

Intramuscular Injection of Sevoflurane Detects Malignant Hyperthermia Predisposition in Susceptible Pigs

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Background: The authors hypothesized that intramuscular sevoflurane injection allows diagnostic differentiation between malignant hyperthermia–susceptible (MHS) and –nonsusceptible (MHN) pigs by measurement of intramuscular lactate and carbon dioxide partial pressure (PCO$_2$), and that dantrolene reduces the sevoflurane-induced PCO$_2$ increase.

Methods: With approval of the local animal care committee, microdialysis probes with attached microtubing for sevoflurane injection were placed in the adductor muscles of nine MHS and six MHN pigs, and PCO$_2$ probes with microtubing were positioned in the triceps muscle of eight MHS and six MHN pigs. After equilibration, sevoflurane boluses at different concentrations and a sevoflurane–dantrolene bolus were injected synchronously. Lactate, pyruvate, and glucose as well as PCO$_2$ were measured spectrophotometrically, and the rate of PCO$_2$ increase was calculated.

Results: Intramuscular sevoflurane injection increased local lactate and PCO$_2$ dose dependently, and significantly higher in MHS than in MHN pigs. Measurement of the rate of PCO$_2$ increase allowed a distinct differentiation between single MHS and MHN pigs. No significant increase in PCO$_2$ was found with sevoflurane and dantrolene.

Conclusions: Local sevoflurane induces a hypermetabolic reaction measured by PCO$_2$ and lactate increases. The reduced PCO$_2$ increase in MHS after sevoflurane and dantrolene injection is likely to be a result of the sevoflurane-mediated calcium release and its antagonism by dantrolene. Sevoflurane may be useful for a less invasive diagnostic test for malignant hyperthermia in humans.

SEVOFLURANE is one of the most common volatile anesthetics used for general anesthesia. Its low blood-gas partition coefficient allows rapid induction of and fast recovery from anesthesia.1 However, sevoflurane is also a triggering agent for malignant hyperthermia (MH), a potentially fatal hypermetabolic syndrome of skeletal muscle.2 In susceptible individuals, an uncontrolled myoplasmic calcium release via a mutated ryanodine receptor results in muscle rigidity, enhanced mitochondrial energy turnover with excessive oxygen consumption, carbon dioxide, and heat production as well as metabolic acidosis.3 MH susceptibility is diagnosed by an invasive in vitro contracture test using halothane and caffeine according to a standardized protocol.4,5 Because halothane might become commercially unavailable in some countries in the future,6 the European Malignant Hyperthermia Group (University of Basel, Switzerland) decided to investigate sevoflurane as a potential substitute.7 Nevertheless, a muscle biopsy for contracture testing is hampered by risks of wound infection, bleeding, and sensory deficit as well as high costs. Recently, a less invasive metabolic test by measurement of intramuscular carbon dioxide partial pressure (PCO$_2$) and lactate after local injection of halothane and caffeine was proposed that allows a differentiation between MH-susceptible (MHS) and MH-nonsusceptible (MHN) individuals.8,9 In comparison with halothane, sevoflurane was found to be less potent in contracture testing for MH and is also suggested to be a less potent trigger for clinical MH.10 In this context, it remains unclear whether intramuscular injection of sevoflurane leads to a local hypermetabolic reaction to use sevoflurane for a less invasive diagnostic test for detecting individuals at risk for MH.

In the current investigation, we hypothesized that local injection of sevoflurane increases intramuscular lactate concentration and the rate of PCO$_2$ increase dose dependently in MHS but not in MHN pigs and that the hydantoin derivative dantrolene inhibits the sevoflurane-induced PCO$_2$ increase.

Materials and Methods

Experimental Protocol

With approval of the local animal care committee (Government of Unterfranken, Würzburg, Germany), homozygous MHS and MHN Pietrain pigs (Farm Will, Mellrichstadt, Germany) weighing 19–37 kg were investigated. The pigs were purchased from two local farmers and were derived from several long-standing colonies. All of the pigs used here were related to one another to varying degrees up to first degree. MH susceptibility or wild type was determined by DNA analysis regarding the presence of a homozygous arginine 615 mutation of the ryanodine receptor indicating MHS.

Anesthesia was induced intravenously with thiopental (14–17 mg/kg) and maintained using a midazolam (0.2–0.4 mg·kg$^{-1}$·h$^{-1}$)+fentanyl (0.01–0.04 mg·kg$^{-1}$·h$^{-1}$) infusion. After tracheal intubation (7.0-mm-ID endotracheal tube; Rüsch, Kernen I.R., Germany), animals were mechanically ventilated (Servo 900C; Siemens, Erlangen, Germany) with 50% oxygen and 50% air. Ventilator settings were adjusted to maintain an end-tidal PCO$_2$ of 30–35 mm Hg (respiratory rate, 12–14 breaths/min; tidal volume, 10–15 ml/kg; positive end-expiratory pressure,
5 mm Hg). Vital signs were monitored by blood pressure monitoring in the saphenous artery and by peripheral oxygen saturation, electrocardiography, and rectal temperature measurements.

Introducer cannulae were placed under ultrasound guidance (SonoSite 180 Plus; SonoSite Inc., Bothell, WA) in parallel to the muscle fibers of the adductor muscles in the hind limb of nine MHS and six MHN animals. Four microdialysis probes (MAB 7; Microbiotech, Stockholm, Sweden) with an attached microtubing catheter (Pajunk, Geisingen, Germany) for single sevoflurane injection were placed at different locations in the muscle at a distance of at least 30 mm and perfused with Ringer’s solution (B. Braun, Melsungen, Germany) at 1 μl/min. The tip of the microtubing was adjusted to 5 mm proximal to the tip of the microdialysis membrane. After 30 min of equilibration, single boluses of 100 μl sevoflurane (Abbott, Wiesbaden Germany) at 3, 7.5, 15, and 28 vol%, dissolved in soybean oil (Intralipid; Baxter, Unterschleißheim, Germany), were injected simultaneously via the microtubing catheter next to the membrane of the microdialysis probes.

In a second series of experiments, cannulae for PCO2 probes (ParaTrend 7+; Diametrics Medical Inc., High Wycombe, Buckinghamshire, United Kingdom) and microtubing catheters were placed under ultrasound guidance in the triceps muscle of the forelimbs. For correct positioning, a sham test using plain Ringer’s solution (B. Braun, Melsungen, Germany) at 1 μl/min was performed. PCO2 decreased and returned to initial baseline levels within 20 min if the probes were placed correctly in the muscle tissue. After equilibration, 100 μl sevoflurane at 2.5, 5, 7.5, and 15 vol% and 100 μl sevoflurane at 7.5 vol% with 0.52 mM dantrolene (Procter & Gamble, Weiterstadt, Germany) dissolved in Ringer’s solution were injected via the microtubing catheter. Because of limited availability of PCO2 probes and monitors, PCO2 experiments were performed as follows. Experiments with 15 vol% sevoflurane and 7.5 vol% sevoflurane−0.52 mM dantrolene were studied in the forelimb of the same animals taking microdialysis samples. Sevoflurane at 2.5, 5, and 7.5 vol% was investigated in another eight MHS and six MHN animals. For technical reasons, measurements failed in a ninth MHS animal. After sevoflurane injection and measurement, PCO2 probes were replaced, and measurements were repeated at a different concentration after ensuring physiologic baseline levels.

Sevoflurane was dissolved in soybean oil, and dantrolene was dissolved in sterile water at room temperature by stirring and injected within 30 min of preparation.

Lactate, pyruvate, and glucose concentrations were measured spectrophotometrically in the dialysate. Samples were collected in intervals of 15 min before and in intervals of 7.5 min after sevoflurane injection. PCO2 was recorded in 1-min intervals. Systemic hemodynamic parameters were monitored throughout the experiment.

In general, different concentrations of sevoflurane were studied synchronously at a distance of at least 30 mm. Previously, no interaction was found between different measurement sites.9

Microdialysis

Flexible microdialysis probes with a molecular cutoff of 15,000 Da, an 80-mm shaft, and a 10-mm semipermeable polyethylenesulfone membrane were used. The inlet of the microdialysis catheter was connected to syringes in a high-precision micro pump (Hamilton Gastight Syringe, Reno, NV; PHD 2000 Syringe Pump, Harvard Apparatus, Holliston, MA) and perfused with Ringer’s solution at a constant flow of 1 μl/min. After the probes were implanted in the tissue, substances passed through the semipermeable membrane from higher to lower concentrations until equilibrium was reached. Samples were collected and analyzed immediately after the experiment by use of a microdialysis auto-analyser (ISCU Clinical Microdialysis Analyzer; CMA, Solna, Sweden) by enzymatic spectrophotometric assays for lactate, pyruvate, and glucose.

Diffusion properties of the membrane were determined before and after every sixth experiment by measurement of the in vitro recovery for lactate at 1 μl/min flow with Ringer’s solution while placed in a 40 μl lactate solution. The in vitro recovery was calculated by the ratio of lactate in the dialysate and the lactate concentration outside the probe. Microdialysis probes with an in vitro recovery for lactate less than 70% were discarded.

Carbon Dioxide Measurements

The fiberoptic sensor used in this study contains an optode to determine PCO2, which is housed within a heparin-coated microporous polyethylene membrane. The cylindrical design of the sensor allows measurements over the entire surface of the probe. Before the first measurement the probe was calibrated in vitro using precision gases of different concentrations in a standardized tonometer solution for 30 min (TrendCare Calibrator; Diametrics Medical Inc.). At the end of every experiment, probes were placed in Ringer’s solution. Probes were reused depending on successful recalibration in vivo and successful sham test using plain Ringer’s solution. For in vitro calibration, probes were placed intravascularly, and carbon dioxide was determined in the same vessel by blood gas analysis and adjusted. The high-cost probes were single use only but designed for use over several days for on-line gas analysis, e.g., in intensive care patients. The rate of PCO2 increase was calculated by the maximal slope of PCO2 starting from baseline PCO2 just before drug application.
Statistical Analysis

Experimental order for MHN and MHS animals was random and determined by availability of pigs independent of MH status. The Kolmogorov-Smirnov test was performed to test data for parametric distribution. Parametric data are displayed as mean and SD. Two-way analysis of variance was applied to test for differences with regard to maximum lactate, pyruvate, and glucose in the microdialysis experiments and for the PCO2 measurements between MHS and MHN pigs and between the used drug concentrations and its interaction. Afterward, the t-test was used for pairwise comparison of the groups. The Bonferroni correction was applied for multiple measurements. A value of P < 0.05 was considered statistically significant.

Results

Biometric and systemic hemodynamic parameters did not differ between MHS and MHN pigs in both groups of animals (table 1).

Dose-Response Effect of Lactate, Pyruvate, and Glucose

Intramuscular lactate, pyruvate, and glucose levels did not significantly differ between MHS and MHN pigs after injection of Ringer’s solution and before sevoflurane application.

Intramuscular injection of sevoflurane resulted in a significant increase of local lactate in MHS compared with MHN animals. However, in the same MHS and MHN animals, results overlapped (fig. 1). Interstitial glucose concentration was not significantly altered by increasing sevoflurane concentrations in both groups, whereas pyruvate and the lactate/pyruvate ratio increased dose dependently in MHS animals (tables 2 and 3).

Dose-Response Effect of Carbon Dioxide

Intramuscular PCO2 concentrations before trigger application did not differ between MHS and MHN individuals.

The rate of PCO2 increase was significantly higher in MHS animals after local injection of 2.5, 5, 7.5, and 15 vol% sevoflurane dissolved in soybean oil compared with MHN. The values of the PCO2 rates did not overlap between diagnostic groups with 5 and 7.5 vol% sevoflurane (fig. 2). The rate of PCO2 increase in MHS was significantly smaller with a mixture of 7.5 vol% sevoflurane and dantrolene compared with solely 7.5 vol% sevoflurane (tables 3 and 4).

Discussion

The current study demonstrates that intramuscular sevoflurane injection increases local metabolism as measured by an increase of lactate and PCO2 in MHS more than in MHN animals. In this context, sevoflurane seems to be a suitable substance for a less invasive metabolic test to diagnose MH susceptibility in humans. The minor PCO2 increase in MHS after sevoflurane and dantrolene injection likely reflects the agonism of sevoflurane on sarcoplasmic calcium release and its antagonism by dantrolene.

The volatile anesthetic sevoflurane increases myoplasmic calcium release via the ryanodine receptor and by inositol 1,4,5-triphosphate stimulation. Excessive and uncontrolled calcium release is suggested to be causative for the triggering potency for MH in susceptible individ-

Table 1. Systemic Hemodynamic Parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>HR, beats/min</th>
<th>MAP, mm Hg</th>
<th>SpO2, %</th>
<th>Rectal Temperature, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microdialysis group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MHSa</td>
<td>75 ± 3</td>
<td>78 ± 6</td>
<td>98 ± 1</td>
<td>36.7 ± 0.5</td>
</tr>
<tr>
<td>MHSb</td>
<td>71 ± 3</td>
<td>83 ± 10</td>
<td>98 ± 1</td>
<td>37.1 ± 0.5</td>
</tr>
<tr>
<td>MHNa</td>
<td>76 ± 6</td>
<td>75 ± 13</td>
<td>99 ± 1</td>
<td>36.8 ± 0.3</td>
</tr>
<tr>
<td>MHNb</td>
<td>72 ± 5</td>
<td>77 ± 10</td>
<td>98 ± 2</td>
<td>37.2 ± 0.3</td>
</tr>
<tr>
<td>PCO2 group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MHSa</td>
<td>75 ± 6</td>
<td>77 ± 8</td>
<td>98 ± 1</td>
<td>36.8 ± 0.5</td>
</tr>
<tr>
<td>MHSb</td>
<td>73 ± 4</td>
<td>79 ± 7</td>
<td>99 ± 1</td>
<td>37.0 ± 0.5</td>
</tr>
<tr>
<td>MHNa</td>
<td>75 ± 10</td>
<td>74 ± 7</td>
<td>98 ± 1</td>
<td>37.0 ± 0.5</td>
</tr>
<tr>
<td>MHNb</td>
<td>72 ± 5</td>
<td>79 ± 5</td>
<td>98 ± 2</td>
<td>37.0 ± 0.4</td>
</tr>
</tbody>
</table>

Systemic hemodynamic parameters before intramuscular application of sevoflurane (A) and at the end of the investigation (B). Data are expressed as mean ± SD. Microdialysis group: n = 9 for malignant hyperthermia susceptible (MHS; 28.3 ± 4.1 kg) and n = 6 for malignant hyperthermia nonsusceptible (MHN; 31.9 ± 3.4 kg); carbon dioxide partial pressure (PCO2) group: n = 8 for MHS (30.4 ± 3.3 kg) and n = 6 for MHN (31.6 ± 2.4 kg). HR = heart rate; MAP = mean arterial blood pressure; SpO2 = peripheral oxygen saturation.

Fig. 1. Maximum intramuscular lactate concentrations after local injection of 100 μl Ringer’s and sevoflurane dissolved in soybean oil at 3, 7.5, 15, and 28 vol% in malignant hyperthermia-susceptible pigs (MHS) and malignant hyperthermia-non-susceptible pigs (MHN).
In previous investigations, injection on skeletal muscle metabolism were excluded and 100 cell damage. Local side effects of pure soybean oil philic carrier allows intravenous anesthesia without similarly to halothane, sevoflurane dissolved in a li-

hypermetabolic state of skeletal muscle. It is likely that observed only in MHS individuals, reflecting the local response effect for lactate and for the lactate/pyruvate concentration.

Similar to halothane and caffeine, in vitro exposure of sevoflurane induces dose-dependent contractures in hu-

man skeletal muscle. In the current study, a dose-response effect for lactate and for the lactate/pyruvate ratio after intramuscular injection of sevoflurane was observed only in MHS individuals, reflecting the local hypermetabolic state of skeletal muscle. It is likely that similarly to halothane, sevoflurane dissolved in a lipophic carrier allows intravenous anesthesia without cell damage. Local side effects of pure soybean oil injection on skeletal muscle metabolism were excluded in previous investigations.

Evaluation of the lactate/pyruvate ratio allows indirect conclusions on the intracellular energy state and energy consumption. The cellular calcium overload induced by ryanodine receptor agonists such as halothane and caffeine increases energy consumption by activation of contractile filaments and initiates a decrease in the adenosine triphosphate/adenosine diphosphate ratio leading to an increase in the free cytoplasmic nicotinamide-adenine dinucleotide/nicotinamide-adenine dinucleotide oxidized ratio, which increases the lactate/pyruvate ratio in MHS. For sevoflurane, similar results were obtained.

The deterioration of intracellular calcium homeostasis in MHS individuals leads to hypoxia, hypercapnia, lactic acidosis, and temperature increase. Because carbon dioxide production is the innate attribute to hypermetabolism, monitoring of the highly diffusible carbon dioxide is a suitable tool to investigate metabolic alterations during metabolic testing in vivo. Intramuscular injection of all studied concentrations of sevoflurane resulted in an increase of the PCO2 rate in MHS but not in MHN pigs.

Lactate does not increase in one animal at 15 and 28 vol% sevoflurane. Several reasons may account for this, i.e., methodologic problems due to misplacement of the microdialysis probe or unknown distribution of the trig-

### Table 2. Maximum Intramuscular Lactate, Pyruvate, and Glucose Concentrations and Lactate/Pyruvate Ratio after 100 μl Ringer’s and 100 μl Sevoflurane Injection

<table>
<thead>
<tr>
<th>Sevoflurane in Soybean Oil</th>
<th>Lactate, mM</th>
<th>Pyruvate, mM</th>
<th>Glucose, mM</th>
<th>Lactate/Pyruvate Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ringer’s</td>
<td>100 μl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 vol%</td>
<td>1.5 ± 0.5</td>
<td>11.1 ± 0.3</td>
<td>9.096</td>
<td></td>
</tr>
<tr>
<td>7.5 vol%</td>
<td>3.8 ± 1.7</td>
<td>9.0 ± 0.2</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>15 vol%</td>
<td>7.8 ± 4.9</td>
<td>9.0 ± 0.2</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>28 vol%</td>
<td>10.2 ± 5.7</td>
<td>12.5 ± 0.5</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD; t test for differences between malignant hyperthermia susceptible (MHS) and malignant hyperthermia nonsusceptible (MHN); P < 0.05; n = 9 for MHS pigs, n = 6 for MHN pigs.

### Table 3. Results (P Values) of Univariate Test of Main and Interaction Effects of Two-way Analysis of Variance with Repeated Measurements

<table>
<thead>
<tr>
<th></th>
<th>Inner Main Effects</th>
<th>Inner Interaction Effects</th>
<th>Diagnosis MHS vs. MHN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate</td>
<td>0.009</td>
<td>0.086</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.069</td>
<td>0.564</td>
<td>0.098</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.019</td>
<td>0.504</td>
<td>0.604</td>
</tr>
<tr>
<td>Lactate/pyruvate ratio</td>
<td>&lt;0.0001</td>
<td>0.011</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PCO2</td>
<td>0.02</td>
<td>0.001</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Analysis performed for each measured substance (lactate, pyruvate, glucose, lactate/pyruvate, and carbon dioxide partial pressure [PCO2], representing the within group variability with five levels (Ringer’s and sevoflurane concentrations) and diagnosis (malignant hyperthermia susceptible [MHS] and malignant hyperthermia nonsusceptible [MHN]) as the between-group variability. In case of sphericity violation, the within-group variability is tested using the conservative Greenhouse-Geisser statistic.

Fig. 2. Individual rate of carbon dioxide partial pressure (PCO2) increase after local injection of sevoflurane dissolved in soybean oil at 2.5, 5, 7.5, and 15 vol% in malignant hyperthermia-susceptible pigs (MHS) and malignant hyperthermia-nonsusceptible pigs (MHN).
ger drug in the interstitial space of the muscle, or even as a physiologic response similarly to MH susceptible patients who do not develop hypermetabolism despite trigger exposition.

In general, this is a quantitative test measuring the metabolic response on exposure to sevoflurane. Similarly to contracture testing, a gray zone including overlapping is expected with this biologic testing system and sensitivity should be increased by repeated measurements as it is done in contracture testing in the European MH protocol, i.e., a single contracture of 2 mN in one out of two halothane and two caffeine contracture tests assigns the patient at risk for MH. Furthermore, this test has not been validated that it is normal in animal models of other muscle diseases. It cannot be excluded that this functional test may lead to a substantial increase in lactate also due to other myopathies than MH, as is the case for the in vitro contracture test.

The hydantoin derivative dantrolene is the only clinically approved drug to treat MH. The cytosolic calcium concentration is reduced by inhibition of sarcoplasmic calcium release without influencing its reuptake.\textsuperscript{16} In vitro, dantrolene reduces muscle contractures in MHS individuals.\textsuperscript{17} However, to date, the exact mode of action has been incompletely understood. Investigations with radioactive ligands localized dantrolene and ryanodine binding sites closely associated with sarcoplasmic reticulum membrane fractions.\textsuperscript{18} Dantrolene inhibition of the sevoflurane-induced sarcoplasmic calcium release in this study is supported by the finding that in an isolated muscle model dantrolene perfusion also reduced caffeine-induced lactate increase in rats.\textsuperscript{19}

In conclusion, intramuscular injection of sevoflurane increases local skeletal muscle metabolism measured by lactate and $P_{\text{CO}_2}$ in MHS compared with MHN individuals. Whereas calculating the rate of $P_{\text{CO}_2}$ increase after injection of 5 and 7.5 vol% sevoflurane allows a differentiation between MHS and MHN animals, local lactate levels overlapped between diagnostic groups. $P_{\text{CO}_2}$ measurement after intramuscular sevoflurane seems to be a suitable method for the development of a less invasive metabolic test to diagnose MH susceptibility. Further validation of this metabolic test in humans needs clear-cut clinical triggers, genotyped probands, and results in patients harboring other neuromuscular diseases as well as results in a second laboratory on the same patient using the identical technique.

References