Sevoflurane Exposure Prevents Diaphragmatic Oxidative Stress During Mechanical Ventilation but Reduces Force and Affects Protein Metabolism Even During Spontaneous Breathing in a Rat Model

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BACKGROUND: Ventilator-induced diaphragmatic dysfunction is associated with the generation of oxidative stress, enhanced proteolysis, autophagy and reduced protein synthesis in the diaphragm. Sevoflurane is a common operating room anesthetic and can be used in the intensive care medicine as well. Besides its anesthetic properties, its use in cardiac ischemia-reperfusion models can maintain protein synthesis and inhibit generation of reactive oxygen species, if used at the beginning of heart surgery. This study has been performed on the hypothesis that sevoflurane might protect against ventilator-induced diaphragmatic dysfunction by preventing the production of oxidative stress.

METHODS: Four-month-old, male Sprague-Dawley rats sedated with sevoflurane (minimal alveolar concentration = 1) were either mechanically ventilated (MV) for 12 hours (n = 8) or allowed to breathe spontaneously (SB) for 12 hours (n = 8). An acutely anesthetized group was used as a control (Con) group (n = 8). After euthanization, diaphragmatic contractile properties, fiber cross-sectional areas, proteolysis (calpain-1 and caspase-3), and oxidative stress (lipid peroxidation, protein oxidation) were examined. After testing for normality, 1-way or 2-way analysis of variance with the Dunnett post hoc test was used to test for significance.

RESULTS: The diaphragm contractile force was similarly reduced at all stimulation frequencies in the SB and MV groups compared with controls. Markers of oxidative stress and fiber cross-sectional areas were unaltered between Con and SB/MV, respectively. The calcium-dependent proteases (calpain-1 and caspase-3) were enhanced in the MV group. The p-AKT/AKT ratio and p-FoxO1/FoxO1 ratio were significantly and similarly reduced after sevoflurane exposure in the SB and MV group compared with Con group.

CONCLUSIONS: Exposure to sevoflurane did not induce oxidative stress. It led to reduction in diaphragmatic force. In the MV group, sevoflurane led to the activation of atrophy signaling pathways. These findings are of particular importance for clinical utilization in intensive care units and question its use, especially during the phases of SB. (Anesth Analg 2015;121:73–80)

Controled mechanical ventilation (CMV) is indispensable for patients with respiratory insufficiency. It is well established that 12 hours of CMV can result in diaphragmatic dysfunction and atrophy, collectively responsible for respiratory failure, increased hospitalization, increased costs, and greater morbidity and mortality. VIDD pathomechanisms include the increase in mitochondrial ROS, reduction in blood flow, and lipid accumulation. In animal models and in humans, down-stream, autophagy, proteolysis, and a reduction in protein synthesis have been shown, with the AKT/mammalian target of rapamycin/FoxO1 pathway a regulating factor. This is especially true of autophagy, which is regulated by the FoxO1 pathway, and has been the focus recently.

Although other volatile anesthetic gases have been studied and have shown inconsistent results in different diaphragmatic models in vivo and in vitro settings, sevoflurane has not been investigated so far. Sevoflurane is used mostly in the anesthetic setting, but its effects on wake up time and ventilation time are still being researched. Sevoflurane is not included in the European guidelines as a standard sedative on intensive care units (ICUs). Research on volatile anesthetics has focused not only on the anesthetic mode of action but also on their effect on apoptosis in heart muscle.

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cells and their potential protective effects on preconditioning in ischemia/reperfusion models. In cardiac muscle, sevoflurane is used as a protective agent in pre- and post-conditioning models due to its interaction with ROS production in mitochondria. In diaphragm muscle, sevoflurane worsens loss of force in fatigued diaphragm, but the cause of this effect remains unclear. Our group has shown that the use of propofol, one of the major anesthetic drugs, induces diaphragmatic atrophy and loss of contractile force during spontaneous breathing (SB) and CMV over 24 hours. These findings question the use of propofol for the diaphragmatic damage, which might influence the ability to wean patients. Different sedative regimens might be less harmful to diaphragm muscle or even protective against VIDD.

It is unknown whether the diaphragmatic atrophic pathways and diaphragm contractility are affected by sevoflurane exposure. We hypothesized that sevoflurane exposure might reduce the effects of VIDD, possibly via an interaction with the generation of oxidative stress. To test this hypothesis, we used our established VIDD rat model of mechanical ventilation using sevoflurane as an anesthetic. We assessed diaphragm function, fiber dimensions, oxidative stress, proteolysis, and markers of protein synthesis.

**METHODS**

The study was approved by the ethical committee (Landesamt für Natur, Umwelt und Verbraucherschutz, LANUV NRW, Germany, reference number: AZ 84-02.04. 2011A277). All surgery was performed under general anesthesia, and all efforts were made to minimize suffering.

**Ventilation and Anesthetic Model**

Healthy, 4-month-old, male Sprague-Dawley rats were separated into 3 groups (each $n = 8$). The first group was mechanically ventilated (MV) for 12 hours under sevoflurane exposure, the second group breathed spontaneously (SB) for 12 hours under sevoflurane exposure, and the third group was a spontaneously breathing, acutely anesthetized control (Con). One animal in the MV group died and was not replaced.

Anesthesia in the sevoflurane groups was initiated by intraperitoneal injection (IP) of 60 mg/kg pentobarbital and sustained by sevoflurane with minimal alveolar concentration $= 1$, which is approximately 2.2 vol% in rats. The Con group received pentobarbital (60 mg/kg IP).

The MV group was tracheotomized, and a pressure-controlled ventilation was performed with a breathing rate of 60 bpm, a positive end-expiratory pressure of 3 cm H$_2$O, a tidal volume of 6 to 7 mL/kg, inspiration/expiration at a ratio of 1:1 to maintain a Pao$_2$ of 70 to 90 mm Hg (Physioflex, Draeger, Luebeck, Germany) and to totally suppress breathing activity.

The second group (SB) was tracheotomized and breathed from a gas reservoir that provided a gas mixture of air, oxygen, and sevoflurane. The expiratory gas concentrations were continuously monitored.

Sevoflurane-exposed animals were continuously monitored by echocardiogram, invasive blood pressure, and a rectal temperature tube. The temperature was maintained at 37°C. Normovolemia was ensured by continuous fluid (saline 0.9%) application of 2 mL/h via a central venous catheter. Arterial blood samples were taken every 3 hours.

In SB and MV, the diaphragm was removed after 12 hours of sevoflurane exposure under sustained respiration and circulation. In the Con group, the diaphragm was removed after tracheostomy and a short ventilation period (maximally 5 minutes) to prevent hypercarbia.

**Functional and Histological Measurements**

Diaphragmatic force production was assessed in vitro using our established protocol, where a diaphragm strip is successively stimulated at 15, 30, 60, 100, and 160 Hz in an organ bath.

For histological assessment of muscle fiber atrophy, a diaphragm strip was embedded in Tissue-Tek® (FA Sakura, Alphen aan den Rijn, Netherlands) and then frozen in liquid butane, which had been precooled in liquid nitrogen.

Diaphragm muscle tissue slices (7 μm) were stained using primary antibodies against type I muscle fibers (A4.480 Developmental Studies Hybridoma Bank, Iowa city, IA), type IIa muscle fibers (SC71, Developmental Studies Hybridoma Bank), dystrophin (Thermo Scientific, Ulm, Germany) and linked to secondary, immunofluorescent antibodies. Fiber cross sections of around 200 muscle fibers per animal were examined by immunofluorescence microscopy and analyzed using the software ImageJ (v1.46k; National Institutes of Health, Bethesda, MA). Both techniques have been used before by our group.

**Overview on Biological Measurements**

Levels of oxidative stress measurements were assessed using the OxyBlot technique (OxyBlot, Millipore, Billerica, MA), which serves a marker of oxidative modifications in proteins and Western blot detection of 4-hydroxynonenal, which is a marker for lipid oxidation.

As a crucial switch between protein synthesis and proteolysis/autophagy serves AKT, which induces protein synthesis in the active, phosphorylated state (p-AKT), while dephosphorylated (AKT), it leads to a decrease in activity of p-FoxO1, which may serve as a translation factor inducing autophagy. Anabolic signaling was assessed by the ratio of p-AKT/AKT and the ratio of p-FoxO1/FoxO1.

Protease activity was detected by measuring the specific breakdown products of αII-spectrin as an indirect measurement of calpain-1 and caspase-3 activity in Western blot technique. Caspase-3 and calpain-1 degrade the 245-kDa αII-spectrin molecule into specific breakdown products, dependent on protease activity.

**Lipid Peroxidation, Proteolytic Signaling, and AKT Pathway**

Thirty milligrams of diaphragm tissue were homogenized on ice in 800 μL of lysis buffer (150 mM sodium chloride, 1.0% NP-40, 0.1% sodium dodecyl sulfate [SDS], 1% sodium deoxycholate, 50 mM Tris-HCl, pH 7.6; all from Sigma-Aldrich, St. Louis, MO) completed by protease inhibitor cocktail tablets (Roche Diagnostics, Mannheim, Germany). Homogenate was then centrifuged through Qiagen-shredder columns (Qiagen, Hilden, Germany) at 2000 rpm (= 425 g) for 2 minutes, and the protein concentration was determined in the supernatants using DC Protein Assay Kit (Bio-Rad Laboratories, Munich, Germany). Equal
amounts of 20 μg total protein in solution were mixed with loading buffer (Laemmli-buffer; 312.5 mM Tris-HCl, pH 6.8, 10% SDS, 50% glycerin, 10% β-mercaptoethanol, <5 mg bromophenol blue; all from Sigma-Aldrich) and heated to 95°C for 5 minutes. Separation of proteins by 10% SDS-polyacrylamide gel was followed by transfer onto a polyvinylidene difluoride membrane (Bio-Rad Laboratories, Hercules, CA), using a standard semi-dry blotting procedure (60 minutes, 25 V). Unspecific binding sites were blocked by 1 hour incubation at room temperature in a blocking solution containing 5% bovine serum albumin (Albumin fraction V; ROTH, Karlsruhe, Germany), before incubation overnight at 4°C with specific antibodies against 4-hydroxy nonenal (ab46545; Abcam, Cambridge, UK), diIspectrin (Santa Cruz Biotechnology, Santa Cruz, CA), phosphorylated and total protein kinase B, phosphorylated and total FoxO1 and glycerinaldehyde-3-phosphat-dehydrogenase as loading control (phospho-AKT: no. 4060, pan-AKT: no. 4691; FoxO1: no. 2880, GAPDH: no. 5174; all from Cell Signaling, Danvers, MA). Nightly incubation was followed by several washing steps (3 x 5 minutes in Tris-buffered saline containing 1% Tween20; Sigma-Aldrich) and incubated with a horseradish-peroxidase-conjugated goat anti-rabbit antibody (no. 7074; Cell Signaling) for 1 hour at room temperature on a shaker. The final reaction was measured by enhanced chemiluminescence (WEST-ZOL Plus Western Blot Detection System; iNTRON Biotechnology, Sangdawon-Dong, Korea) on a detection system (BioDocAnalyze live; Biometra, Goettingen, Germany), and digitalized pictures of the membranes were analyzed densitometrically with the ImageJ software (v1.46k; National Institutes of Health). The results are displayed as integrated density value, relative to glycerinaldehyde-3-phosphat-dehydrogenase.

Protein Oxidation
The detection of protein carbonyls in the diaphragm muscle was performed using a protein oxidation detection kit (OxyBlot, Millipore, Billerica, MA). Diaphragm muscle samples were homogenized using a KPO4 buffer, and the total protein concentration was determined with the Bradford method. Derivatization of the samples, with dinitrophenyl hydrazine, was performed for 15 minutes on 10 μg of total protein after manufacturer’s instructions. The DNP-derivatized proteins were separated on a 12% SDS/polyacrylamide gel, transferred to a polyvinylidene difluoride membrane, and then stained with Ponceau S. After blocking, blots were incubated with the anti-DNP primary and its secondary antibody, both delivered with the detection kit, then developed using chemiluminescence (GE Healthcare, Düsseldorf, Germany) and analyzed with the software package (Bio-1D, Vilber Lourmat, France) of the imaging system (Photo Print, Vilber Lourmat, France). To quantify the amount of oxidation, we defined the oxidative index, which is the ratio between densitometric values of the oxidized proteins and the Ponceau S-stained bands.

Statistical Analysis
The sample size of each n = 8 was chosen because of our experimental experience with this model from recent publications that allows to detect significant differences between MV and Con groups. In this pilot study setup, therefore we chose to use 8 animals per group.

Comparisons between groups for each dependent variable were made by a 1-way analysis of variance (ANOVA), followed by a Tukey post hoc test. We formally tested normality and homogeneity of variance of the residuals by applying Shapiro-Wilk test and Levene test, respectively, with α = 0.05. If any of these 2 assumptions was violated, we performed a Kruskal-Wallis test followed by a Dunn post hoc test instead.

The association between force and frequency in the 3 study groups was evaluated by a general linear mixed model with unstructured covariance matrix, accounting for repeated measurements within individual rats.

RESULTS
Each of the 3 experimental groups consisted of 8 rats. However, due to experimental reasons (missing bands, freezing damage, etc.), there were missing values for some of the variables. The number of individuals for each test is indicated in the figure legends. For all variables except calpain-1 and caspase-3, residuals of the ANOVA models were normally distributed (all P > 0.12) and homogeneity of variances was attained (all P > 0.063).

Arterial blood pressure was stable throughout the whole experimental time. Blood pressures were 116 ± 13 mm Hg (MV) and 110 ± 22 mm Hg (SB) before euthanization. Blood gas data were similar between SB and MV; the Pao2 at the end of the experiment was 103 ± 11 mm Hg (MV) and 110 ± 19 mm Hg (SB); and the Paco2 was 34 ± 4 mm Hg (MV) and 41 ± 8 mm Hg (SB), with a pH of 7.47 ± 0.03 (MV) and 7.42 ± 0.04 (SB). The MV group received 2.2 ± 0.25 vol % of sevoflurane and the SB group 2.1 ± 0.09 vol %, which according to MAC = 1 in rats.

Force Frequency Measurements
The force frequency curve was shifted downward significantly at all stimulation frequencies in the MV and SB groups compared with Con (P = 0.010 for SB versus Con and P = 0.014 for MV versus Con). The interaction between frequency and group was nonsignificant (P = 0.13), indicating that the difference between groups was similar at all stimulation frequencies (Fig. 1). The loss of diaphragm force in the MV and SB groups was similar (e.g., 160 Hz: −20% and −24%, respectively).

Muscle Histology
Histological analysis of diaphragm fiber dimensions did not reveal any significant differences in fiber cross-sectional areas in both SB and MV compared with Con (all ANOVA models have P > 0.17; Kruskal-Wallis test; Table 1).

Lipid Peroxidation
Diaphragmatic 4-hydroxynonenal levels used as an indicator of lipid peroxidation showed no significant differences in
both sevoflurane groups compared with Con (Supplemental Digital Content, http://links.lww.com/AA/B119), although the MV group had somewhat higher levels than the SB group: for MV compared with SB, the difference was 2.46 (95% CI, 0.02–4.89) with Tukey $P = 0.048$.

**Protein Oxidation**
Protein oxidation using OxyBlots did not show any statistically significant differences between the groups ($P = 0.65$; Supplemental Digital Content, http://links.lww.com/AA/B119).

**Calpain-1 and Caspase-3 Activity**
Ratios of the specific breakdown product of αII-spectrin/total αII-spectrin were assessed as markers of calpain-1 and caspase-3 activity, respectively. For calpain-1, the Levene test indicated significant heterogeneity of variances, and for caspase-3 residuals of the ANOVA model were not normally distributed. After log10-transformation, normality of residuals and homogeneity of variances was attained in both variables. Calpain-1 activity (145-kDa breakdown product/245-kDa uncleaved protein) was significantly elevated in MV ($P = 0.021$) but not in SB compared with Con ($P = 0.98$). Similarly, but less clear-cut, caspase-3 activity (120-kDa uncleaved protein) was significantly elevated in MV ($P = 0.048$) compared with Con ($P = 0.98$; Figs. 2 and 3).

**Levels of p-AKT/AKT and p-FoxO1/FoxO1**
Ratio of $p$-AKT/AKT used as a marker of anabolic signaling was significantly lower in MV ($P = 0.0004$) and SB ($P = 0.036$) compared with Con (Fig. 4). There were no statistically significant differences between the groups ($P = 0.65$; Supplemental Digital Content, http://links.lww.com/AA/B119).

**DISCUSSION**

**Overview of Principal Findings**
Our results provide detailed insight in the effects of one major anesthetic drug used in both the operating room and the ICU. Our findings demonstrate a contractile deficit in the diaphragm after 12 hours of sevoflurane exposure, with no additional effect of mechanical ventilation or muscle inactivity.

Sevoflurane did not induce oxidative stress or any atrophy; however, enhanced proteolysis was found after MV, as shown by increased calpain-1 and caspase-3 activity. Finally, reduced levels of the phosphorylated forms of AKT and FoxO1 after sevoflurane exposure suggest an increase in proteolytic signaling and a reduction in protein synthesis independent from diaphragmatic (in-)activity. A brief discussion of these points is given below.

**Sevoflurane Exposure over 12 Hours Reduces Diaphragm Contractile Force**
Our data show that sevoflurane reduces diaphragm contractile force at all stimulation frequencies and to the same extent in the SB group and in the MV group, indicating no additional effect of mechanical ventilation, at least after 12 hours of MV. Interestingly, no fiber atrophy was noticed in the diaphragm, suggesting that the loss of diaphragmatic contractile force was caused by a mechanism other than muscle atrophy. This is important, as we have shown contractile deficit and muscle atrophy after 24 hours of propofol exposure. In fact, sevoflurane has been reported to exert a negative interaction on cross-bridging itself in single cardiac muscle fibers, which reduced force and stiffness of the fibers. Other groups describe a decrease in force due to a reduction in Ca$^{2+}$ availability and sensitivity with sevoflurane. Furthermore, sevoflurane interacts with numerous channels and transporters involved in Ca$^{2+}$ homeostasis, such as the L-type Ca$^{2+}$ channel, Na$^{+}$/Ca$^{2+}$ exchanger, or the Ryanodine receptor in cardiac muscle. It is therefore possible that a channel effect may be responsible for the loss of diaphragmatic contractility in our study independent of the activity level of the diaphragm. The origin of these effects in diaphragm muscle remains unclear and should be investigated in future experiments.

Table 1. Fiber Dimensions of Diaphragm Cross-Sectional Areas

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<th>Control</th>
<th>SB</th>
<th>MV</th>
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<td><strong>Type I</strong></td>
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<td>Mean ($\mu$m$^2$)</td>
<td>1413</td>
<td>1689</td>
<td>1388</td>
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<td>SD</td>
<td>265</td>
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<td><strong>Type IIa</strong></td>
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<tr>
<td>Mean ($\mu$m$^2$)</td>
<td>1413</td>
<td>1689</td>
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<td>SD</td>
<td>265</td>
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<tr>
<td><strong>Type Ilx/b</strong></td>
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<tr>
<td>Mean ($\mu$m$^2$)</td>
<td>4097</td>
<td>1164</td>
<td>4167</td>
</tr>
<tr>
<td>SD</td>
<td>302</td>
<td>363</td>
<td>1413</td>
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Control ($n = 7$); SB ($n = 7$); MV ($n = 6$). Cross-sectional areas of diaphragmatic type I, type IIa, and Type Ilx/b fibers in the controls (Con), spontaneous breathing (SB), and mechanically ventilated (MV) groups. Data are expressed as mean and standard deviation (SD).
Sevoflurane Did Not Induce Oxidative Stress

In the present study, neither lipid peroxidation nor protein carbonyl formation was induced during 12 hours of sevoflurane exposition. This is partially in-line with our hypothesis, as oxidative stress is one of the major pathways activated during VIDD. Our data suggest that sevoflurane, at least during 12 hours of exposure, may hamper the oxidative stress production that commonly develops with CMV.2 By contrast, the use of propofol during 24 hours of MV did not protect against oxidative stress.17 Several publications have reported an interaction between sevoflurane and oxidative stress. Sevoflurane exerted protective effects in ischemia-reperfusion injury via a ROS-dependent activation.26 In preconditioning models, the cytoprotective effects of sevoflurane in cardiomyocytes is linked to short-term ROS production.27
which means that sevoflurane increases ROS in the beginning to attenuate the production in later stages. The direct exposure after the beginning of MV may induce ROS generation and attenuate the ongoing uncoupling of mitochondria due to inactivity. Further experiments need to address this issue in detail by investigating mitochondrial ROS generation. Depending on the statistical model, we found a significant difference between the SB and the MV group, indicating a decrease in lipid peroxidation in SB compared with MV. However, there were no significant differences between SB and Con, so that we can exclude an effect of SB, per se, for both SB groups (Con and SB).

**Sevoflurane Did Not Influence Major Proteolytic Pathways During MV**

Twelve hours of mechanical ventilation under sevoflurane exposure were associated with increased calpain-1 and caspase-3 activation. These data are in agreement with those obtained in ventilated animals anesthetized with pentobarbital. Interestingly, propofol sedation resulted in activation of calpain-1 in ventilated rats, whereas caspase-3 activation was hampered. In the current study, sevoflurane did not activate caspase-3 or calpain-1 in the SB group, and this has also been reported with propofol sedation in rats. However, the increase in calpain-1 and caspase-3 activity during MV but not during SB, emphasizes that the force-reducing effects of sevoflurane are independent of protease activation. It is, however, unclear how the combination of inactivity with sevoflurane may lead to activation of these 2 proteases because sevoflurane did not induce oxidative stress. Also intriguing is the fact that, in cardiac muscle, a decrease in caspase-3 activation has been proposed as one of the mechanisms responsible for the protective action of sevoflurane in preconditioning models. However, the differences between short-lasting sevoflurane action during preconditioning in comparison with ischemia-reperfusion injury are not completely transferable to the continuous exposure during chronic (e.g., 12 hours) and progressive damage in our model.

Finally, the activation of these proteases implies enhanced proteolysis, indicating that sevoflurane, when combined with CMV, activates protein breakdown, although atrophy was not yet detected in the diaphragm.

**Sevoflurane Interacts with the AKT/FoxO1 Pathway**

Exposure to sevoflurane decreased the phosphorylation state of AKT and FoxO1 significantly after 12 hours of sevoflurane exposure in both interventional groups. Active (phosphorylated) AKT plays a crucial role in muscle as a "switch" between protein synthesis by regulating the mammalian target of rapamycin signaling and protein translation. Moreover, phosphorylated AKT depresses muscle proteolysis by preventing FoxO1-induced protein autophagy and induction of ubiquitin ligases. Our results show a decreased activity of AKT in MV and SB and FoxO1 in the MV group after 12 hours of sevoflurane exposure in both groups and suggest that proteolysis and a reduction in protein synthesis may be induced and may lead to muscle atrophy, depending on the exposure time. The ratio of p-FoxO1/FoxO1 in SB was reduced (however with a \( P \) value of 0.059 not significantly changed) but may indicate a direct influence of sevoflurane on this pathway even during SB.

This has been reported in pentobarbital-anesthetized rats and in ICU patients. We recently described a decrease in p-AKT/AKT ratio in the diaphragm of animals sedated with propofol, and this was associated with muscle atrophy. In the latter study, MV was applied for 24 hours. The shorter time span of MV of only 12 hours in the actual study may explain why diaphragm atrophy was not detected, despite the activation of atrophy pathways. Nevertheless, these data suggest a direct influence of sevoflurane on one major pathway of muscle atrophy. During SB, these pathways are not influenced by inactivity and the observed effects are therefore drug-related.

**Clinical Implications**

The benefits of sevoflurane as a volatile sedative are its low blood gas distribution coefficient, its low water solubility, its low mucosal irritability, and its low cost. Can sevoflurane be an alternative to other short-acting drugs, especially propofol, in regard to diaphragmatic pathology? Propofol induces muscle atrophy after 24 hours. Due to different ventilation duration, it is unclear whether sevoflurane would induce atrophy after 24 hours of exposure. The fact remains that diaphragm force is reduced with sevoflurane, and this after only 12 hours of exposure. The major advantage of sevoflurane might be its very short action and low solubility, in contrary to propofol, which accumulates, especially in patients with impaired hepatic function. Sevoflurane does not confer protection against VIDD, in contrast to its described myocardial protection properties.

The clinical relevance of these findings is important not only for ICU patients but also for perioperative use because 12 hours of sevoflurane exposure can often be achieved in surgical procedures, such as in tumor surgery. It is of note that our model describes changes in a functioning organism and is not solely based on an in vitro experiment.

The rising availability of anesthetic-conserving devices in ICUs might also be a reason for a carefully considered long-term usage of sevoflurane. However, during phases of SB, sevoflurane might not be the ideal sedative drug because of its deleterious side effects on diaphragmatic force.

For clinical usage of sevoflurane, it should be remembered that it is not included in the European guidelines as a standard sedative on ICUs, and its application by scavenging devices should be carefully considered for economical reasons. Furthermore, the use of inhaled gases requires skilled hands and an understanding of the pitfalls and side effects of halogenated gases and their mode of application. It has to be kept in mind that our knowledge concerning safety of application is mainly generated in operating rooms.

**Limitations**

It was beyond the aim of this study to evaluate dose-effect relations. We sought to investigate the drug effect of a single dose without the need for an additional sedative, which excludes the application of lower MAC values. Otherwise, the application of sevoflurane doses >1 MAC would be without...
clinical relevance because its renal toxicity is linked to the doses and therefore would be obsolete in many ICU patients. A sophisticated view on the used MAC of 1 would be correct for balanced anesthesia with an opioid and a sedative. The clinical use of 0.5 MAC is suitable for combinations with an opioid. This study used a monosedation of sevoflurane, which was necessary to discriminate the effects of a single drug. No doubt the actual data cannot give an answer on the effects of lower sevoflurane concentrations on diaphragm function.

Both interventional groups could be sedated with equal sevoflurane concentration (i.e., 2.2 ± 0.25 vol % of sevoflurane in MV, 2.1 ± 0.09 vol % in SB). This is important because we had to ensure that the observed effects were not due to different gas concentrations. Nevertheless, the MV group did not breathe spontaneously and the breathing reflex was overruled by the ventilator, resulting in a lower Paco2. We did not investigate the duration of the effect on the contractile deficit although this may be relevant from a clinical point of view.

We did not include a control group in our study, which was SB for 12 hours using pentobarbital as anesthetic. The effects of pentobarbital anesthesia during SB on diaphragmatic function have been extensively investigated35–37 and have revealed a similar diaphragm function compared with acutely anesthetized animals. Following these experiments, we can interpret results of the Con group as a sufficient control for investigating the effects of sevoflurane.

CONCLUSIONS

Our study shows that sevoflurane rapidly and severely reduced diaphragm contractility after only 12 hours of exposure, independent of whether the diaphragm was contracting (SB) or not (CMV). Exposure to sevoflurane did not induce oxidative stress but led to a reduction in diaphragmatic force during SB and MV. These findings are of particular importance for clinical practice in ICUs, and bring into question sevoflurane use, especially during phases of SB.

Sevofluranes application on ICUs should be carefully considered, even though it does not require an anesthesia work station38 because volatile anesthetics and scavenging devices in common ICUs require specialized training for all (often multidisciplinary) participating professionals, who might not be originally experienced in its safe usage.

DISCLOSURES

Name: Thomas Breuer, MD.
Contribution: This author helped conduct the study, analyze the data, and write the manuscript.
Attestation: Thomas Breuer has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
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Contribution: This author helped analyze the data and review the manuscript.
Attestation: Karen Maes has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
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Contribution: This author helped write the manuscript.
Attestation: Rolf Rossaint has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.

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Attestation: Gernot Marx has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
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Contribution: This author performed the statistical analysis of the data.
Attestation: Hans Scheers has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
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Contribution: This author helped conduct the study.
Attestation: Ingmar Bergs has seen the original study data and approved the final manuscript.
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Contribution: This author helped conduct the study and write the manuscript.
Attestation: Christian Bilelevens has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
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Contribution: This author helped analyze the data and write the manuscript.
Attestation: Ghislaine Gayan-Ramirez has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
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Contribution: This author helped design the study, conduct the study, analyze the data, and write the manuscript.
Attestation: Christian S. Bruells has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files.

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REFERENCES

Sevoflurane Reduces Diaphragm Force and Prevents ROS Effects

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