ORIGINAL

Mechanism for propofol inhibition of Na^+, K^+-ATPase activity in rat brain

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ABSTRACT : Propofol is one of the most widely used intravenous anesthetics, however the mechanism of the anesthetic effect is not fully understood. Na^+,K^+-ATPase is an enzyme present in all animal cell membranes and plays essential roles for the maintenance of neuronal excitability. There is a report of propofol inhibition of Na^+,K^+-ATPase activity, but the mechanism is not clearly established. To study the mechanism for propofol inhibition of Na^+,K^+-ATPase purified from whole brains of rats, the effects of propofol on Na^+,K^+-ATPase activity, Na^+-ATPase, and K^+-*p*NPPase activities, which are partial reactions of Na^+,K^+-ATPase were examined. Na^+,K^+-ATPase and Na^+-ATPase activities decreased depending on the concentration of propofol, and were completely inhibited at 1.03 mM. Propofol decreased the maximum activity of Na^+-, K^+-, Mg^{2+}-, and ATP-dependent activation of Na^+,K^+-ATPase activity depending on its concentration, and changed the half maximal concentration for Na^+, K^+, and ATP, but not for Mg^{2+}. Propofol also decreased the maximum activities of Na^+-ATPase and K^+-*p*NPPase, suggesting that propofol inhibits Na^+,K^+-ATPase activity by affecting the whole reaction process of Na^+,K^+-ATPase. The inhibition of Na^+,K^+-ATPase activity by propofol was reversible by dilution of its concentration. These results suggest that propofol reversibly inhibits Na^+,K^+-ATPase activity in a mixed-type inhibition pattern.

Key Words : Intravenous anesthetic, propofol, Na^+, K^+-ATPase

Introduction

Propofol is a short-acting intravenous anesthetic with mainly a sedative effect, widely used for the induction and maintenance of general anesthesia, as well as for sedation of respiratory management in intensive care units and for intravenous sedation in dental therapy. The exact mechanism of general anesthetics including propofol has not been revealed yet, though various studies have been reported [1-7]. Propofol has been reported to have many pharmacological effects [8] : 1) It reduces cerebral blood flow, cerebral metabolic rate, and intracranial pressure. 2) It acts as an antioxidant. 3) It reduces ischemic neuronal injury in animal models of transient global or focal cerebral ischemia. 4) It activates γ-aminobutyric acid ($GABA_A$) receptors directly [8-12].

Na^+, K^+-ATPase is an enzyme present in all animal cell membranes, which translocates sodium and potassium ions across the cell membrane, utilizing the chemical energy of hydrolysis of ATP. Na^+, K^+-ATPase plays important physiological roles [13], and the activity of this enzyme is very sensitive to the influence of various bioregulators, such as cardiac steroids, transition and heavy metals, as well as metal complexes [14, 15]. We hypothesize that the inhibition of Na^+, K^+-ATPase activity by propofol may be related to the state of anesthesia or side effects caused by propofol.

Kutchai *et al.* [16] reported that 73-800 μM propofol inhibited Na^+, K^+-ATPase activity in canine renal medulla, and its IC_{50} was 127 ± 13 μM, however, details still remain to be elucidated. The aim of this study was to investigate the inhibition mechanism of Na^+, K^+-ATPase activity by propofol. We studied the propofol inhibition of Na^+, K^+-ATPase activity and the effects of propofol on

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Na^+-, K^+-, Mg^+-, and ATP- dependent activation of Na^+, K^+-ATPase activity. We also studied the effects of propofol on partial reactions of Na^+, K^+-ATPase, and the reversibility of the inhibiton by dilution of propofol concentration. We show that propofol reversibly inhibits Na^+, K^+-ATPase activity in a mixed-type inhibition pattern.

Materials and Methods

1. Enzyme preparation

The animals and tissue specimens were treated in accordance with the Guidelines of the Experimental Animal Committee, Hokkaido University Graduate School of Dental Medicine. First, microsomes were prepared from the whole brains of rats and the purification of Na^+, K^+-ATPase from microsome was accomplished according to Jorgensen's method [17] with some modifications [18]. The microsome was treated with 0.55 mg of sodium dodecyl sulfate (SDS) per milligram of microsomal protein, and centrifuged on a glycerol density gradient. After centrifugation, we recovered two layers (white cloud and medium) and pellet (button). The protein concentration was estimated by using the Bio-Rad Protein Assay (Bio-Rad Laboratories, CA) according to the manufacture's protocol with bovine serum albumin as a standard. The specific activities of white cloud, medium, and button were 3.11, 4.63, and 0.8 μmol/min/mg protein.

2. Na^+, K^+-ATPase assay

Na^+, K^+-ATPase and Na^+-ATPase activities were determined by the measurement of inorganic phosphate production according to Chifflet's method [19]. Na^+, K^+-ATPase activity was assayed in a total volume 300 μl of reaction mixture containing the enzyme (1 μg white cloud), 25 mM sucrose, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 50 mM tris-HCl at pH 7.41, 160 mM NaCl, 16 mM KCl and 5 mM MgCl2. After pre-incubation, the reaction was started by the addition of 50 μl of 30 mM ATP, allowed to proceed for 30 minutes at 37℃, and then the reaction was stopped by the addition of 12% SDS. In brief, 0.6 ml of the solution containing 3% ascorbic acid, 0.5 N HCl and 0.5% ammonium molybdate was added to the 0.6 ml reaction mixture with SDS, which was left for 3-10 minutes at room temperature. Then, 0.9 ml of a solution containing 2% sodium citrate, 2% sodium metaarsenite and 2% acetic acid was added to the mixture, which was then incubated for 10 minutes at 37℃. The developed color was read at 850 nm spectrophotometrically with a Hitachi U-2000 spectrophotometer. The results are expressed as the mean percentage of enzyme activity relative to the corresponding control value, the data derived from at least 3 experiments, each experiment using 3 samples of propofol.

1) Concentration-dependent inhibition of Na^+, K^+-ATPase activity by propofol

Various concentrations of propofol were added to the above reaction mixture and the effects were observed by assaying Na^+, K^+-ATPase activity.

2) The effect of propofol on the affinities of Na^+, K^+-ATPase for Na^+, K^+, Mg^{2+}, or ATP.

Na^+, K^+-ATPase activity was measured in the reaction mixture containing the enzyme with different concentrations of Na^+, K^+, Mg^{2+}, or ATP. The effects of 0.14, 0.26, or 0.35 mM propofol and dimethyl sulfoxide (DMSO) used as a solvent were observed on the Na^+-, K^+-, Mg^{2+}- or ATP-concentration dependent activation of Na^+, K^+-ATPase activity.

3. The effect of propofol on the affinity of Na^+-ATPase activity for Na^+.

To reveal at which stage of the reaction mechanism propofol had an effect on Na^+, K^+-ATPase, Na^+-ATPase activity, the anterior half partial reaction of Na^+, K^+-ATPase, was measured. Na^+-ATPase activity was measured in the reaction mixture containing the enzyme (5.25 μg medium), 25 mM sucrose, 0.1 mM EDTA, 50 mM tris-HCl at pH 7.41 and 5 mM $MgCl_2$, changing the concentration of Na^+ from 0-20 mM with and without propofol and DMSO. The reaction time was decided to be 1 hour because of weak ATP hydrolys of Na^+-ATPase. Na^+-ATPase activity was also determined by measurement of inorganic phosphate.

4. The effect of propofol on the affinity of K^+-*p*NPPase activity for K^+.

K^+-*p*NPPase activity, posterior half partial reaction of Na^+, K^+-ATPase, was measured in the reaction mixture containing the enzyme (15 μg medium), 25 mM sucrose, 0.1 mM EDTA, 50 mM tris-HCl at pH 7.41 and 5 mM $MgCl_2$, changing the concentration of K^+ from 0-20 mM with and without propofol and DMSO. The reaction was started by the addition of 200 μl of 16 mM *p*NPP, allowed to proceed for 30 minutes at 37℃, and then it was stopped by adding 2 ml of 2% SDS and 1.25% Na_2CO_3. The

developed color was read at 420 nm spectrophotometrically.

5. Reversibility of Na^+, K^+-ATPase activity inhibited by propofol.

Whether the inhibition by propofol was reversible or not was examined as below. At first, the reaction mixture containing the enzyme (1 μg white cloud), 25 mM sucrose, 0.1 mM EDTA, 50 mM tris-HCl at pH 7.41, 160 mM NaCl, 16 mM KCl and 5 mM $MgCl_2$ was pre-incubated with 1.03 mM propofol and 10% DMSO at room temperature or on ice for 30 minutes, and then the propofol and DMSO concentration was reduced by dilution to the concentrations described in Fig. 9. Then Na^+, K^+-ATPase activity was measured as described above.

6. Drugs and chemicals

All the drugs and chemicals used in this study were obtained from Wako Pure (Osaka, Japan). 2, 6-Diisopropylphenol, which is the active ingredient of propofol, has high lipid solubility and little water solubility. Therefore propofol is clinically used as a propofol injection with additives like soybean oil and triglyceride to make an emulsion. However, it is impossible to use an emulsion in this study as turbidity disturbs the measurement of absorbance. We dissolved 2, 6-diisopropylphenol into DMSO as a 0.2% solution and then this solution was diluted with water.

7. Statistics

The data are expressed using the means of at least 3 independent experiments. Statistical assessment of the data was examined by a Student's *t*-test. Differences were considered to be statistically significant when $P<0.05$ and $P<0.01$.

Results

1. Concentration-dependent inhibition of Na^+, K^+-ATPase activity by propofol

Specific activity of Na^+, K^+-ATPase used for ATPase assay was 3.11-4.63 μmol/mg protein/min. Fig. 1 shows the inhibition of Na^+, K^+-ATPase activity in the presence of various concentrations of propofol. The activity decreased depending on the concentration of propofol, and was inhibited completely at 1.03 mM. The concentration that caused half-maximal inhibition (IC_{50}) of Na^+, K^+-ATPase activity was 0.26 mM and the Hill coefficient n was 1.11, analyzed by the Hill equation.

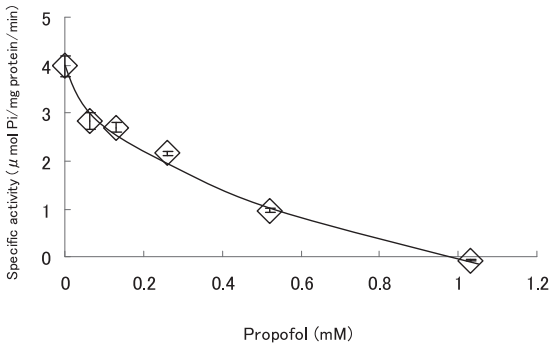

Fig. 1 Concentration-dependent inhibition of Na^+, K^+-ATPase activity by propofol.

Na^+, K^+-ATPase activity was measured in the presence of various concentrations of propofol. The activity decreased depending on the concentration of propofol, and was inhibited completely at 1.03 mM. The concentration that caused half-maximal inhibition of Na^+, K^+-ATPase activity was 0.26 mM. Data have a mean ± SEM ($n \geqq 3$).

2. The effect of propofol on the ATP concentration-dependent activation of Na^+, K^+-ATPase activity.

Fig. 2a shows the effect of propofol on the ATP concentration dependency of Na^+, K^+-ATPase activity. In the absence of DMSO and propofol (◇), the activity increased, depending on the ATP concentration. A double-reciprocal plot of ATP concentration versus ATPase activity resulted in two straight lines (Fig. 2b). By extrapolation of each line to x and y axes, high (0.12 mM) and low (0.48 mM) Km values and corresponding maximum activities (Vmax) were calculated (Fig. 2b and Table 1). Similar experiments were done in the presence of 0.14 (△), 0.26 (○) and 0.35mM (×) propofol or DMSO (□) as controls for propofol solvent (Fig. 2a). The results were analyzed as shown in Fig. 2b, and Km and Vmax values for high and low affinity sites were summarized in Table 1. Propofol decreased Vmax values for both sites depending on its concentration. However, it increased affinities for ATP for both high and low ATP affinity sites (Table 1).

3. The effect of propofol on the Na^+ concentration-dependent activation of Na^+, K^+-ATPase activity.

Fig. 3 shows the effect of propofol on the Na^+ concentration dependency of Na^+, K^+-ATPase activity. In the absence of DMSO and propofol (◇), the activity increased, depending on the Na^+ concentration. The results were analyzed by the Hill equation, and the Na^+ concentration that caused half-maximal activation ($[S]_{0.5}$), maximum activitiy (Vmax) of Na^+, K^+-ATPase activity,

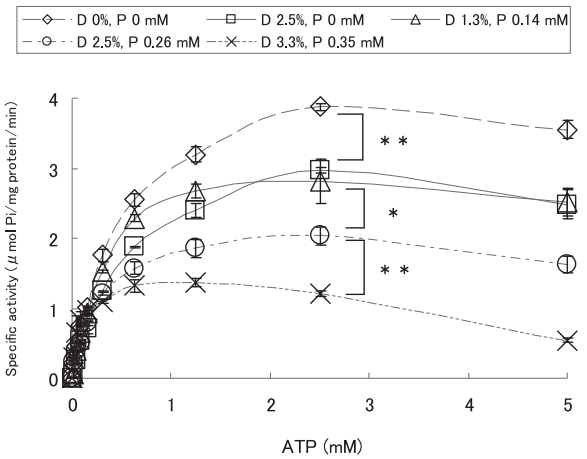

Fig. 2a The effect of propofol on the ATP concentration-dependent activation of Na^+, K^+-ATPase activity.

ATP concentration dependency of Na^+, K^+-ATPase activity was measured in the absence of DMSO and propofol (◇), or in the presence of 2.5% DMSO without propofol (□); 1.3% DMSO and 0.14 mM propofol (△); 2.5% DMSO and 0.26 mM propofol (○); 3.3% DMSO and 0.35mM propofol (×). The results were analyzed as shown in Fig. 2b, and Km and Vmax values for high and low affinity sites are summarized in Table 1. Data have a mean ±SEM (n≧3).

*P<0.05 and **P<0.01 compared with two groups of each by a Student's *t*-test. (◇) vs (□); P<0.01, (△) vs (○); P<0.05, (○) vs (×); P<0.01.

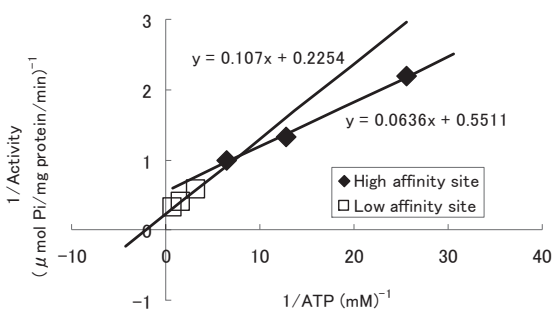

Fig. 2b Double-reciprocal plot of ATP concentration versus ATPase activity.

The results obtained in the experiments of Fig. 2 a were analyzed by a double-reciprocal plot. The double-reciprocal plot of ATP concentration versus Na^+, K^+-ATPase activity resulted in two straight lines. By extrapolation of each line to x and y axes, high and low Km values and corresponding maximum activities (Vmax) were calculated. The concentration of ATP was shown as molar concentration multiplied by 10^4 M. Reaction velocity was expressed as μmol Pi released/ mg protein per min. The Km and Vmax values for high and low affinity sites calculated from each experiment are summarized in Table 1.

Table 1 Effects of propofol on ATP-dependent activation of Na^+, K^+-ATPase activity.

DMSO (%)	Propofol (mM)	High affinity site		Low affinity site	
		Km (mM)	Vmax (μmol/mg/min)	Km (mM)	Vmax (μmol/mg/min)
0	0	0.12	1.82	0.48	4.45
2.5	0	0.35	2.6	0.67	3.82
1.3	0.14	0.12	1.63	0.51	3.99
2.5	0.26	0.06	0.9	0.27	2.22
3.3	0.35	0.02	0.8	0.24	1.76

Km and Vmax values were calculated as shown in Fig. 2b using double-reciprocal plot.

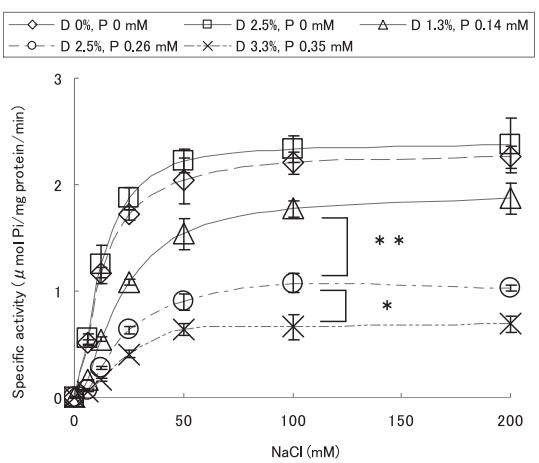

Fig. 3 The effect of propofol on the Na^+ concentration-dependent activation of Na^+, K^+-ATPase activity.

Na^+ concentration dependency of Na^+, K^+-ATPase activity was measured in the absence of DMSO and propofol (◇), or in the presence of 2.5% DMSO without propofol (□); 1.3% DMSO and 0.14 mM propofol (△); 2.5% DMSO and 0.26 mM propofol (○); 3.3% DMSO and 0.35 mM Propofol (×). The results were analyzed by the Hill equation, and the Na^+ concentration that caused half-maximal activation ($[S]_{0.5}$), maximum activitiy (Vmax) of Na^+, K^+-ATPase activity, and the Hill coefficient n are summarized in Table 2. Data have a mean ±SEM (n≧3).

*P<0.05and **P<0.01compared with two groups of each. (△) vs (○); P<0.01, (○) vs (×); P<0.05.

and the Hill coefficient n were calculated as 12.57 mM, 2.26 μmol/mg protein/min and 1.71, respectively (Table 2). Similar experiments were done in the presence of 0.14 (△), 0.26 (○) and 0.35 mM (×) propofol or DMSO (□) (Fig. 3). The results were analyzed by the Hill equation, and $[S]_{0.5}$, Vmax and the Hill coefficient n are summarized in Table 2. DMSO did not change the $[S]_{0.5}$ and Vmax significantly, but propofol decreased both Vmax and affinities for Na^+.

Table 2 Effects of propofol on Na^+-dependent activation of Na^+, K^+-ATPase activity.

DMSO (%)	Propofol (mM)	Hill coefficient	$[S]_{0.5}$ (mM)	Vmax (μmol/mg/min)
0	0	1.71	12.57	2.26
2.5	0	1.91	11.91	2.37
1.3	0.14	1.86	21.16	1.87
2.5	0.26	2.04	21.65	1.03
3.3	0.35	2.14	19.67	0.69

Concentration-response data of Fig. 3 were fitted by nonlinear regression to the Hill equation.
$[S]_{0.5}$ = the concentration with half maximal activity. Vmax = the maximum activity.

4. The effect of propofol on the K^+ concentration-dependent activation of Na^+, K^+-ATPase activity.

Fig. 4 shows the effect of propofol on the K^+ concentration dependency of Na^+, K^+-ATPase activity. In the absence of DMSO and propofol (◇), the activity increased, depending on the K^+ concentration. The results were analyzed by the Hill equation, and the $[S]_{0.5}$ for K^+, Vmax and the Hill coefficient n were calculated as 1.5 mM, 2.19 μmol/mg protein/min and 1.53, respectively (Table 3). Similar experiments were done in the presence of 0.14 (△), 0.26 (○) and 0.35 mM (×) propofol or DMSO (□) (Fig. 4). The results were analyzed by the Hill equation, and $[S]_{0.5}$, Vmax and the Hill coefficient n are summarized in Table 3. DMSO did not change the $[S]_{0.5}$ and slightly increased Vmax, but propofol decreased the Vmax and increased the affinity for K^+.

5. The effect of propofol on the Mg^{2+} concentration-dependent activation of Na^+, K^+-ATPase activity.

Fig. 5 shows the effect of propofol on the Mg^{2+} concentration dependency of Na^+, K^+-ATPase activity. In the absence of DMSO and propofol (◇), the activity increased, depending on the Mg^{2+} concentration. The results were analyzed by the Hill equation, and the $[S]_{0.5}$ for Mg^{2+}, Vmax and the Hill coefficient n were calculated as 0.43 mM, 2.23 μmol/mg protein/min and 1.15, respectively (Table 4). Similar experiments were done in the presence of 0.14 (△), 0.26 (○) and 0.35 mM (×) propofol or DMSO (□) (Fig. 5). The results were analyzed by the Hill equation, and $[S]_{0.5}$, Vmax and the Hill coefficient n are summarized in Table 4. DMSO did not change the $[S]_{0.5}$ significantly but increased Vmax. Propofol also did not change the $[S]_{0.5}$ significantly but decreased the Vmax, depending on its concentration.

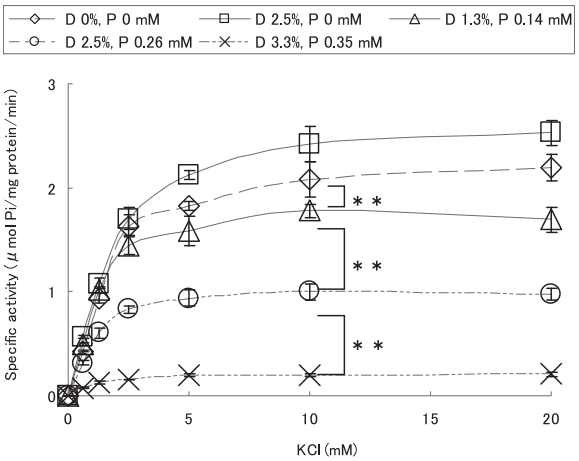

Fig. 4 The effect of propofol on the K^+ concentration-dependent activation of Na^+, K^+-ATPase activity.

K^+ concentration dependency of Na^+, K^+-ATPase activity was measured in the absence of DMSO and propofol (◇), or in the presence of 2.5% DMSO without propofol (□); 1.3% DMSO and 0.14 mM propofol (△); 2.5% DMSO and 0.26 mM propofol (○); 3.3% DMSO and 0.35 mM propofol (×). The results were analyzed by the Hill equation, and the $[S]_{0.5}$ for K^+, Vmax, and the Hill coefficient n are summarized in Table 3. Data have a mean ± SEM (n≧3).
**P<0.01compared with two groups of each. (◇) vs (△); P<0.01, (△) vs (○); P<0.01, (○) vs (×); P<0.01.

Table 3 Effects of propofol on K^+-activation of Na^+, K^+-ATPase activity.

DMSO (%)	Propofol (mM)	Hill coefficient	$[S]_{0.5}$ (mM)	Vmax (μmol/mg/min)
0	0	1.53	1.5	2.19
2.5	0	1.54	1.5	2.53
1.3	0.14	1.5	1.09	1.78
2.5	0.26	1.67	0.99	1
3.3	0.35	1.75	0.96	0.21

Concentration-response data of Fig. 4 were fitted by nonlinear regression to the Hill equation like Table 2.

Table 4 Effects of propofol on Mg^{2+}-dependent activation of Na^+, K^+-ATPase activity.

DMSO (%)	Propofol (mM)	Hill coefficient	$[S]_{0.5}$ (mM)	Vmax (μmol/mg/min)
0	0	1.15	0.43	2.23
2.5	0	1.37	0.47	2.7
1.3	0.14	1.13	0.44	1.59
2.5	0.26	1.51	0.5	1.25
3.3	0.35	1.61	0.49	1.01

Concentration-response data of Fig. 5 were fitted by nonlinear regression to the Hill equation like Table 2, and 3.

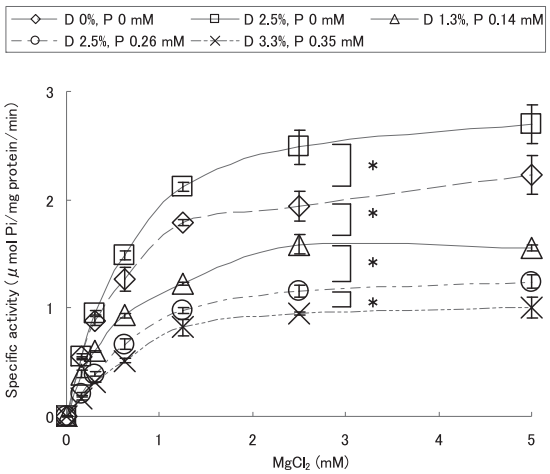

Fig. 5 The effect of propofol on the Mg^{2+} concentration-dependent activation of Na^+, K^+-ATPase activity.

Mg^{2+} concentration dependency of Na^+, K^+-ATPase activity was measured in the absence of DMSO and propofol (◇), or in the presence of 2.5% DMSO without propofol (□); 1.3% DMSO and 0.14 mM propofol (△); 2.5% DMSO and 0.26 mM propofol (○); 3.3% DMSO and 0.35 mM propofol (×). The results were analyzed by the Hill equation, and the $[S]_{0.5}$ for Mg^{2+}, Vmax, and the Hill coefficient n are summarized in Table 4. Data have a mean ± SEM (n≧3).

*P<0.05 compared with two groups of each. (□) vs (◇); P<0.05, (◇) vs (△); P<0.05, (△) vs (○); P<0.05, (○) vs (×); P<0.05.

6. Concentration-dependent inhibition of Na^+- ATPase activity by propofol

Fig. 6 shows the inhibition of Na^+-ATPase activity in the presence of various concentrations of propofol. The data obtained by Na^+-ATPase activity tended to scatter compared with Na^+, K^+-ATPase, because of the unsTableness of Na^+-ATPase. The activity decreased, depending on the concentration of propofol, and was inhibited completely at 1.03 mM. IC_{50} of Na^+-ATPase inhibition was about 0.26 mM (Fig.6).

7. The effect of propofol on the Na^+ concentration-dependent activation of Na^+-ATPase activity.

Fig. 7 shows the effect of propofol on the Na^+ concentration dependency of Na^+-ATPase activity. In the absence of DMSO and propofol (◇), the activity increased, depending on the Na^+ concentration ($[S]_{0.5}$ ≈2 mM), and Vmax was attained at 5 mM. Similar experiments were done in the presence of 0.14 mM propofol (□) or DMSO (△) (Fig. 7). Na^+-ATPase activity also increased, depending on the concentration of Na^+, and Vmax was attained at 5 mM. Both DMSO and propofol decreased the Vmax, but DMSO decreased $[S]_{0.5}$ for Na^+ ($[S]_{0.5}$ ≈1 mM), and propofol increased it ($[S]_{0.5}$ ≈3mM).

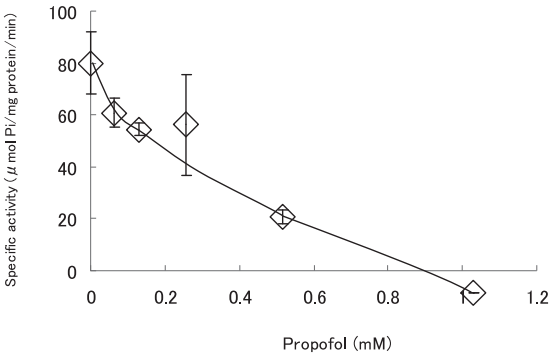

Fig. 6 Concentration-dependent inhibition of Na^+- ATPase activity by propofol.

Na^+-ATPase activity was measured in the presence of various concentrations of propofol. The activity decreased depending on the concentration of propofol, and was inhibited completely at 1.03 mM. The concentration that caused half-maximal inhibition of Na^+-ATPase activity was 0.26 mM. Data have a mean ± SEM (n≧3).

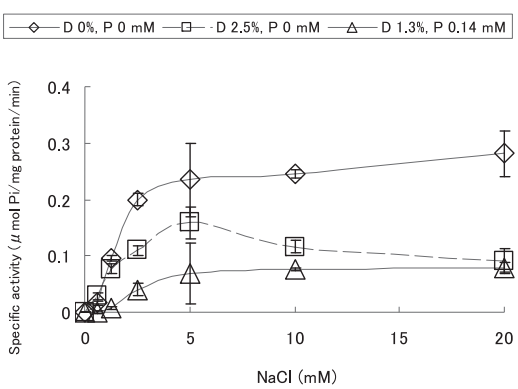

Fig. 7 The effect of propofol on the Na^+ concentration-dependent activation of Na^+-ATPase activity.

Na^+ concentration dependency of Na^+-ATPase activity was measured in the absence of DMSO and propofol (◇), or in the presence of 2.5% DMSO without propofol (□); 1.3% DMSO and 0.14mM propofol (△). Data have a mean ± SEM (n≧3).

8. The effect of propofol on the K^+ concentration-dependent activation of K^+-*p*NPPase activity.

Fig. 8 shows the effect of propofol on the K^+ concentration dependency of K^+-*p*NPPase activity. In the absence of DMSO and propofol (◇), the activity increased, depending on the K^+ concentration. The results were analyzed by the Hill equation, and the $[S]_{0.5}$ for K^+, Vmax and the Hill coefficient n were calculated as 2.43 mM, 908 nmol/mg protein/min, and 1.86 respectively (Table 5). Similar experiments were done in the presence of 0.14 (△), 0.26 (○) and 0.35 mM (×)

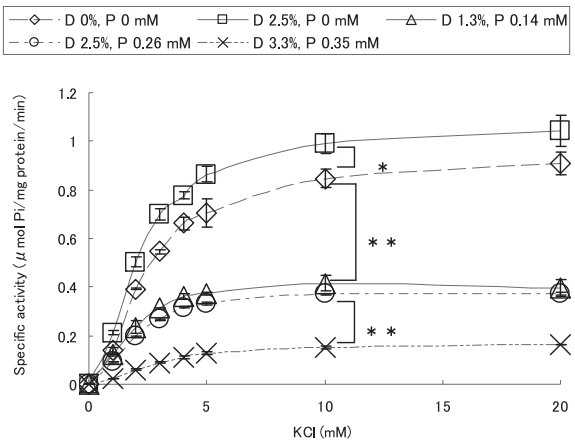

Fig. 8 The effect of propofol on the K^+ concentration-dependent activation of K^+-*p*NPPase activity.

K^+ concentration dependency of K^+-*p*NPPase activity was measured in the absence of DMSO and propofol (◇), or in the presence of 2.5% DMSO without propofol (□); 1.3% DMSO and 0.14mM propofol (△); 2.5% DMSO and 0.26 mM propofol (○); 3.3% DMSO and 0.35 mM propofol (×). The results were analyzed by the Hill equation, and the $[S]_{0.5}$ for K^+, Vmax, and the Hill coefficient n are summarized in Table 5. Data have a mean ±SEM (n≧3).

*P<0.05and **P<0.01compared with two groups of each. (□) vs (◇); P<0.05, (◇) vs (△); P<0.01, (○) vs (×); P<0.01.

Table 5 Effects of propofol on K^+-dependent activation of K^+-pNPPase activity.

DMSO (%)	Propofol (mM)	Hill coefficient	$[S]_{0.5}$ (mM)	Vmax (nmol/mg/min)
0	0	1.86	2.43	908
2.5	0	1.85	2.1	1041
1.3	0.14	1.97	1.62	415
2.5	0.26	2.49	2.04	372
3.3	0.35	1.9	2.61	163

Concentration-response data of Fig. 8 were fitted by nonlinear regression to the Hill equation like Table 2-4.

propofol or DMSO (□) (Fig. 8). The results were analyzed by the Hill equation, and $[S]_{0.5}$, Vmax, and the Hill coefficient n were summarized in Table 5. DMSO slightly decreased the $[S]_{0.5}$ and increased Vmax. Propofol decreased the Vmax, depending on its concentration but increased the affinity for K^+ at 0.14 (△) and 0.26(○) mM, and then decreased it at 0.35 mM (×).

9. Reversibility of propofol inhibition of Na^+, K^+-ATPase activity by decreasing its concentration.

As described in Materials and Methods, Na^+, K^+-ATPase was exposed to 1.03 mM propofol at which concentration the activity was completely inhibited, and

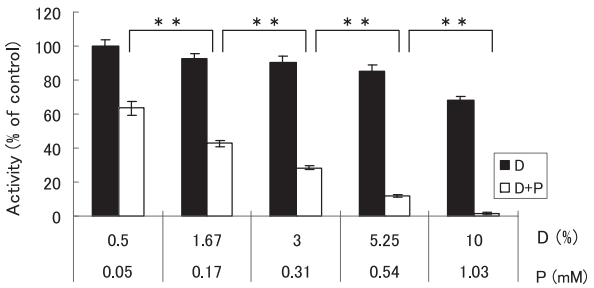

Fig. 9 Reversibility of propofol inhibition of Na^+, K^+-ATPase activity by decreasing its concentration .

At first, Na^+, K^+-ATPase was exposed to 1.03 mM propofol and 10% DMSO (open bar) or 10% DMSO without propofol as a control for solvent (closed bar). In the presence of 1.03 mM propofol and 10% DMSO (open bar), Na^+, K^+-ATPase activity was inhibited completely (See also Fig. 1 and text). In the presence of 10% DMSO (closed bar), about 35% of Na^+, K^+-ATPase activity was inhibited. Then propofol and/or DMSO concentration was reduced to the final concentrations shown on the graph by dilution and then Na^+, K^+-ATPase activity was measured. The activity was recovered depending on the degree of dilution and was about 60% at 0.05 mM propofol. Data have a mean ±SEM (n≧3).

**P<0.01 compared with two groups.

then after dilution of propofol Na^+, K^+-ATPase activity was measured (Fig. 9). Na^+, K^+-ATPase activity was recovered to about 60% at 0.05 mM and 40% at 0.17 mM propofol after dilution. The extent of activity recovery was dependent on the propofol concentration after dilution. A difference in the temperature, being on ice or at room temperature during preincubation did not affect the results.

Discussion

1. Mechanism for propofol inhibition of Na^+, K^+-ATPase activity

There is a report about the effect of propofol on Na^+, K^+-ATPase activity by Kutchai *et al.* [16] which showed propofol inhibition of Na^+, K^+-ATPase activity depending on its concentration. However, they did not study the inhibition mechanism. As Na^+, K^+-ATPase plays essential roles for the maintenance of neuronal excitability, its inhibition may be related to a change in brain function. For this reason, we studied the inhibition mechanism for propofol inhibition of Na^+, K^+-ATPase activity.

ATP hydrolysis by Na^+, K^+-ATPase requires the presence of Na^+, K^+ and Mg^{2+} [13, 20]. In the Post-Albers reaction sequence, the enzyme binds ATP in the presence of Na^+ and Mg^{2+}, hydrolyze ATP and forms a

phosphorylated enzyme (EP) with γ-phosphate of ATP as a reaction intermediate. Then Na^+-bound EP (called E1P) releases Na^+ to the outside of the cell and changes the conformation to E2P, which is sensitive to K^+. In the absence of K^+, E2P is dephosphorylated spontaneously and releases phosphate. This reaction is called Na^+-ATPase activity, as a partial reaction of Na^+, K^+-ATPase. In the presence of K^+, K^+ binds to E2P and accelerates the dephosphorylation. After the release of phosphate Na^+, K^+-ATPase forms a K^+-bound enzyme (KE2). KE2 hydrolyzes *p*-nitrophenyl phosphate (*p*NPP) and this activity is called K^+- *p*NPPase as a partial reaction of Na^+, K^+-ATPase activity [21]. To release K^+ from KE2 and start a new cycle, relatively high concentrations of ATP is necessary (low affinity site), however low concentrations of ATP is enough to form EP (high affinity site) [22]. Mg^{2+} is essential for an ATPase reaction. As expected from these reaction sequences, Na^+, K^+, Mg^{2+} and ATP modulates the Na^+, K^+-ATPase activity.

In order to study at which step propofol inhibits Na^+, K^+-ATPase activity, we examined the effect of propofol on Na^+, K^+, Mg^{2+} and ATP dependent activation of Na^+, K^+-ATPase activity. Propofol decreased all Vmax values for Na^+ (Fig 3 and Table 2), K^+ (Fig 4 and Table 3), Mg^{2+} (Fig 5 and Table 4) and ATP (Fig 2 and Table 1) dependent activation of Na^+, K^+-ATPase activity. These results suggest that propofol affects the whole reaction sequence of Na^+, K^+-ATPase, not a specific step. Propofol increased the affinity of Na^+, K^+-ATPase for both high and low ATP and K^+, decreased it for Na^+ and did not change it for Mg^{2+} significantly. In brief, propofol decreased the Vmax and also changed the affinities of Na^+, K^+-ATPase for Na^+, K^+, Mg^{2+} and ATP. These results suggest that the inhibition pattern of Na^+, K^+-ATPase by propofol is a mixed-type, but not competitive or non- competitive.

We studied the effects of propofol on Na^+-ATPase (Fig 7) and K^+- *p*NPPase (Fig 8 and Table 5) activities, which are partial reactions of Na^+, K^+-ATPase activity. Propofol decreased the Vmax and the affinity for Na^+ of Na^+-ATPase activity. Propofol also decreased the Vmax and changed the affinity for K^+ of K^+- *p*NPPase activity. These results support the above suggestions obtained from the effect of propofol on Na^+, K^+-ATPase activity.

As far as we know, this is the first report about the inhibition mechanism for Na^+, K^+-ATPase by propofol.

2. Reversibility of propofol inhibition of Na^+, K^+-ATPase activity

By diluting propofol concentration, the Na^+, K^+-ATPase activity which was inhibited nearly completely by 1.03 mM propofol, was recovered depending on the degree of dilution (Fig. 9). This result suggests that the effect of propofol is reversible *in vitro*, and we may suppose that the inhibition of Na^+, K^+-ATPase activity by propofol is also reversible in vivo, which is necessary to wake a human from anesthesia.

3. Propofol concentration for the inhibition of Na^+, K^+-ATPase activity and anesthesia.

Propofol inhibited Na^+, K^+-ATPase and Na^+-ATPase activities depending on its concentration (Figs. 1 and 6). For complete inhibition, 1.03 mM propofol was necessary, however approximately 29% of Na^+, K^+-ATPase or 24% of Na^+-ATPase activity was inhibited in the presence of 65 μM propofol.

Though there are some discrepancies about propofol concentrations necessary for anesthesia, Kazama *et al.* [23] measured the propofol concentrations of plasma obtained from the patients during the time that the patient was under anesthesia using high-performance liquid chromatography. They reported that the propofol concentrations at which 50% of the patients did not respond to skin incisions, peritoneum incisions or abdominal wall retractions, were 72, 96 or 109 μM, respectively. These results suggest that almost 30% of Na^+, K^+-ATPase activity may be inhibited at the range of plasma concentrations found by Kazama *et al.* during anesthesia and that this inhibition may be related to the anesthesia. Higuchi *et al.* [24] reported that propofol suppressed a hyperpolarization-activated inward current by approximately 10-20% in similar concentrations, supporting our results. The role of Na^+, K^+-ATPase inhibition for the state of anesthesia remains to be studied.

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