Anesthesiology 2009; 111:584–90

In Vitro *Effects of Propofol and Volatile Agents on Pharmacologically Induced Chloride Channel Myotonia*

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Background: **Anesthetic choice for patients with chloride channel myotonia remains under debate. The authors have, therefore, investigated the *in vitro* effects of various anesthetic agents on pharmacologically induced chloride channel myotonia.**

Methods: **Functionally viable (> 10 mN force generation) rectus abdominis muscle preparations obtained from normal swine were investigated using *in vitro* muscle contracture test baths. During continuous 0.1-Hz supramaximal electrical stimulation, the chloride channel blocker 9-anthracenecarboxylic acid (64 μM) was added before the addition of propofol or one of three volatile anesthetics. The concentration of propofol in either Intralipid (n = 11) or dimethyl sulfoxide (n = 10) was doubled every 10 min (from 4–512 μM). The concentration of halothane (n = 8), isoflurane (n = 8), and sevoflurane (n = 8) was doubled from 0.25 vol% up to the maximum dose according to calibrated vaporizers. Control muscle bundles were either untreated (n = 30) or exposed to 9-anthracenecarboxylic acid (n = 19).**

Results: **The myotonic reactions induced by 9-anthracenecarboxylic acid were reversed by high-dose (> 64 μM) propofol ($P < 0.01$). Halothane, isoflurane, or sevoflurane each enhanced the myotonic reactions at 5.4 ($P < 0.001$), 0.21 ($P < 0.01$), and 0.5 minimum alveolar concentrations ($P < 0.05$), respectively.**

Conclusions: **The authors' *in vitro* data imply that propofol administration for general anesthesia may be better suited for patients with chloride channel myotonia *versus* volatile anesthetics. In isolated swine skeletal muscle bundles, propofol elicited a reversal of 9-anthracenecarboxylic acid–induced chloride channel myotonia, whereas volatile anesthetics further increased the associated myotonic reactions.**

ANESTHETIC choice for patients with various neuromuscular disorders remains in many cases a matter of debate, as complications such as malignant hyperthermia (MH)–like episodes or rhabdomyolysis have been reported (*i.e.*, in association with various anesthetics that are MH-triggering agents). Furthermore, both central core disease and King-Denborough syndrome have been found to be associated with triggered MH episodes.[1,2] Importantly, propofol with its lipid carrier (composed of long-chain fatty acids) has been reported to adversely affect mitochondrial fatty acid oxidation, and thus has been associated with a propofol-infusion syndrome in normal patients as well as those with mitochondrial myopathies.[2,3] Another group of myopathies that has been associated with anesthetic complications includes myotonic muscle disorders such as Thomsen's disease and Becker type myotonia, both of which are considered to originate from decreased chloride conductance.[4] In skeletal muscle, chloride channels are essential to stabilize membrane potential and prevent recurring depolarizations. Nevertheless, although chloride channel myotonic disorders were shown to be genotypically unrelated to MH,[5] it remains unclear what anesthetic choice is optimal for these patients.[6] To date, there exist reports of aggravating and improving myotonia associated with the use of either propofol or volatile agents.[7–9]

The aim of the present study was to investigate the *in vitro* effects of propofol and inhalational anesthetic agents on pharmacologically induced (with 9-anthracenecarboxylic acid [9-AC]) chloride channel myotonia.

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Materials and Methods

After approval from the Institutional Animal Care and Use Committee of the University of Minnesota (Minneapolis, Minnesota), 12 castrated male Yorkshire crossbreed swine with a mean weight of 85.8 ± 8.8 kg were pretreated with an intramuscular injection of a mixture of 250 mg tiletamine and 250 mg zolazepam (Telazol, Fort Dodge Animal Health, Fort Dodge, IA). After anesthesia with 5–7 mg/kg IV thiopental (Gensia Pharmaceuticals, Inc., Irvine, CA), their tracheae were intubated and lungs mechanically ventilated with an air-oxygen mixture. Shortly thereafter, rectus abdominis muscle biopsies were obtained (within 15 min) and samples placed in preoxygenated Krebs-Ringer solution (gassed with carbogen, 95% O_2 and 5% CO_2) and then immediately transferred to a dissecting dish (with continuous gassing). Using a dissecting microscope, the muscle biopsies were dissected into small muscle bundles of intact fibers from tendon to tendon.[10,11] Next, they were transferred to *in vitro*, temperature-controlled (37 ± 0.1°C) muscle experimental chambers (containing Krebs-Ringer solution bubbled continuously with carbo-

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Received from the Department of Surgery, University of Minnesota, Minneapolis, Minnesota. Submitted for publication December 10, 2008. Accepted for publication May 14, 2009. Support was provided solely from institutional and/or departmental sources. Presented in part at the European Malignant Hyperthermia Group Annual Meeting, May 30, 2008, Lund, Sweden, and at the Annual Meeting of the European Society of Anaesthesia, June 2, 2008, Copenhagen, Denmark.

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gen); each bundle was independently stimulated with supramaximal electrical field stimulation pulses of 1 ms duration at a frequency 0.1 Hz, as previously described.[12,13] To identify the optimal muscle lengths, length-tension relationships were derived (bundles were stretched) according to the standardized protocol for *in vitro* contracture testing for MH susceptibility.[14] An average of 23 ± 8 muscle bundles was obtained from each pig for these experiments. After maintaining a stable baseline for approximately 10-15 min, muscle bundles were pharmacologically challenged according to the study protocol (see below in the section Investigation Protocols). Each muscle bundle length was measured after optimal lengths had been determined (as the distance between the suture ties that hold the muscle in the tissue bath). Muscle mass was determined as the wet weight of the specimen after removing it from the bath and cutting it at the sutures. Next, the specimens were patted dry and weighed immediately. Cross-sectional areas were estimated as follows[15]: Area (mm^2) = muscle mass (mg)/(fiber length [mm] × muscle density [mg/mm^3]) using 1.06 mg/mm^3 as the density of skeletal muscle.[16]

Pharmacologic Agents

The investigated and administered drugs were 9-AC (Sigma-Aldrich GmbH, Steinheim, Germany), a specific voltage-gated chloride channel blocker[17]; propofol in Intralipid (Disoprivan 1%; Astra Zeneca, Plankstadt, Germany); pure propofol (pharmaceutical sample; B. Braun, Melsungen, Germany); Intralipid (Intralipid 10%; Fresenius Kabi, Stans, Switzerland); dimethyl sulfoxide (DMSO; Fluka, Buchs, Switzerland); halothane (Sigma-Aldrich, St. Louis, MO); isoflurane (MINRAD Inc., Bethlehem, PA); sevoflurane (Abbott Laboratories, North Chicago, IL); acetazolamide (Bedford Laboratories, Bedford, OH); lidocaine (ICN Biochemicals Inc., Aurora, OH), and procainamide (Sigma-Aldrich GmbH, Buchs, Switzerland). The Krebs-Ringer solution consisted of 118.1 mM NaCl, 3.4 mM KCl, 1.2 mM KH_2PO_4, 1.0 mM $MgSO_4$-($7H_2O$), 11.0 mM d-glucose, 25.0 mM $NaHCO_3$, and 2.5 mM $CaCl_2$-($2H_2O$). The chloride-free Krebs-Ringer solution consisted of 118.1 mM $NaNO_3$, 3.4 mM KNO_3, 1.2 mM KH_2PO_4, 1.0 mM $MgSO_4$-($7H_2O$), 11.0 mM d-glucose, 25.0 mM $NaHCO_3$, and 2.5 mM $CaSO_4$-($2H_2O$).

Investigation Protocols

Dose–Response Curves. Dose–response curves were established for 9-AC in DMSO (n = 5), propofol in Intralipid (n = 5), propofol in DMSO (n = 5), acetazolamide (n = 5), lidocaine (n = 5), and procainamide (n = 5) at concentrations of 4, 8, 16, 32, 64, 128, 256, and 512 μM. DMSO (n = 5) and Intralipid alone (n = 5) were each tested according to the volume used in the groups where they served as the solvent. The volatile anesthetics halothane (n = 4), isoflurane (n = 4), and sevoflurane (n = 4) were tested at 0.25, 0.5, 1.0, 2.0, and 4.0 vol% up to the maximum dose allowable (4.5, 5.8, and 8.0 vol% for halothane, isoflurane, and sevoflurane, respectively) by a given vaporizer (Dräger, Drägerwerk AG, Lübeck, Germany).[13] Before entry into the flowmeters, the concentration of the volatile agents was continuously sampled by an inline servo gas monitor (Datex Ohmeda 5530 Agent Monitor, Datex-Ohmeda, Inc., Louisville, CO).

Investigations of the Effects of Anesthetics and Potential Mechanisms of Myotonia. For the various pharmacologic groups, muscle bundles were pretreated with 9-AC (64 μM) for 10 min,[18] followed by a challenge of increasing concentrations of propofol in Intralipid (n = 11), propofol in DMSO (n = 10), Intralipid (n = 10), DMSO (n = 10), halothane (n = 8), isoflurane (n = 8), sevoflurane (n = 8), acetazolamide (n = 10), lidocaine (n = 10), or procainamide (n = 10) in the concentrations according to the study protocol. In addition, we tested the effects of caffeine (n = 4) at 0.5 mM and 1.0 mM, respectively. Control muscle bundles were either untreated (n = 30) or bathed in 9-AC alone (n = 19). To investigate a direct effect of chloride, additional muscle bundles were incubated in a chloride-free Krebs-Ringer solution for 40 min. Thereafter, the muscle bundles were exposed to increasing concentrations (every 10 min) of propofol in Intralipid (n = 12) or Intralipid alone (n = 12). As appropriate controls, muscle bundles were incubated in chloride-free Krebs-Ringer solution (n = 12) or normal Krebs-Ringer solution (n = 12).

Data Collection and Data Analysis

The following variables, representing skeletal muscle contractile function, were recorded: Peak forces, areas under the force curves (AUCs), contraction times, half-relaxation times, and baseline forces (preloads). As such, all values were normalized to relative baseline forces and then expressed as percents of change. AUCs were measured for a period of 9.5 s after muscle stimulation (fig. 1). We waited for 8 min after each treatment to achieve full effects of a given agent and then collected the data during the subsequent 60 s (*i.e.*, six twitch responses per collection period); this data then was used for later analyses. All data collection was automated employing LabView (ADEPT, Adept Scientific, Hertfordshire, United Kingdom). For relative anesthetic comparisons, concentrations of the volatile agents were calculated as minimum alveolar concentration (MAC) equivalents.

Statistical Analysis

For measurement with respect to time, data were analyzed using two-way ANOVA with repeated measures, and one-way ANOVA were used for determining differences between drug groups at each time point. Bonferroni *post hoc* tests were used to identify intergroup differences. To estimate relative changes, the appropriate control groups were used. All data are presented in this report as mean ± SEM, except the muscle charac-

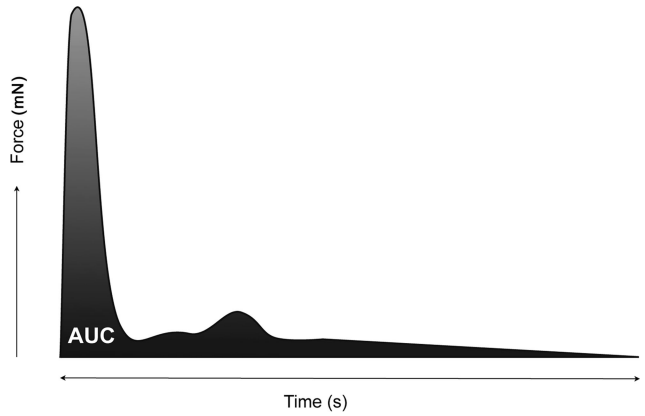

Fig. 1. Illustration of a typical twitch waveform after treatment of the muscle bundles with the chloride channel blocker 9-anthracenecarboxylic acid (9-AC; 64 μM). After stimulation, area under the force curve (AUC) was recorded for 9.5 s and analyzed. Forces were recorded in mN.

teristics data, which is given in mean ± SD; a $P < 0.05$ was considered statistically significant. All statistical analyses were performed using a Prism software package (GraphPad Software, La Jolla, CA).

Results

Dose–Response Curves

Length, masses, or cross-sectional areas of the muscle bundles used in the various treatment groups did not differ between groups. In the dose–response experiments, the mean values of the muscle bundles' characteristics were 30.5 ± 4.0 mm, 179.3 ± 42.0 mg, and 5.58 ± 1.3 mm^2 (see tables 1 and 2, Supplemental Digital Content 1, which list the characteristics of the specific muscle bundles of the various treatment groups used in this study, http://links.lww.com/ALN/A537). All control muscle bundles with no treatment elicited both significant decreases in peak forces and AUCs over time ($P < 0.001$). All administered drugs led to significantly

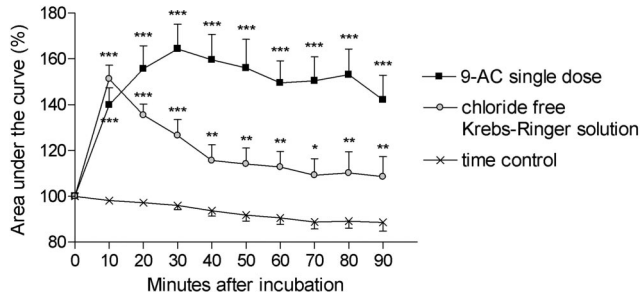

Fig. 2. Myotonia induced by the chloride channel blocker 9-anthracenecarboxylic acid (9-AC; 64 μM) (n = 19) or chloride-free Krebs-Ringer solution (n = 12) and time control (n = 30). Data are mean ± SEM. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ *versus* time control.

altered values of peak forces, AUCs, contraction times, and half relaxation times, as summarized in table 1. As compared to time controls, 9-AC led to significantly increased AUCs, starting at concentrations of 16 μM ($P < 0.05$); the dose response curve was sigmoidal in shape (see tables 3–7, Supplemental Digital Content 1, http://links.lww.com/ALN/A537, which are tables providing the entire data set of the dose–response experiments using normal skeletal muscle bundles. The different treatment groups and the indices of skeletal muscle performance, including peak force [mN], AUC [g*s], contraction time [s], half relaxation time [s], and baseline tension [mN] are provided).

Myotonia Induced by Either 9-AC or Chloride-free Krebs-Ringer Solutions

In the experiments with induced myotonia, the mean values of the muscle bundles' characteristics were 31.9 ± 4.1 mm, 191.8 ± 67.0 mg, and 5.73 ± 2.0 mm^2. The chloride channel blocker 9-AC (64 μM) led to significantly increased peak forces for up to 50 min after its administration, as compared with controls ($P < 0.05$). The same treatment significantly increased AUCs during the entire study period as compared with controls, starting at 10 min after administration (fig. 2; $P < 0.001$).

Table 1. Effects of Study Drugs and Volatile Agents on Skeletal Muscle Performance

	Peak Force	AUC	Contraction Time	Half Relaxation Time	Baseline Tension
9-AC	(16 μM)*	(16 μM)†	—	—	—
Propofol/intralipid	512 μM*	512 μM‡	256 μM*	512 μM*	—
Propofol/DMSO	128 μM†	128 μM†	—	512 μM†	—
Intralipid	—	—	—	—	—
DMSO	512 μM‡	512 μM*	—	(64 μM)†	—
Lidocaine	32 μM*	32 μM†	64 μM†	128 μM‡	—
Procainamide	—	—	—	—	—
Acetazolamide	—	—	—	—	—
Isoflurane	1.7 MAC‡	3.3 MAC†	—	—	—
Sevoflurane	(0.5 MAC)†	—	—	—	—
Halothane	—	—	—	—	(6.1 MAC)†

Concentration (μM) of the study drugs and minimum alveolar concentration (MAC) of the volatile agents, at which a significant decrease in the indices of skeletal muscle performance was observed. ( ) = significant increasing effect; — = no effect.

* $P < 0.01$, † $P < 0.05$, and ‡ $P < 0.001$ *vs.* time control.

9-AC = 9-anthracenecarboxylic acid; AUC = area under the force curve; DMSO = dimethyl sulfoxide.

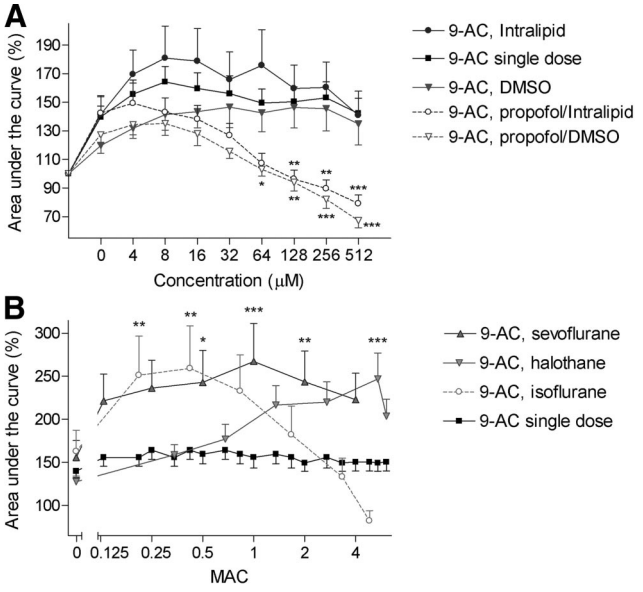

Fig. 3. Effects of anesthetics on chloride channel–related myotonia. (*A*) Influence of Intralipid (n = 10), DMSO (n = 10), propofol/Intralipid (n = 11), propofol/DMSO (n = 10), and (*B*) sevoflurane (n = 8), halothane (n = 8), and isoflurane (n = 8) on 9-AC (64 μM)–induced myotonia. Data are mean ± SEM. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ *versus* 9-AC single dose. 9-AC = 9-anthracenecarboxylic acid; DMSO = dimethyl sulfoxide; MAC = minimum alveolar concentration.

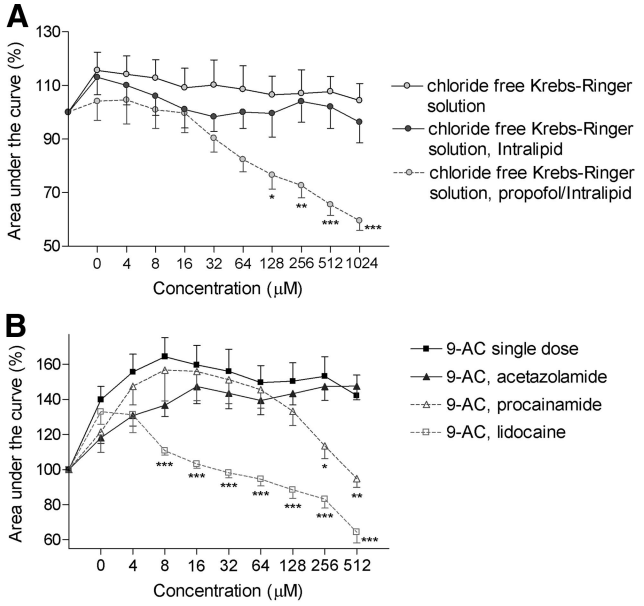

Fig. 4. Potential mechanisms involved in the effects of the different anesthetic agents on chloride channel–related myotonia. (*A*) The effects of Intralipid (n = 12) and propofol/Intralipid (n = 12) in a chloride-free environment. (*B*) The effects of the chloride channel modulator acetazolamide (n = 10) and the sodium channel modulators procainamide (n = 10) and lidocaine (n = 10) on 9-AC (64 μM)–induced myotonia. Data are mean ± SEM. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ *versus* chloride free solution or 9-AC single dose. 9-AC = 9-anthracenecarboxylic acid.

There were no differences in contraction times and half relaxation times between the two groups. Ninety minutes after 9-AC administration (64 μM) the baseline force values were significantly higher ($P < 0.01$). After incubation in chloride-free Krebs-Ringer solution, the muscle bundles showed increased peak forces and AUCs during the entire study period (fig. 2; $P < 0.05$). In addition, half relaxation times began to increase significantly 80 min after incubation in the chloride-free medium ($P < 0.05$). Contraction times and baseline force values were not different for muscle samples in either normal or chloride-free Krebs-Ringer solutions.

Influences of Different Drugs on 9-AC–induced Myotonia

The myotonic reactions, indicated by the increased values of AUCs after treatments with 9-AC, were reversed by the subsequent administrations of either propofol in DMSO or propofol in Intralipid, starting at 64 μM ($P < 0.05$) and 128 μM ($P < 0.01$), respectively (fig. 3A). This was not the case with either Intralipid or DMSO administration alone. Challenges with halothane, isoflurane, and sevoflurane increased the myotonic reactions, starting at 5.4 ($P < 0.001$), 0.21 ($P < 0.01$), and 0.5 MAC ($P < 0.05$), respectively (fig. 3B). Interestingly, the higher concentrations of the inhalational anesthetic agents appeared to somewhat reverse the elevated myotonic reaction. While isoflurane started to decrease myotonia at 0.83 MAC, sevoflurane and halothane only did so at their maximum dosages (4.0 MAC and 6.1 MAC) (fig. 3B). Importantly, myotonia induced by chloride-free Krebs-Ringer solution was reversed by the administration of propofol in Intralipid at 128 μM (fig. 4A; $P < 0.05$). Similarly, the sodium channel blockers lidocaine and procainamide both decreased the myotonic effects significantly at concentrations of 8 μM ($P < 0.001$) and 256 μM ($P < 0.05$), respectively (fig. 4B). However, the administration of lidocaine increased peak forces at 128 μM and 256 μM concentrations in the same setting. It was observed that treatment with the chloride channel activator acetazolamide had no effects on the induced myotonia (fig. 4B). In contrast, caffeine administration significantly increased the myotonic reactions of exposed muscle bundles ($P < 0.05$). The effects of these agents on the contractile features of the 9-AC–pretreated muscles are summarized in table 2. (See tables 8–12, Supplemental Digital Content 1, http://links.lww.com/ALN/A537, which are tables listing the entire data set of the dose response experiments using myotonic [9-AC–pretreated] skeletal muscle bundles. The different treatment groups and the indices of skeletal muscle performance, including peak force [mN], AUC [g*s], contraction time [s], half relaxation time [s] and baseline tension [mN] are provided.)

Discussion

We have investigated the *in vitro* effects of propofol and various volatile anesthetics on pharmacologically

Table 2. Modulation of Myotonic Muscle Performance by Study Drugs and Volatile Agents

	Peak Force	AUC	Contraction Time	Half Relaxation Time	Baseline Tension
Propofol/intralipid	—	128 μM*	64 μM†	64 μM†	512 μM†
Propofol/DMSO	256 μM*	64 μM†	—	128 μM†	128 μM†
Intralipid	—	—	—	—	—
DMSO	—	—	(256 μM)*	—	—
Lidocaine	(128 μM)†	8 μM‡	64 μM*	16 μM†	—
Procainamide	—	256 μM†	(64 μM)†	—	—
Acetazolamide	(256 μM)†	—	(64 μM)†	—	512 μM†
Caffeine	(0.5 mM)‡	(0.5 mM)†	(0.5 mM)†	(0.5 mM)‡	—
Isoflurane	1.7 MAC*	(0.2 MAC)*	—	(0.4 MAC)*	3.3 MAC†
Sevoflurane	(0.3 MAC)†	(0.5 MAC)†	—	—	—
Halothane	(0.7 MAC)‡	(5.4 MAC)‡	—	(5.4 MAC)†	—

Concentration (μM or mM) of the study drugs and minimum alveolar concentration (MAC) of the volatile agents, at which a significant decrease in the indices of myotonic skeletal muscle performance was observed. The muscle bundles were treated with the chloride channel blocker 9-anthracenecarboxylic acid (9-AC; 64μM) to induce myotonia. ( ) = significant increasing effect, — = no effect.

* $P < 0.01$, † $P < 0.05$, and ‡ $P < 0.001$ *vs.* 9-AC single dose.

AUC = area under the force curve; DMSO = dimethyl sulfoxide.

induced chloride channel myotonia. The primary findings of this study were that propofol administration reversed the pharmacologically induced myotonia, whereas the volatile agents halothane, isoflurane, and sevoflurane were associated with aggravated myotonic reactions.

Under normal physiologic conditions, the influx of chloride is considered to rapidly stabilize the sarcolemmal membrane potential after a depolarization; *e.g.*, induced action potential within the muscle fiber membrane. Clinically, in patients with myotonia congenita, impaired chloride conductance because of altered chloride channels results in membrane hyperexcitability, *i.e.*, the myogenic production of repetitive action potentials, which in turn prolongs the overall contractions within their skeletal muscles. In the experiments presented here, we observed that in these isolated muscle bundles we could reduce fiber chloride conductances, either by pharmacologically blocking specific chloride conductance with 9-AC[18] or by lowering extracellular chloride concentration. As expected, these treatments led to disturbed repolarizations with afterdepolarizations, and thus repetitive aftercontractions (myotonia).[19]

Propofol

Importantly, it was observed here that propofol dramatically reversed these drug-induced myotonic reactions at concentrations above 64 μM. This effect was considered part as a result of the activity of propofol itself; *i.e.*, because neither Intralipid nor DMSO alone altered the relative degrees of induced myotonia. Consistent with these findings, there are several previous reports citing the direct myorelaxant effects of propofol on skeletal muscle.[20–22] Thus, in our study, to determine whether voltage-gated chloride channels were primarily involved in these responses, we tested the relative effects of the voltage-gated chloride channel activator acetazolamide.[23,24] We observed that the treatment with acetazolamide induced minimal or no such antimyotonic effects in such *in vitro* experiments. This may suggest that chloride anions are not primarily involved in these antimyotonic responses. Furthermore, propofol administration resulted in the same efficacies in resolving the myotonic reactions in a chloride-free environment.

In general, it is considered that human skeletal muscle sodium channels are blocked by propofol in a voltage-dependent manner.[25,26] This action on the voltage-gated sodium channels may have been responsible for the antimyotonic effects of propofol that were observed in the present studies. Therefore, we also tested the relative effect of the sodium channel inhibitors lidocaine and procainamide. Interestingly, these two agents were associated with reversals of the myotonic reactions in our experiments as well.[27,28] This may be further evidence that the antimyotonic actions of propofol are mainly a result of modulations of voltage-gated sodium channels within the sarcolemmal membranes of skeletal muscle.

Volatile Agents

We observed here that the volatile anesthetic agents halothane, isoflurane, and sevoflurane each significantly increased the myotonia induced by 9-AC. Of potential clinical interest was the present observation that both isoflurane and sevoflurane administration induced enhanced myotonic responses at clinically relevant doses: 0.21 MAC and 0.5 MAC, respectively. This may in part have been because of the expected increase in the intracellular release of calcium into skeletal muscle cells after exposure to inhalational agents.[29] More specifically, like caffeine, volatile anesthetics are known to act agonistically on the skeletal muscle type 1 ryanodine receptor and lead to increased calcium release from the sarcoplasmic reticulum. This phenomenon was supported by our findings that both caffeine and volatile agents were associated with increased myotonic reactions in our experiments; *i.e.*, the sarcoplasmic release of cal-

cium through ryanodine receptors may have been at least partially involved in these responses. In addition, the known abilities of isoflurane to inhibit the voltage-gated sodium channels of skeletal muscle could explain the favorable effect of isoflurane on 9-AC–induced myotonia at higher concentrations; in addition, isoflurane administration has been associated with decreased peak force values at concentrations above 1.7 MAC.[30]

We consider here that our experiments support the view that in muscles that elicit hyperexcitability, volatile anesthetics may secondarily increase overall muscle metabolism and could therefore be potentially dangerous; *i.e.*, patients affected by myotonic syndromes may elicit aggravated myotonia causing hypermetabolic events (when extreme, this may even mimic episodes of malignant hyperthermia).[31,32] However, to date there are no comparative clinical or *in vivo* animal studies on these issues, and we therefore have to rely on occasional and often anecdotal case reports. It is of interest to note that in some of these reports, propofol in myotonic patients is not without observed side effects; there have been variable responses, marked sensitivity to its depressant effects, and even precipitation of myotonia reported with its use.[8,33]

There are several potential limitations of our experimental approaches. First, in these isolated muscle bundles there is no vascular perfusion, and thus if diameters are too large, adequate oxygenation of centralized fibers may be limited. In addition, it is not exactly clear how *in vitro* test bath concentrations of administered agents apply to blood concentrations when employed *in vivo*. Nevertheless, it has been proposed that in experiments in which volatile anesthetics are administered for the *in vitro* contracture testing for the determination of MH susceptibility, such responses will occur at clinically relevant concentrations. Therefore, we postulate that the concentrations of volatile anesthetics used in this study may also be considered to be clinically relevant. It is less clear whether the results with propofol administration should be interpreted somewhat differently. It may be proposed that higher concentrations of propofol would likely be needed to simulate the situation *in vivo*, given that propofol had no effect at clinically relevant concentrations and even showed a reversal of chloride channel–induced myopathy at high concentrations. Finally, half relaxation times represent the established marker for myotonia.[19] There are publications describing changes in relaxation times with smaller changes in twitch amplitudes or AUCs in humans with myotonia.[34,35] In our study model, we were not able to significantly increase the half relaxation times of the muscle bundles by using 9-AC, irrespective of dosage. However, in some of the aforementioned studies the biggest differences between normal and myotonic patients were in the second half of the relaxation phases. Consistent with these results of the indices of skeletal muscle performances observed in our experimental model, AUC seemed to be the most sensitive marker for an elicited myotonic reaction. Furthermore, if we considered here changes in half relaxation times as the primary measures of elicited myotonia, the primary findings would remain the same. Although 9-AC did not alter half relaxation times, propofol was associated with shortening of half relaxation times, whereas the volatile agents isoflurane and halothane increased half relaxation times.

In conclusion, according to our *in vitro* data, general anesthesia with propofol seems to be the better choice for use in a patient who may have myotonic activity. However, we emphasize that it is difficult to conclude from these *in vitro* studies employing swine skeletal muscle bundles that one can assume relative safety of these agents in patients. Again, we have not studied the relative effects of these protocols on muscles of myotonic patients directly. Regardless of the chosen anesthetic and the likely preferred choices, the anesthesiologist must consider responses in individual patients as the anesthetic proceeds.

The authors would like to thank Allison Dwileski, B.S. (Administrative Assistant, Department of Anesthesia, University Hospital, University of Basel, Switzerland) and Monica Mahre, B.S. (Lab Services Coordinator, Department of Surgery, University of Minnesota, Minneapolis, Minnesota) for their editorial assistance and help with manuscript submission.

References

1. Driessen JJ: Neuromuscular and mitochondrial disorders: What is relevant to the anaesthesiologist? Curr Opin Anaesthesiol 2008; 21:350–5
2. Kinder Ross A: Muscular dystrophy *versus* mitochondrial myopathy: The dilemma of the undiagnosed hypotonic child. Paediatr Anaesth 2007; 17:1–6
3. Wolf A, Weir P, Segar P, Stone J, Shield J: Impaired fatty acid oxidation in propofol infusion syndrome. Lancet 2001; 357:606–7
4. Pusch M: Myotonia caused by mutations in the muscle chloride channel gene CLCN1. Hum Mutat 2002; 19:423–34
5. Newberg LA, Lambert EH, Gronert GA: Failure to induce malignant hyperthermia in myotonic goats. Br J Anaesth 1983; 55:57–60
6. Lehmann-Horn F, Iaizzo PA: Are myotonias and periodic paralyses associated with susceptibility to malignant hyperthermia? Br J Anaesth 1990; 65:692–7
7. Hayashida S, Yanagi F, Tashiro M, Terasaki H: [Anesthetic managements of a patient with congenital myotonia (Becker type)]. Masui 2004; 53:1293–6
8. Johnson GW, Chadwick S, Eadsforth P, Hartopp I: Anaesthesia and myotonia. Br J Anaesth 1995; 75:113
9. Heiman-Patterson T, Martino C, Rosenberg H, Fletcher J, Tahmoush A: Malignant hyperthermia in myotonia congenita. Neurology 1988; 38:810–2
10. Moulds RF, Denborough MA: Procaine in malignant hyperpyrexia. Br Med J 1972; 4:526–8
11. Nelson TE, Austin KL, Denborough MA: Screening for malignant hyperpyrexia. Br J Anaesth 1977; 49:169–72
12. Larach MG: Standardization of the caffeine halothane muscle contracture test. North American Malignant Hyperthermia Group. Anesth Analg 1989; 69: 511–5
13. Iaizzo PA, Wedel DJ, Gallagher WJ: *In vitro* contracture testing for determination of susceptibility to malignant hyperthermia: A methodologic update. Mayo Clin Proc 1991; 66:998–1004
14. Ording H, Brancadoro V, Cozzolino S, Ellis FR, Glauber V, Gonano EF, Halsall PJ, Hartung E, Heffron JJ, Heytens L, Kozak-Ribbens G, Kress H, Krivosic-Horber R, Lehmann-Horn F, Mortier W, Nivoche Y, Ranklev-Twetman E, Sigurdsson S, Snoeck M, Stieglitz P, Tegazzin V, Urwyler A, Wappler F: *In vitro* contracture test for diagnosis of malignant hyperthermia following the protocol of the European MH Group: Results of testing patients surviving fulminant MH and unrelated low-risk subjects. The European Malignant Hyperthermia Group. Acta Anaesthesiol Scand 1997; 41:955–66
15. Segal SS, Faulkner JA: Temperature-dependent physiological stability of rat skeletal muscle *in vitro*. Am J Physiol 1985; 248:C265–70
16. Cooper TE, Trezek GJ: Correlation of thermal properties of some human tissue with water content. Aerosp Med 1971; 42:24–7

17. Dutka TL, Murphy RM, Stephenson DG, Lamb GD: Chloride conductance in the transverse tubular system of rat skeletal muscle fibres: Importance in excitation-contraction coupling and fatigue. J Physiol 2008; 586:875–87

18. Kwieciński H, Lehmann-Horn F, Rüdel R: Drug-induced myotonia in human intercostal muscle. Muscle Nerve 1988; 11:576–81

19. Iaizzo PA, Lehmann-Horn F: The correlation between electrical afteractivity and slowed relaxation in myotonia. Muscle Nerve 1990; 13:240–6

20. Marsch SC, Schaefer HG: External ophthalmoplegia after total intravenous anaesthesia. Anaesthesia 1994; 49:525–7

21. Borgeat A, Dessibourg C, Rochani M, Suter PM: Sedation by propofol in tetanus–is it a muscular relaxant? Intensive Care Med 1991; 17:427–9

22. Ummenhofer WC, Kindler C, Tschalèr G, Hampl KF, Drewe J, Urwyler A: Propofol reduces succinylcholine induced increase of masseter muscle tone. Can J Anaesth 1998; 45:417–23

23. Eguchi H, Tsujino A, Kaibara M, Hayashi H, Shirabe S, Taniyama K, Eguchi K: Acetazolamide acts directly on the human skeletal muscle chloride channel. Muscle Nerve 2006; 34:292–7

24. Ruff RL: Sour on the inside, calm on the outside: How acetazolamide may stabilize membrane excitability. Muscle Nerve 2006; 34:263–4

25. Haeseler G, Störmer M, Bufler J, Dengler R, Hecker H, Piepenbrock S, Leuwer M: Propofol blocks human skeletal muscle sodium channels in a voltage-dependent manner. Anesth Analg 2001; 92:1192–8

26. Haeseler G, Karst M, Foadi N, Gudehus S, Roeder A, Hecker H, Dengler R, Leuwer M: High-affinity blockade of voltage-operated skeletal muscle and neuronal sodium channels by halogenated propofol analogues. Br J Pharmacol 2008; 155:265–75

27. Chen Z, Ong BH, Kambouris NG, Marbán E, Tomaselli GF, Balser JR: Lidocaine induces a slow inactivated state in rat skeletal muscle sodium channels. J Physiol 2000; 524(Pt 1):37–49

28. Zamponi GW, Sui X, Codding PW, French RJ: Dual actions of procainamide on batrachotoxin-activated sodium channels: Open channel block and prevention of inactivation. Biophys J 1993; 65:2324–34

29. Nelson TE, Sweo T: $Ca2^{+}$ uptake and $Ca2^{+}$ release by skeletal muscle sarcoplasmic reticulum: Differing sensitivity to inhalational anesthetics. ANESTHESIOLOGY 1988; 69:571–7

30. OuYang W, Hemmings HC: Isoform-selective effects of isoflurane on voltage-gated Na^{+} channels. ANESTHESIOLOGY 2007; 107:91–8

31. Lehmann-Horn F, Iaizzo PA, Franke C, Hatt H, Spaans F: Schwartz-Jampel syndrome: II. Na^{+} channel defect causes myotonia. Muscle Nerve 1990; 13:528–35

32. Haberer JP, Fabre F, Rose E: Malignant hyperthermia and myotonia congenita (Thomsen's disease). Anaesthesia 1989; 44:166

33. White RJ, Bass SP: Myotonic dystrophy and paediatric anaesthesia. Paediatr Anaesth 2003; 13:94–102

34. Michel P, Sternberg D, Jeannet PY, Dunand M, Thonney F, Kress W, Fontaine B, Fournier E, Kuntzer T: Comparative efficacy of repetitive nerve stimulation, exercise, and cold in differentiating myotonic disorders. Muscle Nerve 2007; 36:643–50

35. Moxley RT 3rd, Logigian EL, Martens WB, Annis CL, Pandya S, Moxley RT 4th, Barbieri CA, Dilek N, Wiegner AW, Thornton CA: Computerized hand grip myometry reliably measures myotonia and muscle strength in myotonic dystrophy (DM1). Muscle Nerve 2007; 36:320–8