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Increase of Glutamate Uptake in Astrocytes

A Possible Mechanism of Action of Volatile Anesthetics

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Background: **Glutamate is the most ubiquitous excitatory neurotransmitter in the vertebrate central nervous system. Astrocytes play an important role in terminating glutamatergic neurotransmission by removing released glutamate from the synaptic cleft. The authors examined the effects of several anesthetics on the glutamate uptake activity of astrocytes.**

Methods: **Cultured astrocytes from hippocampi of rat embryos were incubated with solution containing [^{3}H]glutamate, which was pre-equilibrated with 0–4% halothane at 37°C. The uptake activity was evaluated as the amount of radioactivity per cell of protein.**

Results: **When the reaction solution was equilibrated with 4% halothane, glutamate uptake increased to about 165% of the control. The effect of halothane was dose-dependent, and a significant augmentation (30–50%) of glutamate uptake was observed at a range in clinical use concentrations (1–2%). On the other hand, the uptake of γ-aminobutyric acid, an inhibitory transmitter, was hardly affected by 1–4% halothane. The effect of halothane on glutamate uptake was also examined in neuron-rich culture, and similar augmentation was observed, although the extent was less than that in astrocyte culture. Biochemical subcellular fractions (*i.e.*, glial plasmalemmal vesicles and synaptosomes) were also examined, however, only slight (not significant) increase was detected in the glutamate uptake activity. Other volatile anesthetics, such as enflurane, isoflurane, and sevoflurane, also enhanced glutamate uptake, whereas the intravenous anesthetics ketamine and pentobarbital showed no effect on glutamate uptake.**

Conclusions: **The increase of glutamate uptake by astrocytes in the presence of volatile anesthetics potentially attenuates excitatory synaptic transmission in the entire central nervous system, a finding that may explain in part the action of volatile anesthetics. (Key words: Anesthetics, volatile: halothane. Brain, cells: astrocytes; primary cultures. Brain, excitatory neurotransmitters: glutamate. Pharmacokinetics, uptake: glutamate.)**

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GLUTAMATE is the most ubiquitous free amino acid in brain tissue.[1] Therefore, compared with other neurotransmitters such as catecholamines and acetylcholine, the recognition of glutamate as a neurotransmitter was delayed. Glutamate has been established as the most important fast-acting neurotransmitter in the vertebrate central nervous system.[2] Glutamate that is released from presynaptic terminals as a transmitter should be scavenged to terminate synaptic transmission. The presynaptic terminals themselves can uptake again a part of glutamate from the synaptic cleft, however, a large part of glutamate is removed by astrocytes, which are the most abundant cell type in the central nervous system and whose many fine processes surround each synapse. Only astrocytes in the brain possess glutamine synthase that catalyzes the reaction from glutamate and ammonium to glutamine using energy of adenosine triphosphate. The glutamine synthesized in astrocytes can be transferred to neurons. Thus astrocytes play important roles not only for structural and trophic supports of neurons but also for termination of excitatory glutamatergic neurotransmission.[3-5]

Many different effects of volatile anesthetics on the central nervous system have been reported, but nearly all involve neurons, whose membranes and cytosolic proteins are directly affected by anesthetics.[6-11] Only a few studies have reported anesthetic effects on astrocytes.[12] However, *in vivo*, anesthetics should exert their effects on neurons and astrocytes. Therefore, we

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examined the effects of several anesthetics on the glutamate uptake activity of primary cultured astrocytes from rat hippocampus.

Materials and Methods

Cell Culture

This study was done in compliance with the Guide for Animal Experimentation at Ehime University, School of Medicine, Ehime, Japan. Hippocampal astrocytes were prepared as described previously.[13] Briefly, 19-day-old Wistar rat embryos were removed from deeply anesthetized mothers with ether and decapitated. The hippocampi were dissected out under microscope and cut into 1-mm^3 blocks and treated with 5 ml 0.25% trypsin (Difco, Detroit, MI) in Ca^{2+}, Mg^{2+}-free phosphate-buffered saline containing 5.5 mM glucose for 15 min at 37°C. Trypsinization was stopped by adding 5 ml horse serum supplemented with 0.1 mg/ml DNase I (DN-25, Sigma Chemical Co., St. Louis, MO). The tissue was centrifuged at 100*g* for 5 min and resuspended by gentle trituration with 5 ml Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Grand Island, NY) containing 10% fetal calf serum, 100 mg/l streptomycin, and 5 × 10^5 unit/l penicillin. After filtering with lens-cleaning paper (Fuji Photo Co., Tokyo, Japan), the cells were plated on a polyethylenimine-coated 90-mm dish (Nunc Inc., Naperville, IL) at a density of 4-8 × 10^4/cm^2. Cultures were maintained in a 5% carbon dioxide humidified incubator at 37°C. At day 7 of culture, cells were placed on polyethylenimine-coated plastic cover slips (Thermanox, 13-mm diameter, Nunc Inc.) or 96-well plates (Sumitomo, Tokyo, Japan) at a density of 4-5 × 10^4/cm^{-2} to eliminate neuronal cells. Cells were cultured for 14 days with the medium changed every 3 days and then used for the experiments. Approximately 90% of the cells were immunoreactively positive to an astrocyte marker, glial fibrillary acidic protein.

Hippocampal neurons were prepared as described previously.[13] Briefly, hippocampal cells from 17-day-old Wistar rat embryos were prepared by a similar method, as described before for astrocytes, until the centrifugation step. Then the tissue was triturated with 5 ml DMEM containing 5% normal horse serum and 10% fetal calf serum. After filtering, the cells were plated on polyethylenimine-coated 13-mm diameter plastic cover slips at a density of 3-4 × 10^5/cm^{-2}. After 24 h, the medium was replaced with a serum-free medium that consisted of DMEM and supplement 14 of 15 mM Hepes, 30 nM Na2SeO2, 20 nM progesterone, 5 mg/l human transferrin, 5 mg/l bovine insulin, 100 mg/l streptomycin, and 5 × 10^5 units/l penicillin.[14] At day 4 of culture, the cells were exposed to 10-20 μM cytosine arabinoside for 24 h. Cells were cultured for 12 days, with the medium changed every other day, and then used in the experiment. Approximately 65% of the cells were immunoreactively positive to a neuronal marker, microtubule-associated protein 2, and 30% of cells were positive for glial fibrillary acidic protein.

Glutamate Uptake Measurement and Halothane Application

Gas-flow Method. The astrocytes cultured on cover slips were preincubated with Hepes-buffered Krebs-Ringer solution (HKR; 130 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 1 mM NaH_2PO_4, 5.5 mM glucose, 50 mM Hepes, with *p*H adjusted to 7.4 with NaOH) in four-well plates for 20-30 min in a carbon dioxide incubator. The four-well plate was transferred onto a dry bath (PDM-41, Yamato Co., Tokyo, Japan) to maintain temperature at 37°C. The reaction solution, containing either 5 μM [^{3}H]glutamate (1 mCi/l) or 5 μM [^{14}C]glutamate (1 mCi/l) plus 2.5 μM gamma-[^{3}H]aminobutyric acid (GABA; 3 mCi/l) for simultaneous uptake assay, had been bubbled and equilibrated with 0-4% halothane gas at 37°C for 15 min at a rate of 30 ml/min. The uptake reaction was started by replacing the preincubation HKR with the reaction solution. Each well was sealed with a rubber stopper, followed by introduction of 0-4% halothane gas into the air phase through needles to maintain a constant halothane partial pressure of the reaction solution throughout the reaction period. The reaction was stopped by washing the cells twice with 1.5 ml ice-cold HKR. The cells were solubilized with 0.2 ml 0.2 M NaOH, and radioactivity was measured in a liquid scintillation counter. The amounts of glutamate, GABA, or both that were taken up in the cells were normalized with respect to milligrams of cell protein, which was measured by the method of Bradford,[15] using CBB-color solution (Nacalai Tesque, Kyoto, Japan) and bovine serum albumin as a standard.

As reported previously,[16] during these conditions, more than 90% of the uptake activity was inhibited by 100 μM (2S,3S,4R)-2-(carboxycyclopropyl)glycine (CCG-III), a potent specific inhibitor for a high-affinity glutamate transporter.

Dilution Method. The liquid volatile anesthetics, halothane, enflurane, isoflurane, or sevoflurane were

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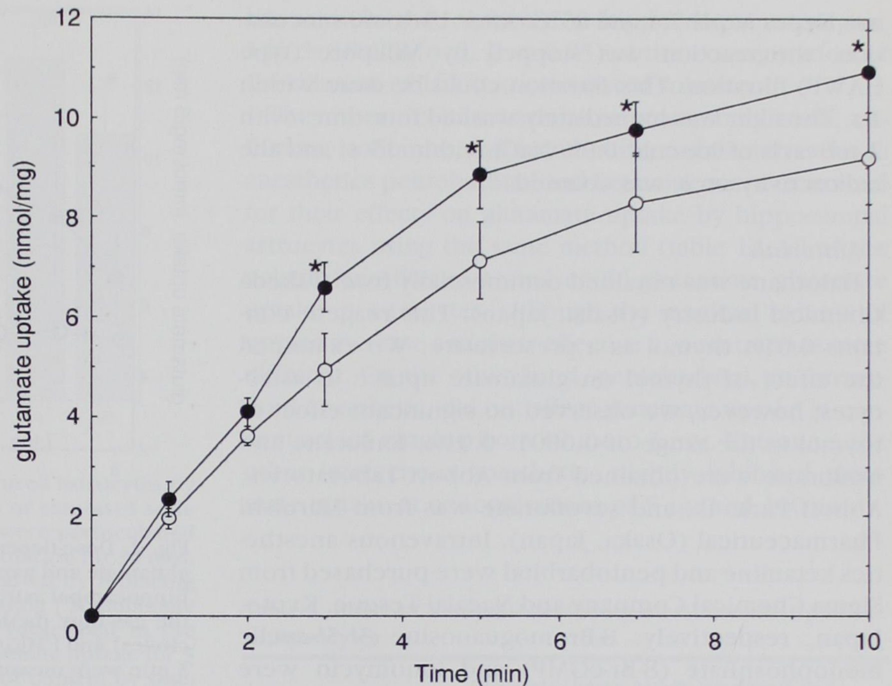

Fig. 1. Effect of halothane on the time course of glutamate uptake by cultured hippocampal astrocytes. The reaction medium containing [^{3}H]glutamate was equilibrated with 0% (open circles) or 2% (closed circles) halothane using the gas-flow method (mean ± SD; n = 5). *P < 0.05 with corresponding time points of control, 0% halothane, by the Student's *t* test.

mixed with five volumes of HKR in a sealed glass tube and left at room temperature at least for 2 h. This volatile anesthetic-saturated HKR was diluted with HKR at 1:32 to 1:1 concentrations and placed in microcentrifuge tubes with polyethylene caps. The reaction solutions were prepared immediately before reaction by mixing with the same volume of 10 μM [^{3}H]glutamate (2 mCi/l) containing HKR and the diluted volatile anesthetic solution. Cultured astrocytes on 96-well plates, which were preincubated with 100 μl HKR for 30 min in a carbon dioxide incubator, were placed in a dry bath to maintain the temperature at 37°C. Then the uptake reaction was started by replacing the preincubation HKR with 50 μl reaction solution, described previously. Five minutes later, the reaction was stopped by washing the wells three times with 150 μl ice-cold HKR. The amounts of glutamate taken up by the cells were measured using the method described previously. Intravenous anesthetics pentobarbital and ketamine were added to the reaction solutions.

Glutamate Uptake in Glial Plasmalemmal Vesicles

Glial plasmalemmal vesicles (GPVs) and synaptosomes from rat hippocampus were prepared as described previously.[17] Briefly, Wistar rats were decapitated during deep anesthesia with ether. The brains were removed rapidly, and hippocampi were then removed. Tissue homogenate with 10 volumes 0.32 M sucrose and 1 mM ethylenediamine tetraacetic acid was centrifuged at 1,000*g* for 10 min. The supernatant was layered onto a four-step discontinuous gradient composed of 20%, 10%, 6%, and 2% Percoll, and the tubes were centrifuged at 33,500g_{max} for 5 min. The turbid layer between 2% and 6% Percoll was diluted and centrifuged at 1,000*g* for 20 min to remove myelin components. The supernatant was further centrifuged at 33,500*g* for 40 min. The precipitate was washed twice with 0.32 M sucrose, 1 mM ethylenediamine tetraacetic acid, 0.25 mM dithiothreitol, and 20 mM Hepes (*p*H 7.4) by the centrifugations and was used as the GPV fraction. Synaptosomes were collected from the interfacial layer of 10–20% Percoll by centrifugation at 33,500*g* for 40 min and washed in the same way. These fractions were kept on ice and used for experiments within 4 h.

Glutamate uptake was measured by a Millipore filtration method,[17] and the effects of halothane were studied by the dilution method, as described previously. Usually the reaction was started by adding 10 μl of the sample (approximately 1 mg protein/ml) to 190 μl of a reaction medium containing 1 μM [^{3}H]glutamate, 140 mM NaCl, 5 mM KCl, 1 mM CaCl2, 1 mM MgCl2, and 20

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mM Hepes at *p*H 7.4 and 25°C. After 15- to 60-s incubation, the reaction was stopped by Millipore (type HAWP) filtration. This filtration could be done within 1 s. The filter was immediately washed four times with 2 ml each of ice-cold 0.9% NaCl within 15 s, and the radioactivity on it was counted.

Materials

Halothane was obtained commercially from Takeda Chemical Industry (Osaka, Japan). This reagent contains 0.01% thymol as a preservative. We examined the effect of thymol on glutamate uptake in astrocytes; however, we observed no significant effect of thymol in the range of 0.0001-0.01%. Enflurane and isoflurane were obtained from Abbott Laboratories, Abbott Park, IL, and sevoflurane was from Maruishi Pharmaceutical (Osaka, Japan). Intravenous anesthetics ketamine and pentobarbital were purchased from Sigma Chemical Company and Nacalai Tesque, Kyoto, Japan, respectively. 8-Bromoguanosine 3′,5′-cyclic monophosphate (8-Br-cGMP) and ionomycin were supplied by Boehringer-Mannheim GmbH (Mannheim, Germany). N6,2′-O-dibutyryladenosine 3′,5′-cyclic monophosphate; 12-O-tetradecanoylphorbol-13-acetate; and staurosporine were obtained from Sigma Chemical Company. L-[2,3-^{3}H]glutamic acid, L-[^{14}C]glutamic acid, and g-[2,3-^{3}H]aminobutyric acid were supplied from DuPont/NEN, Wilmington, DE.

Data Analysis

For the limitation of the experimental scale, we did an experiment using cells prepared from 12-14 embryos in a mother rat. The number of data is the number of wells or coverslips. We confirmed similar results repeatedly at least three times using the other cell preparations. For statistical analysis of the data, Student's *t* test or one-way analysis of variance followed by Fisher's least significant difference tests were used.

Results

Halothane's Effect on Glutamate Uptake in Cultured Astrocytes by the Gas-flow Method

Figure 1 shows the time course of glutamate uptake in cultured hippocampal astrocytes in the absence and presence of halothane. Halothane was administrated by the gas-flow method, in which the concentration of halothane was equilibrated with 2% gas-phase partial pressure during nearly the entire experiment. Without

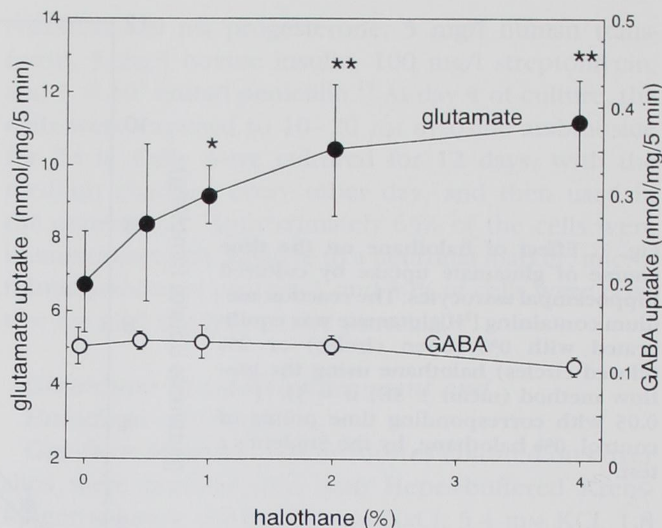

Fig. 2. Dose-dependent effect of halothane on the uptake of glutamate and gamma aminobutyeric acid (GABA) by cultured hippocampal astrocytes. Halothane (0–4%) was applied using the gas-flow method. The amounts of [^{14}C]glutamate (closed circles) and [^{3}H]GABA (open circles) taken up by the cells for 5 min were measured simultaneously with the double-labeled method (mean ± SD; n = 4). *$P < 0.05$, **$P < 0.01$ with the value of control, 0% halothane, by one-way analysis of variance followed by Fisher's paired least-squares test.

halothane, the amount of glutamate taken up into the cells increased almost linearly for 5 min, and then the rate slowed. In the presence of 2% halothane, the uptake amount of glutamate increased slightly faster than the control within 2 min, and the difference became significant at 3 min thereafter. Therefore, we usually examined the increase of glutamate uptake at the reaction time of 5 min.

We measured the effect of halothane on glutamate uptake, changing the concentration of halothane (fig. 2). Increased glutamate uptake exhibited a dose-dependent relation with the gas-phase partial pressure of halothane. When the reaction solution was equilibrated with 1% halothane, the extent of the increase of glutamate uptake was 134% of the control and was 164% with 4% halothane. Figure 2 also shows the effect of halothane on GABA uptake, which was measured simultaneously with the glutamate uptake assay. The GABA uptake was, on the other hand, not affected by 1-4% halothane.

The effect of halothane was also examined in a neuron-rich culture. A similar augmenting effect on glutamate uptake was observed in primary cultured hippocampal neurons. The effect was significant but smaller than that observed in astrocytes: 144 ± 14% (n = 4, *P*

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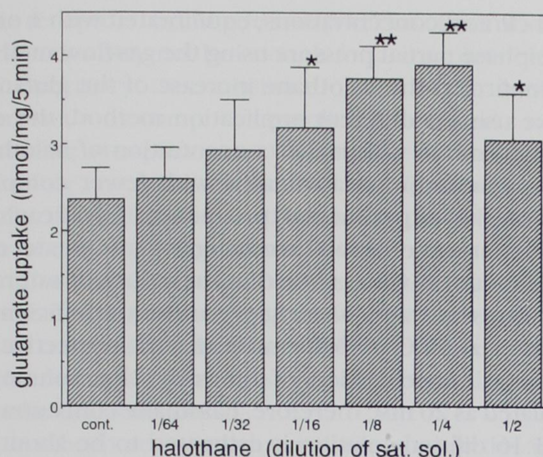

Fig. 3. Increase of glutamate uptake of cultured astrocytes by halothane applied by the dilution method of saturated solution. The cells cultured on a 96-well plate were preincubated with normal Hepes-buffered Krebs-Ringer solution for 30 min. The glutamate uptake reaction was then started by replacing the reaction solution with solution containing [^{3}H]glutamate and various concentrations of halothane (described in the text). Values (mean ± SD; n = 5) show glutamate uptake for 5 min. *$P < 0.05$, **$P < 0.01$ with the value of control by one-way analysis of variance followed by Fisher's paired least-squares test.

< 0.05) at 4% halothane, with no significant enhancement at halothane concentrations less than 2%. An effect on GABA uptake was not observed in the range of halothane concentrations of 0–4%.

Halothane's Effect on Glutamate Uptake in Astrocytes Measured by the Dilution Method

We confirmed the increase of glutamate uptake by halothane using the other application method. As shown in figure 3, we observed the similar increasing effect of halothane by the dilution method (described in Materials and Methods). This increasing effect was observed in a dose-dependent manner up to 1:4 dilution of halothane-saturated solution, and glutamate uptake increased to a maximum of about 150% of the control. Significant enhancement was observed at concentrations greater than 1:16 dilution. At the concentration of 1:2 dilution of halothane-saturated solution, the uptake activity decreased. The reason for the decrease is unknown; however, a high concentration of halothane may affect the cells in a nonspecific manner.

The effect of halothane also was examined in primary cultured astrocytes from rat cortex. A similar increase by halothane in glutamate uptake was also observed in cortex astrocytes. The extent of the increase was nearly the same as that observed in hippocampal astrocytes; 146 ± 38% (n = 5, $P < 0.05$) at the concentration of 1:4 dilution of halothane-saturated solution.

The other volatile anesthetics commonly used, enflurane, isoflurane, and sevoflurane, and the intravenous anesthetics pentobarbital and ketamine were examined for their effects on glutamate uptake by hippocampal astrocytes using the same method (table 1). All of the volatile anesthetics tested could potentiate glutamate uptake in astrocytes, although the maximal extents of uptake increase and their effective concentrations were different. On the other hand, pentobarbital, an intravenous anesthetic, did not affect glutamate uptake at concentrations ranging from 30 μM to 3 mM. Ketamine, the other intravenous anesthetic, slightly inhibited glutamate uptake at concentrations of 5 μM and 100 μM.

Table 1. Effect of Various Anesthetics on Glutamate Uptake in Astrocytes

	Glutamate Uptake Activity (nmol/mg/5 min)		
	0	1/16 Saturation	1/4 Saturation
Halothane (n = 8)	2.37 ± 0.36 (1.0)	3.18 ± 0.69* (1.34)	3.90 ± 0.37† (1.65)
Enflurane (n = 4)	1.91 ± 0.18 (1.0)	3.56 ± 0.48† (1.87)	2.96 ± 0.96* (1.55)
Isoflurane (n = 5)	2.28 ± 0.15 (1.0)	3.24 ± 0.11† (1.42)	3.30 ± 0.27† (1.45)
Sevoflurane (n = 5)	2.28 ± 0.15 (1.0)	2.83 ± 0.24† (1.24)	3.08 ± 0.42† (1.35)
Concentration	0 μM	30 μM	300 μM
Pentobarbital (n = 8)	2.11 ± 0.49 (1.0)	1.99 ± 0.49 (0.94)	1.99 ± 0.18 (0.94)
Concentration	0 μM	50 μM	100 μM
Ketamine (n = 12)	2.77 ± 0.64 (1.0)	2.43 ± 0.60 (0.88)	2.12 ± 0.19 (0.76)

Four different kinds of volatile anesthetics were applied to the cells cultured on a 96-well plate by the dilution method, while two intravenous anesthetics were simply added. Values (mean ± SD) show glutamate uptake for 5 min and those in parentheses are the ratios to control value.

* $P < 0.05$, †$P < 0.01$ with the value of control.

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In primary astrocyte cultures, we considered the possibility that the extent of halothane augmentation of glutamate uptake may be changed by some drugs that modulate intracellular signaling, including ionomycin (100 nM), N6,2′-O-dibutyryladenosine 3′,5′-cyclic monophosphate (100 nM to 1 mM), 8-Br-cGMP (10 μM to 1 mM), 12-O-tetradecanoylphorbol-13-acetate (100 nM), and staurosporine (10 nM). However, all of these drugs showed no significant effect on the augmentation of glutamate uptake by 2% halothane (data not shown).

Halothane's Effect on Glutamate Uptake in Glial Plasmalemmal Vesicles and Synaptosomes

The effects of halothane on glutamate uptake were studied in subcellular fractions, GPVs, and synaptosomes, which were prepared from rat hippocampal homogenate. The glutamate uptake activities of GPV and synaptosomes were slightly enhanced, at 114 ± 17% and 113 ± 13% (n = 8), respectively, at halothane concentrations ranging from 1:8 to 1:64 dilution, but the differences were not significant.

Reversible Effects of Halothane on Glutamate Uptake

To determine whether the observed effect is reversible, cultured astrocytes that had been preincubated with halothane by the gas-flow method were assayed for glutamate uptake with or without halothane. The activity of the cells preincubated with 2% halothane for 5 min increased significantly in the presence of 2% halothane to 125 ± 4.2% ($n = 4$, $P < 0.05$) of the control (without halothane in preincubation and reaction solutions). On the other hand, in the absence of halothane in reaction solutions, the activity of these cells returned and rather decreased to 91.5 ± 7.4% ($n = 4$) of the control.

Discussion

The mechanism of anesthesia caused by volatile anesthetics has not been clarified, although many different effects of these agents have been reported. To elucidate the anesthetic mechanism of volatile anesthetics, the effect should be observed at a clinically relevant range of the concentrations; however, only a few effects have been reported at this range.[9,18–20] In the present study, we showed that commonly used volatile anesthetics potentiated glutamate uptake in astrocytes. Halothane enhanced the uptake activity in a dose-dependent manner at clinical concentrations, equilibrated with 1 or 2% of gas-phase partial pressure using the gas-flow method. We confirmed the halothane increase of the glutamate uptake using a different application method, the dilution method, in which the concentration of halothane is not reliable but is associated with fewer complications of the experimental procedure. Significant enhancement was observed at concentrations greater than 1:16 dilution. If we assume that the values of saturated vapor pressure and water/gas partition coefficient of halothane at 25°C are 290 mmHg and 1.2, respectively,[21] halothane concentration of the saturation solution is calculated as 20 mM; therefore, halothane concentration in a 1:16 diluted solution is estimated to be about 1.2 mM. If we assume further that the value of water/gas partition coefficient of halothane at 37°C is 0.63, this value of 1.2 mM is approximately equivalent to 4.2% gas-phase partial pressure and is four times greater than that observed with the gas-flow method. However, that actual halothane aqueous concentration should be lower than the estimated value because of evaporation from the reaction solution during assay procedures and of adsorption on the surface of assay equipment. The inconsistency between the two methods may be due to the loss of halothane in the dilution method.

Using the dilution method, we observed increase that was similar to that by the other volatile anesthetics, enflurane, isoflurane, and sevoflurane. The maximal extents of uptake increase and their effective concentrations were different. To discuss the differences among these anesthetics, knowing the accuracy of the measurements is not enough, and further extensive measurements may be needed. On the other hand, the intravenous anesthetics pentobarbital and ketamine did not enhance the glutamate uptake. These results suggest that the increase of glutamate uptake in astrocytes is a common characteristic of these volatile anesthetics but not of intravenous anesthetic.

In nearly all regions of the brain, astrocytes surround the synapses and scavenge the released neurotransmitter to terminate the synaptic signal transmission. It is thought that the decay of glutamate concentration in the synaptic cleft contributes to the decay time course of the excitatory postsynaptic current and that the activity of glutamate transporters may be reflected in the time course of the excitatory postsynaptic current decay.[5,22,23] The potentiation by volatile anesthetics of glutamate uptake by perisynaptic astrocytes may cause a rapid decrease in the glutamate concentration in the cleft and may decrease the action of postsynaptic gluta-

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mate receptors. In a single synapse, this effect of volatile anesthetics may not be enough for a complete block of the excitatory transmission. In the brain, however, many neurons compose complicated networks *via* uncountable synapses so as to amplify or unify signals. Therefore, even if the extent of the signal reduction is small in a single synapse, the resulting reduction in the sum of excitatory signals may become large enough to contribute to anesthesia. Further, GABA uptake was not affected by halothane, suggesting that the inhibitory signal transmission becomes more dominant than the excitatory signal transmission in the brain during such anesthetic conditions.

It has been reported that volatile anesthetics inhibit Ca^{2+}-adenosinetriphosphatase activity on erythrocyte membrane ghosts[24] and synaptosomes,[25] suggesting a direct effect on membrane proteins. Halothane may enhance glutamate uptake activity of subcellular membrane fractions. It has been reported that synaptosomal glutamate uptake is not influenced by anesthetics[26]; therefore, we examined the halothane effect not only on synaptosomes but also on GPV, glia-rich plasma membrane fractions prepared from brain homogenate. However, we could not observe any significant increase of glutamate uptake activity in GPV and synaptosomes. These results suggest that the halothane effect is not caused by a direct action on transporter protein in membranes and that cell structure is needed for the action of anesthetics. The targets of volatile anesthetics in astrocytes, such as intracellular signaling systems, might have been lost during GPV preparation.

Glutamate transporters are phosphorylated by protein kinase C and protein kinase A, and the former activator enhances glutamate uptake in astrocytes.[27] We studied the effect of some modulating drugs for intracellular signaling systems. However, we detected no significant effect on halothane increase of glutamate uptake. It is unlikely that the target of volatile anesthetics is such intracellular signaling systems.

Further investigation is needed to determine the mechanisms of glutamate uptake increase by volatile anesthetics. There remain at least two possibilities.

1) Primary cultured astrocytes have gap junctions among cells, and volatile anesthetics halothane and isoflurane close the gap junctions at 1 minimum alveolar concentration.[12] The detailed mechanisms are unknown, however, the permeability of gap junction might be involved in the increase of glutamate uptake.
2) Astrocytes have a high activity of glutamine synthase, which catalyzes the reaction to form glutamine from glutamate and ammonium.[28] Volatile anesthetics might enhance the activity of glutamine synthase and reduce the intracellular concentration of glutamate. This might potentiate indirectly the glutamate uptake efficiency in the astrocytes.

In conclusion, we have shown that volatile anesthetics induce astrocytes to increase their scavenging activity for an excitatory neurotransmitter, glutamate, but not for an inhibitory one, GABA, which may result in the depression of excitatory transmission of glutamate in the global central nervous system. We propose the possibility that the increase of glutamate uptake into astrocytes is the mechanism of action of volatile anesthetics.

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