# *Extracellular Magnesium Ion Modifies the Actions of Volatile Anesthetics in Area CA1 of Rat Hippocampus* In Vitro

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*Background:* Magnesium ion ($Mg^{2+}$) is involved in important processes as modulation of ion channels, receptors, neurotransmitter release, and cell excitability in the central nervous system. Although extracellular $Mg^{2+}$ concentration ($[Mg^{2+}]_o$) can be altered during general anesthesia, there has been no evidence for $[Mg^{2+}]_o$-dependent modification of anesthetic actions on neural excitability in central nervous system preparations. The purpose of current study was to determine whether the effects of volatile anesthetics are $[Mg^{2+}]_o$-dependent in mammalian central nervous system.

*Methods:* Extracellular electrophysiologic recordings from CA1 neurons in rat hippocampal slices were used to investigate the effects of $[Mg^{2+}]_o$ and anesthetics on population spike amplitude and excitatory postsynaptic potential slope.

*Results:* The depression of population spike amplitudes and excitatory postsynaptic potential slopes by volatile anesthetics were significantly dependent on $[Mg^{2+}]_o$. The effects were attenuated in the presence of a constant $[Mg^{2+}]_o$/extracellular $Ca^{2+}$ concentration ratio. However, neither *N*-methyl-D-aspartate receptor antagonists nor a non–*N*-methyl-D-aspartate receptor antagonist altered the $[Mg^{2+}]_o$-dependent anesthetic-induced depression of population spikes. Volatile anesthetics produced minimal effects on input–output (excitatory postsynaptic potential–population spike) relations or the threshold for population spike generation. The effects were not modified by changes in $[Mg^{2+}]_o$. In addition, the population spike amplitudes, elicited *via* antidromic (nonsynaptic) stimulation, were not influenced by $[Mg^{2+}]_o$ in the presence of volatile anesthetics.

*Conclusions:* These results provide support that alteration of $[Mg^{2+}]_o$ modifies the actions of volatile anesthetics on synaptic transmission and that the effects could be, at least in part, a result of presynaptic $Ca^{2+}$ channel–related mechanisms.

THE magnesium ion ($Mg^{2+}$) is the fourth most abundant cation in the mammalian body. Its role in energy transformation and cell excitability is important for cellular function.[1–4] In the central nervous system (CNS), $Mg^{2+}$ is known to compete with calcium ion ($Ca^{2+}$) at calcium channels,[5] modulate neurotransmitter release from presynaptic terminals,[6] and regulate the activation of *N*-methyl-D-aspartate (NMDA) receptors at negative membrane potentials.[7]

Since clinical studies have demonstrated that extracellular $Mg^{2+}$ concentration $[Mg^{2+}]_o$ is altered in the perioperative period,[8] and that there is a negative correlation between intravenous administration of Ringer solution and $[Mg^{2+}]_o$,[9] it was important to test whether alteration of $[Mg^{2+}]_o$ can influence the actions of general anesthetics on electrophysiologic properties in the CNS. Mody *et al.*[10] studied the effects of halothane on evoked field potentials of rat hippocampal slices in the presence and absence of extracellular $Mg^{2+}$ but failed to demonstrate a significant relation between anesthetic actions and $[Mg^{2+}]_o$. However, they applied a relatively high concentration of halothane that produced complete depression of field potentials and did not examine in detail the $[Mg^{2+}]_o$-dependent changes in the presence of various $[Mg^{2+}]_o$. Their results warranted a study of the influences of $[Mg^{2+}]_o$ on the actions of general anesthetics in the CNS and the determination of the mechanism(s) of $[Mg^{2+}]_o$-related modification of anesthetic actions.

Recent investigations indicated that excitatory glutamate-mediated transmission is one of the important target sites for general anesthetics.[11] It has been well known that Schaffer collateral fiber input to CA1 pyramidal neurons in the hippocampus is a monosynaptic pathway consisting of glutamate synapses, and that stimulation of the pathway produces field potentials. The field excitatory postsynaptic potential (EPSP) is a recording of the current flow into dendrites of pyramidal neurons, whereas the soma field potential is represented by the population spike (PS). In the current study, we examined the relation between $[Mg^{2+}]_o$ and the effects of two volatile anesthetics (sevoflurane and isoflurane) on evoked field EPSPs and PSs of CA1 pyramidal neurons in rat hippocampal slices.

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## Material and Methods

### Preparation

After approval had been obtained from the Animal Research Committee of Toyama Medical and Pharmaceutical University, male Wistar rats (weight, 100–200 g) were anesthetized with sevoflurane and decapitated. The technique for preparing rat hippocampal slices was identical to the methods previously described by Hirota and Roth.[12] The brain was rapidly removed, and the dissected hippocampus was sliced in cold artificial cerebrospinal fluid (ACSF) transversely to its longitudinal axis

(400 $\mu$m thick) with a Rotorslicer DTY–7700 (DSK, Osaka, Japan). Slices were placed on a nylon mesh at a liquid–gas interface in a recording chamber at 37°C. A humidified gas mixture (95% $O_2$–5% $CO_2$) was applied to the chamber at a flow rate of 1 l/min. ACSF was continuously perfused at a rate of 90 ml/h. Slices were incubated for 90 min without electrical stimulation.

*Electrophysiologic Techniques*

Bipolar nichrome stimulating electrodes were placed in the region of stratum radiatum to activate Schaffer-collateral inputs to CA1 pyramidal neurons. Glass micro-electrodes (3–5 M$\Omega$ filled with 2 M NaCl) were placed in the cell body region of CA1 neurons and stratum radiatum to record the evoked field PSs and EPSPs, respectively. Stimuli were applied *via* the alveus hippocampi to produce antidromic responses of pyramidal neurons in the absence of synaptic transmission. The minimum stimulus intensity (5–10 V) that elicited a maximum PS amplitude was used to evoke a response. Square-wave stimuli (5–10 V, 0.05 ms, 0.1 Hz) were delivered using a SEN-3301 stimulator (Nihon Kohden, Tokyo, Japan). Field potentials were amplified (Nihon Kohden MEZ-8301), filtered (1 Hz to 10 kHz), and digitally converted (100 kHz) using an iNet system (GWI, Somerville, MA) and stored on a Macintosh computer (Apple, Cupertino, CA) for later analysis.

*Data Analysis*

The PS amplitudes were measured from peak positive to peak negative. EPSP slopes were calculated by fitting digitized data points between onset and peak negativity to a linear function (dV/dt).[13] Results were collected in a group of five, averaged, and stored as a single record. All preparations used in this study showed control variability less than 5% during the initial data acquisition period and after washout of anesthetics.

*Drug Administration*

The general anesthetics, sevoflurane and isoflurane, were applied as vapors to the tissue chamber *via* the prewarmed and humidified 95% $O_2$–5% $CO_2$ gas stream above the slices, using the appropriate vaporizers, Tec 3 (Omeda, Steeton, West Yorkshire, United Kingdom) and Forawick (Muraco, Tokyo, Japan), respectively. Concentrations, expressed as volume percent (vol%), refer to dial settings on the vaporizer. The vaporizers had been previously calibrated with an anesthetic gas analyzer (Capnomac; Datex, Helsinki, Finland). Volatile anesthetics were applied for a minimum of 5 min to reach equilibrium, as the preliminary experiments demonstrated that the onset time for sevoflurane and isoflurane effects were less than 30 s and that maximum effects reached a plateau within 3 min (not shown). Concentrations of sevoflurane and isoflurane in the perfusate of the recording chamber were determined using gas chroma-

tography (Shimazu, Kyoto, Japan). The concentrations of anesthetics in solution were found to be linear (0.64 and 0.55 mM per 1 vol%, respectively) up to 5.0 vol%. Recovery responses were recorded at least 10 min after washout of anesthetic-equilibrated ACSF from the chamber. In this study, the concentrations of sevoflurane and isoflurane that depressed PS amplitudes and EPSP slopes to 50% of control in normal (ACSF) condition were used, except for the antidromic experiments. The NMDA receptor antagonists (DL-2-amino-5-phosphonovaleric acid [AP-5] and (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine [MK-801]) and the non-NMDA receptor antagonist (6-Cyano-7-nitroquinoxaline-2,3-dione [CNQX]) were dissolved into ACSF and applied as perfusate for 20 min. Stock solutions of CNQX (1 mM) were prepared in dimethyl sulfoxide and diluted in ACSF before it was perfused into the chamber. The concentration of dimethyl sulfoxide used in the experiments alone did not affect the field potentials. In some experiments, concentrations of $Mg^{2+}$ and $Ca^{2+}$ in the perfusate were altered by changing the concentrations of $MgSO_4$ and $CaCl_2$ in ACSF; the modified perfusate was also applied for 20 min.

*Solution and Drugs*

The composition of the ACSF was 124 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1.25 mM $NaH_2PO_4$, 2 mM $MgSO_4$, 26 mM $NaHCO_3$, and 10 mM glucose, prepared in purity water. The $Mg^{2+}$ and $Ca^{2+}$ concentrations in cerebrospinal fluid previously reported in rats are 0.53–0.63 mM[14] and 0.75–0.98 mM,[15] respectively. It is necessary to use higher concentrations of $[Mg^{2+}]_o$ (2.0 mM) in normal ACSF for slice preparations because many of the afferents and efferents are disrupted during the slicing procedure. This results in preparations that produce spontaneous spiking. The ACSF was precooled (8–10°C) and saturated with a 95% $O_2$–5% $CO_2$ gas mixture before use (pH 7.3–7.5). Sevoflurane and isoflurane were donated by Dinabot (Osaka, Japan) and Maruishi Pharmaceutical Co. (Osaka, Japan), respectively. Most of the chemicals were obtained from Sigma (St. Louis, MO), except for MK-801 and CNQX, which were obtained from Tocris Cookson (Bristol, United Kingdom).

*Statistical Analysis*

Experiments were conducted on 61 hippocampal slices prepared from 48 rats. Anesthetic effects are expressed as percent of control (normalized to the value in the absence of anesthetic), and the mean $\pm$ SD was calculated based on measures from at least four slices. Statistical differences among groups of different $[Mg^{2+}]_o$ or extracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_o$) were determined by one-way analysis of variance with Bonferroni-corrected *post hoc* analyses. Differences between the effects of anesthetics or antagonists on the field potential parameters in various $[Mg^{2+}]_o$ were deter-

mined by two-way factorial analysis of variance (Statview 4.0; Abacus Concepts, Berkeley, CA). A *P* value < 0.05 was considered significant. Linear and quadratic functions were fitted to the data using the SigmaPlot program (Jandel Scientific, San Rafael, CA).

## Results

In normal $[Mg^{2+}]_o$ (2.0 mM), the magnitudes of EPSP slopes and PS amplitudes of CA1 pyramidal neurons in response to stimulation of the Schaffer collateral fibers are 24 ± 11 v/s and 7.4 ± 1.5 mV (n = 5), respectively. Changing the perfusate to low (0.5 mM) enhanced the EPSP slopes and the PS amplitudes to 114 ± 10% and 119 ± 11% of those in each normal $[Mg^{2+}]_o$, respectively (n = 4). Higher $[Mg^{2+}]_o$ (4.0 mM) reduced the signals to 66 ± 3% and 62 ± 11%, respectively (n = 5). Table 1 summarizes the alterations of PS amplitudes in various $[Mg^{2+}]_o/[Ca^{2+}]_o$ conditions.

Figure 1 shows representative recordings of the effects of sevoflurane (3.0 and 5.0 vol%) on the evoked PSs (fig. 1A) and EPSPs (fig. 1B), respectively, in various $[Mg^{2+}]_o$ concentrations. In normal $[Mg^{2+}]_o$, sevoflurane reduced the PS amplitudes and the EPSP slopes in a reversible manner. The inhibitory actions of sevoflurane on PSs and EPSPs were attenuated in the $[Mg^{2+}]_o$-free condition but were enhanced in high $[Mg^{2+}]_o$. Figure 2 summarizes the relations between $[Mg^{2+}]_o$ and the effects of sevoflurane and isoflurane on the PS amplitude (fig. 2A) and the EPSP slopes (fig. 2B). The inhibitory actions on PS amplitudes in the presence of sevoflurane and isoflurane were largely dependent on $[Mg^{2+}]_o$ ($r^2$ = 0.92 and 0.90; $P$ < 0.0001 and $P$ < 0.0001, respectively), whereas the EPSP slopes in the presence of sevoflurane and isoflurane were loosely correlated with $[Mg^{2+}]_o$ ($r^2$ = 0.79

**Table 1. The Amplitudes (mV) of Population Spikes (PSs) in Various Extracellular Mg$^{2+}$/Ca$^{2+}$ Concentrations**

| Ca$^{2+}$ Concentration (mM) | Mg$^{2+}$ Concentration (mM) | | |
|---|---|---|---|
| | Low (0.5) | Normal (2.0) | High (4.0) |
| Low (1.0) | 6.5 ± 1.6 | 10.4 ± 1.3* | NA |
| Normal (2.0) | 8.4 ± 1.9 | 7.4 ± 1.5 | 4.7 ± 1.6* |
| High (4.0) | NA | 11.6 ± 0.8* | 8.2 ± 1.2 |

Amplitudes (mV) are expressed as mean ± SD (n = 4–7).

* $P$ < 0.05 *versus* the value in normal Mg$^{2+}$/Ca$^{2+}$ concentration (Mg$^{2+}$, 2.0 mM; Ca$^{2+}$, 2.0 mM) by Bonferroni *post hoc* test.

NA = not available.

and 0.39; $P$ < 0.0001 and 0.05, respectively). The data points for PSs and EPSPs of sevoflurane were not significantly different from those of isoflurane.

A series of experiments was then conducted to study the mechanism(s) of the $[Mg^{2+}]_o$-dependent depression of PS amplitudes by volatile anesthetics. First, the role of presynaptic Ca$^{2+}$ channels in the $[Mg^{2+}]_o$-dependent events was examined. Because Mg$^{2+}$ competes with Ca$^{2+}$ at calcium channels, the ratio of $[Mg^{2+}]_o$ to $[Ca^{2+}]_o$ in the perfusate was maintained at a constant, *i.e.*, $[Ca^{2+}]_o$ was adjusted in proportion to $[Mg^{2+}]_o$. Figure 3 demonstrates the poor correlation between $[Mg^{2+}]_o$ and anesthetic-induced depression, indicating that the $[Mg^{2+}]_o$-dependent effects could be suppressed during conditions in which a ratio of $[Mg^{2+}]_o$ to $[Ca^{2+}]_o$ was fixed.

To examine the involvement of the NMDA receptor in the $[Mg^{2+}]_o$-dependent anesthetic depression, the effects of NMDA receptor antagonists, AP-5 ($10^{-4}$ M) and MK-801 ($4 \times 10^{-5}$ M), were studied on the $[Mg^{2+}]_o$-dependent depression produced by isoflurane (0.7 vol%). The concentrations of the antagonists have



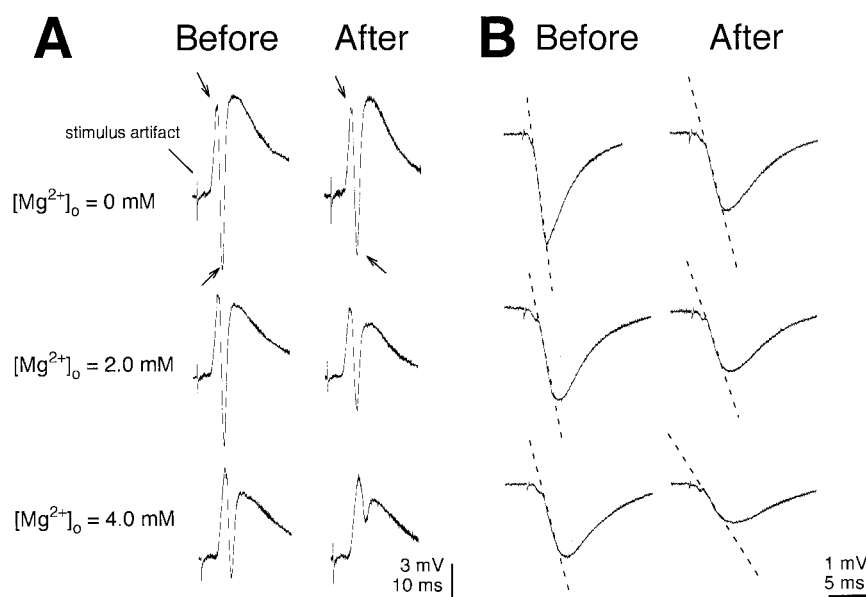**Fig. 1. Sevoflurane (3.0 vol%) reduces population spike amplitude (*A*; indicated by arrows) and excitatory postsynaptic potential slope (*B*; indicated by broken lines) in different extracellular Mg$^{2+}$ concentrations.**
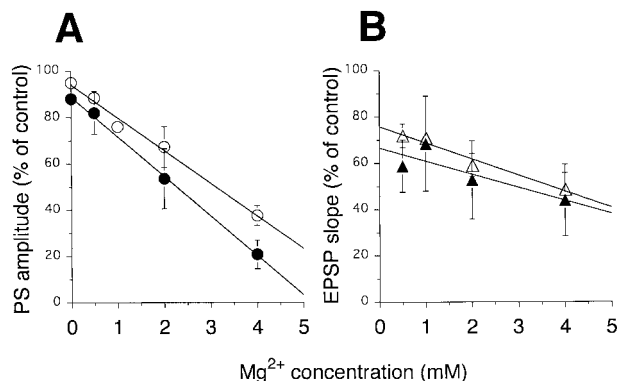
A  Before  After  B  Before  After

stimulus artifact

$[Mg^{2+}]_o$ = 0 mM

$[Mg^{2+}]_o$ = 2.0 mM

$[Mg^{2+}]_o$ = 4.0 mM

3 mV
10 ms

1 mV
5 ms

**Fig. 2.** (*A*) **Relations between the amplitudes of population spikes (PSs) and extracellular Mg$^{2+}$ concentrations ([Mg$^{2+}$]$_o$) in the presence of sevoflurane (3.0 vol%; open circles) and isoflurane (0.7 vol%; closed circles). (*B*) Relations between the slope of excitatory postsynaptic potentials (EPSPs) and [Mg$^{2+}$]$_o$ in the presence of sevoflurane (5.0 vol%; open triangles) and isoflurane (2.0 vol%; closed triangles). Points (mean ± SD) represent percent of control (normalized to the value in the absence of anesthetic at each [Mg$^{2+}$]$_o$) for five determinations from separate slices. Data points were fitted to a linear function (continuous lines).**

been reported to completely block the NMDA receptor in hippocampal preparations *in vitro*[16,17] and have been used as pharmacologic tools in electrophysiologic experiments.[18] AP-5 or MK-801 alone had no consistent effect on the shape of EPSP and PS. The anesthetic-induced depression of PS amplitudes was well correlated with [Mg$^{2+}$]$_o$ in the presence of AP-5 ($r^2 = 0.83$; $P < 0.0001$; fig. 4) and MK-801 ($r^2 = 0.99$; $P < 0.0003$), suggesting that blockade of NMDA receptors had no effect on the [Mg$^{2+}$]$_o$-dependent effects. In addition, the participation of the non-NMDA receptor antagonist was examined. The complete blockade of the non-NMDA receptor diminishes field potentials, since the non-NMDA receptor is
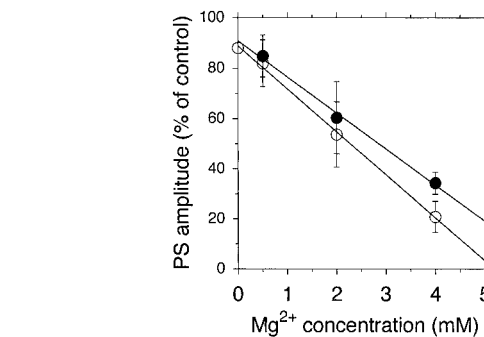


**Fig. 4. The effects of DL-2-amino-5-phosphonovaleric acid (AP-5; 10$^{-4}$ M) on the relations between extracellular Mg$^{2+}$ concentration ([Mg$^{2+}$]$_o$) and isoflurane (0.7 vol%)-induced inhibition of population spikes (PSs). Open circles = the artificial cerebrospinal fluid (no antagonist); closed circles = AP-5. Data points (mean ± SD) are expressed as percent of control (normalized to the value in the absence of anesthetic at each [Mg$^{2+}$]$_o$) for five determinations from separate slices. Data points were fitted to a linear function (continuous lines).**

primarily responsible for the orthodromic activation of pyramidal neurons. A relatively low concentration of CNQX ($5 \times 10^{-7}$ M) was used in the current study: CNQX alone reduced the PS amplitudes from $9.1 \pm 4.4$ mV to $7.1 \pm 2.8$ mV (n = 5). CNQX failed to modify the [Mg$^{2+}$]$_o$-dependent inhibition ($r^2 = 0.99$; $P < 0.0003$) in the presence of isoflurane (0.7 vol%).

In subsequent experiments, two independent recording electrodes were placed in the dendrite and soma regions of CA1 pyramidal neurons to simultaneously record EPSPs and PSs, respectively. The relations between the EPSP slopes and the PS amplitudes describe an input–output function for pyramidal neurons. The input–output relations at different stimulus intensities were then studied to address whether the postsynaptic events were involved in the [Mg$^{2+}$]$_o$-dependent volatile
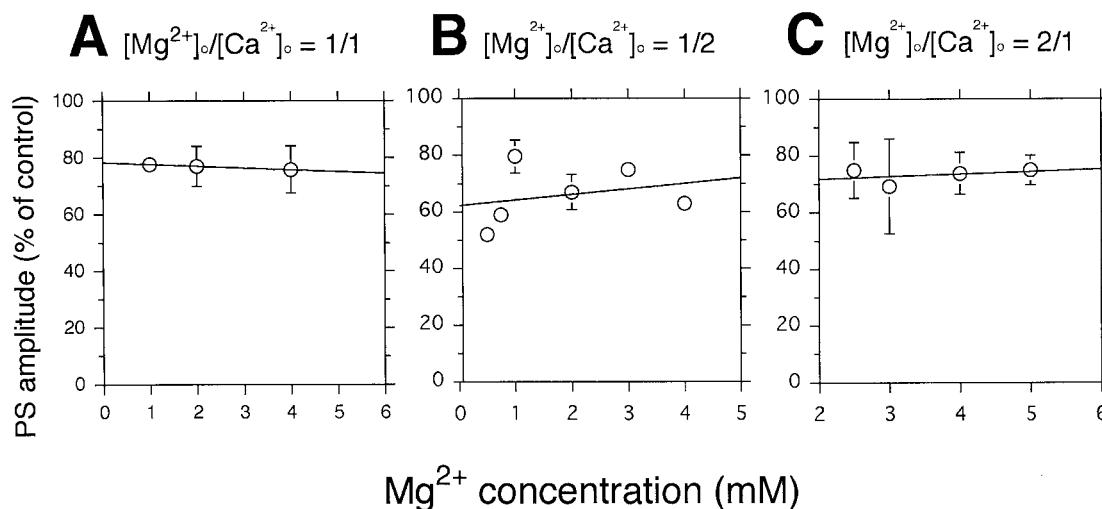


**Fig. 3. Sevoflurane (3.0 vol%)-induced depression of population spike amplitude was not related to extracellular Mg$^{2+}$ concentration ([Mg$^{2+}$]$_o$) under the fixed ratio of Mg$^{2+}$ to Ca$^{2+}$ ([Mg$^{2+}$]$_o$/[Ca$^{2+}$]$_o$) in the perfusate. The values of r$^2$ are (*A*) 0.038, (*B*) 0.002, and (*C*) 0.17, respectively. Data points (mean ± SD) are expressed as percent of control (normalized to the value in the absence of anesthetic at each [Mg$^{2+}$]$_o$) for five determinations from separate slices. Data points were fitted to a linear function (continuous lines).**

anesthetic depression. Figure 5 shows representatives of the effects of volatile anesthetics on input–output curves in low, normal, and high $[Mg^{2+}]_o$. In normal $[Mg^{2+}]_o$, PS amplitudes steeply increased as a function of EPSP slopes. Neither sevoflurane nor isoflurane produced consistent effects on the configuration of the input–output curves and on the EPSP-axis intersections (threshold for PS generation) in the range of 0.5 to 4.0 mM of $[Mg^{2+}]_o$. The threshold did not exhibit the $[Mg^{2+}]_o$-dependent modification in the absence and presence of volatile anesthetics (fig. 6).

The $[Mg^{2+}]_o$-dependent anesthetic inhibition was also tested on antidromically elicited PSs in response to the activation of nonsynaptic pathways. The baseline amplitudes of antidromic PSs at 0.5, 2.0, and 4.0 mM of $[Mg^{2+}]_o$ were $2.7 \pm 1.2$, $2.3 \pm 1.3$, and $2.6 \pm 1.5$ mV ($n = 4$–7), respectively. Figure 7 shows that the effects of sevoflurane and isoflurane on antidromic PSs were not dependent on $[Mg^{2+}]_o$, suggesting that the soma or axons of pyramidal neurons are not responsible for the $[Mg^{2+}]_o$-dependent anesthetic inhibition.

## Discussion

We have demonstrated that $[Mg^{2+}]_o$ can modify the inhibitory actions of volatile anesthetics on field potentials elicited by stimulation of monosynaptic pathways in mammalian CNS preparations. Because the input–output analysis showed that neuronal excitation in PS steeply increases as a function of the EPSP amplitude (fig. 5), the $[Mg^{2+}]_o$-dependent effects were more evident on PS amplitudes than on EPSP slopes in the current study.

It is known that increasing total divalent cation decreases cell excitability,[5] and the alteration of $[Mg^{2+}]_o$ itself modified the parameters of field potentials in the current study. Previous work reported that an intravenous infusion of $Mg^{2+}$ produced neither anesthesia nor analgesia in two human volunteers, even when their serum $Mg^{2+}$ concentration reached 7.3–7.7 mM (11 times the normal value).[19] Aldrete *et al.*[20] observed a sleep-like state in mongrel dogs after intravenous administration of $Mg^{2+}$. However, they concluded that the
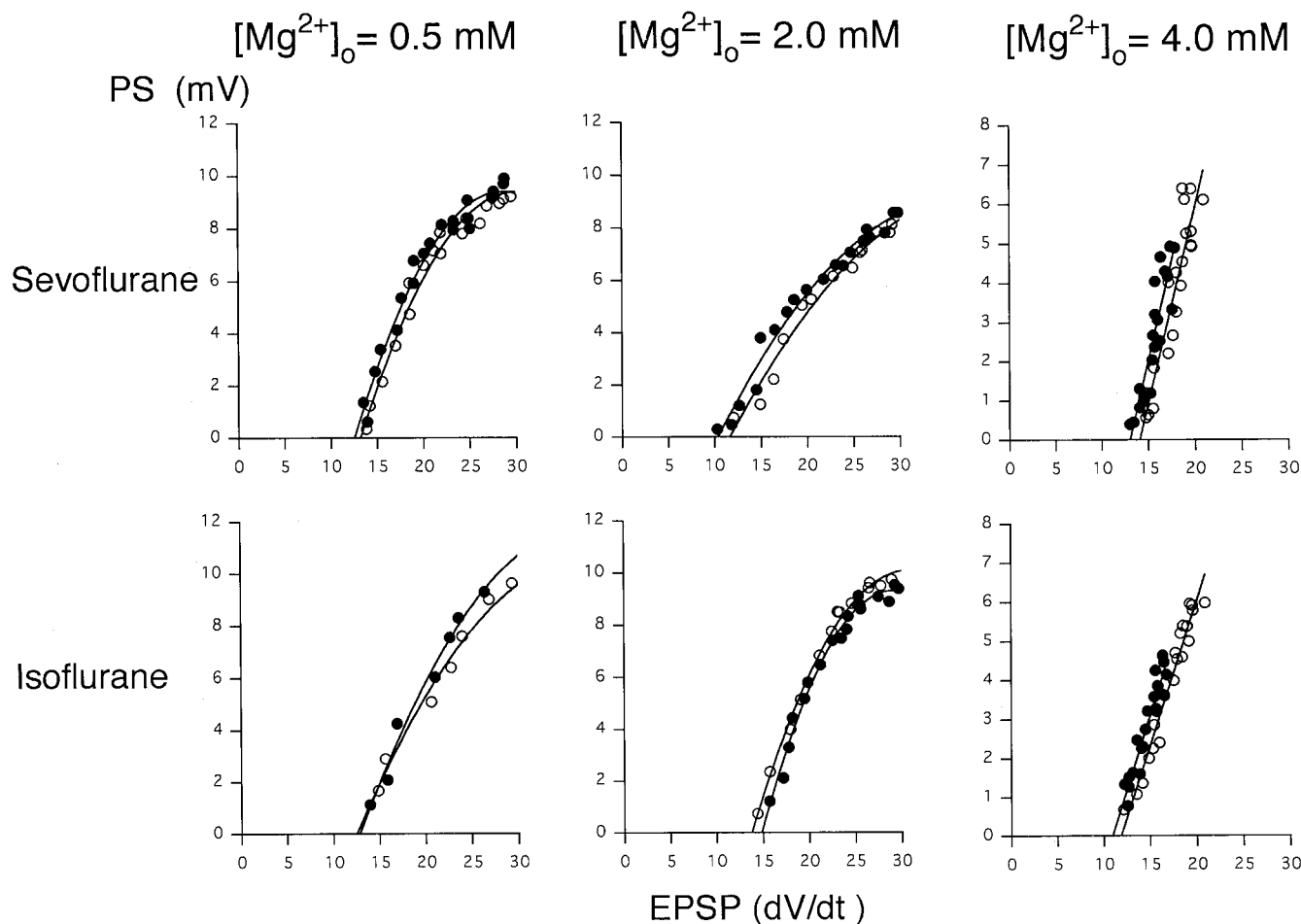
Fig. 5. The excitatory postsynaptic potential (EPSP) and the population spike (PS) of CA1 pyramidal neurons were simultaneously recorded to represent effects of sevoflurane (3.0 vol%) and isoflurane (0.7 vol%) on the input–output curves in various extracellular $Mg^{2+}$ concentrations ($[Mg^{2+}]_o$). Open circles = in the absence of anesthetic; closed circles = in the presence of anesthetic. Data points were fitted to a linear or quadratic function (continuous lines).
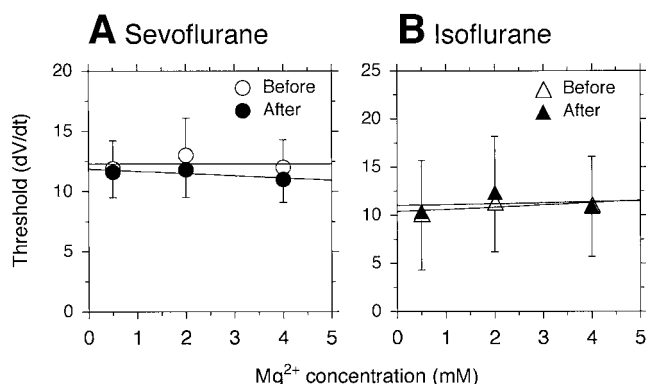
## A Sevoflurane



## B Isoflurane



**Fig. 6. Relations between the threshold for population spike generation and extracellular $Mg^{2+}$ concentrations in the absence (before) and presence (after) of sevoflurane (3.0 vol%, *A*) and isoflurane (0.7 vol%, *B*). The threshold was determined at a x-axis (dV/dt) intersection of the input–output curve (fig. 5) and expressed as mean ± SD (n = 5). Data points were fitted to a linear function (continuous lines). The $r^2$ values for open circles, closed circles, open triangles, and closed triangles are 0.001, 0.028, 0.003, and $8 \times 10^{-7}$, respectively.**

cardiovascular depression and respiratory muscle paralysis by high $[Mg^{2+}]_o$ impaired the level of consciousness. Taken together with the current results, $Mg^{2+}$ *per se* could not produce anesthesia or analgesia, but could probably modify the actions of volatile anesthetics in CNS. Our study was designed to test whether the $[Mg^{2+}]_o$-dependent modification of anesthetic actions could arise from (1) presynaptic $Ca^{2+}$ channels, (2) NMDA receptors, (3) postsynaptic mechanisms, or (4) neural excitability of pyramidal cell soma.

Presynaptic $Ca^{2+}$ channels regulate neurotransmitter (glutamate) release in the CNS. Isoflurane has been reported to block $Ca^{2+}$ channels in hippocampal pyramidal neurons,[21] and these could be a major target site for volatile anesthetics in the CNS. The current results showed that the $[Mg^{2+}]_o$ dependence was attenuated
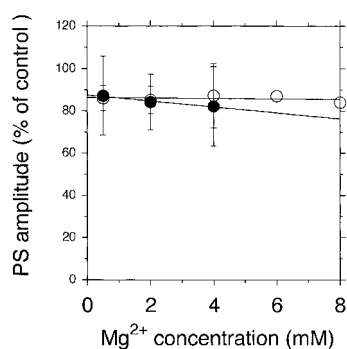


**Fig. 7. The actions of volatile anesthetics on antidromically evoked population spikes (PSs) are not affected by the change in extracellular $Mg^{2+}$ concentrations ($[Mg^{2+}]_o$). The PSs were elicited in response to stimulation of the alveus hippocampi. Open circles = sevoflurane (5.0 vol%); closed circles = isoflurane (5.0 vol%). Points (mean ± SD) represent percent of control (normalized to the value in the absence of anesthetic at each $[Mg^{2+}]_o$) for four and seven determinations from separate slices, respectively. Data points were fitted to a linear function (continuous lines).**

under the fixed $[Mg^{2+}]_o/[Ca^{2+}]_o$ ratio, suggesting that the competition between $Mg^{2+}$ and $Ca^{2+}$ at the presynaptic $Ca^{2+}$ channels modify the volatile anesthetic depression of the $Ca^{2+}$ currents. Presynaptic $Ca^{2+}$ channels could be, at least in part, responsible for the $[Mg^{2+}]_o$-dependent anesthetic inhibition.

$Mg^{2+}$ is known to regulate activation of NMDA receptors in a voltage-dependent manner.[6,7] Tramer *et al.*[22] showed in clinical trials that intravenous administration of magnesium sulfate reduced the postoperative pain score, and suggested that high concentrations of $[Mg^{2+}]_o$ can modify analgesic effects caused by NMDA receptor–mediated mechanisms. However, the results of the current study demonstrate that neither NMDA nor non-NMDA receptors are involved in the $[Mg^{2+}]_o$-dependent modification of anesthetic effects.

Input–output (EPSP–PS) relations were examined to investigate whether the postsynaptic events, such as inhibitory postsynaptic potentials, were involved in the $[Mg^{2+}]_o$-dependent modification of anesthetic actions. Because the inhibitory postsynaptic potential has a latency to onset of 3 ms or less in CA1 of rat hippocampus,[23] the feedforward inhibitory postsynaptic potential could modulate PS generation. If the inhibitory postsynaptic potential is modified in a $[Mg^{2+}]_o$-dependent manner, the alteration of application of $[Mg^{2+}]_o$ would affect the input–output curve and shift the threshold for PS generation in the presence of volatile anesthetics. However, the current results did not support this concept. Sevoflurane and isoflurane had minimal effects on the curves and thresholds, and the effects were not modified by changing $[Mg^{2+}]_o$. In addition, the actions of volatile anesthetics on antidromically elicited responses were not $[Mg^{2+}]_o$-dependent. Taken together, it is unlikely that the postsynaptic events or the excitability of cell soma are responsible for the $[Mg^{2+}]_o$-dependent anesthetic actions.

It has been reported that serum $Mg^{2+}$ concentration can be altered during general anesthesia.[8,9] Considering that the cerebrospinal fluid concentration of $Mg^{2+}$ is regulated *via* active transport mechanisms, a small amount of $Mg^{2+}$ could cross the blood–brain barrier.[24] The alteration of cerebrospinal fluid $Mg^{2+}$ concentration is found to be highly correlated with serum $Mg^{2+}$ concentration,[25] and systemically administered $Mg^{2+}$ reduces minimum alveolar concentration of volatile anesthetic in rats *in vivo*.[26] Hence, it might be possible that $[Mg^{2+}]_o$-dependent modification of volatile anesthetic actions can take place during clinical anesthesia.

In conclusion, the current study provides evidence that alteration of $[Mg^{2+}]_o$ can modify the actions of volatile anesthetics on stratum radiatum synaptic input to CA1 pathway in rat hippocampus *in vitro*. The results suggest that $[Mg^{2+}]_o$-dependent anesthetic inhibition could be, in part, a result of the modification of presynaptic $Ca^{2+}$ channels.

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