Sevoflurane Directly Excites Locus Coeruleus Neurons of Rats

Yutaka Yasui, M.D.,* Eiji Masaki, M.D., Ph.D.,† Fusao Kato, Ph.D.‡

Background: Sevoflurane, an anesthetic showing high incidence of emergence agitation in human patients, especially in children, increases noradrenaline release in the preoptic area in the rat brain. The clinically observed frequency of emergence agitation with sevoflurane is significantly reduced by drugs activating α_2 adrenoceptors. The locus coeruleus (LC) is a source nucleus of widely spreading noradrenergic projections in the central nervous system and is also known as one of the principal targets of some α_2-adrenoceptor agonists, such as dexmedetomidine. The authors analyzed the effects of sevoflurane and other anesthetics on the membrane current of the LC neurons to study the mechanism of the paradoxical "excitatory" effects of the anesthetics.

Methods: Effects of volatile and nonvolatile anesthetics on the membrane potential and currents of LC neurons in pontine slices of the rat were evaluated. Action potential–dependent transmission was suppressed with tetrodotoxin.

Results: Sevoflurane at 5% (measured concentration in the recording chamber, 0.5 mM) induced an early-phase inward current in most of LC neurons in a robust manner, which significantly increased the firing frequency in the absence of tetrodotoxin under current clamp recording. Preadministration of dexmedetomidine (1–3 nM) occluded this increase in firing frequency with sevoflurane. This inward current was inhibited by a gap junction inhibitor carbenoxolone and was not observed with nonvolatile general anesthetics and in non-LC neurons examined.

Conclusions: The excitatory current activated by sevoflurane in LC neurons, likely to be mediated by gap junction–related mechanisms, might be one of the potential cellular mechanisms underlying paradoxical excitatory effect of sevoflurane.

RECENT advances in electrophysiologic and molecular biologic techniques have identified various types of molecular targets underlying the depressant effects of inhalation anesthetics. These include potentiation of receptor channels mediating fast inhibitory transmission such as γ-aminobutyric acid type A and glycine receptors,[1] attenuation of excitatory synaptic transmission,[2] and activation of background leak potassium channels.[3,4] All of these mechanisms identified to date point to a powerful depressant effect of the inhalation anesthetics on central nervous system (CNS) excitability.

Besides the well-analyzed depressant effect, the inhalation anesthetics have been known to exert transient paradoxical "excitatory" effects in the animals and human patients, especially in children. In particular, paradoxical agitation at the emergence of inhalation anesthesia, which is called *hyperexcitation*, *emergence agitation*, and *emergence delirium* (for review, see Vlajkovic and Sindjelic[5]), is of important and serious clinical concern. The cellular mechanism of such excitatory effects remains unidentified.[5] In particular, it remains unknown whether anesthetics inducing such excitatory behavior could exert direct excitatory effect on central neurons in the absence of synaptic influences.

To address this issue, we recorded the membrane current and potential of the locus coeruleus (LC) neurons in brainstem slices and analyzed the effects of inhalation and nonvolatile anesthetics thereon. We targeted LC in the pons for the following reasons. First, the neurons in the LC send divergent noradrenergic projections to the widely distributed structures in the CNS, including the cortex, thalamus, and hippocampus, and control their excitability in a one-to-many manner, allowing the LC to play a central role in the global control of CNS excitability.[6,7] Second, sevoflurane and isoflurane increase noradrenaline release in the preoptic area in rats,[8] suggesting that these inhalation anesthetics stimulate noradrenaline-releasing systems in the CNS. Third, the incidence of agitation in human patients is markedly reduced by premedication of the α_{2A}-adrenoceptor agonists, such as dexmedetomidine, which exerts depressant effects by suppressing of LC neuron excitability through activation of LC α_{2A} adrenoceptors.[9]

Materials and Methods

Slice Preparation

The manipulation of the animals conformed to the Guiding Principles for the Care and Use of Animals in the Field of Physiologic Sciences of the Physiologic Society of Japan (1988) and was approved by the Animal Care Committee of The Jikei University School of Medicine, Tokyo, Japan. Transverse brainstem slices from Wistar rats (2–3 weeks old) were prepared according to a method described previously.[10] Briefly, the lower brainstem was dissected out during deep ketamine anesthesia

(100–150 mg/kg intraperitoneal), and one or two transverse slices of 400 μm thickness containing the LC were cut in the ice-cold "cutting" artificial cerebrospinal fluid (ACSF).[10] The slices were first incubated in a holding chamber with "standard" ACSF[10] at 37°C for 30–45 min. The slices were then kept at room temperature (approximately 25°C) in the same chamber for 0.5–3 h until the recording. A slice was transferred to a recording chamber (approximately 0.4 ml volume) and submerged in and continuously superfused at a rate of 1–2 ml/min with the HEPES-buffered ACSF (*c.f.* Sirois *et al.*[3]) composed of 140 mM NaCl, 2 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM HEPES, and 10 mM D-glucose (pH 7.4 or pH 6.5 with NaOH or HCl; bubbled with air; osmolarity, approximately 310 mOsm/kg). Tetrodotoxin citrate (TTX; Alomone, Jerusalem, Israel) was also added to the ACSF at a concentration of 1 μM to completely block action potential generation and subsequent synaptic transmission except for the membrane potential recordings. In a part of the experiment, $[Na^+]_o$ is reduced to 40 mM by N-methyl-D-glucamine (NMDG; Sigma Aldrich, Tokyo, Japan) replacement.

Patch Clamp Recordings

The whole cell transmembrane current was recorded from neurons in the LC, the mesencephalic trigeminal nucleus (MeV), and the facial nucleus (VII). The neurons were visually identified with an upright microscope equipped with oblique illumination condenser lens (BX-50; Olympus, Tokyo, Japan). Recordings were made in healthy-looking 172 LC neurons in 172 slices from 152 rats, 4 MeV neurons in 4 slices, and 9 VII neurons in 9 slices. The patch clamp electrodes were made from borosilicate glass pipettes (1B120F-4; World Precision Instruments, Sarasota, FL). The composition of the intracellular solution was 120 mM gluconic acid potassium, 6 mM NaCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, 2 mM ATP Mg, 0.5 mM GTP Na, 5 mM EGTA, 10 mM HEPES hemisodium, and 12 mM Na_2 phosphocreatine (pH 7.2 as adjusted with KOH; osmolarity, approximately 310 mOsm/kg). The tip resistance of the electrode was 3–8 MΩ. The membrane potential was held at −70 mV during the recordings, except where otherwise stated. The membrane potentials were shown with appropriate correction of the estimated liquid-junction potentials with each solution. The membrane current was recorded with an Axopatch 200B amplifier (Axon Instruments, Sunnyvale, CA) or CEZ-2400 (Nihon-Kohden, Tokyo, Japan), low-pass filtered at 2 kHz, and sampled at 4 kHz with a PowerLab interface (AD Instruments Japan, Nagoya, Japan) together with the holding potential and the timing signal from the electromagnetic valve controller used for the drug application (VC-6; Warner Instruments, Holliston, MA). The microscope images were captured with a charge-coupled device camera (IR-1000; Dage-MTI, Michigan City, IN) and saved on a personal computer. All recordings were made at room temperature (20°–25°C).

Drugs and Their Application

Propofol (Diprivan; AstraZeneca, Osaka, Japan), pentobarbital sodium (Nembutal; Abbott Laboratories, Chicago, IL), and dexmedetomidine hydrochloride (Maruishi, Osaka, Japan) were dissolved in HEPES-buffered ACSF. The following blockers were also dissolved in HEPES-buffered ACSF: a voltage-dependent Na channel blocker, TTX, a nonselective blocker of transient receptor potential (TRP) and related channels, gadolinium chloride (Gd^{3+}; Sigma Aldrich), a P2 purinoceptor blocker, suramin (Sigma Aldrich) and pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonic acid tetrasodium (PPADS; Sigma Aldrich), gap junction blockers, carbenoxolone (Sigma Aldrich), 1-octanol (Sigma Aldrich), 18α-glycyrrhetiic acid (Sigma Aldrich), and lanthanum chloride (La^{3+}; Sigma Aldrich). All perfusate were applied locally with a glass pipette (ID, 0.7 mm), the tip of which was placed approximately 2–5 mm upstream of the recording electrode, ensuring a faster rise of the anesthetic concentration and faster exchange of the solution around the recorded cells than that achieved with bath application. The plateau concentration was attained within 10–20 s after the opening of the electromagnetic valve, the on–off timing of which is indicated in the figures. Volatile anesthetics (sevoflurane and isoflurane, Maruishi; halothane, AstraZeneca) were vaporized with air using appropriate calibrated vaporizers (Sevotec 3, Ohmeda, Steeton, United Kingdom; Forawick, Muraco Medical, Tokyo, Japan; and Fluotec 3, Ohmeda, for sevoflurane, isoflurane, and halothane, respectively) and continuously bubbled into the HEPES-buffered ACSF. Throughout the text, the concentration of volatile anesthetics is expressed as the partial pressure in percentage read from the vaporizer. The actual aqueous concentrations of sevoflurane in the ACSF sampled from the recording chamber used for electrophysiologic recordings, as analyzed with gas chromatography (GC-14B; Shimadzu, Kyoto, Japan), were 0.49, 0.33, and 0.12 mM with the vaporizer partial pressure of 5, 3, and 1%, respectively.[11]

Data Analysis and Statistical Analysis

The recorded membrane current was analyzed offline with an Igor Pro 5 (WaveMetrics, Lake Oswego, OR). Data are presented as mean ± SEM. Statistical comparisons were made with the Wilcoxon test, analysis of variance (ANOVA) with appropriate *post hoc* comparisons, Mann–Whitney U test, Student paired t test, and Fisher exact probability test with SPSS 11.5 for Windows (SPSS, Tokyo, Japan). $P < 0.05$ was considered significant.
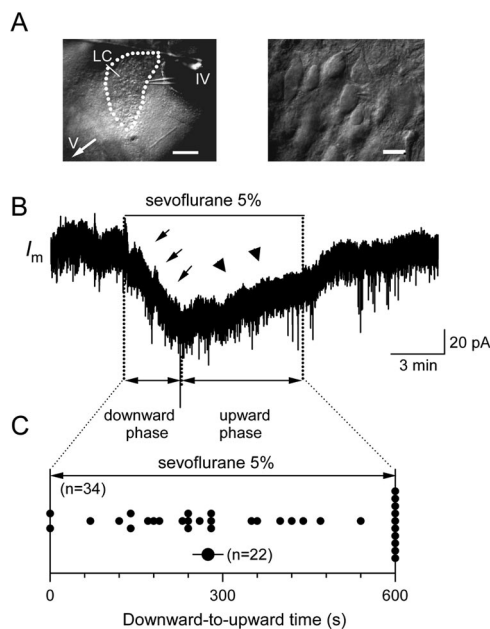
Fig. 1. Sevoflurane induced excitatory responses in the locus coeruleus (LC) neurons. (*A*) Microscopic images of the rat brain stem slice containing LC region (*left*) and LC neurons (*right*). IV = fourth ventricle; V = the ventral direction. *Scale bars*: 200 μm (*left*) and 20 μm (*right*). (*B*) The membrane current of the LC neuron held at −70 mV in the presence of tetrodotoxin showing the biphasic responses to sevoflurane. Sevoflurane (5%) was applied at the horizontal bar. (*C*) Summary of the time at which the downward phase turned to upward during 10-min application of 5% sevoflurane (n = 34).

Results

Sevoflurane Excites LC Neurons

We recorded membrane potential and transmembrane currents from 172 LC neurons identified visually (fig. 1A) and electrophysiologically.[12–14] Under current clamp recording, all 172 neurons examined showed strong A-current–like delayed excitation upon injection of depolarizing step current pulse (change in V_m, from approximately −80 mV to approximately −50 mV) and were devoid of h-current–like sag rebound, which is the typical membrane potential response of neurons expressing h-current conductance,[15] upon injection of hyperpolarizing current pulse (from resting potential to approximately −90 mV). In all 172 LC neurons recorded, stable and strong oscillation in the membrane potential or membrane current was observed. These characteristics are in accord with the previously reported characteristics of the LC neurons.[12–14] After these visual and electrophysiologic identifications of LC neurons, we analyzed the effects of anesthetics on the membrane current under voltage clamp in 156 neurons and those on the membrane potential in 16 neurons. To analyze the mechanism underlying this early and transient depolarization by sevoflurane, we measured the membrane current (I_m) of the LC neurons at a holding potential of −70 mV in the presence of TTX. Application of TTX (1 μM) abolished the spontaneous oscillations in

the membrane current in a similar manner to those already reported.[16] Sevoflurane (5%) rapidly induced a downward shift of I_m trace (peak plateau amplitude of the downward shift; −33.1 ± 4.8 pA; arrows in fig. 1B, I_m) in 32 of 34 LC neurons examined. In 69% of these neurons (n = 22), this downward shift was followed by a subsequent late upward shift toward preapplication level (arrowheads in fig. 1B, I_m). In 22 of these neurons, this upward shift appeared (*i.e.*, the shift became from downwardly to upwardly in the course of sevoflurane application) within 10-min application time. The average time at which the downward shift turned to an upward shift was 275 ± 26 s from the onset of sevoflurane application (fig. 1C) in these neurons. In another 31% (n = 10) of the neurons, this downward shift lasted for the whole span of 10-min application of sevoflurane (filled circles at 600 s in fig. 1C). In the remaining 2 neurons, only the upward shift was observed during 10 min of sevoflurane application (two filled circles at 0 s in fig. 1C). This downward shift in response to sevoflurane is not a specific consequence of the use of HEPES-buffered external solution, which was used in this study for stable manipulation of its pH, because a similar downward shift of 40–80 pA could be observed in the LC neurons recorded with bicarbonate-buffered ACSF.[14] These changes in the membrane currents by sevoflurane were reproducible in an essentially similar manner upon repeated application of sevoflurane.

We then asked whether such direct excitatory current in the LC neurons by sevoflurane excites these neurons to a level sufficient to generate action potentials. This series of experiments was performed in the absence of TTX to measure the action potential frequency. In all 16 neurons in which the membrane potential was measured, 5% sevoflurane depolarized the membrane potential, which occasionally increased the spontaneous firing frequency. Figure 2A shows an example of a neuron in which sevoflurane depolarized the neuron and increased firing frequency (figs. 2A-1 and -2). The average depolarization as measured at the trough of the spontaneous oscillation was 5.2 ± 0.9 mV ($P < 0.001$, paired *t* test; n = 16; range, 1.5–13.8 mV; fig. 2B). Accordingly, the firing frequency was significantly increased by 5% sevoflurane from 0.07 ± 0.03 to 0.35 ± 0.14 spikes/s at the peak effect (n = 10; $P < 0.05$, Wilcoxon test; fig. 2C; fig. 2C contains results from all 16 neurons to which 5% sevoflurane was applied under current clamp mode, but this statistics was made with 10 neurons that generated action potential before and/or after 5% sevoflurane application; the remaining 6 neurons showed no action potential before, during, or after application of sevoflurane [indicated with an arrow in fig. 2C] and were therefore not included in the statistics). Figure 2D summarizes the average time course of the effect of 5% sevoflurane on the firing frequency in 6 neurons, which we continuously observed up to 15 min after the onset
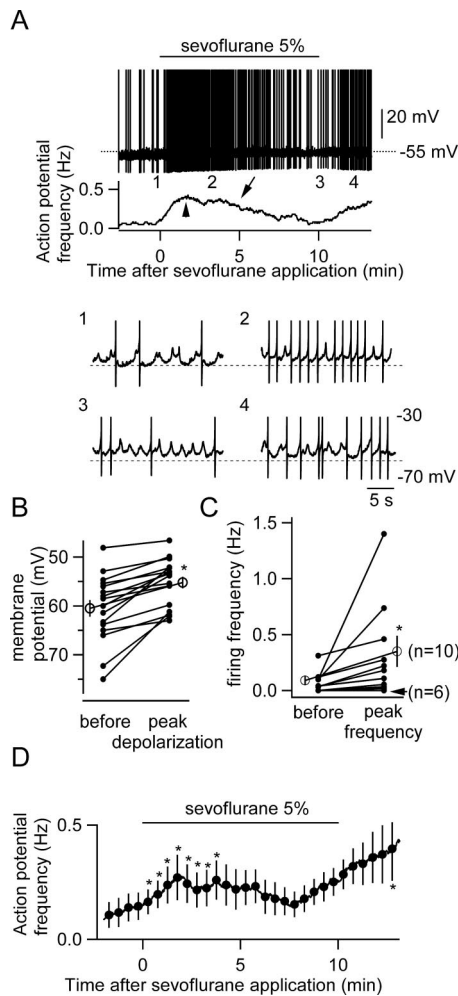
A

sevoflurane 5%

20 mV

−55 mV

Action potential frequency (Hz)

0.5

0.0

1   2   3   4

0   5   10

Time after sevoflurane application (min)

1   2

3   4   −30

−70 mV

5 s

B   C

membrane potential (mV)

50

60

70

before   peak
       depolarization

firing frequency (Hz)

1.5

1.0

0.5

0.0

*

(n=10)

(n=6)

before   peak
       frequency

D

Action potential frequency (Hz)

0.5

0.0

sevoflurane 5%

* * * * *
 * *   *

*

0   5   10

Time after sevoflurane application (min)

Fig. 2. Sevoflurane increased action potential frequency of locus coeruleus neurons. (*A*) The membrane potential recording of locus coeruleus neurons (*above*) and changes in action potential frequency (*below*). Sevoflurane (5%) was applied at the *horizontal bar*. Traces 1–4 show the time-extended traces at points 1–4 in the above trace in *A*. Action potentials are truncated at +30 mV for the clarity in *A*. The trough resting potential level before sevoflurane application is indicated with a *broken horizontal line* in 1–4. (*B*) Summary of the effect of sevoflurane (5%) on the membrane potential. *Filled circles* show the values in each neuron (n = 16), and *open circles* and *vertical bars* indicate the mean values and SEs for 16 neurons. * *P* < 0.05, paired *t* test. (*C*) Summary of the effect of 5% sevoflurane on the firing frequency of locus coeruleus neuron. *Filled circles* show the values in each neuron (n = 16), and *open circles* and *vertical bars* indicate the mean values and SEs for 10 neurons (6 neurons devoid of firing both before and after application were not included in the mean and SE calculation, indicated with an *arrow*). * *P* < 0.05, paired *t* test. In *B* and *C*, the mean resting membrane potential was kept spontaneously (*i.e.*, without any current injection; n = 9) or manually (with continuous current injection of −28 to +40 pA; mean, 12.3 ± 10 pA; n = 7). (*D*) The time course of the changes in action potential frequency with sevoflurane. Each *point* and *vertical bar* represent mean and SE of 6 neurons. Sevoflurane (5%) was applied at the *horizontal bar*. * *P* < 0.05 *versus* mean action potential frequency during 1 min immediately before sevoflurane application, paired *t* test; n = 6.

of sevoflurane application without any other manipulation. Sevoflurane at 5% significantly ($P < 0.05$, paired t test; n = 6) increased the firing frequency during ap-

proximately the first 4 min (asterisks in fig. 2D). A slight but significant increase in firing frequency was observed also after removing sevoflurane. These results indicate that sevoflurane significantly depolarized the LC neurons and significantly increased their firing frequency.

Differential Effects of Acidification on the Downward and Upward Shifts of Membrane Current of LC Neurons by Sevoflurane

What are the mechanisms underlying these excitatory responses of the LC neurons to sevoflurane? To address this issue, we sought to isolate the components underlying the downward and upward shifts of I_m by sevoflurane. One of the principal molecular substrates for the depressant effect of inhalation anesthetics identified to date is the pH-sensitive background potassium channel (TASK), a subtype of KCNK channel family. TASK channels are expressed in LC, and their activation by halothane results in outward K^+ current.[3] Because the TASK channels are suppressed by acidification, we examined the effect of low-pH perfusate on the shifts in membrane current by sevoflurane.

Bath acidification to pH 6.5 caused an inward shift of the membrane current in good accord with its inhibitory effect on TASK channels in LC neurons as described by Sirois *et al.*[3] Figure 3A shows a typical example of the responses of a neuron to sevoflurane at pH 7.4 (left) and pH 6.5 (right). The upward shift (arrowhead) was strongly suppressed at pH 6.5, whereas the downward I_m shift (arrows) was not suppressed at pH 6.5 (figs. 3A and B). At pH 6.5, the downward I_m shift lasted for whole span of sevoflurane application in 75% of the neurons out of 15 (figs. 3A and B). At pH 7.4, amplitudes of both the downward and the upward shifts by sevoflurane were dependent on its concentration (fig. 3C). At pH 6.5, the amplitude of the upward shift, but not that of the downward one, caused by 3% and 5% sevoflurane was significantly smaller than that at pH 7.4 (fig. 3C; $P < 0.05$, U = 15.0 and U = 173.0, respectively). Based on these concentration–response curves, we compared the fraction of "responding" neurons to sevoflurane with downward and upward I_m shifts at different pHs. A shift in I_m larger than 5 pA (dotted lines in fig. 3C) was considered to be "responding." The sevoflurane-induced downward shift of I_m was observed in 95% of the neurons examined both at pH 7.4 and 6.5 (fig. 3D). In contrast, the fraction of neurons showing the upward shift of I_m was significantly smaller in acidic condition (71% to 25% of the neurons at pH 7.4 and pH 6.5, respectively; fig. 3D). In contrast, there was no significant change in the amplitude of the sevoflurane-induced downward I_m shift by acidification at all concentrations examined (fig. 3C). The marked pH sensitivity of the late upward I_m shift is in good accord with the reported properties of the TASK conductance.[3] Based on these observations, we attribute this upward I_m shift to activa-
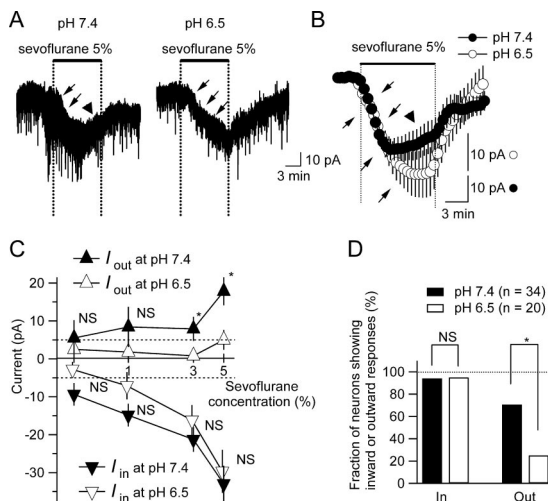
Fig. 3. Acidification attenuated the sevoflurane-induced outward component (*arrowheads*), but not the early inward component (*arrows*). (*A*) The membrane current of a locus coeruleus neuron exposed to sevoflurane recorded at pH 7.4 (*left*) and 6.5 (*right*). (*B*) Averaged time course of the membrane current trajectories of locus coeruleus neurons exposed to sevoflurane; recordings at pH 7.4 (n = 34) and 6.5 (n = 20). Each *point* and *vertical bar* represent mean and SE. (*C*) Concentration dependency of the inward component amplitude (I_{in}) and the outward component amplitude (I_{out}) at pH 7.4 and pH 6.5, respectively. *Abscissa* indicates the sevoflurane concentration (in log scale), which was tested at 0.4, 1, 3, and 5%. The amplitude of the inward component was defined as the shift in I_m from the beginning of application to the point at which downward became upward (figs. 1B and C). The amplitude of the outward component was defined as the shift in I_m from the point at which downward became upward to the end of the application (10 min from the beginning in all cases). * $P < 0.05$, Mann–Whitney U test. NS = not significantly different between the values at pH 7.4 and those at pH 6.5. The number of cells are 5, 7, 10, and 34 for 0.4, 1, 3, and 5%, respectively, for the recordings at pH 7.4 and 4, 6, 8, and 20, respectively, for the recordings at pH 6.5. (*D*) Fraction of neurons showing inward (in) or outward (out) phases in response to 5% sevoflurane at different pH values. * $P < 0.05$, Fisher exact probability test.

tion of TASK-like conductance and call this component *sevoflurane-induced outward current* hereafter. These results indicate that the membrane current trajectory in response to sevoflurane (as typically shown in fig. 1B) is composed of at least two different components: unidentified aberrant excitatory downward component and TASK-related pH-sensitive outward component.

Ionic Mechanism and Pharmacologic Characteristics of Sevoflurane-induced Current

To our knowledge, such direct excitatory response to anesthetics has never been documented in the CNS neurons. What is the nature of this early excitatory downward shift of I_m in the LC neurons? Does it result from activation of unidentified inward ionic conductance? To address this issue, we took advantage of this pH sensitivity of the sevoflurane-induced outward current; the slice was perfused with the extracellular solution of pH 6.5 to maximally reduce the possible influence of the outward current. The membrane potential was held at
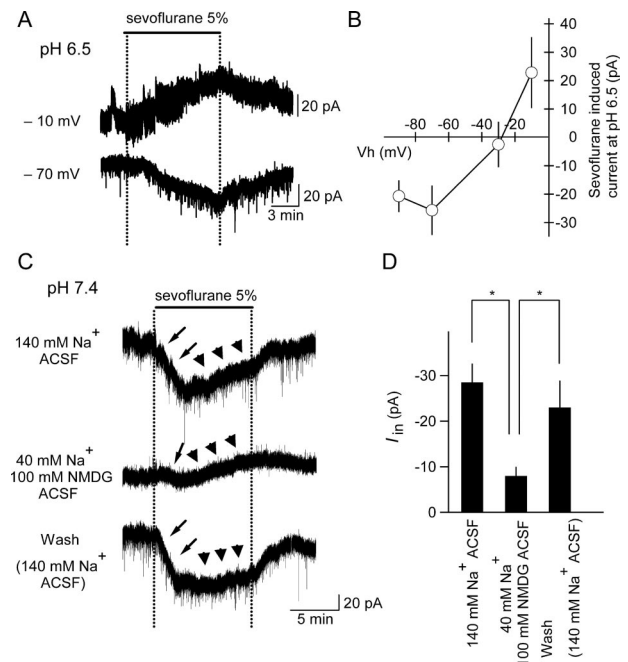

Fig. 4. The sevoflurane-induced early inward current was dependent on extracellular Na^+ concentration. (*A*) Effect of sevoflurane at various holding potentials measured at pH 6.5. *Upper* and *lower traces* were the current traces recorded at −10 and −70 mV, respectively. (*B*) Current–voltage relations of the sevoflurane-induced current. *Abscissa*, holding membrane potential at which the membrane potential was clamped during sevoflurane application. *Open circles* and *vertical bars* show the mean amplitude and SE of the current induced by 5% sevoflurane (n = 20 for −70 and −90 mV, n = 3 for −10 and −30 mV). (*C*) *Upper*, *middle*, and *lower traces* were the membrane current traces recorded at extracellular Na^+ concentrations of 140, 40, and 140 mM, respectively, at pH 7.4. *Arrows* = early inward current; *arrowheads* = late outward current. (*D*) Summary of I_{in} at different extracellular Na^+ concentrations (n = 10). * $P < 0.05$; Mann–Whitney U test. ACSF = artificial cerebrospinal fluid. NMDG = *N*-methyl-D-glucamine.

−90 to −10 mV for 5–15 min to stabilize the membrane current. The amplitude of the downward shift by sevoflurane became smallest at approximately −30 mV of the holding potentials examined, beyond which this shift became upward (figs. 4A and B). Figure 4B demonstrates membrane-potential dependency of this inward component. This result indicates that the downward shift by sevoflurane at a holding potential of −70 mV resulted from activation of this outwardly rectifying current reversing at approximately −30 mV. These lines of evidence indicates that the "downward shift" of I_m in response to sevoflurane is likely to result from a sevoflurane-activated inward current. This current–voltage relation suggests a principal involvement of mixed cationic conductance. Because the estimated equilibrium potential for Cl^- in this recording condition is −63 mV, involvement of anion-selective conductance is unlikely.[17,18]

A simple application of the Goldman-Hodgkin-Katz solution with the used concentrations of the internal and external solutions and the recorded reversal potential of

-30 mV gives an estimate of 0.27:1 for the relative ratio of permeability (P_{Na}:P_K) for this sevoflurane-activated conductance. In the condition where $[Na^+]_o$ is reduced to 40 mM by NMDG replacement, the reversal potential of this conductance is estimated to be -58.8 mV, assuming that the P_{Na}:P_K ratio does not change in this external solution. This reduction in $[Na^+]_o$ to 40 mM is expected to reduce the driving force through this conductance at a holding potential of -70 mV from 40 mV (70 to 30) to 11.2 mV (70 to 58.8), *i.e.*, 28% of the amplitude at recordings with 140 mM $[Na^+]_o$. In good accord with this estimation, the inward current with sevoflurane at 40 mM $[Na^+]_o$ was significantly decreased to a mean value of 27.5% of that at 140 mM $[Na^+]_o$ (n = 10; figs. 4C and D; $P < 0.05$, U = 4.0). The sevoflurane-induced outward current was not significantly affected by reduction in $[Na^+]_o$ (17.4 ± 4.7 pA at 140 mM $[Na^+]_o$; 16.8 ± 5.2 pA at 40 mM $[Na^+]_o$; n = 10; $P = 0.78$, U = 46.5). These results suggest that sevoflurane activates conductance that is permeable to both Na and K.

What is the nature of this unidentified conductance that is specifically activated by sevoflurane? The following series of manipulations that might affect a series of conductance reported to be expressed in the LC neurons did not apparently affect the amplitude of sevoflurane-induced inward current: (1) Deprivation of extracellular Ca^{2+} with no added $CaCl_2$ and 0.2 mM EGTA (n = 3); (2) application of 10 μM Gd^{3+} (n = 3) to block TRP and related-channels[19]; and (3) application of P2 receptor antagonists suramin (100 μM) and PPADS (40 μM)[14] (n = 3 for each). We then examined the effects of agents that affect gap junction channels because the LC neurons express connexin proteins (isoforms 32, 36, and 43),[20] the molecular elements forming gap junction channels and/or hemichannels, which underlie the neuron-to-neuron and neuron-to-glia communications in LC.[21] Carbenoxolone (100 μM), an agent that suppresses gap junction opening in LC[21] and hemichannels,[22] markedly reduced sevoflurane-induced inward current (fig. 5A, arrows; and figs. 5B and C; to 26.2 ± 11.7% of that in the absence of carbenoxolone; n = 5; $P < 0.01$, U = 0) without affecting the late outward current (fig. 5A, arrowheads; and figs. 5B and C; $P = 0.075$, U = 4.0). We also examined the effect of octanol at a concentration suppressing gap junction channel opening.[23] Octanol (200 μM) significantly reduced the amplitude of sevoflurane-induced inward current without affecting the late outward current (fig. 5C; reduced to 60.6 ± 21.6% of the inward current in the absence of octanol; n = 5; $P < 0.05$, U = 2.0). Other two types of agents that are shown to suppress gap junction opening in some tissues, 18α-glycyrrhetinic acid (40 μM)[24] and La^{3+} (100–300 μM),[25] also attenuated the sevoflurane-induced inward current, which, however, was not significant (18α-glycyrrhetinic acid, reduced to 71.7 ± 10.9% of the control; n = 4; U = 5.0; $LaCl_3$, reduced to 74.7 ± 16.2%; n = 4; U = 4.0). These
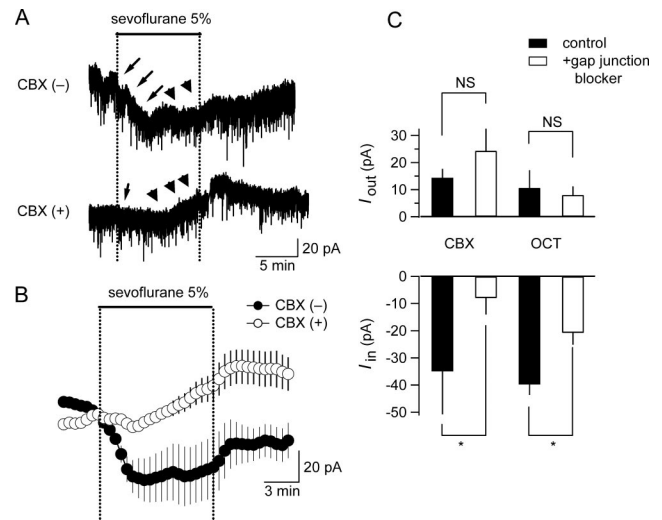

Fig. 5. The sevoflurane-induced early inward current was inhibited by gap junction blockers. (*A*) The effect of carbenoxolone (CBX; 100 μM) on the response to sevoflurane. *Top* and *bottom traces* show the responses to sevoflurane (5%) in the absence (CBX(−)) and presence (CBX(+)), respectively, of carbenoxolone. *Arrows* = early inward current; *arrowheads* = late outward current. (*B*) Averaged time course of the membrane current trajectories of five neurons, onto which sevoflurane was applied in the absence (*filled circles*; CBX(−)) and presence (*open circles*; CBX(+)) of carbenoxolone. Mean ± SE of five neurons. (*C*) The effects of carbenoxolone (CBX) and 1-octanol (OCT) on the outward component amplitude (I_{out}, *top*) and the inward component amplitude (I_{in}, *bottom*). Mean ± SE. * $P < 0.05$ *versus* values in the absence of carbenoxolone or octanol (n = 5), Mann–Whitney U test. NS = not significantly different.

pharmacologic properties, together with the current–voltage relation of this inward current as shown in figure 4B, point to an involvement of a specific type of gap junction channels and/or hemichannels with less permeability to large molecules such as NMDG in the generation of the inward current by sevoflurane.

Sevoflurane Produced Largest Inward Current between Various Classes of Anesthetics

In the clinical practice, it has been reported that the incidence of inhalation anesthetic–induced agitation depends on the type of agent. In addition, unlike inhalation anesthetics, intravenous anesthetics do not exert "excitatory" effects in humans and animals. We therefore examined whether other types of anesthetics could activate the early inward current in LC neurons. First, we compared the effects of three types of volatile anesthetics. Sevoflurane at 3% and 5% evoked the largest average inward current among three volatile anesthetics (fig. 6A, bottom). For example, the amplitude of inward current with 5% sevoflurane was markedly and significantly larger than that with 5% halothane ($P < 0.01$, ANOVA with *post hoc* Tukey honest significant difference [HSD] test, $F_{10,85} = 4.951$), which is in marked contrast to the significantly larger outward current with 5% halothane ($P < 0.05$, ANOVA with *post hoc* Tukey HSD test, $F_{10,85} = 3.463$) than that with 5% sevoflurane. Second, we
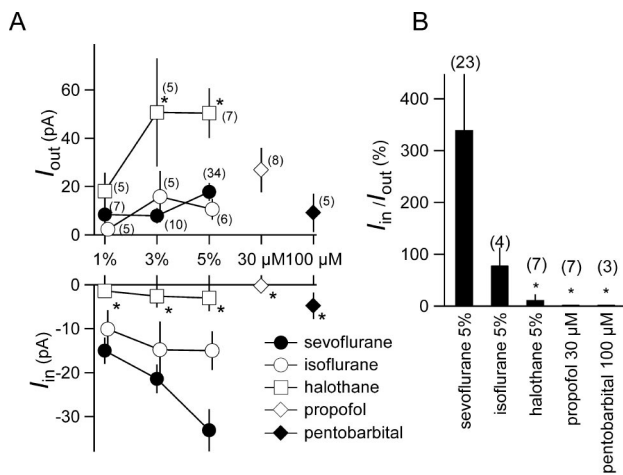
Fig. 6. (*A*) Summaries of the inward component amplitude (I_{in}, *bottom*) and the outward component amplitude (I_{out}, *top*) evoked by various anesthetics at different concentrations (for the volatile anesthetics). *Markers* and *vertical bars* show mean amplitude and SE. * $P < 0.05$, one-way analysis of variance with *post hoc* multiple comparisons *versus* the value with 5% sevoflurane (5%), except for the comparison between 5% sevoflurane and pentobarbital, in which one-way analysis of variance was made. The difference was not significant for all data points without * *versus* 5% sevoflurane. The *numbers in parentheses* indicate the number of neurons. The data for sevoflurane are the same as in those in figure 3C and are plotted for comparison. (*B*) The amplitude of inward current normalized by that of the outward current produced by each volatile anesthetic. The neurons showing outward current responses smaller than 5 pA were excluded from this normalization comparison because too-small outward current result in unstable estimates by division. The *numbers in parentheses* indicate the number of neurons analyzed. * $P < 0.05$, Mann–Whitney U test, compared with sevoflurane (5%).

examined whether noninhalation anesthetics could also activate a similar inward component. No detectable inward component was observed with propofol (30 μM; n = 8; fig. 6A) despite a large outward current, which was not significantly from that with 5% sevoflurane (P = 0.99, ANOVA with *post hoc* Tukey HSD test, $F_{10,85}$ = 3.463). Pentobarbital (100 μM; n = 5; fig. 6A) activated only small outward and inward currents, supporting its principal potentiating effects on the γ-aminobutyric acid type A receptors.[26] In summary, the amplitude of the inward current with 5% sevoflurane was significantly larger than that with any concentration of halothane, propofol, or pentobarbital (fig. 6A, bottom; ANOVA with *post hoc* Tukey HSD test, $F_{10,85}$ = 4.951).

These anesthetics exert anesthetic effects of different intensity even at the same concentration. To overcome this potential problem and make meaningful comparisons, we calculated the "excitatory-to-inhibitory ratio" by normalizing the amplitude of the inward component to that of the outward component generated in the same neurons by each volatile anesthetic application. This normalized value could be regarded as an index of "excitatory" property of each anesthetics compared with their "inhibitory" properties (fig. 6B). The average of the normalized inward current was largest with sevoflurane,

followed by isoflurane. Halothane produced only small normalized inward current (fig. 6B). This small effect of halothane is not due to the high concentration used compared with the minimum alveolar concentration (MAC) in rats, because 1% (n = 5) and 3% (n = 5) of halothane only produced negligibly small normalized inward current (< 10%; data not indicated). The normalized inward current by propofol was negligibly small. As mentioned in the previous paragraph, pentobarbital did not produce measurable outward current, making it impossible to estimate the normalized inward current amplitude and not included in figure 6B. In summary, the normalized inward current with 5% sevoflurane was significantly larger than that with 5% halothane, 30 μM propofol, and 100 μM pentobarbital (fig. 6B; P < 0.01, U = 17.0; P < 0.01, U = 7.0; and P < 0.05, U = 3.0).

Activation of α_{2A} Receptors Prevents LC Neuron Firing in Response to Sevoflurane

In human children, the incidence of agitation and delirium with inhalation anesthetics, especially with sevoflurane, is significantly reduced by drugs, such as dexmedetomidine and clonidine,[5,27–30] that selectively suppress the excitability of LC neurons through activating α_{2A} receptors in the LC.[9,31] To examine whether dexmedetomidine could counteract the excitatory effect of sevoflurane in the same neurons recorded in the brain slices isolated from other structures, we analyzed the effect of dexmedetomidine on the sevoflurane-induced depolarization and inward current in the absence and presence of TTX, respectively. Dexmedetomidine (1–3 nM) significantly hyperpolarized all LC neurons examined (figs. 7A and B; from −53.3 ± 0.9 mV to −69.4 ± 4.2 mV; by 16.2 ± 1.6 mV; n = 5; P < 0.05, paired *t* test). This result is in good accord with a previous report showing that dexmedetomidine opens potassium conductance in LC neurons.[32] Sevoflurane (5%) depolarized these neurons in the presence of dexmedetomidine by 8.8 ± 2.7 mV, which brought the membrane potential to −63 ± 4.3 mV (n = 5; P < 0.02 *vs.* the value before sevoflurane, paired *t* test) at the maximum effect of sevoflurane, a value below the threshold. Subsequently, in the presence of dexmedetomidine, the significant increase in the firing frequency by sevoflurane was not observed (fig. 7C; P = 0.32, Wilcoxon test; n = 5). Then, we recorded the membrane current in the presence of tetrodotoxin under voltage clamp at −70 mV. The sevoflurane-induced inward current measured in the presence of dexmedetomidine was −24.0 ± 4.9 pA (n = 5), a value not significantly different from that in the absence of dexmedetomidine (as stated in the first paragraph of the Results, −33.1 ± 4.8 pA; P = 0.583). These results indicate that the hyperpolarization by dexmedetomidine counteracts the pro-firing excitatory effect of sevoflurane on LC neurons in the brain slice.
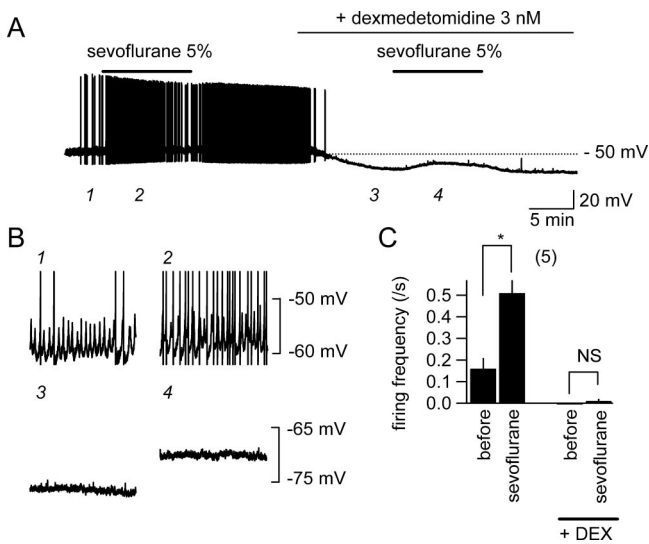
Fig. 7. Dexmedetomidine suppressed the increase in the firing frequency by sevoflurane. (*A*) The membrane potential recording of a locus coeruleus neuron exposed to sevoflurane at the *thick horizontal bars* in the absence (*left*) and presence (*right*) of 3 nM dexmedetomidine (*thin horizontal bar*). (*B*) 1–4 are time-extended traces taken at the points 1–4 in the trace in *A*. (*C*) The effect of sevoflurane on the maximum action potential frequency in the absence and presence of dexmedetomidine (+DEX; 1–3 nM). Mean and SE of 5 neurons. * *P* < 0.05 Mann–Whitney U test. NS = not significantly different.

Sevoflurane-induced Inward Current Was Specific to LC Neurons

Is such excitation by sevoflurane always observed in the central neurons that are sensitive to sevoflurane? We examined whether such excitatory effect of sevoflurane could also be observed in other types of neurons expressing volatile anesthetic–sensitive TASK conductance,[3,33] by analyzing its effect in the neurons in the VII and the MeV, as representatives of the neural structures underlying motor and sensory functions, respectively, under the identical condition to that for the LC recording. Sevoflurane at 5% activated an outward current at pH 7.4 that became significantly smaller at pH 6.5 in all neurons examined in the MeV and VII (fig. 8; Mann–Whitney U test, $P < 0.05$, U = 0.0 and $P < 0.01$, U = 0.0). In contrast, the inward component was absent or negligibly small in the both nuclei (figs. 8B and C).

Discussion

These results indicate that, in addition to the already well-documented potentiation of the background leak potassium channels,[3,34,35] inhalation anesthetics directly activate an inward current that excites the LC neurons even in the absence of synaptic input. This is the first demonstration, to our knowledge, of the direct excitatory effect of inhalation anesthetics on a type of central neurons recorded in the brain slices. The characteristics of this excitation are summarized as follows: (1) It occurs as a result of sevoflurane-induced persistent inward
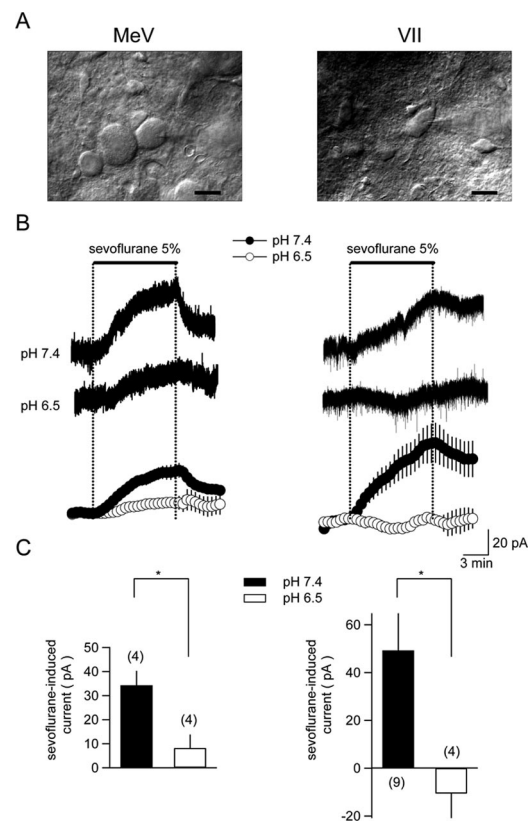

Fig. 8. Effects of sevoflurane on neurons in the mesencephalic trigeminal nucleus (MeV) and the facial nucleus (VII). (*A*) Microscopic images of MeV neurons (*left*) and VII neurons (*right*) recorded. (*B*) Representative data from MeV and VII neurons showing responses of sevoflurane. *Upper* and *middle traces* were measured at pH 7.4 and pH 6.5, respectively. *Bottom*, averaged time course of membrane current trajectories. Each *point* and *vertical bar* represent mean and SE. (*C*) The changes in sevoflurane-induced current at different pHs. * $P < 0.05$, Mann–Whitney U test between the data at pH 6.5 and pH 7.4. The *numbers in parentheses* indicate the number of neurons analyzed.

current which is resistant to TTX, indicating a direct effect on LC neurons. (2) Unlike the sevoflurane-induced late outward current, this inward current was resistant to acidification, suggesting that this inward current occurs through a mechanism distinct from that involved in the anesthetic-evoked activation of TASK channels. (3) This inward current reversed at approximately −30 mV, suggesting an involvement of nonselective cationic conductance. (4) This inward current was suppressed by carbenoxolone and octanol, suggesting a possible involvement of gap junction– or hemichannel–related mechanisms. (5) Sevoflurane generated much larger inward current than isoflurane and halothane when compared with their potential to generate outward currents. (6) Finally, such inward current was not observed in the motor neurons in the VII and sensory neurons in MeV, arguing for an LC-specific effect. Based on these findings, here we propose a plausible scenario that volatile anesthetics, especially sevoflurane, directly activates hemichannel– or gap junction–related conductance spe-

cifically expressed on LC neurons, leading to their excitation. Because the LC plays an important role in controlling the overall activity level of the whole brain through its divergent noradrenergic projections,[7] we propose that this direct excitation of the LC neurons by inhalation anesthetics plays at least a part of central roles in the excitatory effect of inhalation anesthetics in humans and animals.

Molecular Identity of the Sevoflurane-activated Conductance

Until now, a number of electrophysiologic studies have identified mechanisms for the *inhibitory* effects of inhalation anesthetics, including activation of background K^+ channels,[3] potentiation of anionic conductance,[1] and attenuation of cationic conductance.[2] None of these provide satisfactory explanation for its direct *excitatory* effect (depolarizing and inward current-generating) identified in this study. In particular, this conductance was activated earlier than the pH-sensitive outward current, which is likely to be due to background TASK channels,[3] further supporting that the molecular mechanisms underlying these currents are separate.

The pharmacologic properties and its reversal potential indicative of nonselective conductance of this inward current are in good accord with the properties of gap junction channels and hemichannels. Indeed, the LC shows the highest level of connexin expression in the adult brain that forms gap junctions between neurons and glial cells.[20,21] The opening and closing of these gap junctions determine the excitability of the whole LC network.[16] The electrotonic coupling between neurons and glial cells through gap junctions gives rise to spontaneous and synchronous membrane current oscillation of LC neurons,[16] which we observed in all LC neurons examined in the current study (see Results). The connexins can also form hemichannels on the membrane surface, which are not coupled to the counterpart connexins on other cells to form gap junctions (for review, see Spray *et al.*[36]) and are able to function as surface conductance. It is therefore likely that sevoflurane opened these membrane-expressed hemichannels in the LC to generate nonselective current. However, our results are in part against this interpretation. First, La^{3+}, Gd^{2+}, and 18α-glycyrrhetinic acid, possible blockers of hemichannels in the astrocytes,[36] suppressed the sevoflurane-induced inward current only partially. Second, the sevoflurane-induced inward current was attenuated by replacement of approximately two thirds of extracellular Na^+ with NMDG (molecular weight, 195 Da), which is unlikely for the large conductance NMDG-permeable hemichannels.[22] Third, we did not observe outflow of lucifer yellow in response to sevoflurane from the LC cells into which this fluorescent dye had been injected *via* patch-electrode (unpublished observations by Y. Yasui, M.D., E. Masaki, M.D., Ph.D., and F. Kato,

Ph.D., March 8–14, 2007, in experiments performed at the Department of Neuroscience, The Jikei University School of Medicine, Tokyo, Japan; see Thompson *et al.*[37]). Fourth, despite dense expression of connexins 26, 32, 36, and 43 and formation of gap junction,[38] the neurons in the MeV did not show any sign of sevoflurane-activated inward current. Fifth, the spontaneous oscillation, an activity thought to result from gap junction–mediated electrical coupling between LC neurons and glial cells,[16] was rather attenuated by sevoflurane.[11] Taken together, the current results show that the sevoflurane-activated conductance might have functional relations with yet unidentified membrane mechanisms related to channels formed with connexin or pannexin proteins.[37] However, the current limitations of the pharmacologic tools do not allow identification of the molecular nature of this conductance at this moment, which remains to be identified in the future studies.

Distinct Potency of Inhalation Anesthetics in Excitatory and Inhibitory Effects

Sevoflurane was strongest and halothane was weakest in inducing the inward excitatory component of three volatile anesthetics. This order contrasts with that of the MAC for the anesthetic effect, *i.e.*, halothane exerts anesthetic effects at the smallest concentration whereas a larger concentration is required with sevoflurane.[39] Despite a large inward current with 5% sevoflurane (approximately twice MAC in rats), the inward current with 5% isoflurane (approximately threefold MAC in rats) and that with 5% halothane (approximately fivefold MAC in rats) were significantly smaller (fig. 6), indicating that such excitatory effect is not exclusively but highly specific to sevoflurane. It is not likely that the too-high concentration masked the excitatory effect because 1% and 3% halothane produced almost no detectably small inward shift (fig. 6A). As stated in the Results, intravenous anesthetics did not exert excitatory effects at the concentrations exerting inhibitory effects. Such specificity of sevoflurane again supports that the excitatory effect of sevoflurane results from a specific activation of a gap junction– or hemichannel–related conductance though mechanisms entirely independent of the anesthetic effect. Interestingly, the order in generating inward current for these three volatile anesthetics, especially the fact that the inward current with sevoflurane was larger than that with halothane, is in complete accord with the previous clinical studies showing that sevoflurane provokes the emergence agitation at the highest frequency in children among these anesthetics and that intravenous anesthetics do not exert excitatory effects.[40–43]

Clinical Implications of the Direct Excitation by Sevoflurane in LC Neurons

The results presented here indicate that a specific excitation of LC neurons by inhalation anesthetics, espe-

cially sevoflurane, plays an important role in this paradoxical excitation because of the following three reasons. First, dexmedetomidine, an α_{2A} agonist exerting its sedative effect through activating α_{2A} receptors in the LC[9,31] prominently prevents the emergence agitation and emergence delirium by sevoflurane in human patients.[27,28] Such prevention of emergence agitation is reported also with another α_{2A} agonist clonidine.[29,30] In support of this, also in our slice preparations, dexmedetomidine markedly hyperpolarized LC neurons and prevented their firing in response to sevoflurane-induced depolarization. Second, the order of the inhalation anesthetics in generating LC neuron excitation was in complete accord with the incident of clinically observed "agitation" in children, strongly supporting that this direct excitation of LC neurons by sevoflurane plays an important role in clinically observed excitatory effect. Third, LC neurons are the major source of noradrenaline in the CNS and are primarily implicated in control of vigilance and arousal.[6] Sevoflurane and isoflurane increase noradrenaline release in preoptic area in rats,[8] leading these authors to postulate that these inhalation anesthetics stimulate noradrenergic neurons in the LC, which is a consistent idea to our hypothesis. Taken together, our findings point to a central role of excitatory conductance densely expressed in LC neurons in the inhalation anesthetic–induced excitatory behaviors. The current results indicate that the inward currents are activated earlier or faster than the pH-sensitive outward current. It remains an open question, however, how such excitatory effect becomes more predominant after cessation of sevoflurane, as seen in the emergence agitation. This might require analysis of recovery kinetics of distinct components *in vivo*, by recording unitary discharges from nonanesthetized animals *in vivo*, and will be an important future subject. Our results do not necessarily rule out the possibility that such conductance is also expressed in other ascending systems, such as serotonergic and cholinergic systems; however, the potent suppression of emergence delirium and agitation by dexmedetomidine in human patients supports a potential role of the excitatory effect on the LC neurons in the inhalation anesthetic–induced paradoxical behavior. Development of drugs that selectively suppress this sevoflurane-activated conductance would be promising in preventing serious undesired consequences of paradoxical agitation and delirium.

References

1. Franks NP, Lieb WR: Molecular and cellular mechanisms of general anaesthesia. Nature 1994; 367:607–14

2. MacIver MB, Mikulec AA, Amagasu SM, Monroe FA: Volatile anesthetics depress glutamate transmission *via* presynaptic actions. ANESTHESIOLOGY 1996; 85:823–34

3. Sirois JE, Lei Q, Talley EM, Lynch C III, Bayliss DA: The TASK-1 two-pore domain K$^+$ channel is a molecular substrate for neuronal effects of inhalation anesthetics. J Neurosci 2000; 20:6347–54

4. Lesage F: Pharmacology of neuronal background potassium channels. Neuropharmacology 2003; 44:1–7

5. Vlajkovic GP, Sindjelic RP: Emergence delirium in children: Many questions, few answers. Anesth Analg 2007; 104:84–91

6. Aston-Jones G, Chiang C, Alexinsky T: Discharge of noradrenergic locus coeruleus neurons in behaving rats and monkeys suggests a role in vigilance. Prog Brain Res 1991; 88:501–20

7. Aston-Jones G, Chen S, Zhu Y, Oshinsky ML: A neural circuit for circadian regulation of arousal. Nat Neurosci 2001; 4:732–8

8. Anzawa N, Kushikata T, Ohkawa H, Yoshida H, Kubota T, Matsuki A: Increased noradrenaline release from rat preoptic area during and after sevoflurane and isoflurane anesthesia. Can J Anaesth 2001; 48:462–5

9. Correa-Sales C, Rabin BC, Maze M: A hypnotic response to dexmedetomidine, an alpha 2 agonist, is mediated in the locus coeruleus in rats. ANESTHESIOLOGY 1992; 76:948–52

10. Shigetomi E, Kato F: Action potential-independent release of glutamate by Ca^{2+} entry through presynaptic P2X receptors elicits postsynaptic firing in the brainstem autonomic network. J Neurosci 2004; 24:3125–35

11. Masaki E, Kawamura M, Kato F: Attenuation of gap-junction-mediated signaling facilitated anesthetic effect of sevoflurane in the central nervous system of rats. Anesth Analg 2004; 98:647–52

12. Williams JT, Marshall KC: Membrane properties and adrenergic responses in locus coeruleus neurons of young rats. J Neurosci 1987; 7:3687–94

13. Ishimatsu M, Williams JT: Synchronous activity in locus coeruleus results from dendritic interactions in pericoerulear regions. J Neurosci 1996; 16:5196–204

14. Masaki E, Kawamura M, Kato F: Reduction by sevoflurane of adenosine 5′-triphosphate-activated inward current of locus coeruleus neurons in pontine slices of rats. Brain Res 2001; 921:226–32

15. McCormick DA, Pape HC: Properties of a hyperpolarization-activated cation current and its role in rhythmic oscillation in thalamic relay neurones. J Physiol 1990; 431:291–318

16. Alvarez VA, Chow CC, Van Bockstaele EJ, Williams JT: Frequency-dependent synchrony in locus ceruleus: Role of electrotonic coupling. Proc Natl Acad Sci U S A 2002; 99:4032–6

17. Yang J, Isenberg KE, Zorumski CF: Volatile anesthetics gate a chloride current in postnatal rat hippocampal neurons. FASEB J 1992; 6:914–8

18. Wu J, Harata N, Akaike N: Sevoflurane-induced ionic current in acutely dissociated CA1 pyramidal neurons of the rat hippocampus. Brain Res 1994; 645:303–8

19. Minke B, Cook B: TRP channel proteins and signal transduction. Physiol Rev 2002; 82:429–72

20. Van Bockstaele EJ, Garcia-Hernandez F, Fox K, Alvarez VA, Williams JT: Expression of connexins during development and following manipulation of afferent input in the rat locus coeruleus. Neurochem Int 2004; 45:421–8

21. Alvarez-Maubecin V, Garcia-Hernandez F, Williams JT, Van Bockstaele EJ: Functional coupling between neurons and glia. J Neurosci 2000; 20:4091–8

22. John SA, Kondo R, Wang SY, Goldhaber JI, Weiss JN: Connexin-43 hemichannels opened by metabolic inhibition. J Biol Chem 1999; 274:236–40

23. Jahromi SS, Wentlandt K, Piran S, Carlen PL: Anticonvulsant actions of gap junctional blockers in an *in vitro* seizure model. J Neurophysiol 2002; 88:1893–902

24. Ye ZC, Wyeth MS, Baltan-Tekkok S, Ransom BR: Functional hemichannels in astrocytes: A novel mechanism of glutamate release. J Neurosci 2003; 23:3588–96

25. Contreras JE, Sanchez HA, Eugenin EA, Speidel D, Theis M, Willecke K, Bukauskas FF, Bennett MV, Saez JC: Metabolic inhibition induces opening of unapposed connexin 43 gap junction hemichannels and reduces gap junctional communication in cortical astrocytes in culture. Proc Natl Acad Sci U S A 2002; 99:495–500

26. Parker I, Gundersen CB, Miledi R: Actions of pentobarbital on rat brain receptors expressed in *Xenopus* oocytes. J Neurosci 1986; 6:2290–7

27. Ibacache ME, Munoz HR, Brandes V, Morales AL: Single-dose dexmedetomidine reduces agitation after sevoflurane anesthesia in children. Anesth Analg 2004; 98:60–3

28. Guler G, Akin A, Tosun Z, Ors S, Esmaoglu A, Boyaci A: Single-dose dexmedetomidine reduces agitation and provides smooth extubation after pediatric adenotonsillectomy. Paediatr Anaesth 2005; 15:762–6

29. Kulka PJ, Bressem M, Tryba M: Clonidine prevents sevoflurane-induced agitation in children. Anesth Analg 2001; 93:335–8

30. Tesoro S, Mezzetti D, Marchesini L, Peduto VA: Clonidine treatment for agitation in children after sevoflurane anesthesia. Anesth Analg 2005; 101:1619–22

31. Nelson LE, Lu J, Guo T, Saper CB, Franks NP, Maze M: The alpha2-adrenoceptor agonist dexmedetomidine converges on an endogenous sleep-promoting pathway to exert its sedative effects. ANESTHESIOLOGY 2003; 98:428–36

32. Chiu TH, Chen MJ, Yang YR, Yang JJ, Tang FI: Action of dexmedetomidine on rat locus coeruleus neurones: Intracellular recording *in vitro*. Eur J Pharmacol 1995; 285:261–8

33. Talley EM, Solorzano G, Lei Q, Kim D, Bayliss DA: CNS distribution of members of the two-pore-domain (KCNK) potassium channel family. J Neurosci 2001; 21:7491–505

34. Patel AJ, Honore E, Lesage F, Fink M, Romey G, Lazdunski M: Inhalational anesthetics activate two-pore-domain background K^+ channels. Nat Neurosci 1999; 2:422–6

35. Talley EM, Bayliss DA: Modulation of TASK-1 (Kcnk3) and TASK-3 (Kcnk9) potassium channels: Volatile anesthetics and neurotransmitters share a molecular site of action. J Biol Chem 2002; 277:17733–42

36. Spray DC, Ye ZC, Ransom BR: Functional connexin "hemichannels": A critical appraisal. Glia 2006; 54:758–73

37. Thompson RJ, Zhou N, MacVicar BA: Ischemia opens neuronal gap junction hemichannels. Science 2006; 312:924–7

38. Honma S, De S, Li D, Shuler CF, Turman JE Jr: Developmental regulation of connexins 26, 32, 36, and 43 in trigeminal neurons. Synapse 2004; 52:258–71

39. Koblin DD: Mechanisms of action, Miller's Anesthesia, 6th edition. New York, Churchill Livingstone, 2005, pp 105–30

40. Aono J, Ueda W, Mamiya K, Takimoto E, Manabe M: Greater incidence of delirium during recovery from sevoflurane anesthesia in preschool boys. ANESTHESIOLOGY 1997; 87:1298–300

41. Cravero J, Surgenor S, Whalen K: Emergence agitation in paediatric patients after sevoflurane anaesthesia and no surgery: A comparison with halothane. Paediatr Anaesth 2000; 10:419–24

42. Bortone L, Ingelmo P, Grossi S, Grattagliano C, Bricchi C, Barantani D, Sani E, Mergoni M: Emergence agitation in preschool children: Double-blind, randomized, controlled trial comparing sevoflurane and isoflurane anesthesia. Paediatr Anaesth 2006; 16:1138–43

43. Uezono S, Goto T, Terui K, Ichinose F, Ishguro Y, Nakata Y, Morita S: Emergence agitation after sevoflurane *versus* propofol in pediatric patients. Anesth Analg 2000; 91:563–6