Isoflurane and Sevoflurane Interact with the Nicotinic Acetylcholine Receptor Channels in Micromolar Concentrations

Michaela Scheller, M.D.,* Johannes Buzler, M.D.,† Hajo Schneck, M.D.,‡ Eberhard Kochs, M.D.,‡ Christian Franke, M.D., Ph.D.§

Background: This study was performed to elucidate and compare the effects of sevoflurane and of isoflurane on the nicotinic acetylcholine receptor of mouse myotubes. The experiments were done with special reference to anesthetic concentrations considerably less than those used for clinical anesthesia.

Methods: The patch-clamp technique was used to record acetylcholine-activated currents from the embryonic type of the nicotinic acetylcholine receptor in the outside–in mode. A piezo-driven liquid filament switch was used for the ultrafast application of acetylcholine alone or in combination with isoflurane or sevoflurane. In addition, the patches were preexposed to either anesthetic, preceding the activation with acetylcholine.

Results: The current elicited by acetylcholine was reduced reversibly and in a concentration-dependent manner by both anesthetics, which were equally effective. Preexposure of the patches to isoflurane or sevoflurane showed an additional inhibition that was present at micromolar concentrations. The time courses of current decay could be fitted by single exponentials for isoflurane. At higher concentrations of sevoflurane, the current decay became biexponential. In contrast to isoflurane, sevoflurane increased the time constants of desensitization when applied in low concentrations.

Conclusion: At the nicotinic acetylcholine receptor, isoflurane and sevoflurane act primarily through the same mechanisms: both affect the open and the closed state of the channels in concentrations equal to and less than those encountered clinically. The kinetics of desensitization, however, are altered in a different manner. Thus there may be several different sites of interaction. (Key words: Anesthesia: mechanism. Anesthetics: isoflurane; sevoflurane. Equipment: fast application system; patch clamp. Receptors: nicotinic acetylcholine.)

THE principal mechanism of general anesthesia is thought to be related to altered neuronal synaptic transmission, such as to modifications of ion channel proteins. The nicotinic acetylcholine receptor channel (nAChR) belongs to the superfamily of ligand-activated ion channels. Its structure and function are very well characterized. This channel serves as a model for biophysical investigations such as patch-clamp studies. The members of the superfamily show similarity in structure and considerable amino acid sequence homology.|| They are expressed in all parts of the central nervous system. Anesthetics have been shown to interact with the nAChR, glutamate and gamma-aminobutyric acid receptors as well as with voltage-activated ion channels. Most of the studies of the nAChR investigated changes in single-channel kinetics in the presence of acetylcholine when combined with anesthetics. With a technique for rapid perfusion of acetylcholine to outside-out patches, showed that isoflurane and other general anesthetics (ether, propofol) produce diverse effects on the open and the closed state of the nAChR. These authors used concentrations of the respective substance, which were comparable with or moderately to considerably greater than the concentrations observed during clinical anesthesia.

Our study investigates the mechanisms of action of the relatively new volatile anesthetic sevoflurane and of isoflurane on the simultaneous opening of many single channels of the nAChR, activated by a saturating concentration of acetylcholine. The embryonic nAChR of mouse myotubes was used for the experiments, because a protocol for the reaction between acetylcholine and this ion channel was developed previously.
aim of the study was to compare the effects of isoflurane and of sevoflurane on fast activation and desensitization kinetics as well as on the closed, unliganded state, and to determine whether both anesthetics act through the same or different mechanisms. A rapid application system was used to apply the agonist in fast pulses. This approach avoids desensitization of the channels in the continuous presence of acetylcholine and allows for preexposure of membrane patches to anesthetics before activation by acetylcholine. The experiments were performed over a wide range of concentrations of the anesthetics (10⁻² to 10⁻⁷ M).

**Materials and Methods**

For the study, cultured mouse myotubes were used. Cells were prepared as described earlier. Enzymatically and mechanically dissociated muscles were kept in culture and incubated at 37°C, 5% carbon dioxide for 10 to 14 days. During this period, the myotubes express the embryonic type of the nAChR.

The patch-clamp technique was used to record currents activated by acetylcholine. Acetylcholine binding to nAChRs generates conformational changes followed by opening of the ion channels, thus allowing current to flow across the membrane patches. A tight electrical seal with a resistance of several GΩ formed between the membrane and a patch-clamp electrode allows this transmembrane current to resolve in the pA range. Recordings were performed in the outside-out mode by standard methods. To match the rapid kinetics of direct ligand-activated ion channels and to imitate the physiologic transmitter release at the neuromuscular junction, an ultrafast liquid filament switch technique was used to apply acetylcholine alone or combined with isoflurane (Forene; Deutsche Abbott GmbH, Wiesbaden, Germany) or sevoflurane (Maruishi Pharmaceutical Co., Osaka, Japan) to the outside-out patches. The substances were applied to the patches using a liquid filament, discharged by a tube connected to a piezo crystal. The solution in the tube was switched from an acetylcholine-containing solution to an acetylcholine-plus-anesthetic-containing solution using a manual valve (about 10 s are usually required for an exchange of solutions within the tube). The reservoirs containing anesthetic solutions were sealed to prevent evaporation. The patches were placed at the interface between this filament and a bathing solution (extracellular solution alone or combined with isoflurane or sevoflurane).

When the piezo is voltage activated, the tube is moved up and down onto or away from the patch. This technique allows for exchange of solutions within 200-300 μs. The compounds were applied repetitively as single pulses lasting 800 ms. A 10-s interval between the pulses guaranteed full recovery of the channels from desensitization. A saturating concentration of 10⁻⁴ M acetylcholine was used. The application of 10⁻⁴ M acetylcholine results in an open probability of 0.95; that is, almost all ion channels on a patch (typically 40-90 channels) are activated with this concentration.

In a first series of experiments, acetylcholine was applied pulsewise alone or combined with various concentrations of isoflurane or sevoflurane with the liquid filament switch. In these series, neither anesthetic was added to the bathing solution.

In a second series, the patches were pre-exposed to isoflurane or sevoflurane for a period of about 1 min (the bathing solution was switched to extracellular solution with isoflurane or sevoflurane added; the exchange time was about 10 to 40 s and could not be determined precisely). In a next step, acetylcholine combined with the same concentration of isoflurane or sevoflurane was applied repetitively with the liquid filament switch. Ten to 15 single recordings were obtained for each condition.

The polyethylene tubes of the application system were rinsed carefully with extracellular solution after every application of an anesthetic. Furthermore, anesthetic concentrations added to the acetylcholine-containing solution or to the bathing solution were increased starting at the lowest concentrations, thereby excluding artifacts by retaining isoflurane or sevoflurane within the tubing or other parts of the apparatus from previous measurements. In all experiments, holding potentials of −20 mV to −50 mV were applied. Pipettes were fabricated from borosilicate glass using a two-step vertical puller (Zeitz Instruments, Augsburg, Germany) and heat polished. The resulting tip diameters were 0.5–2 μm, with a resistance of 2 to 5 MΩ. Currents were recorded with an EPC 9 patch-clamp amplifier (List Electronics, Darmstadt, Germany), digitized at 20 kHz, and stored on a video tape (modified Sony PCM-501ES, Tokyo, Japan). Data were low-pass filtered at 2 kHz and analyzed offline on a microcomputer (Hewlett-Packard, Palo Alto, CA). For the measurements, the culture medium was replaced by an extracellular solution containing 162 mM NaCl, 5.3 mM KCl, 0.67 mM NaH₂PO₄, 0.22 mM KH₂PO₄, 15 mM N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid, 5.6 mM glucose, and 2 mM...
CaCl$_2$. The extracellular solution was adjusted to a pH of 7.4 with NaOH. Patch electrodes were filled with a pseudointracellular solution containing 140 mM KCl, 2 mM MgCl$_2$, 11 mM ethyleneglycol-bis (oxyethylenenitrile) tetraacetic acid, 10 mM N-2-hydroxyethylpipеразине-N-2-ethanesulfonic acid, and 10 mM glucose. Intracellular solution was adjusted to a pH of 7.3 with KOH and to an osmolality of 340 mosm with mannitol. Acetylcholine was dissolved in extracellular solution shortly before the experiment. Saturated solutions of isoflurane or sevoflurane were prepared by adding aliquots of the respective anesthetic to extracellular solution and by stirring in a closed glass bottle for at least 3 h. Concentrations were determined by gas chromatography. The maximal solubility in physiologic saline at room temperature was 15 mM for isoflurane and 4.87 mM for sevoflurane. Well-defined concentrations were prepared by diluting the saturated solutions. To control the concentrations, especially in the micromolar range, different concentrations of isoflurane were prepared appropriately, the probes were passed through the application system, collected, and analyzed by gas chromatography. The differences between the calculated and the measured concentrations were less than 15%. All experiments were performed at room temperature (20 - 23°C). Assuming a partition coefficient of approximately 1.2 at room temperature, 4.5 x 10$^{-6}$ M isoflurane probably corresponds to 1.3% in the vapor phase. By introducing the aqueous-gas partition coefficient of 0.52 recently suggested by Park and coworkers, 25 3.5 x 10$^{-6}$ M sevoflurane would correspond to about 1.7 vol% at room temperature, or 2.3 vol%, respectively, at 37°C. Assuming a minimum alveolar concentration (MAC) value of about 0.9 vol% for sevoflurane at 37°C, 2.3 vol% equals 1.2 MAC. The range of concentrations used in our study was 10$^{-7}$ to 10$^{-4}$ M sevoflurane.

Results

In part one of the study, the outside-out patches were first exposed to pulses of 10$^{-4}$ M acetylcholine alone. Then increasing concentrations of isoflurane or sevoflurane were added to the acetylcholine pulse. Ten to 15 single pulses were applied repetitively, and the currents elicited were averaged. The upper trace of figure 1A shows a typical average current activated by 10$^{-4}$ M acetylcholine: At the start of the pulse, the current rapidly increases to a peak amplitude of approximately 48 pA within less than 1 ms, corresponding to the superposition of about 40 single channel openings (the holding potential was -20 mV). The current then decreases to a low steady-state value due to desensitization. 18,20 Time courses of desensitization could be fitted by single exponentials for 10$^{-4}$ M acetylcholine (semilogarithmic plot in figure 1B, upper trace). 19 At this concentration, the time constants of desensitization vary between 20 and 100 ms from patch to patch. 19,25 The time constants may also decrease slightly during the lifetime of an outside-out patch. Adding isoflurane or sevoflurane to the acetylcholine pulse reduced the peak current amplitude in a concentration-dependent manner. Figure 1 shows this reduction for isoflurane, and figure 2 shows it for sevoflurane. Figures 3A and B show the evaluation of experiments of the type of figures 1 and 2. The relative peak current amplitude activated by 10$^{-4}$ M acetylcholine combined with various concentrations of isoflurane or sevoflurane was plotted. Approximately 50% of the channels were blocked by 1.5 x 10$^{-3}$ M isoflurane and 1.5 x 10$^{-3}$ M sevoflurane.

To test reversibility, control pulses of 10$^{-4}$ M acetylcholine alone were applied to the respective outside-out patch after applying isoflurane or sevoflurane. Currents returned to reference levels almost completely (after a period of about 1 min) and reached relative values of 0.91 ± 0.11 (n = 23) of the first control, indicating almost complete reversibility (figs. 1 and 2, bottom traces). This minor reduction is probably due to a nonspecific rundown of the peak current amplitude during the lifetime of the patch.

Isoflurane and sevoflurane are similar in their dose-response curves but different in their effects on the kinetics of current decay. Complex effects on the current decay could be observed after applying 10$^{-4}$ M acetylcholine combined with sevoflurane to the nAChR. At concentrations between 4.9 x 10$^{-7}$ M and 4.9 x 10$^{-6}$ M sevoflurane, the time constants of current decay increased considerably (e.g., semilogarithmic plot shown in fig. 2B). At 4.9 x 10$^{-7}$ M and 4.9 x 10$^{-6}$ M sevoflurane, the current amplitude remained unchanged; that is, no obvious block was present. Because of the variation of the time constants of current decay, the relative increase of the constants was calculated. The relative values were 1.35 ± 0.38 (n = 13), 1.85 ± 0.36 (n = 10), 1.41 ± 0.37 (n = 12), and 1.55 ± 0.64 (n = 6) at 4.9 x 10$^{-7}$ M, 4.9 x 10$^{-6}$ M, 4.9 x 10$^{-5}$ M,
and $4.9 \times 10^{-4}$ M sevoflurane, respectively. Thus the effect was concentration independent.

When the concentration of sevoflurane was increased, the current decay showed two exponential components: Starting at $1.5 \times 10^{-4}$ M sevoflurane, an initial, faster component of current decay of $9.39 \pm 2.92$ ms ($n = 4$) appeared, followed by the slower component with approximately the time constant of desensitization. Such a biexponential decay of currents indicates an open-channel block. With increasing concentrations of sevoflurane, the time constant of the initial, faster component decreased to $4.47 \pm 2.64$ ms ($n = 9$) at $4.9 \times 10^{-4}$ M (e.g., 3 ms in fig. 2B) and to $0.9 \pm 0.59$ ms ($n = 10$) at $4.9 \times 10^{-3}$ M sevoflurane (e.g., 0.4 ms in fig. 2B), and the relative amplitude of the slow component decreased, showing the increasing blocking effect (fig. 3C). With isoflurane, the current decay remained at single exponential levels. At concentrations as great as $4.5 \times 10^{-4}$ M, the time course of the current decay did not change considerably. In contrast to sevoflurane, isoflurane decreased the relative time constants of current decay starting at $4.5 \times 10^{-4}$ M (e.g., semilogarithmic plot in fig. 1B), an effect that was considerable at high concentrations. The relative time constants were $0.97 \pm 0.14$ ($n = 12$), $0.69 \pm 0.15$ ($n = 15$), $0.42 \pm 0.22$ ($n = 6$), and $0.29 \pm 0.23$ ($n = 6$) at $4.5 \times 10^{-4}$ M, $1.5 \times 10^{-3}$ M, $3 \times 10^{-3}$ M, and $1.5 \times 10^{-2}$ M isoflurane, respectively.

Additional experiments were performed to determine whether the channels blocked by isoflurane or by sevoflurane would reopen when the blocker was removed. Short pulses of acetylcholine in the range of several milliseconds avoid complete desensitization of the nAChR, and high concentrations of the anesthetics block most of the channels during these pulses. Thus 10-ms pulses of $1.5 \times 10^{-2}$ M isoflurane or of $4.9 \times 10^{-3}$ M sevoflurane combined with $10^{-4}$ M acetylcholine were applied repetitively (in ten patches). At the beginning of the pulses, short current spikes were elicited (fig. 4). When the isoflurane or sevoflurane was removed, the current increased again and then finally decreased to zero (fig. 4). These reopenings were faster.
for isoflurane than for sevoflurane, indicating a faster dissociation from the site of action for isoflurane. A similar increase of currents after short pulses was recently observed after applying well-known open channel blockers to the nAChR.\textsuperscript{25}

In the second part of the study, effects of the anesthetics on the closed, unliganded state of the channels were investigated. Thus the patches were constantly exposed to isoflurane or sevoflurane for a period of about 1 min by adding different concentrations of the respective anesthetic to the background bathing solution of the application system. The addition of isoflurane or sevoflurane to the bathing solution allows for equilibration of the patch with the respective anesthetic concentration. Then the channels were exposed to pulses of $10^{-4}$ M acetylcholine combined with isoflurane or sevoflurane in the identical concentration as present in the bathing solution.

Figure 5 shows the procedure of the protocol. The first set of traces represents the reference peak currents with $10^{-3}$ M acetylcholine. The second set shows that micromolar concentrations of isoflurane (fig. 5A) or sevoflurane (fig. 5B), given only pulse-wise in combination with acetylcholine, do not change the peak current amplitudes. After addition of these concentrations to the bathing solution and preexposure of the patch, however, the currents elicited by $10^{-4}$ M acetylcholine were reduced by more than 50\% (fig. 5, third traces). The control currents with $10^{-4}$ M acetylcholine alone (after switching to extracellular solution without anesthetic) revealed reversibility of this effect (fig. 5, bottom traces).

The relative reduction of the peak current amplitude after preexposure of the patches to different concentrations of isoflurane or sevoflurane is plotted in figure 6. The evaluation of data from 34 patches for isoflurane and 25 patches for sevoflurane revealed an additional inhibition at all concentrations added. The inhibition of closed, unliganded channels already started at $1.5 \times 10^{-7}$ M isoflurane ($4.9 \times 10^{-7}$ M sevoflurane) and reached about 50\% at micromolar concentrations—those approximately 1,000 times lower than concentra-
ISOFLURANE AND SEVOFLURANE BLOCK ACETYLCHOLINE RECEPTORS

A

B

C

Fig. 3. (A) Concentration dependency of the block of the nicotinic acetylcholine receptor, activated by $10^{-4}$ M acetylcholine, given in combination with different concentrations of isoflurane (27 patches) or (B) sevoflurane (37 patches). Each point represents the mean ± SD of the relative peak amplitude (relative peak amplitude activated by $10^{-4}$ M acetylcholine = 1). (C) Concentration-dependent decrease of the relative amplitude of the slow component of current decay of sevoflurane (relative peak amplitude activated by $10^{-4}$ M acetylcholine = 1).

10$^{-3}$ M sevoflurane), however, the inhibition was nearly complete. In this setting, the kinetics of current decay were not altered considerably. The current decay remained at single exponential levels at all concentrations of isoflurane and at low concentrations of sevoflurane. At higher concentrations of sevoflurane, at which open-channel block is present, biexponential decays were observed.

Discussion

The present results provide evidence that both sevoflurane and isoflurane inhibit the nAChR primarily through the same mechanisms.

The experiments in the first part of the study show that isoflurane and sevoflurane, when given only during the pulse of $10^{-4}$ M acetylcholine, reduce the peak current amplitude reversibly and in a concentration-dependent manner. The dose-response curves with the same IC50 values (figs. 3A, B) show that the anesthetics are equally effective at the nAChR. The IC50 of $1.5 \times 10^{-5}$ M isoflurane compares well to the IC50 of about $10^{-3}$ M isoflurane reported by Dilger and colleagues at the nAChR of clonal BC3H-1 cells with the application of $10^{-4}$ M acetylcholine. These concentrations of anesthetics are in the range of those used for clinical anesthesia.

Anesthesiology, V 86, No 1, Jan 1997

Fig. 4. (A) Currents from one outside-out patch elicited by pulses (duration 10 ms) of $10^{-4}$ M acetylcholine combined with $4.9 \times 10^{-3}$ M sevoflurane (holding potential $-50$ mV) or (B) $1.5 \times 10^{-3}$ M isoflurane (recordings from another patch, holding potential $-30$ mV). The first trace in each figure shows a single recording. The traces represent each the average from 10–15 single recordings. (For details, see text.)
The following observations indicate an open-channel block mechanism by isoflurane and by sevoflurane: The current decay could be fitted by two exponentials with the addition of sevoflurane, starting at concentrations of $1.5 \times 10^{-3}$ M. The initial, faster component of decay demonstrates the block of open channels, where sevoflurane can only bind to channels already opened by acetylcholine, thus preventing ions from passing the pore. This concentration-dependent block is reflected by the decreasing time constant (fig. 2B) of this component with increasing concentrations of sevoflurane. The following slower component of decay reflects the equilibrium between the blocked and the unblocked state, thus corresponding to desensitization in the continuous presence of $10^{-3}$ M acetylcholine. With the addition of isoflurane, however, the current decay remains at single exponential levels. Isoflurane dissociates too quickly from the binding site at the nAChR to allow for discrimination between the two exponential components (block of open channels and desensitization).

On removal of either anesthetic and acetylcholine after short pulses, the current increased again due to a transition of the blocked state to the open state. This was previously shown for the open-channel blockers physostigmine and procaine. From single channel measurements on the nAChR with isoflurane, a “flicker” block (frequently repeated binding and dissociation of a blocker to and from the open state) has been observed. For a sequential open-channel block, an increase of...
ISOFLURANE AND SEVOFLURANE BLOCK ACETYLCHOLINE RECEPTORS

the burst duration is expected. However, isoflurane decreased the burst duration in a concentration-dependent manner. Therefore the sequential open-channel blocking protocol had to be extended. A simple open-channel block is also not sufficient to explain all effects observed in the present experiments with the fast-application technique. Sevoflurane, for example, increased the time constants of current decay, an effect that already occurred at $4.9 \times 10^{-7}$ m and was not related to concentration. Isoflurane, in contrast, decreased these time constants. Especially at high concentrations, isoflurane strongly accelerated the current decay. Different variations of rate constants of the isomerization from the open state to the desensitized state might underlie these observations. At least two different sites of action of the nAChR should account for the block on the one hand and for the alterations of the current decay on the other.

At high concentrations of halothane and enfurane, a reduction of miniature endplate currents and a biphasic decay was observed in mouse diaphragm, indicating an open-channel block. The prolonged tail of the decay observed in these experiments corresponds to prolonged burst duration by open-channel blockers.

In the second part of the study, the constant application of isoflurane or sevoflurane, preceding the activation with $10^{-4}$ m acetylcholine, revealed an interaction of both compounds with the closed, unliganded state of the channel. The experiments showed an additional inhibition of the current that is much more effective than the open-channel block. Concentrations of isoflurane and sevoflurane used for clinical anesthesia are higher by a factor of 100 to 1,000.

This effect was tested in a considerable number of patches (34 patches for isoflurane, 25 for sevoflurane), most of which were exposed to both perfusion protocols (fig. 5). Furthermore, the effect was reversible on control applications with acetylcholine alone (fig. 5, last traces). The shape of the dose-response curves is similar for isoflurane and for sevoflurane (fig. 6). The reduction of the current amplitude is concentration dependent for the lowest effective concentrations. Between $1.5 \times 10^{-6}$ m and $1.5 \times 10^{-4}$ m isoflurane ($4.9 \times 10^{-6}$ m and $4.9 \times 10^{-4}$ m sevoflurane), however, the effect seems to reach saturation. The increasing slope at concentrations $> 1.5 \times 10^{-4}$ m isoflurane ($4.9 \times 10^{-4}$ m sevoflurane) may reflect the additional open-channel block shown for these concentrations.

Considering the binding of either anesthetic to the closed, unliganded state of the channels, inducing a new blocked state, the dose-response curves would be expected to be concentration dependent. Similar to the prolongation of the desensitization kinetics by sevoflurane, which also started at $4.9 \times 10^{-7}$ m, the effects observed after preexposure are not closely related to concentration. By analogy, the interaction with the closed channels could be explained by modified desensitization rate constants from the unliganded, closed state to an unliganded, desensitized state. Results suggesting that volatile anesthetics either enhance the rate of desensitization or stabilize the desensitized form have been reported earlier.

Dilger and colleagues also reported an interaction of isoflurane with the closed state of the same embryonic mouse model nAChR: After continuous exposure of outside-out patches of BC3H-1 cells to $10^{-3}$ m isoflurane, a reduction of about 50% of the macroscopic current occurred throughout the 5 ms after agonist application. In the present study, the preexposure to $10^{-3}$ m isoflurane resulted in a reduction of the current of about 70%; that is, the protocol revealed an additional inhibition compared with the block of 40% when $10^{-3}$ m isoflurane is given only during the acetylcholine pulse. One explanation for the lack of additional effect could be that the authors used $10^{-2}$ m acetylcholine (compared with $10^{-3}$ m for the present experiments). A higher concentration of acetylcholine may relieve the effect of isoflurane. In addition, a decrease of the peak current of about 15% is reported if pulses of $10^{-3}$ m acetylcholine are applied during continuous exposure of the patch to $2.5 \times 10^{-4}$ m isoflurane (two experiments), in contrast to the decrease of about 45% (nine experiments) with this concentration in the present experiments. According to the authors, there was considerable variability in the relative peak currents from patch to patch. In the discussion, they state that perhaps the concentration of isoflurane was not as well controlled during rapid perfusion as they had hoped. The effects of concentrations of isoflurane less than $2.5 \times 10^{-4}$ m on closed channels were not shown.

The embryonic nAChR of mouse myotubes is more sensitive to low concentrations of acetylcholine and its agonists than is the adult type. Furthermore, this embryonic type of preparation also appears to be very sensitive to low concentrations of volatile anesthetics. Isoflurane and sevoflurane interact with both the open and the closed, unliganded state of the nAChR of mouse myotubes. The observation that these two states of the channel are affected by isoflurane corresponds with those by Dilger and colleagues, who reported

Anesthesiology, V 86, No 1, Jan 1997

Downloaded From: http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931824/ on 10/15/2018
effects of isoflurane, ether, and propofol on the open and the closed state of the nACHr of BC3H-1 cells. The present results provide evidence that at the nACHr both volatile anesthetics act primarily via the same mechanisms. The actions on desensitization kinetics, however, are different for isoflurane and sevoflurane, respectively, and are not in accord with a simple open-channel block. Thus there are probably two or more different sites of interaction at the channel protein.

Furthermore, in equilibrium with isoflurane or with sevoflurane, the embryonic nACHr of mouse myotubes seems to be a highly sensitive target site for these anesthetics. Because the inhibition of the closed state of the nACHr in this series of experiments is not strongly related to concentration, variation of rate constants of desensitization from the closed, unliganded state might account for these additional effects.

The diverse patterns of interaction suggest a direct action of the anesthetics on the ion channel protein itself. Otherwise, equipotent concentrations of anesthetics would affect the channels uniformly. Several studies support a protein interaction of anesthetics.6,31–40 Anesthetics might bind to the open state of the receptor, “plugging” the channel or to a different site of the protein or the protein lipid interface, inducing a new state, which does not allow ions to pass through (allosteric effect). It has been shown previously that membrane lipids such as cholesterol play a modulatory role in anesthetic interactions.41 An indirect disturbance of protein surrounding lipids, leading to, for example, a variation of rate constants, might still add to direct actions on the protein.

The authors thank Monika Hammel for expert technical assistance in preparing and maintaining the cultured cells. They also thank Heribert Husmann (Max-Planck-Institut für Kohleforschung, Mulheim/Ruhr) and Professor v. Meyer (Institut für Gerichtsmedizin der Ludwig-Maximilian-Universität, München) for providing the GCG measurements.

References

17. Reheberg B, Xiao YH, Duch DS: Central nervous system sodium channels are significantly suppressed at clinical concentrations of volatile anesthetics. Anesthesiology 1996; 84:1223–1233.
23. Franke C, Hatt H, Parnas H, Dudel J: Recovery from rapid

Anesthesiology, V 86, No 1, Jan 1997
desensitization of nicotinic acetylcholine receptor channels on mouse muscle. Neurosci Lett 1992; 140:169-72
27. Neher E, Steinbach JH: Local anaesthetics transiently block currents through single acetylcholine-receptor channels. J Physiol 1978; 399:663-78
28. Raines DE, Rankin SE, Miller KW: General anaesthetics modify the kinetics of nicotinic acetylcholine receptor desensitization at clinically relevant concentrations. Anesthesiology 1995; 82:276-87
35. Dubois BW, Evers AS: 3F-NMR spin-spin relaxation (T2) method for characterizing volatile anesthetic binding to proteins. Analysis of isoflurane binding to serum albumin. Biochemistry 1992; 31:7067-76