, and the current caused by the transport of Cl^- is about 10 times larger than that of K^+ (or Na^+). The ion transport across the BLM is facilitated in this manner. Therefore, the variation of the membrane potential brings about the permeation of ions. As for the nervous transmission, the membrane potential is immediately changed when the Na^+ channel opens.[1,2] Although the myelin sheath is usually regarded as an insulator, the conductivity of the myelin sheath is assumed to be increased with an increase of relative permittivity of the inside of the myelin sheath by the addition of inhalation anesthetics. Therefore, it is considered that the transmission of the change in the membrane potential between the end terminals of the nervous cell is prevented by the current leak in the myelin sheath.

Conclusions

In the present paper, the facilitated ion transport between two aqueous phases across the planar BLM by the addition of inhalation anesthetics was electrochemically identified.

In conclusion, the proposed mechanism of the ion transport is reasonable to explain the experimental characteristics. This result will contribute to understand and utilize biological reactions concerning ion transports across cell membranes.

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