

## *Xenon Does Not Trigger Malignant Hyperthermia in Susceptible Swine*

Gebhard Froeba, M.D.,\* Thomas Marx, M.D.,\* Johannes Pazhur, M.D.,† Christoph Baur, M.D.,\* Stefan Baeder, B.S.,‡ Enrico Calzia, M.D.,\* Hans M. Eichinger, Ph.D.,§ Peter Radermacher, M.D., Ph.D.,|| Michael Georgieff, M.D., Ph.D.#

**Background:** Xenon is a noble gas with anesthetic properties currently under investigation for use in humans. This study was performed to evaluate whether xenon may trigger malignant hyperthermia in susceptible swine.

**Methods:** Nine malignant hyperthermia-sensitive swine (Pietrain) were initially anesthetized with pentobarbital and then ventilated with 70% xenon in oxygen for 2 h. Heart rate, mean arterial pressure, cardiac output, body temperature, arterial and mixed-venous blood gases, and plasma catecholamine and lactate levels were measured every 10 min both during xenon-oxygen ventilation and after a 30-min xenon washout phase followed by subsequent administration of halothane (1% inspired) and succinylcholine (3 mg/kg intravenous). During the investigation, no malignant hyperthermia-specific therapy was instituted.

**Results:** Xenon exposure did not induce any changes in metabolic and hemodynamic parameters nor elevations of the plasma catecholamine levels indicative for an episode of malignant hyperthermia. By contrast, in all animals, within 20 min after the administration of halothane and succinylcholine, ful-

minant and fatal malignant hyperthermia episodes were initiated.

**Conclusions:** The authors conclude that xenon does not trigger malignant hyperthermia in susceptible swine. (Key words: Ryanodine receptor; volatile anesthetics.)

MALIGNANT hyperthermia (MH) is a potentially lethal, frequently autosomal-dominant, pharmacogenetic disorder of skeletal muscle that can be triggered by volatile anesthetics and succinylcholine.<sup>1-3</sup> Xenon, a noble gas with anesthetic properties offers many potential advantages over routinely used gases.<sup>4-6</sup> The improvement of closed-circuit anesthesia<sup>7,8</sup> and the development of a xenon-recycling device<sup>9</sup> have renewed the interest in xenon, and as a result xenon is currently under investigation for use in humans.

The possibility of xenon triggering MH in susceptible individuals has not been investigated previously. Therefore, this study tested whether xenon may trigger MH in the purebred Pietrain swine model of MH, considered susceptible to MH verified by genetic analysis.<sup>10-12</sup>

### Material and Methods

Nine MH-susceptible (MHS) swine from a colony of Pietrain swine ( $31 \pm 5$  kg body weight) were studied. The study protocol was approved by the animal care committee of the administration district and was performed in accordance with the legal regulations for use of laboratory animals.

#### *Genetic Testing*

All pigs had been tested for the presence of the *RYR1arg-615-cys* mutation by restriction analysis on genomic DNA. This mutation is associated with an abnormally high release of calcium in the skeletal muscle, mediated *via* an abnormal ryanodine receptor specific for porcine MH.<sup>11,12</sup> Genomic DNA was isolated from blood preserved in edetic acid. A restriction enzyme

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\* Staff Anesthetist.

† Resident in Anesthesiology.

‡ Medical Engineer.

§ Professor of Agriculture, Lehrstuhl für Tierzucht der Technischen Universität München, Germany.

|| Professor of Anesthesiology.

# Professor of Anesthesiology and Chairman.

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Address reprint requests to Dr. Froeba: Universitätsklinik für Anästhesiologie, Universität Ulm, Steinhövelstr. 9, D-89075 Ulm/Donau, Germany. Address electronic mail to: gebhard.froeba@medizin.uni-ulm.de

assay was used to test for the presence of mutations in the ryanodine receptor gene on chromosome 6.<sup>11</sup>

#### *Anesthesia and Surgical Preparation*

Each animal was fasted overnight with free access to water. All swine were premedicated with 4 mg/kg intramuscular azaperone 1 h before surgical preparation. Anesthesia was induced with 8 mg/kg pentobarbital sodium *via* ear vein. The swine was stabilized in the dorsal recumbent position, and an endotracheal tube was inserted into the trachea during spontaneous respiration. Muscle paralysis was then achieved with alcuronium dichloride (0.25 mg/kg). Ventilation was performed with a standard semiclosed circuit anesthesia machine (Cicero, Draegerwerk AG, Luebeck, Germany) modified to provide for xenon application, with 70% nitrogen in oxygen and a fresh gas flow of 1 L/min. Ventilatory settings throughout the preparation and recovery periods were tidal volume, 10–14 ml/kg (adjusted in order to achieve an arterial carbon dioxide tension of 37–43 mmHg); ventilator frequency, 12/min; and positive end-expiratory pressure, 5 cm H<sub>2</sub>O. Anesthesia was maintained using a continuous intravenous infusion of pentobarbitone sodium (0.14–0.20 mg · kg<sup>-1</sup> · min<sup>-1</sup>) and one dose of 0.01 mg/kg buprenorphine at the beginning of the surgical preparation. Depth of anesthesia was controlled by continuous electroencephalographic monitoring (Neurotrac, Interspec, Conshohocken, PA). The spectral edge frequency was always below 15 Hz; the median power frequency was 5–10 Hz. In previous experiments this pentobarbital infusion rate provided full anesthesia.<sup>13,14</sup> Ringer's solution (8 mg · kg<sup>-1</sup> · h<sup>-1</sup>) was continuously administered as maintenance fluid. A lead-II electrocardiogram was monitored, and catheters were advanced *via* femoral cutdown into the femoral artery and vein. A 7-French thermistor-tipped flotation catheter was inserted *via* an external jugular vein cutdown into the pulmonary artery. Body temperature was monitored simultaneously by a rectal probe and from the thermistor of the pulmonary artery catheter. Temperature was maintained between 36.5°C and 37.5°C using a heat pad during preparation and recovery period.

#### *Measurements*

Systemic and pulmonary artery pressures, as well as central venous pressure, were continuously recorded using calibrated pressure transducers (model 1290A, Hewlett Packard, Rockville, MD) aligned at right atrial level. Cardiac output was measured using the thermodilution technique (Hewlett Packard HP M1166A). The

values recorded are the means of triplicate injections of 10 ml of ice-cold saline randomly spread over the respiratory cycle. Inspiratory and expiratory oxygen concentrations were measured continuously by the machine-integrated monitor (Draegerwerk AG), as were carbon dioxide and halothane concentrations. Concentrations of xenon, oxygen, and nitrogen in the inspiratory limb of the ventilator circle were measured by mass spectrometry (Fisons Gaslab 3000, Birmingham, UK). The mass spectrometer was calibrated before each experiment with a mixture of 70% xenon, 20% oxygen, and 10% nitrogen. The effective working range of the mass spectrometer was 1–100%, with an error of  $\pm 1.0\%$  and a measurement interval of 4 s. For the measurement of minute volume, a rotating vane flowmeter was used.

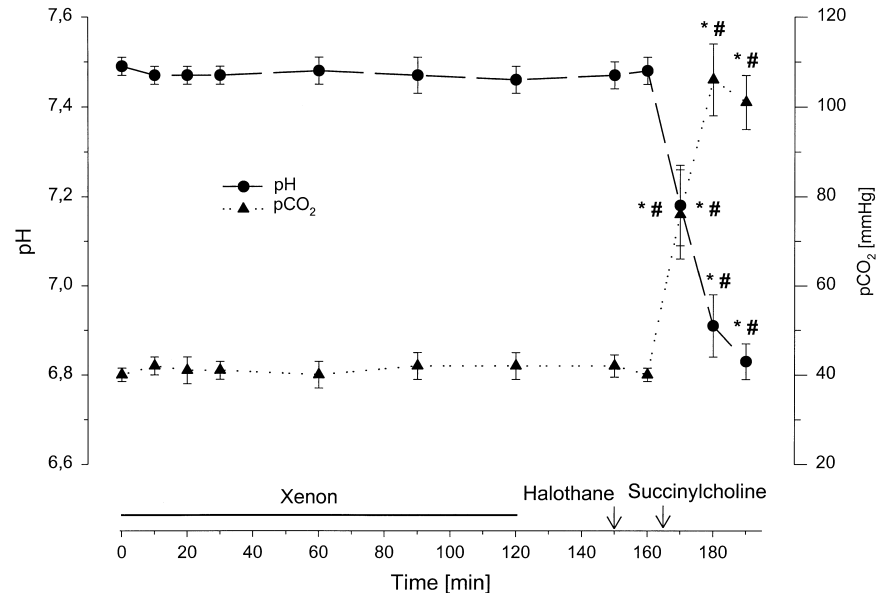
Arterial and mixed venous blood samples were obtained for measurement of arterial and mixed venous hemoglobin oxygen saturation and total hemoglobin concentration (Cooximeter IL 682 calibrated for swine blood, Instrumentation Laboratory, Lexington, MA). Arterial lactate concentrations were enzymatically measured in duplicate using an automatic analyzer (YSI Model 2300 STAT, Schlag Wissenschaftliche Messinstrumente, Bergisch-Gladbach, Germany). The O<sub>2</sub> content, arterial-venous O<sub>2</sub> content difference (avD<sub>O<sub>2</sub></sub>), and O<sub>2</sub> consumption ( $\dot{V}_{O_2}$ ) were calculated using the following formulae: O<sub>2</sub> content = (hemoglobin concentration  $\times$  1.39)  $\times$  O<sub>2</sub> saturation + (O<sub>2</sub> tension  $\times$  0.0031); avD<sub>O<sub>2</sub></sub> = arterial O<sub>2</sub> content – mixed venous O<sub>2</sub> content;  $\dot{V}_{O_2}$  = avD<sub>O<sub>2</sub></sub>  $\times$  cardiac index. Furthermore, arterial blood samples for the measurement of norepinephrine and epinephrine concentrations were obtained simultaneously. Plasma catecholamine concentrations were then measured using reversed-phase high-performance liquid chromatography with electrochemical detection. The lower limit of detection was 15 pg/ml.<sup>15</sup>

#### *Experimental Protocol*

After instrumentation, animals were allowed to recover for 1 h. During that period the pentobarbitone infusion was reduced to 0.09 (0.06–0.12) mg · kg<sup>-1</sup> · min<sup>-1</sup>, in order to avoid any potential hemodynamic depression induced by the additional xenon administration. Ventilation was continued using the ventilatory settings used before the experiment, and these were not changed throughout the whole experiment. After control data (0 min) had been obtained, the heat pad used to maintain temperature was switched off, and xenon (70% inspired) was administered. Blood sampling and cardiac output measurement were repeated every 10 min for

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**Fig. 1.** Time course of pH and arterial carbon dioxide tension during xenon administration, followed by halothane and succinylcholine. Data are presented as medians as well as the 25th and the 75th percentiles of nine observations. \* $P < 0.05$  vs. baseline data (times 0 min, 150 min, respectively); # $P < 0.05$  vs. xenon group.



2 h. After 2 h, xenon was discontinued and allowed to wash out over a period of 30 min. In a complementary investigation, a washout phase of 20 min reduced the expiratory xenon concentration to levels below 1%.<sup>16</sup> After a second set of control data (0 min), the animals ( $n = 9$ ) were connected to a second standard semiclosed circuit anesthesia machine (Cicero, Draegerwerk AG) in order to avoid contamination with halothane during the phase of xenon exposure. Then halothane (1% inspired, end-tidal concentration 0.7%) was administered, followed 15 min later by succinylcholine 3 mg/kg intravenously. Blood sampling and cardiac output measurement was repeated every 10 min. The animals were studied until death.

#### Statistical Analysis

Values are expressed as median as well as 25th and 75th percentiles if not stated otherwise. After exclusion of normal distribution, within-group comparisons were analyzed with a Friedmann rank sign analysis of variance and a subsequent Wilcoxon-Wilcox test for multiple comparisons. Intergroup differences were analyzed using a Mann-Whitney U test for independent samples with  $\alpha$ -adjustment according to Bonferroni. Statistical significance was considered at  $P < 0.05$  and  $P < 0.01$  (0.05:10) for intergroup differences, respectively.

#### Results

All pigs were found to be homozygous for the *RYR1arg-615-cys* mutation. There were no differences

between the two baseline measurements before xenon or halothane administration. The metabolic and gas-exchange responses during xenon or halothane and succinylcholine administration are shown in figures 1–4. The hemodynamic responses to a prolonged 2-h xenon administration are presented in table 1.

Xenon anesthesia slightly reduced both heart rate ( $P < 0.05$ ) and cardiac index ( $P < 0.05$ ); systemic arterial and pulmonary arterial pressure remained unaffected (table 1). None of the hemodynamic (table 1), gas-exchange, or metabolic parameters (figs. 1–4) was further influenced during the xenon application.

Except for a modest decrease in mean arterial pressure (from 102 [87–111] to 82 [68–89] mmHg;  $P < 0.05$ ), halothane produced no discernible effect within the initial 10 min of exposure. After succinylcholine administration at 15 min, abrupt and progressive changes characteristic of MH were observed in all animals (figs. 1–4). Rigidity occurred in all animals. All animals died between 50 and 60 min after halothane administration in cardiac arrest caused by high potassium levels (8.7–10.1 mM).

#### Discussion

The purpose of the present study was to investigate whether xenon may trigger MH in MHS swine. The key finding was the complete absence of any hemodynamic, gas-exchange, or metabolic response indicative of MH during 2 h of xenon administration.

Porcine MH, triggered by halothane in combination

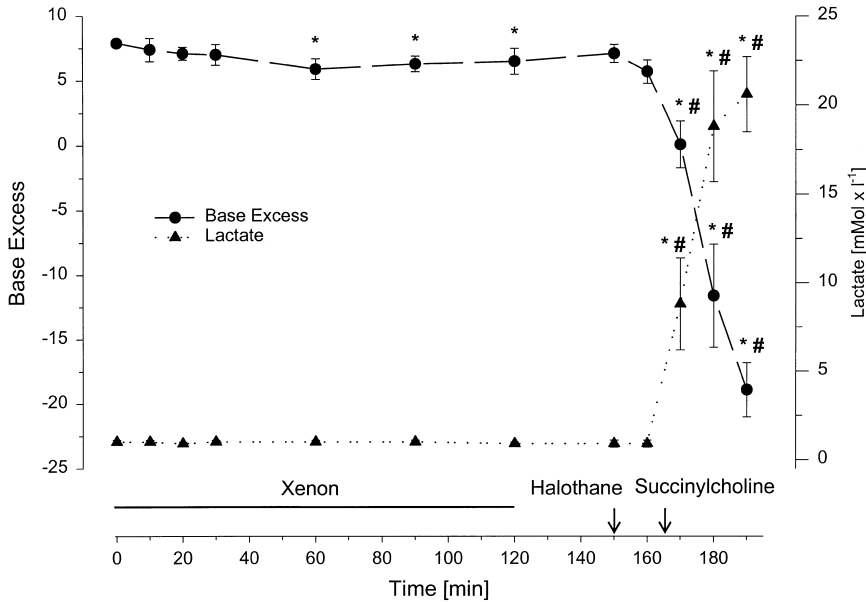


Fig. 2. Time course of base excess and lactate during xenon administration, followed by halothane and succinylcholine. Data are presented as medians as well as the 25th and the 75th percentiles of nine observations. \**P* < 0.05 vs. baseline data (times 0 min, 150 min, respectively); #*P* < 0.05 vs. xenon group.

with succinylcholine, which has been well investigated in Landrace, Pietrain, and Poland China pigs,<sup>17</sup> is characterized by generalized muscular rigidity, dynamic alterations of cardiovascular and metabolic parameters (e.g., arterial carbon dioxide tension > 60 mmHg), and increased body temperature.<sup>18</sup> Hemodynamic changes show a biphasic course: tachycardia as an early sign of onset in connection with severe arrhythmia, increased cardiac index, and decreased mean arterial pressure; subsequently, cardiac index decreases approximately

40–50 min after beginning anesthesia with triggering anesthetics.<sup>18</sup> We also observed all these hemodynamic changes typical for MH during the administration of halothane and succinylcholine.

Xenon anesthesia only caused a slight decrease of heart rate and cardiac index, probably as a result of deeper anesthesia, relative to the pentobarbital background. In previous investigations, this dosage of pentobarbitone, in combination with 70% xenon in oxygen, guaranteed an adequate level of anesthesia to tolerate a

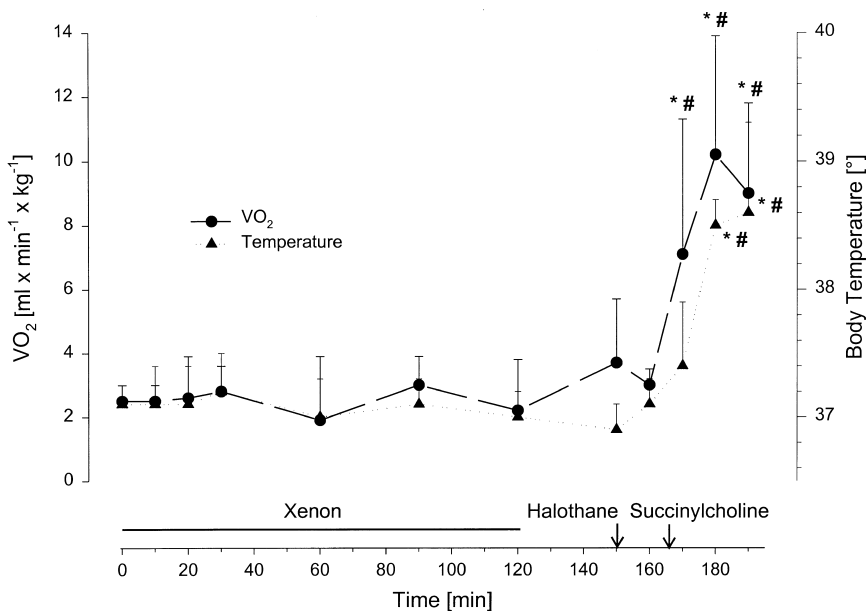
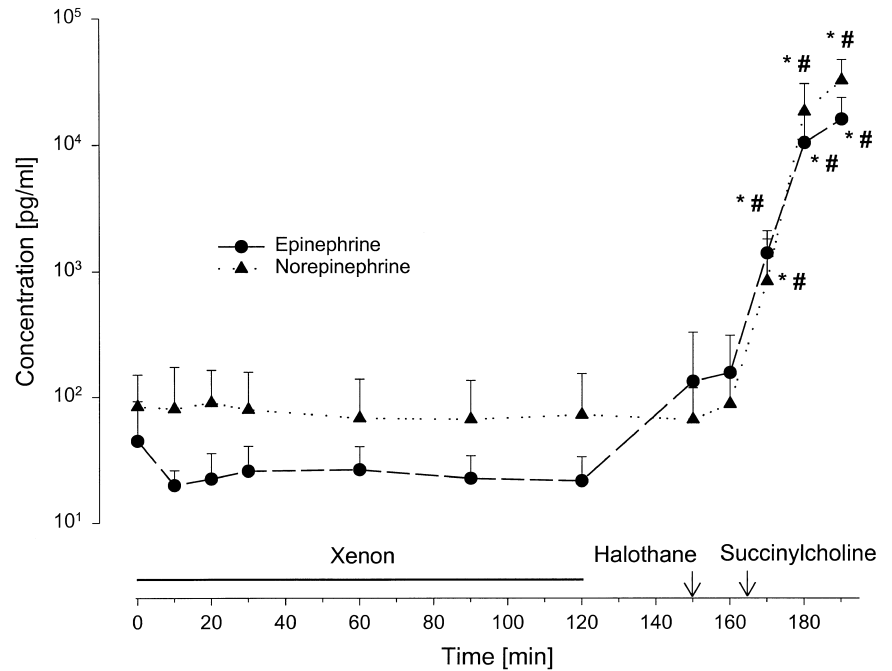


Fig. 3. Time course of oxygen consumption ( $\dot{V}_{O_2}$ ) and body temperature during xenon administration, followed by halothane and succinylcholine. Data are presented as medians as well as the 25th and the 75th percentiles of nine observations. \**P* < 0.05 vs. baseline data (times 0 min, 150 min, respectively); #*P* < 0.05 vs. xenon group.

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**Fig. 4. Epinephrine and norepinephrine plasma concentration of during xenon administration, followed by halothane and succinylcholine. Data are presented as medians as well as the 75th percentiles of nine observations. \* $P < 0.05$  vs. baseline data (times 0 min, 150 min, respectively); # $P < 0.05$  vs. xenon group.**



surgical procedure without hemodynamic depression<sup>13,16</sup> The reduction of the heart rate is the only known cardiovascular effect of xenon.<sup>5,6</sup> No hemodynamic changes typical for MH were observed during xenon administration.

In contrast, there was an abrupt two- to four-fold increase in whole-body oxygen consumption within 5 min after administration of succinylcholine, in conjunction with a rapid increase in arterial carbon dioxide tensions (from 80 to 100 mmHg) and increased lactate concentrations (eightfold), leading to a marked acidosis ( $pH$ : 6.6 to 7.1). Similar findings as well as a comparable increase in plasma catecholamine concentrations have also been described for porcine MH<sup>19</sup> by other investigators. The reason for the latter, *i.e.*,

whether they result from primary or secondary sympathetic stimulation, is still a matter of debate.<sup>19</sup>

Skeletal muscle is the primary source of heat production during MH, resulting from the uncoupling of the energy metabolism<sup>17-21</sup> The first increase in temperature beyond control values is normally seen in the muscle, 5 min after succinylcholine administration.<sup>19</sup> Increases in rectal or blood temperature are more variable; usually they do not occur until 10-15 min after succinylcholine administration,<sup>3,17,21</sup> just as the course of blood temperature in the second part of our experiment.

In our investigation the body temperature of 37.0°C was less than the normal resting temperature of pigs (38.0°C). Previous studies showed that the initiation and prolifera-

**Table 1. Hemodynamic Parameters in Malignant Hyperthermia-susceptible Swine (n = 9) before and during Xenon Exposure (70% Inspired)**

	0 min	60 min	90 min	120 min
HR (beats/min)	94 (87-99)	73 (71-76)*	72 (67-75)*	70 (64-72)*
MAP (mmHg)	110 (105-115)	108 (94-110)	102 (92-106)	96 (79-106)*
MPAP	21 (19-22)	18 (17-21)	19 (17-22)	20 (17-22)
CI (ml · min <sup>-1</sup> · kg <sup>-1</sup> )	125 (103-134)	84 (75-93)*	80 (75-91)*	81 (73-86)*

Data are given as median and 25th and 75th percentiles.

HR = heart rate; MAP = mean arterial pressure; MPAP = mean pulmonary arterial pressure; CI = cardiac index.

\*  $P < 0.05$  vs. baseline data (time 0).

tion of MH might be altered by a reduced core temperature, in particular between 33 and 35°C.<sup>2,21</sup> In contrast to the contracture amplitude *in vitro*,<sup>21</sup> however, the onset of MH *in vivo*,<sup>2,21</sup> like the adenosine triphosphate-dependent Ca<sup>2+</sup> uptake *in vitro*,<sup>21</sup> is not influenced at 37°C.<sup>2,21</sup>

During 2 h of xenon exposure there was no change in any gas-exchange or metabolic parameter indicative of MH. It could be argued that exposure time was not long enough. It should be underscored, however, that not only did 2 h of xenon exposure not lead to hemodynamic or metabolic changes specific of MH, but the administration of halothane and succinylcholine resulted in rapid and progressive hemodynamic and metabolic derangements characteristic of MH in all animals, leading to cardiac arrest within 50–60 min after halothane administration. Together with the fact that we used purebred MHS Pietrain swine based on genetic records, the typical course of porcine MH after triggering with the classical triggering agents halothane and succinylcholine hence underlines the validity of our model.

Although it is tempting to conclude from our results that xenon is not a trigger of MH, the transfer of our findings to humans must be made cautiously, in particular because of the different genetic MH locus of MHS humans and MHS pigs. Porcine MH is always associated with a single point mutation for the ryanodine receptor located on chromosome 6, but in only 50% of humans is MH linked to the ryanodine receptor gene. Furthermore, porcine MH is inherited in an autosomal-recessive pattern, unlike human MH, which is autosomal-dominant.<sup>11</sup> It should be noted, however, that recent *in vitro* investigations of muscle fibers of MHS patients that were exposed to xenon (1 MAC) instead of halothane during the *in vitro* contracture test demonstrated that there was no elevation of the baseline fiber tension.<sup>22</sup> A pathologic tension elevation would have been characteristic for the onset of a MH-associated intracellular calcium release, which, in fact, could be provoked by replacing xenon with halothane. Therefore, the authors conclude that xenon does not trigger MH in humans.<sup>22</sup>

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