Xenon Does Not Impair the Responsiveness of Cardiac Muscle Bundles to Positive Inotropic and Chronotropic Stimulation

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Background: Most volatile anesthetics exhibit a direct myocardial depressant effect. This side effect often limits their applicability in patients with impaired cardiac function. Xenon is a new gaseous anesthetic that did not show any adverse cardiovascular effects in clinical and experimental studies. The authors tested the hypothesis that xenon does not affect myocardial contractility or the positive inotropic effect of isoproterenol, calcium, and increase in pacing rate in isolated guinea pig ventricular muscle bundles.

Methods: Thin ventricular muscle bundles from guinea pig hearts with a mean diameter of 0.4–0.45 mm were prepared under stereomicroscopic control. Force of contraction and contraction times were studied in muscles superfused with medium equilibrated with either 65% xenon and 35% oxygen (xenon group), 1.2% isoflurane in oxygen (isoflurane group), or 65% nitrogen and 35% oxygen (control group). In addition, the positive inotropic effects of calcium, isoproterenol (10⁻¹⁰ – 3 × 10⁻⁸ M) and increasing frequency (0.5–2 Hz) were studied during xenon and isoflurane exposure.

Results: In contrast to isoflurane, xenon did not alter myocardial force of contraction or contraction times. The positive inotropic effect of isoproterenol, calcium, and increasing pacing frequencies did not differ between the muscles exposed to xenon and the control group. Isoflurane elicited the expected negative inotropic effect (30% reduction of force of contraction) but did not impair the response to inotropic stimuli.

Conclusions: Xenon does not alter myocardial contractility and the response to inotropic stimuli such as calcium, isoproterenol, or increase in pacing frequency in isolated guinea pig ventricular muscle bundles.

IN contrast to volatile anesthetics, xenon did not show any adverse cardiovascular effects in clinical and experimental studies thus far. Despite its advantageous properties, xenon has not been used routinely in clinical practice because it is more expensive than all other gas and intravenous anesthetics. Recently, the development of low-flow anesthesia and recycling techniques have reduced the potential costs of xenon anesthesia and rekindled the interest in its clinical use.

Volatile anesthetics are known to exert cardiodepressant effects by interactions with various calcium channels and transporters. In clinical use, these effects on ion channels result in a depression of cardiac output and blood pressure. In contrast, blood pressure remains unchanged or even increases during xenon anesthesia. This clinical observation is confirmed by a recent investigation showing that xenon does not alter myocardial contractility in isolated Langendorff preparations. However, in one study, a small negative inotropic effect was found.

The increase in blood pressure that is reported occasionally during xenon anesthesia may be caused by elevated endogenous catecholamines. However, Boomsma et al. found no difference in blood pressure in the presence of higher plasma noradrenaline and lower adrenaline concentrations compared with nitrous oxide anesthesia. The myocardial response to catecholamines and physiologic inotropic stimuli under the influence of xenon anesthesia has not been investigated until now.

In the current study, we investigated the effect of xenon in comparison to isoflurane on the myocardial contractility. In addition, we tested the hypothesis that xenon does not affect the positive inotropic effect of isoproterenol and calcium or the positive force-frequency relation in isolated guinea pig ventricular muscle bundles.

Materials and Methods

The study was performed in accordance with the guidelines of the Animal Care Committee of the University Hospital RWTH (Aachen, Germany) and the German law concerning the care and use of animals.

Contractions of Guinea Pig Ventricular Muscle Bundles

Male guinea pigs (pearl bright white) were killed by a blow to the head, followed by opening of both carotid arteries. The hearts were excised and placed in a bathing solution containing 136 mM NaCl, 3.5 mM KCl, 1.2 mM KH₂PO₄, 1.1 mM MgSO₄, 2.5 mM CaCl, 10 mM glucose, 10 mM HEPES, 6 mM 2,3-butanediole-monoxime (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany), adjusted to pH 7.4 with NaOH and gassed with 100%
oxygen. Thin ventricular muscle bundles (mean diameter, 0.4 – 0.45 mm) were then prepared from the opened ventricles under stereomicroscopic control and connected to force transducers. The buffer contained 2,3-butanedione-monoxime to protect the muscles during the preparation procedure. Before the experimental protocol, all muscles were prestretched to a diastolic tension of 0.4 mN, and the buffer medium was changed to the same solution as described above, but without 2,3-butanedione-monoxime. The force of contraction (i.e., active force) was continuously measured with inductive force transducers and recorded using a microcomputer and data evaluation software (Föhr Medical Instruments, Seelheim, Germany). The muscles were driven electrically at 1 Hz with rectangular impulses (5 ms) of a voltage of 5–10% above threshold. The force of contraction of each muscle was allowed to reach a steady state. During this time (10–30 min), the resting force was increased until the force of contraction was maximal (i.e., until the sarcomeric length was optimal), and the buffer was continuously bubbled with 100% oxygen. All experiments were conducted at a temperature of 30°C.

Force of contraction, time to peak tension (TPT), and time to 90% relaxation (TR90) were studied in muscles superfused with medium equilibrated with either 65% nitrogen and 35% oxygen (control group), 65% xenon and 35% oxygen (xenon group, 0.92 minimum alveolar concentration [MAC] xenon), or 1.2% isoflurane in oxygen (isoflurane group, 1 MAC isoflurane). Furthermore, the inotropic effects of calcium (10.8 mM), increasing stimulation frequency (0.5–2 Hz), and isoproterenol (10−10 – 3 × 10−8 m) were studied in each experimental group.

Isoflurane was received from Abbott GmbH (Wiesbaden, Germany). Xenon was a gift from Messer-Griesheim GmbH (Krefeld, Germany). Isoproterenol was obtained from Boehringer (Mannheim, Germany).

Force of contraction TPT and TR90 were determined after 20 min of equilibration for each anesthetic. After this period, the muscles received one inotropic stimulus: either isoproterenol, calcium, or an increasing pacing frequency.

Concentration–response curves for isoproterenol (10−10 – 3 × 10−8 m) were determined by adding the drug cumulatively to the organ chambers after the muscles had equilibrated to the anesthetic for at least 20 min. This part of the experimental protocol was performed at a pacing frequency of 2 Hz.

Calcium was elevated from 2.5 to 10.8 mM. Before establishing the experimental protocol used, we examined the concentration-dependent effect of calcium at control conditions. Calcium at 10.8 mM results in a maximum inotropic effect without development of diastolic dysfunction. Therefore, this dose is suitable for evaluation of the calcium efficacy in the chosen experimental setting. The data for statistical analysis were taken after 3 min, when the maximum inotropic response was reached.

To examine the force–frequency relation, the stimulation frequency was increased from 0.5 to 2 Hz in steps of 0.5 Hz.

To exclude relevant core ischemia in the xenon group, muscle performance during reduced oxygen tension was studied in 15 muscle bundles. Active force of contraction was 8.0 ± 4.5 mN/mm2 at baseline control values; TPT and TR90 were 166.6 ± 15.9 and 244.4 ± 16.3 ms, respectively. Oxygen was then reduced from 100 to 35% (65% nitrogen). As reported by other investigators, reduced oxygen tension neither changed the force of contraction nor the times of contraction and relaxation. Twenty minutes after reduction of oxygen, the active force of contraction was 7.5 ± 4.1 mN/mm2, and TPT and TR90 were 170.8 ± 20.1 and 251.6 ± 25.8 ms, respectively (no significant difference to 100% oxygen). The reaction to calcium, isoproterenol, and changing stimulation frequency did not differ significantly between 100 and 35% oxygen (data not shown).

Equilibration of Anesthetics in Crystallloid Buffer Medium

Isoflurane (1.2%; 1 MAC in humans) was added to oxygen using a vaporizer (Isotec 3; Ohmeda, Madison, WI) and bubbled into the organ bath. Gas chromatographic measurements revealed a full equilibration of the anesthetic in the buffer solution within 5 min. For xenon, the buffer was prepared as follows: to shorten the equilibration time, the buffer was gassed under elevated ambient pressure (2 bar) by the xenon–oxygen mixture (65% xenon + 35% oxygen). The pressure was then released, and the organ baths were filled with the prepared buffer and continuously bubbled with 65% xenon and 35% oxygen. The xenon content of buffer samples was determined by nuclear magnetic resonance spectrometry (Bruker, AMX 300; Bruker Electronic GmbH, Rheinstetten, Germany) at a temperature of 24°C with a measurement frequency of 83.3 MHz. Before the measurement was started, 3–20 µl of an alkaline solution of a manganese high-spin complex were added to the xenon containing buffer as a relaxation accelerator. Spectra were recorded by accumulating 1,024 scans with a relaxation delay of 2–4 s. The xenon content in buffer prepared in the way described above was 0.06 ml xenon per milliliter buffer at 24°C. Considering the relation between temperature and solubility, the xenon content in buffer at 30°C is approximately 10–15% less. This result is in accordance with published solubilities of xenon in water and body fluids (i.e., full equilibration of 65% or 0.92 MAC xenon).

Statistical Analysis

All data in text and figures, apart from EC50 values, are expressed as mean ± SD. Examinations for normality
were performed before further statistical analysis in all
groups (Kolmogorov-Smirnov test and D'Agostino skew-
ness test). P values < 0.05 were considered significant.
Comparisons between groups were performed by anal-
ysis of variance followed by Tukey-Kramer multiple com-
parisons test. The force of contraction in these experi-
ments was normalized, as the absolute values varied
markedly throughout the population of muscle bundles.
Comparisons within one group between baseline values
and values obtained after anesthetic exposure or be-
tween values obtained with 100% oxygen and 35% oxy-
gen were performed using the two-tailed Student paired t
test. EC_{50} values are given as median and extremes.
Comparisons in these data were performed using the
Kruskal-Wallis test.

Statistical analysis was performed using Graphpad In-
stat (Graphpad Software, San Diego, CA) and NCSS
(NCSS Statistical Software, Kaysville, UT).

**Results**

We studied thin ventricular guinea pig muscles that
were paced electrically at a frequency of 1 Hz. The mean
diameter of the muscles was 0.44 ± 0.09 mm. Among
the muscle groups, there was no significant difference in
mean diameter, resting tension, and force of contraction
at the end of the equilibration time (baseline conditions).
For pooled data, the mean force of contraction at base-
line conditions was 14.1 ± 12.4 mN/mm².

**Contractions under the Influence of Xenon and
Isoflurane**

During 20 min of superfusion with buffer medium
equilibrated with 65% xenon, neither the maximum
force of contraction nor the TPT or TR_{90} were altered
compared with the control group. As reported by other
investigators, isoflurane exhibited a negative inotope-
ic effect that was maximal after 5 min of gas exposure
and was completely reversible at the end of gas expo-
sure. Isoflurane (1.2 vol%) resulted in a reduction of the
force of contraction by 30% (fig. 1A). TPT was slightly
shortened by isoflurane (fig. 1B). This observation was
statistically significant compared with the xenon muscle
group, but not in comparison to the control muscle
group. The time to 90% relaxation remained unchanged.

**Force-Frequency Relation**

The effect of xenon or isoflurane on the force-fre-
quency relation is illustrated in figures 2A–C. The force
of contraction is normalized to the value obtained after
equilibration with the respective anesthetic. Because of
this normalization, the negative inotropic effect of isoflu-
rane, although significant in absolute values, cannot be
seen in this diagram. For all groups, the staircase was
positive up to a pacing frequency of 1.5 Hz and flattened
or even became slightly but not significantly negative
from 1.5 to 2 Hz. TPT and TR_{90} decreased at increasing
pacing frequencies (figs. 2B and C). Again, TPT in the
isoflurane group was shorter than in the other muscles,
whereas TR_{90} was not altered by this anesthetic. Xenon
did not influence the contraction times compared with
control.

**Influence of Xenon and Isoflurane on the Positive
Inotropic Effect of Isoproterenol**

Isoproterenol induced a dose-dependent positive inotope-
effect in all trial groups (fig. 3A). The median EC_{50}
values were 0.36 nM (range, 0.05–9.8 nM) for the xenon
group, 1.3 nM (range, 0.09–2.9 nM) in control muscles,
and 0.34 nM (range, 0.04–1.6 nM) in isoflurane-exposed
muscles (no significant difference according to the
Moreover, isoproterenol induced a significant reduction in TPT and TR90 at concentrations greater than 10^{-9} M (figs. 3B and C). Again, muscles exposed to isoflurane showed a shorter TPT and TR90 (significant vs. xenon group). This effect vanished at isoproterenol concentrations greater than the nanomolar range.

**Influence of Xenon and Isoflurane on the Positive Inotropic Effect of Calcium**

Calcium (10.8 mM) induced a positive inotropic effect that reached its maximum after 2–3 min. The maximal active force was enhanced to 175.0 ± 30.3% in the xenon group (n = 8), 205.2 ± 35.7% in the isoflurane group (n = 10), and 174.6 ± 35.2% in control muscles (n = 8) compared with values at a calcium concentration of 2.5 mM. This effect was not significantly different between the experimental groups. The TPT remained unchanged after stimulation with calcium, whereas TR90 shortened (table 1). Again, xenon did not alter the myocardial response to the inotropic stimulation.

**Discussion**

The current study demonstrates that 65% xenon (0.92 MAC in humans) does not alter myocardial contractility, whereas 1.2% isoflurane (1 MAC in humans) reduced the myocardial force development by 30% in isolated guinea pig ventricular muscle bundles. Neither xenon nor isoflurane affected the response to inotropic stimulation with isoproterenol, calcium, and increase in pacing rate.

Commonly used volatile anesthetics are known to decrease myocardial contractility and lower systemic vascular resistance. Volatile anesthetics have been investigated in detail with regard to their direct myocardial effects. Halothane, isoflurane, enfurane, and sevoflurane inhibit the sarcolemmal calcium current (I_{Ca}). In addition, enfurane and halothane activate the sarcoplasmic reticulum calcium release channel, which decreases the Ca^{2+} load of the sarcoplasmic reticulum, resulting in a depletion of calcium available for excitation-contraction coupling. In contrast, the noble gas anesthetic xenon is expected to be associated with less or no cardiodepressant side effects.

Based on clinical studies and case reports, 65–70% xenon did not depress myocardial function. First experimental studies in animals confirmed the clinical results. Xenon at 65% did not influence arterial and left

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*The use of the MAC definition in a study on isolated myocardium is limited for the following reasons: (1) MAC is defined as concentration that inhibits certain cerebral functions (reaction to skin incision); however, the cerebral sensitivity to anesthetics is not correlated to the myocardial sensitivity to anesthetics. (2) The MAC value depends on the investigated species (for xenon: 71% in humans, 161% in rats, 96% in mice). Therefore, in the current study, clinically typical and equipotent concentrations of xenon and isoflurane were chosen.

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ventricular pressure in isoflurane-anesthetized healthy or cardiomyopathic dogs\textsuperscript{20}. Unfortunately, the effects of xenon had to be studied during isoflurane anesthesia, as the MAC for xenon in dogs is superatmospheric (119%). Nevertheless, this remains the only study to investigate xenon in a model of heart failure thus far.

Because most anesthetics alter calcium ion currents in myocardial cells, this site is of special interest for experimental studies on xenon. Effects on the cellular calcium homeostasis were reported in 1995, when Franks et al.\textsuperscript{21} proved the inhibition of the plasma membrane calcium-adenosine triphosphatase in rat brain synaptic membrane vesicles by xenon. This channel is found in human as well as guinea pig myocardial tissue but does not play a major role in myocardial contraction. Recently, Stowe et al.\textsuperscript{8} investigated cardiac effects of xenon using a guinea pig Langendorff model and whole cell patch clamp of sodium, L-type calcium, and inward rectifier K\textsuperscript{+} channel\textsuperscript{8,22,23}. In agreement with our results, no significant change of myocardial contractility and no significant change of the investigated ion fluxes were observed. Although xenon does not appear to alter contractility of isolated myocardium, it might interact with the classic physiologic mechanisms controlling contractile force \textit{in vivo}. For example, xenon might influence myocardial contractility by altering the response to physiologic variations of sympathetic tone or fluctuations in heart rate.

The influence of xenon anesthesia on the positive inotropic effect of catecholamines, calcium, or other substances has not been investigated until now. Interactions of anesthetics with catecholamines are of special clinical interest, as these substances are used in hemodynamic instability during anesthesia where unexpected drug interactions may lead to fatal outcome. Volatile anesthetics have been reported to alter the myocardial response to \(\beta\)-adrenergic stimulation, but experimental results remain conflicting. The potency of isoproterenol to increase the force of contraction of isolated muscle preparations was reduced by halothane but remained unchanged by isoflurane.\textsuperscript{13} On the other hand, desflurane significantly enhanced the positive inotropic effect

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**Table 1. TPT and TR\textsubscript{90} at 2.5 mM Compared to 10.8 mM Calcium under the Influence of Xenon and Isoflurane**

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<th>2.5 mM Calcium</th>
<th>10.8 mM Calcium</th>
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<tbody>
<tr>
<td>TPT (ms)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 8)</td>
<td>172.0 $\pm$ 27.3</td>
<td>174.0 $\pm$ 25.9</td>
</tr>
<tr>
<td>Xenon (n = 8)</td>
<td>164.0 $\pm$ 23.2</td>
<td>172.1 $\pm$ 18.2</td>
</tr>
<tr>
<td>Isoflurane (n = 10)</td>
<td>149.6 $\pm$ 15.5</td>
<td>164.8 $\pm$ 12.7</td>
</tr>
<tr>
<td>TR\textsubscript{90} (ms)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 8)</td>
<td>262.1 $\pm$ 17.1</td>
<td>248.8 $\pm$ 25.8*</td>
</tr>
<tr>
<td>Xenon (n = 8)</td>
<td>259.1 $\pm$ 17.9</td>
<td>245.4 $\pm$ 25.6*</td>
</tr>
<tr>
<td>Isoflurane (n = 10)</td>
<td>278.6 $\pm$ 9.1</td>
<td>250.7 $\pm$ 11.2*</td>
</tr>
</tbody>
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* \(P < 0.05\) vs. 2.5 mM calcium.

TPT = time to peak tension; TR\textsubscript{90} = time to 90% relaxation.
of dobutamine in rat myocardium,24 and isoflurane and sevoflurane were reported to potentiate the positive inotropic effect of isoproterenol in rat papillary muscles.25

This is the first study to demonstrate that xenon does not interfere with the positive inotropic effect of isoproterenol and calcium. In addition, the physiologic force-frequency relation of guinea pig ventricular muscle bundles is unaffected during xenon exposure.

The following points must be considered when the clinical relevance of our results is assessed. First, our experiments were conducted at a temperature of 30 °C. Myocardial muscle bundles in crystalloid buffer medium that are oxygenated by diffusion should be studied at hypothermia to prevent core ischemia when the oxygen tension is lowered to approximately 250 mmHg11 (i.e., oxygen tension in the xenon and control group). Hypothermia decreases the activity of myocardial enzymes and ion pumps. This results in a slower contraction cycle and therefore increases the times for contraction and relaxation. At higher pacing rates, the slow calcium transport may lead to an incomplete relaxation. This effect is believed to be responsible for the flattened force–frequency staircase at frequencies greater than 2 Hz in the current study. This observation corresponds to the results published by Mattheussen et al.26 Second, it should be noted that hypothermia decreases the MAC of volatile anesthetics. For xenon, a correlation between MAC and temperature can be supposed, but the subject has not been studied so far. Therefore, MAC values have not been corrected for hypothermia in the current study. Finally, temperature effects must be considered in the discussion of the positive inotropic effect of isoproterenol. Riishede and Nielsen-Kudsk27 described a left shift of the dose–response curve for β-adrenoceptor agonists but a reduction of the maximum increase of the contraction amplitude at lowered temperature (27 °C compared with 37 °C). These results are in accordance with our observations and may explain the low EC50 for isoproterenol in the current study.

In summary, the current study demonstrates that xenon does not affect myocardial contractility or the physiologic response to positive inotropic and chronotropic stimuli. The lack of negative inotropic effects of xenon may be beneficial for patients with impaired cardiac functions who poorly tolerate the cardio depressant side effects of common volatile anesthetics.

The authors thank Miriam Vogel (Veterinary Surgeon, Department of Cardiology, University Hospital RWTH Aachen, Aachen, Germany) for technical assistance; Messer-Griesheim GmbH (Krefeld, Germany) for provision of xenon; the Department of Anorganic Chemistry (University of Cologne, Cologne, Germany) for nuclear magnetic resonance analysis of dissolved xenon; and the Department of Forensic Medicine (University Hospital RWTH Aachen, Aachen, Germany) for mass spectrometric analysis of dissolved isoflurane.

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