Excitatory and Inhibitory Actions of Isoflurane on the Cholinergic Ascending Arousal System of the Rat

Hai-long Dong, M.D., Ph.D.,* Satoru Fukuda, M.D., Ph.D.,† Eri Murata, D.V.M., M.S.,‡ Takashi Higuchi, M.D., Ph.D.§

Background: The cholinergic arousal systems are known to critically regulate the state of consciousness. The aim of this study was to determine the effect of isoflurane on the inhibitory or excitatory neurotransmitters efflux in important nuclei within the cholinergic arousal system using in vivo intracerebral microdialysis.

Methods: The efflux of glutamate, γ-aminobutyric acid (GABA), or acetylcholine in the posterior hypothalamus (PH), the basal forebrain (BF), and the somatosensory cortex (S1BF) of rats was detected using intracerebral microdialysis under an awake condition and at 0.5–2.0 minimum alveolar concentration (MAC) isoflurane anesthesia. The intrabasalis perfusion of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and N-methyl-D-aspartate on the cortical acetylcholine effluxes was also examined under both conditions.

Results: Isoflurane had no influence on the glutamate and GABA efflux in the PH, whereas in the BF, it dose-dependently increased glutamate efflux and decreased GABA efflux. A transient increase in glutamate efflux at 1.0 MAC and a decrease in GABA at 0.5–1.5 MAC were observed in the S1BF. Isoflurane dose-dependently decreased acetylcholine efflux in the S1BF. Perfusion of the BF with AMPA increased acetylcholine efflux in the S1BF with electroencephalographic activation during 0.75 MAC isoflurane anesthesia, suggesting an inhibitory action of isoflurane on AMPA receptors in the BF. However, N-methyl-D-aspartate had no effect on these parameters.

Conclusion: Isoflurane induces both excitatory and inhibitory actions in the cholinergic arousal system. The predominant inhibitory action of isoflurane over its excitatory action at the BF would result in the decrease in the acetylcholine efflux in the S1BF.

The cholinergic pathway is the most powerful cortical activation system and causes acetylcholine release in the somatosensory cortex (S1BF). It has been shown that acetylcholine is one of the major excitatory neurotransmitters in the central nervous system (CNS), and the activity of cholinergic neurons has long been postulated to play a key role in regulating the state of consciousness. There are two cholinergic ascending cortical activation pathways: a dorsal pathway from the brain stem that innervates the thalamus and a ventral pathway that ascends through the subthalamus and the posterior hypothalamus (PH) toward the basal forebrain (BF). The cholinergic pathway of the ventral ascending activating system, which is composed of the PH, the BF, and the S1BF, is known to be an important regulator of the state of consciousness during natural sleep–wake cycle. Among these nuclei, the BF plays a predominant role in this system.

Cholinergic activation by the administration of selective agonists or cholinesterase inhibitors has been shown to produce cortical activation and increase the level of activity required for unconsciousness during anesthesia, including that induced by isoflurane. However, the neurotransmitter changes at the level of individual nuclei in this ventral cholinergic ascending arousal system during different stages of isoflurane anesthesia have not been clarified. General anesthetics, like most other neuroactive drugs, modulate synaptic transmission in the CNS. Elucidation of the effects of general anesthetics on the release of the major excitatory transmitter glutamate and major inhibitory transmitter γ-aminobutyric acid (GABA) is essential to understanding the neurophysiologic sequelae of presynaptic anesthetic actions. Elucidating the changes in neurotransmitters in the ventral cholinergic ascending arousal system during arousal–anesthesia regulation and different stages of isoflurane anesthesia is imperative to pursue a detailed understanding of the mechanism underlying general anesthesia. Compared with other volatile anesthetics, isoflurane has been shown to have inhibitory actions in in vitro brain experiments. For example, presynaptic inhibitory effects of the anesthetics on glutamate release have been demonstrated directly at the neurochemical level using isolated nerve terminals. In further studies, isoflurane has been observed to inhibit depolarization-induced glutamate efflux from isolated nerve terminals.

The findings of Westphalen and Hemmings also support the selective presynaptic inhibition of glutamate release via Na⁺ channel blockade by clinical concentrations of isoflurane. Taken together, these observations argue in favor of a significant effect on discrete neuronal systems/pathways. However, it is yet to be clarified whether the actions of isoflurane on efflux of neurotransmitters in the brain in vivo are similar to in vitro observations, especially in some important nuclei related to the cholinergic arousal ascending system. Therefore, the primary aim of the current study was to investigate whether isoflurane alters the excitatory and/or inhibitory neurotransmitters changes in the cholinergic ascending arousal system with in vivo intracerebral microdialysis in freely moving rats.
In contrast to the inhibitory actions of isoflurane in these previous in vitro studies, our current study showed that glutamate efflux was dose-dependently increased in the basal forebrain during isoflurane anesthesia. There are cholinergic neurons in the BF that innervate the S1BF. Among the glutamate receptors, N-methyl-D-aspartate (NMDA) and non-NMDA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid [AMPA]) receptors play a major role in excitatory synaptic neurotransmissions. Therefore, to clarify the role of glutamate and glutamate receptors in the BF during isoflurane anesthesia, we compared the changes in cortical acetylcholine efflux caused by intrabasalis administration of glutamate receptor agonists (AMPA and NMDA) with a dual-probe microdialysis technique in awake and isoflurane-anesthetized rats.

Materials and Methods

Animals

Male Wistar rats, weighing 270–320 g (aged 7–11 weeks), were purchased from Shizuoka Laboratory Animal Center (Shizuoka, Japan). They were housed at a constant temperature (24 ± 0.5°C) with a relative humidity (60 ± 2%) on a light-controlled schedule (light on between 6:00 AM and 6:00 PM) and had free access to food and water. The experimental protocols were approved by the Animal Care Committee of Fukui University (Fukui, Japan).

Surgical Operations

During pentobarbital anesthesia (50 mg/kg intraperitoneal), rats underwent surgery for implantation of electrodes for electroencephalographic recording and placement of a guide cannula for the microdialysis probe (membrane length, 2 mm; OD, 0.5 mm; cutoff size, 20 kd; Eicom, Kyoto, Japan). A guide cannula (OD, 0.6 mm) was directed stereotaxically into the nucleus as follows. The coordinates of the microdialysis probe tip according to the atlas of Paxinos and Watson were as follows: PH: anteroposterior, −3.8 mm; lateral, 0.5 mm; dorsoventral, 9.0 mm from bregma; BF (nucleus basalis and substantia innominate); anteroposterior, −1.4 mm; lateral, 2.5 mm; dorsoventral, 8.5 mm; S1BF: anteroposterior, −1.4 mm, lateral, 5.0 mm, dorsoventral, −2.8 mm. As for the perfusion of AMPA or NMDA in the BF and measurement of acetylcholine in the S1BF, the coordinates of the BF were the same, but the microdialysis probe for the S1BF was inserted at a 30° angle from the dura. Thus, the tip of the microdialysis probe was positioned as follows: anteroposterior, −1.4 mm; lateral, 5.0 mm; dorsoventral, −2.8 mm from bregma.

Five stainless steel screws for epidural electroencephalographic recording were implanted. The leads were connected to a socket, which was fixed to the skull together with the electrodes and the microdialysis guide cannula using dental cement. One day before the experiments, the rats were anesthetized with 1.8% isoflurane, and a heparinized saline-filled polyethylene catheter (MRE-040; Eicom) was inserted into the femoral artery for the measurement of arterial blood pressure and heart rate.

Experimental Protocols

After 5–7 days of recovery from the surgical implantation of electroencephalographic electrodes and microdialysis cannula insertion, the rats were transferred from their own home cage to a cylindrical cage (internal diameter, 25 cm; height, 50 cm; volume, 25 l) for multichannel infusions and electrical recordings (Nejiren; Osaka Microsystems, Osaka, Japan). They were connected to the electroencephalographic recording cable for adaptation to the experimental conditions. The twisting of liquid lines was avoided by an automated floor rotating system controlled by a twist degree detector, allowing free movement of the animal. During measurement, the stylet of the microdialysis guide cannula was replaced by a microdialysis probe. The probe was continuously perfused with Ringer’s solution (140 mM NaCl, 3 mM KCl, 1.3 mM CaCl₂; pH 7.4).

Experiment 1: Glutamate and GABA Changes in the PH, BF, and S1BF during Isoflurane Anesthesia

These experiments were conducted in 18 rats (6 rats in each group). The microdialysis probes inserted into the PH, the BF, or the S1BF were perfused with the Ringer’s solution at a flow rate of 2 μl/min. In our preliminary experiments, we observed that glutamate and GABA concentrations became stable 2 h after insertion of the microdialysis probe. Therefore, after the 2-h equilibration with the perfusion solutions, the samples (20 μl) were collected every 10 min and immediately stored at −80°C until analyzed.

After 1 h of control dialysate collection, 0.5 minimum alveolar concentration (MAC, 0.6%) of isoflurane was added to the oxygen gas mixture for 1 h. Subsequently, the concentration of isoflurane was increased stepwise from 1.0 MAC (1.2%) to 1.5 MAC (1.8%) to 2.0 MAC (2.4%). Every inhalation concentration of isoflurane was sustained for 1 h. The concentration reached a plateau within 1–2 min and became stable because the outlet of the gas tubing was placed at the bottom of the cylindrical cage and a high flow rate (3 l/min) of oxygen containing isoflurane immediately filled with the atmosphere around the rat. The concentration of isoflurane was continuously measured by a sidestream capnometer (CAPNOX; Colin, Aichi, Japan). The 1.2% isoflurane was considered to be 1.0 MAC as reported previously.

The efflux of glutamate and GABA in the PH, the BF, and the S1BF during isoflurane anesthesia was expressed as percent control of the average of the first six samples.
during the awake condition. The blood pressure, blood gas, and electroencephalogram were monitored at each condition (blood gas was monitored 30 min after the condition change during the experiment).

Experiment 2: Acetylcholine Changes in the S1BF during Isoflurane Anesthesia

Six animals were used in this experiment. The microdialysis probes inserted into the nucleus of the S1BF were the same type as mentioned above. The inlet of the dialysis probe was connected to the perfusion pump, and the outlet of the probe was connected to the automated sample injector with polyethylene tubing. The internal standard, 50 nM isopropylhomocholine, delivered by the perfusion pump at a flow rate of 0.25 μl/min, was connected into the perfusate tube just proximal to the injection valve through a three-way joint. The dialysis probe was perfused continuously with the Ringer’s solution containing eserine (10−7 M) at a rate of 1.0 μl/min.

The perfusate samples obtained for the first 2 h after implantation of the probe were discarded. The dialysates were then collected every 20 min to the sample loop in the automated sample injector, which was on-line to the high-performance liquid chromatography system (Eicom). The protocol for the stepwise increase in the concentration of isoflurane was the same as described in experiment 1. Every inhalation concentration of isoflurane was sustained for 1 h, during which three samples were collected every 20 min. The blood pressure, blood gas, and electroencephalogram were also monitored during the experiment.

Experiment 3: Effects of AMPA or NMDA Perfusion into the BF on Cortical Acetylcholine Efflux and Electroencephalogram in the Awake and Isoflurane Anesthetic Conditions

This experiment was performed to examine the potential for differential effect of intrabasalis perfusion of AMPA (Sigma-Aldrich, Inc., St. Louis, MO) or NMDA (Sigma-Aldrich Inc.) in stimulating cortical acetylcholine efflux in the awake and isoflurane-induced anesthetic conditions. Twenty rats were assigned randomly to two groups: awake and isoflurane-induced anesthetic groups. In the awake group, after collecting the control dialysate measurements, the rats were anesthetized with 0.75 MAC (0.9%) of isoflurane for 1 h, during which three samples were collected. The concentration of isoflurane was kept constant at 0.75 MAC during the perfusion of AMPA (n = 5) or NMDA (n = 5). The acetylcholine efflux in the S1BF was also measured simultaneously. The correlation coefficient between changes in acetylcholine efflux and the percent changes in the power bands of the electroencephalogram was analyzed during AMPA perfusion.

Glutamate and GABA Assays

Glutamate and GABA were assayed by high-performance liquid chromatography with fluorescence detection, using the precolumn derivatization method with ortho-phthaldialdehyde. The retention times of glutamate and GABA were approximately 21 and 43 min, respectively. The concentrations of glutamate and GABA were calculated from peak area with the aid of external standards. The values of glutamate and GABA were expressed as percent control of prevalue before the isoflurane anesthesia. For the derivatization, we used 2-mercaptoethanol as an essential thiol compound. Because the retention time for GABA was approximately 43 min and late-eluting peaks were observed, washing processes using stepwise elution method were used to minimize the total analysis time. The mobile phase consisted of 0.1 M phosphate buffer (pH 2.5), 600 ml; 400 ml methanol; and 5 mg EDTA-2Na (40 v/v% methanol solution). Washing solution consisted of 0.1 M phosphate buffer (pH 2.5), 50 ml; 450 ml methanol; and 2.5 mg EDTA-2Na (90 v/v% methanol solution). Just after GABA was eluted, the mobile phase was switched to the washing solution, and late-eluting peaks were washed out from the separation column. For the measurement of glutamate and GABA, all of the derivatization processes were performed automatically by automatic sampling injector (231 XL; Gilson, Roissy Ch de Gaulle, France). In the 231XL, samples were kept at 10°C in the vials placed in the thermostatic rack, and the reaction time was accurately set at 2.5 min. Because of these accurate controls for reaction conditions, both glutamate and GABA peaks showed good reproducibility and linear standard curve even if the decomposition of the products in the separation column has occurred. Moreover, it has been reported recently that the GABA–ortho-phthaldialdehyde derivative is stable enough in the reversed-phase separation column even at retention times as long as 60 min. We used three points (10−7, 5 × 10−7, and 10−6 M) to generate the standard curve. The process for making the standard curve was repeated two times before and after every sample measurements to judge the validity of measurements. The detection limits for glutamate and GABA were 15 fmol/injection and 50 fmol/injection at a signal-to-noise ratio of 3, respectively.

Recovery rates for the microdialysis probes for glutamate and GABA were 15.5 ± 3.6 and 14.6 ± 4.3%,
respectively. The representative chromatograms for glutamate and GABA are shown in figures 1A and B.

**Acetylcholine Assay**

Acetylcholine in the dialysates was measured using the on-line high-performance liquid chromatography system (HTEC-500; Eicom) containing an immobilized enzyme column and electrochemical detector. The pump delivered the mobile phase (50 mM Na₂HPO₄, 0.1 mM EDTA, 0.03% SDS; pH adjusted to 8.2 with phosphate acid) at a rate of 150 μl/min. Data were collected and analyzed using Powerchrome software (Castle Hill, New South Wales, Australia). The retention times of isopropylhomocholine (IPHC; internal standard) and acetylcholine are approximately 9.5 and 11 min, respectively. For acetylcholine measurement, the retention times of isopropylhomocholine (IPHC; internal standard) and acetylcholine are approximately 9.5 and 11 min, respectively. The system was calibrated for each experiment using standard solutions containing 0.1, 0.5, and 1 pmol acetylcholine. The detection limit was approximately 10 fmol at a signal-to-noise ratio of 3. Quantification of the collected acetylcholine was evaluated with the internal standard (isopropylhomocholine) peak area. The recovery rate for the microdialysis probes for acetylcholine was 29.5 ± 5.2%. The representative chromatograms for acetylcholine are shown in figure 1C.

In all microdialysis studies, the experimental stability for the measurement of glutamate, GABA, or acetylcholine was confirmed by the fact that the coefficient of variance of these data during the mean initial 60-min dialysate collections was within the 10% range. When the stability of the measurement was confirmed, we used this as a basal value.

**Electroencephalographic Recording and Analysis**

The electroencephalogram was analyzed while different concentrations of AMPA or NMDA perfusion were administered in the awake and isoflurane anesthetic groups in experiment 3. The measured time points were 20 min after each condition was changed. The time points were selected for comparable electroencephalographic analysis. The electroencephalographic signal was on-line digitized at a sampling rate of 200 Hz and subjected to off-line spectral analysis. Power spectra were computed for consecutive 2.56-s epochs and 0.39-Hz frequency bins by using a SleepSign® analysis system (version 2; Kissei Comtec Co., Ltd., Matumoto, Japan). In this study, the electroencephalographic global frequency band (0–30 Hz) was divided into four frequency bands: δ (0.5–4 Hz), θ (4–8 Hz), α (8–13 Hz), and β (13–30 Hz) bands.

**Histologic Verification**

The correct placement of the tip of the microdialysis probe in the nucleus was confirmed for each animal by histologic examination after completion of the experiment. The animals were further anesthetized with excess sodium pentobarbital and perfused transcardially with the saline followed by the 10% formalin in 0.1M phosphate buffer (pH 7.4). Sections containing the track of the microdialysis probe were stained with neutral red and visually inspected for evidence of a dialysis probe–induced lesion. Digitized sections were compared with coronal plates from a rat brain atlas18 to determine the stereotaxic coordinates for each dialysis site. The representative histologic probe placements in the brain slices are shown in figure 2.

**Statistical Analysis**

All data are expressed as percent control of the basal values and represent mean ± SEM. In all studies, the results were analyzed by analysis of variance, and when significant F values were obtained, post hoc comparisons were performed using the Fisher protected least significant difference test. Changes in relative power in each of the bands (electroencephalogram) were assessed over the different

---

Fig. 1. Representative chromatograms for glutamate (A), γ-aminobutyric acid (GABA; B), and acetylcholine (ACH; C). These chromatograms were obtained from brain samples. The abscissa indicates the time after sample injection (min). The ordinate axis indicates amplitude (mV). The retention times of glutamate (A) and GABA (B) are approximately 21 and 43 min, respectively. For acetylcholine measurement, the retention times of isopropylhomocholine (IPHC; internal standard) and acetylcholine are approximately 9.5 and 11 min, respectively.
groups using a one-way analysis of variance. Comparisons of the changes in acetylcholine efflux during the AMPA perfusion between the awake and isoflurane anesthetic groups were performed using a univariate analysis of variance. The correlation coefficient between changes in acetylcholine efflux and the spectrum parameters in the power of the cortical electroencephalogram was analyzed using Pearson correlation analysis. All statistical analyses were performed using SPSS 11.0 (SPSS Inc., Chicago, IL), and a 5% probability of type I errors was used to determine statistical significance. In all cases, $P < 0.05$ was taken as the level of significance.

**Results**

*Glutamate and GABA Changes in the PH, BF, and S1BF during Isoflurane Anesthesia*

The positions of all the tips of the probes were correctly located in each sample (figs. 3–5). The heart rate was increased at 1.5 and 2.0 MAC isoflurane inhalation ($P < 0.05$, vs. control). The mean arterial blood pressure was decreased at 1.5 and 2.0 MAC isoflurane inhalation ($P < 0.05$, vs. control). There were no statistically significant differences in blood gas measurements between control (basal condition) and each isoflurane anesthetic condition.

The basal glutamate effluxes ($\times 10^{-5}$ M) in the PH, the BF, and the S1BF were $6.63 \pm 0.83$ (n = 6), $0.31 \pm 0.07$ (n = 6), and $0.32 \pm 0.03$ (n = 6), respectively. The basal GABA effluxes ($\times 10^{-7}$ M) in the PH, the BF, and the S1BF were $0.92 \pm 0.24$ (n = 6), $1.12 \pm 0.30$ (n = 6), and $0.81 \pm 0.34$ (n = 6), respectively. The changes in the glutamate and GABA effluxes at different MAC in isoflurane anesthesia in the PH, the BF, and the S1BF are shown in figures 6A, B, and C, respectively.

There was no significant change in the glutamate and GABA effluxes in the PH during isoflurane inhalation (fig. 6A). In contrast, isoflurane significantly increased the
The glutamate efflux in the S1BF was increased to 145.1 ± 21.8% at 1.0 MAC isoflurane (F_{4,25} = 2.846, P = 0.045) and then returned to 90.3 ± 3.6% at 2.0 MAC isoflurane (fig. 6C, left). Meanwhile, the GABA efflux in the S1BF was significantly decreased to 79.1 ± 7.2% at 0.5 MAC, 75.1 ± 5.1% at 1.0 MAC, and 80.7 ± 6.8% at 1.5 MAC (F_{4,25} = 3.539, P = 0.025) and then returned to 90.0 ± 5.0% at 2.0 MAC isoflurane (fig. 6C, right).

Acetylcholine Changes in the S1BF during Isoflurane Anesthesia

The hemodynamics and blood gas changes were the same as in experiment 1. The spontaneous cortical acetylcholine efflux in all animals (n = 6) during the three baseline samples was 0.86 ± 0.04 (× 10^{-7} M). In the current study, isoflurane significantly suppressed acetylcholine efflux in the S1BF in a dose-related manner (F_{4,25} = 101.625, P < 0.001) as shown in figure 7.

Effects of AMPA or NMDA Perfusion into the BF on Cortical Acetylcholine Efflux and Electroencephalogram in the Awake and Isoflurane Anesthetic Groups

For the heart rate, mean arterial blood pressure, and blood gas measurements, there were no statistically significant differences between each concentration of AMPA or NMDA perfusion in two groups. Perfusion of AMPA at 30, 100, and 300 μM in the BF significantly increased the acetylcholine effluxes in the S1BF (F_{5, 24} = 7.859, P < 0.001 in awake group and F_{5, 24} = 8.151, P < 0.001 in anesthesia group) in both groups (fig. 8). The maximal increase in the acetylcholine efflux by AMPA perfusion was at 100 μM AMPA in both groups (172.4 ± 10.7% in awake group and 204.2 ± 15.0% in anesthetic group). The changes in the acetylcholine efflux in the S1BF were not different between the two groups (univariate analysis, F_{1,5} = 2.882, P = 0.095).

The changes in electroencephalographic power after AMPA perfusion in the awake and 0.75 MAC isoflurane groups were presented in figures 9A and B (n = 5/group). The baseline power in the electroencephalogram recorded during the awake condition was the same for all of the animals in both groups. However, there was no statistically significant change in power bands of the electroencephalogram after AMPA perfusion in the awake group.

After 0.75 MAC isoflurane inhalation, the δ power significantly increased, whereas the θ, α, and β powers significantly decreased (fig. 9B, lower). The powers of four bands during isoflurane inhalation were significantly different from that in the awake condition (P < 0.05). After AMPA perfusion, there was a significant change in each power on the electroencephalogram after AMPA perfusion in the awake group.
The relation between the percent control of the acetylcholine efflux and the changes in the δ band showed a significant negative correlation ($r = -0.68$, $P < 0.01$; fig. 10A). In contrast, a significant positive correlation was observed between the percent control of increased cortical acetylcholine efflux and the increased power activity in the θ ($r = 0.66$, $P < 0.01$), β ($r = 0.56$, $P < 0.01$), and α bands ($r = 0.49$, $P < 0.01$; figs. 10B–D).

Perfusion of NMDA in the BF did not induce a significant change in acetylcholine effluxes in the S1BF in either group ($n = 5$/group). There was no significant change in electroencephalogram power bands after NMDA perfusion in either group.

**Discussion**

To our knowledge, the current study is the first to demonstrate that there are changes in the extracellular neurotransmitters (glutamate, GABA, and acetylcholine) in the PH, the BF, and the S1BF, which have been shown to have an important role for the ventral cholinergic ascending arousal system, from the awake state to isoflurane-induced anesthesia in the rat. Our findings

---

**Fig. 4.** Histologic localization of microdialysis probe sites in the basal forebrain and the somatosensory cortex in α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) groups (dual-probe technique). Open triangles (awake group) and rhombuses (anesthesia group) represent the location of the tip of the microdialysis probe for AMPA perfusion into the basal forebrain. Closed circles (awake group) and open circles (anesthesia group) represent the location of the tips of the microdialysis probe for measurement of acetylcholine in the somatosensory cortex. Coronal sections were adopted from the atlas of Paxinos and Watson, with permission from Elsevier. Number at the bottom right indicates distance from bregma along the anteroposterior axis.

**Fig. 5.** Histologic localization of microdialysis probe sites in the basal forebrain and the somatosensory cortex in N-methyl-D-aspartate groups (dual-probe technique). Open triangles (awake group) and rhombuses (anesthesia group) represent the location of the tip of the microdialysis probe for N-methyl-D-aspartate perfusion into the basal forebrain. Closed circles (awake group) and open circles (anesthesia group) represent the location of the tips of the microdialysis probe for measurement of acetylcholine in the somatosensory cortex. Coronal sections were adopted from the atlas of Paxinos and Watson, with permission from Elsevier. Number at the bottom right indicates distance from bregma along the anteroposterior axis.
suggest that isoflurane has both excitatory and inhibitory actions on the cholinergic arousal system, and then the predominance of inhibitory relative excitatory actions of isoflurane may result, at least in part, in the induction of isoflurane anesthesia.

Nitz and Siegel found in cats that most PH neurons decrease unit discharge during slow-wave sleep on the electroencephalogram and a selective increase in GABA efflux without change in glutamate efflux. They suggested that disfacilitation of an excitatory glutamatergic PH input may not be a major factor underlying the decrease in the PH unit activity linked to slow-wave sleep. However, in our study, glutamate and GABA effluxes were unchanged in the PH, suggesting that glutamate and GABA efflux in the PH might not contribute to the induction of isoflurane anesthesia.

The inhibitory actions of isoflurane on the CNS have been reported in many in vitro studies. It has been reported that glutamate release was inhibited by isoflurane using Drosophila larval neuromuscular junction and isolated rat cerebrocortical nerve terminals. As for the synaptic action of isoflurane, it has also been demonstrated that isoflurane is capable of depressing glutamate-mediated fast excitatory synaptic transmission and/or facilitation of fast inhibitory synaptic transmission mediated by GABA and glycine. In contrast to
these inhibitory actions of isoflurane, MacIver and Roth demonstrated that isoflurane increased CA1 neuronal excitability and produced postsynaptic depression of dentate neurons. It has also been demonstrated that isoflurane transiently increased the immediate-early gene c-fos messenger RNA expression in rat brain, indicating that isoflurane can stimulate, rather than suppress, the CNS. However, this study did not report on what kind of neurons are involved in the excitatory action of isoflurane. These findings suggest that isoflurane may induce different actions depending on the neurons located in the CNS. Whereas these depressant effects of isoflurane on some neurons in the CNS may be related to the isoflurane-induced anesthesia, the current finding of increased excitatory glutamate efflux in the cholinergic ascending arousal system may contribute to our understanding of the basic mechanism of anesthesia.

The results of the current study show a biphasic response to isoflurane of glutamate efflux in the S1BF (fig. 6C). The glutamate efflux was high at low isoflurane (1.0 MAC) concentration and returned to the basal level at 2 MAC isoflurane. In contrast, the GABA efflux decreased and reached lowest values at 1.0 MAC isoflurane. The mechanism for these responses of glutamate and GABA effluxes in the S1BF to isoflurane remains unclear. However, it is noteworthy that isoflurane could produce biphasic effects on population spike amplitudes in hippocampal synapse.27

In the current study, isoflurane dose-dependently suppressed acetylcholine efflux (fig. 7). The findings were consistent with that of the previous study.29,30 It has been reported that the BF neurons contain acetylcholine and directly innervate the cortex.31 In the current study, we found that isoflurane induced the increases in the excitatory transmitting glutamate in the BF, whereas it decreased the acetylcholine efflux in the cortex. Hence, this decrease in the efflux of acetylcholine in the cortex induced by isoflurane is a contradictory phenomenon despite the isoflurane-induced increase in the glutamate in the BF, because the increases in the glutamate would induce the increases in the acetylcholine in the cortex. The current study showed that AMPA perfusion to the BF increased the acetylcholine efflux in the S1BF with electroencephalographic activation during 0.75 MAC isoflurane anesthetic conditions (figs. 8 and 9), whereas perfusion of the NMDA did not. These results indicate that cholinergic BF neurons can be excited by glutamergic inputs via glutamate receptors but are more readily excited by activation of the AMPA subtype. The antagonistic actions of AMPA on the decrease in cortical acetylcholine efflux by isoflurane (fig. 8) suggest that isoflurane has its inhibitory actions on the AMPA receptors at the BF.

In the current study, AMPA was observed to induce increase in acetylcholine efflux in both awake and isoflurane-induced anesthetic conditions (fig. 8), whereas the percent changes in power bands were unaffected by AMPA as shown in figure 9A in the awake conditions. Rasmussen et al.32 reported that a combination of both NMDA and AMPA antagonists abolished the increase in cortical acetylcholine release without reducing desynchronization. This observation suggests that the dissociation between increased cortical acetylcholine release and electroencephalographic desynchronization indicates that the activity of corticopetal basal forebrain cholinergic neurons is neither necessary nor sufficient to produce electroencephalographic desynchronization. Instead, it is likely that the nucleus basalis can presum-
ably affect the electroencephalogram by its projections to the thalamus. The dissociation between the increase in the acetylcholine efflux without a concomitant change in electroencephalogram after AMPA perfusion in the awake conditions requires further investigation.

During anesthesia, it has been demonstrated that microinjection of glutamate into the BF of halothane-anesthetized rats evokes acetylcholine efflux in the ipsilateral neocortex.33 Also, Fournier et al.34 have previously reported that AMPA and NMDA glutamate receptor agonists produced concentration-dependent increases in acetylcholine efflux with electroencephalographic activation in urethane-anesthetized rats and that AMPA was more potent than NMDA in producing the effects. These reports are consistent with the current observations in that intrabasalis injection of AMPA decreased δ power but increased θ, α, and β powers. The findings suggest that the cortical activity in terms of the electroencephalogram may be dependent on the inhibitory action of isoflurane on AMPA receptors at the BF. This is further supported by the observation in this study of the positive correlation between β, α, and θ powers and acetylcholine efflux and the negative correlation between δ power and acetylcholine efflux (fig. 10). In previous literature and ours, the decrease in acetylcholine efflux with electroencephalographic suppression resulting from isoflurane anesthesia suggests that one of the neuronal mechanisms of isoflurane anesthesia is dependent, at least in part, on suppression of cholinergic activities. In the current study, we focused on the effect of isoflurane on the neurotransmitter changes in the cholinergic ascending arousal system. However, there are other arousal systems, such as noradrenergic, serotonergic, histaminergic, and so forth.36 It remains to be determined whether isoflurane may alter the neurotransmission mediated by these alternative pathways at various nuclei in these arousal systems. Further studies are necessary to explore the composite details of the neuronal mechanism of isoflurane anesthesia.

Based on the observations of the current study, we propose that the possible inhibitory action of isoflurane at AMPA receptors may overcome its glutamate-dependent excitatory action at the BF resulting in a decrease in the acetylcholine efflux in the S1BF when the animal is exposed to isoflurane. This finding may contribute, at least in part, to a further understanding of the mechanisms underlying isoflurane-induced anesthesia.

Fig. 10. Correlation between percent control of acetylcholine efflux and the relative each wave power to total electroencephalographic power in isoflurane anesthetized rats. Scatter plot shows the significant negative correlation between the percent control of acetylcholine efflux and the percent δ-wave power (A). In contrast, significant positive correlation between the percent control of acetylcholine efflux and the θ, α, or β-wave power were observed (B, C, and D, respectively).
The authors thank Chuma Okere, Ph.D. (Department of Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, Pennsylvania), for reviewing the manuscript.

References

22. Rea K, Cremer TE, Westerink BH: BPIIC conditions are critical for the detection of GABA by microdialysis. J Neurochem 2005; 94:672–9
36. Jones BE: Arousal systems. Front Biosci 2003; 8:s438–51