Xenon and Isoflurane Reduce Left Ventricular Remodeling after Myocardial Infarction in the Rat

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ABSTRACT

Background: Xenon and isoflurane are known to have cardioprotective properties. We tested the hypothesis that these anesthetics positively influence myocardial remodeling 28 days after experimental perioperative myocardial infarction and compared their effects.

Methods: A total of 60 male Sprague–Dawley rats were subjected to 60 min of coronary artery occlusion and 120 min of reperfusion. Prior to ischemia, the animals were randomized for the different narcotic regimes (0.6 vol% isoflurane, 70 vol% xenon, or intraperitoneal injection of s-ketamine). Acute injury was quantified by echocardiography and troponin I. After 4 weeks, left ventricular function was assessed by conductance catheter to quantify hemodynamic compromise. Cardiac remodeling was characterized by quantification of dilatation, hypertrophy, fibrosis, capillary density, apoptosis, and expression of fetal genes (α/β *myosin heavy chains,* α*-skeletal actin, periostin, and sarco/endoplasmic reticulum* Ca^{2+}*-ATPase*).

Results: Whereas xenon and isoflurane impeded the acute effects of ischemia-reperfusion on hemodynamics and myocardial injury at a comparable level, differences were found after 4 weeks. Xenon in contrast to isoflurane or ketamine anesthetized animals demonstrated a lower remodeling index (0.7 ± 0.1 *vs.* 0.9 ± 0.3 and 1.0 ± 0.3 g/ml), better ejection fraction (62 ± 9 *vs.* 49 ± 7 and 35 ± 6%), and reduced expression of β*-myosin heavy chain* and *periostin*. The effects on hypertrophy, fibrosis, capillary density, and apoptosis were comparable.

Conclusions: Compared to isoflurane and s-ketamine, xenon limited progressive adverse cardiac remodeling and contractile dysfunction 28 days after perioperative myocardial infarction.

What We Already Know about This Topic

- Previous studies have demonstrated that xenon and isoflurane have cardioprotective properties.
- However, little is known about the long-term effects of xenon and isoflurane on cardiac remodeling after perioperative myocardial infarction. This study determined the long-term effects of anesthetic preconditioning on cardiac remodeling after perioperative myocardial infarction using a rat model of left ventricular ischemia-reperfusion injury.

What This Article Tells Us That Is New

- Compared to isoflurane, xenon limited adverse cardiac remodeling and contractile dysfunction after perioperative myocardial infarction.

PERIOPERATIVE myocardial infarction is a serious adverse event during surgery and is associated with high complication and mortality rates.[1] Left ventricular remodeling following myocardial infarction leads to progressive hypertrophy, chamber dilatation, and ultimately heart failure. Treatments that block the β-adrenergic and renin–angiotensin–aldosterone system, as well as mechanical support, can limit or reverse this process.[2,3] The extent of the remodeling process is highly dependent on the initial infarct size and thus could be limited by myocardial salvage achieved by reperfusion therapy.[4] As shown in short-term studies, anesthetic preconditioning not only reduces initial myocardial injury,[5,6] but also prevents activation of the deleterious gene expression profile.[7] However, long-term effects of anesthetic preconditioning on cardiac remodeling with respect to long-term function after transient ischemia have yet to be identified. Therefore, we used a rat model of left ventricular ischemia-reperfusion (IR) injury to examine the effects of isoflurane and xenon anesthesia on cardiac function, structure, and expression of fetal genes after 4 weeks.

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Materials and Methods

Animals

A total of 60 male Sprague–Dawley rats (10–12 weeks old, 350–420 g) were purchased from Charles River Laboratories (Charles River, Sulzfeld, Germany). All of the experiments were performed in accordance with the German legislation governing animal studies, followed *The Principles of Laboratory Animal Care* (National Institute of Health, publication No. 85-23, revised in 1996), and had prior approval by the governmental animal care and use office (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen, Recklinghausen, Germany, Protocol No. 9.93.2.10.35.07.210).

Instrumentation and Protocol on Day 0

After induction of anesthesia by an intraperitoneal injection of 200 mg/kg s-ketamine (Pfizer, Berlin, Germany), a three-lead electrocardiogram was installed, and the first echocardiographic examination with a 10-MHz probe (Vivid I, GE, Solingen Germany) was performed. The animals were orally intubated and ventilated with 30% oxygen and 70% nitrogen in a pressure-controlled mode with a positive end-expiratory pressure of 5 cm H_2O using a closed-circuit anesthesia machine to control the end-tidal carbon dioxide concentration (Physioflex®, Draeger, Luebeck, Germany). The left jugular vein was catheterized with a 22G cannula (Leader Flex, Vygon, Aachen, Germany) for fluid replacement (10 ml • kg^{-1} • h^{-1} Ringer's solution) and blood withdrawal. The anesthetic depth was evaluated every 30 min by tail pinch. In case of limb movement or an increase in heart rate (HR), 50 mg/kg s-ketamine was injected intraperitoneally. Blood pressure was monitored by a 1.4F pressure catheter (SPR-671, Millar Instruments, Houston, TX) through the right femoral artery. The animals were placed on a feedback-controlled heating pad to maintain the body temperature at 37°C (TCAT-2 controller; Physitemp, Clifton, NJ). After a left lateral thoracotomy between the fourth and fifth rib, a ligature was placed around the left anterior descending coronary artery. Subsequently, the animals were randomly divided into four groups, using a sealed envelope system (see fig. 1). Identification numbers provided by the animal facility were used as pseudonyms to blind the assessor for future investigations and analysis. The treatment groups received 0.43 minimum alveolar concentration (MAC) of the anesthetic gases, respectively, 0.6 vol% isoflurane (Forene, Abbott, Wiesbaden, Germany) or 70 vol% xenon (LENOXe, Air Liquide Medical GmbH, Düsseldorf, Germany), as a substitute for nitrogen. The control and sham groups received additional s-ketamine to maintain anesthesia. After 1 h, the left anterior descending coronary artery was occluded for 1 h, except in the sham group. After 2 h of reperfusion, the wounds were closed in layers and anesthesia was stopped. A second echocardiogram was performed after the animals were extubated and sufficient spontaneous breathing was achieved. For postoperative analgesia, the wounds were infiltrated with 1 ml 0.5% ropivacaine (Astra Zeneca, Wedel, Germany). Metamizole (Ratiopharm, Ulm, Germany) was injected intraperitoneally (20 mg/kg) and added to the tap water (10 mg/ml/kg) for 3 days. The rats were transferred to a warm isolation chamber (Vetario S10 Intensive Care Unit, Brinsea, Sandford, United Kingdom) for recovery.

Instrumentation on Day 28

Anesthesia was induced with a reduced intraperitoneal dosage of s-ketamine (120 mg/kg). A three-lead electrocardiogram was obtained and a third echocardiographic examination performed. The animals were intubated and ventilated as on the first day. The right jugular vein was catheterized, and a 2F conductance catheter (SPR-869, Millar Instruments, Houston, TX) was inserted via the right carotid artery into the left ventricle. Parallel conductivity was determined by three intravenous injections of 100 µL 10% saline. Pressure volume loops were recorded during apnea and gradual, short-term reduction of preload. To this end, the inferior caval vein was compressed with a cotton bud through a small subxiphoidal incision. After hemodynamic measurements and blood withdrawal, the animals were killed by intravenous injection of potassium chloride and the heart was excised for further analysis.

Hemodynamic Calculations

All signals were collected at a sampling rate of 1000 Hz using a data acquisition system (Power Lab 8/30, ADInstruments, Colorado Springs, CO). Time intervals of 10 s were analyzed with dedicated software (LabChart 6 Pro, ADInstruments GmbH, Spechbach, Germany; Circlab 2010, Paul Steendijk, Leiden, The Netherlands) at the following time points (see fig. 1): At baseline (T0), 50 min after application of inhalative narcotics just before start of ischemia (T1), 50 min after induction of ischemia (T2), 110 min after start of reperfusion (T3), and 60 min after instrumentation on day 28 (T4). The HR was derived from the electrocardiogram, and the systolic arterial pressure (sAP) from the pressure signal of the conductance catheter. The left ventricular end-diastolic volume (EDV) and ejection fraction (EF) were calculated using the volume signal of the conductance catheter after calibration for parallel conductance and slope factor α. Parallel conductance was obtained by the hypertonic saline method.[8] Slope factor α was calculated as the ratio of cardiac output (CO) determined by conductance and by echocardiography. Myocardial contractility was quantified by the slope of the preload recruitable stroke work (S_{PRSW}), which equates to the linear relation of EDV and stroke work, obtained from five consecutive caval occlusions.[9,10]

Echocardiography

B-mode recordings with a frame rate of 190/s from short- and long-axis parasternal views were analyzed using post-processing software (EchoPAC PC V108.1.4, GE Healthcare, Munich, Germany). CO was calculated using the aortic

velocity time integral (long-axis view), cross-sectional area, and HR.[11] The left ventricular end-diastolic diameter and wall thickness of the posterior wall were measured in the M-mode of the short-axis view. Two-dimensional speckle tracking (short-axis view) was used to determine the global (RS_{global}) and anterior radial strain (RS_{ant}). The extent of hypo-/akinesia, which reflects the area at risk, was identifiable by a low radial strain (<14.5%) and was expressed as the percentage of the circumference.[12]

Blood Samples

The serum concentration of troponin I (210-2-HS, Life Diagnostics, West Chester, PA) was measured by an enzyme-linked immunosorbance assay on day 0 after 120 min of reperfusion to quantify the initial myocardial injury.

Histology

After removal, the heart was weighed and immediately frozen at −20°C for 5–10 min and cut into five slices perpendicular to the left ventricular long axis. The heart weight was normalized to the tibia length and EDV (remodeling index).[13] The infarcted area of the left ventricle was determined after incubation in 1% triphenyl tetrazolium chloride at 37°C for 10 min, as described previously.[14] Afterward, samples from the non-infarcted (remote) myocardium of the medial left ventricle were either fixed in paraformaldehyde and embedded in paraffin or snap-frozen in liquid nitrogen and stored at −80°C. Cross-sections were stained with Sirius red (Direct Red 80, Sigma–Aldrich, St. Louis, MO) to quantify the degree of ventricular fibrosis. The ratio of collagen deposition was averaged from five areas using image analysis software (Image J, National Institute of Health, Bethesda, MD).[15] Staining with fluorescein-conjugated wheat germ agglutinin (W834, Molecular Probes, Eugene, OR) was used to determine the cross-sectional area of cardiomyocytes in remote areas of the left ventricle. The results from 25 to 50 single planimetric measurements were averaged.[16] Capillaries were counted after staining using isolectin IB_4 (I21414, Molecular Probes) and Alexa Fluor 568 (S11226, Molecular Probes) in five remote areas of the left ventricle.[17]

Messenger RNA Quantification by Real-Time Polymerase Chain Reaction

Total RNA was extracted from samples of the remote myocardium using a commercial extraction kit (NucleoSpin RNA/Protein, Machery-Nagel, Düren, Germany). Total RNA (250 ng) was reverse transcribed into complementary DNA using a high-capacity reverse transcription kit (Applied Biosystems, Carlsbad, CA). The polymerase chain reaction (PCR) was performed using 50 ng of DNA, PCR master mix (TaqMan universal PCR mix, Applied Biosystems), and specific TaqMan probes (Applied Biosystems) for α-*myosin heavy chain* (α-*MHC*, Rn00568304_m1), β-*MHC* (Rn00568328_m1), α-*skeletal actin* (Rn01426628_g1), *periostin* (Rn01494627_m1), *sarco/endoplasmic reticulum* Ca^{2+}*-ATPase* (Rn01499544_m1), and *caspase-3* (Rn00563902_m1) on a PCR system (StepOne-Plus, Applied Biosystems). Relative quantity values were calculated according to the ΔΔCt method, which reflects the differences in the threshold for each target gene relative to the housekeeping gene *glyceraldehyde-3-phosphate dehydrogenase* (Rn99999916_s1) and expression in normal uninjured hearts. The expression of α-*MHC* was calculated as the percentages of β-*MHC* copies.

Statistics

The results are presented as the mean and SD (Prism 5.01, GraphPad Software, La Jolla, CA). Differences between groups were investigated for significant changes using one-way ANOVA. If the null hypothesis could be rejected, a two-sided Dunnett *post hoc* test was applied to compare means of the sham, control, or xenon group with the control group as reference. For repeated measurements, a two-way ANOVA was used to investigate significant effects of time (within subject factor) and group (between subject factor). Greenhouse–Geisser correction was used if sphericity could not be assumed. In addition to the Dunnett *post hoc* test, a contrast analysis was applied to compare differences between consecutive time points. Levels of significance were displayed for $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$ (SPSS 19, IBM, Ehningen, Germany).

Results

A total of 60 animals were included in the study and randomly assigned to either the sham group (n = 12), control group (n = 17), isoflurane group (n = 15), or xenon group (n = 16). During initial anesthesia induction, thus before the start of the experimental protocol at T0, all animals of different groups received a comparable amount of s-ketamine (256 ± 7 mg/kg). During the remaining time on day 0, the animals in the control and sham groups required 55 ± 16 and 37 ± 13 mg/kg s-ketamine, respectively. The rats in the isoflurane group did not require further s-ketamine; whereas the rats in the xenon group required a reduced averaged dosage of 19 ± 5 mg/kg s-ketamine to maintain adequate anesthesia. In all, 46 animals survived the acute IR injury. Fourteen rats died from bleeding, cardiac failure, or recurrent ventricular fibrillation and 10 animals remained in the sham group and 12 in the control, isoflurane, and xenon group, respectively. Incidence of complications and thus acute survival were not different between groups (table 1).

Acute Effects (T0–T3)

During the preconditioning period (T0–T1), HR and sAP decreased in all groups. Ischemia led to a further reduction in sAP with an accompanying increase in HR. During reperfusion sAP recovered, whereas HR further increased. After perfusion (T3), a significant reduction in CO, left ventricular end-diastolic diameter, RS_{global}, and RS_{ant} compared to T0 could be observed. RS_{ant} in the sham and xenon groups

Table 1. Complications

	Total	Control	Isoflurane	Xenon	Sham
Number of animals	60	17	15	16	12
Survivors (4 wk)	46	12	12	12	10
Causes of death (d 0)	14	5	3	4	2
Bleeding	5	1	1	1	2
Cardiac failure	7	3	2	2	0
Ventricular fibrillation	2	1	0	1	0

Distribution of animals according to experimental groups and causes of death at day 0 during instrumentation.

remained significantly higher compared to that in the control group (table 2). The sizes of the hypo-/akinetic areas assessed by radial strain after IR amounted approximately 45% of the left ventricle in the control with no differences compared to that in the isoflurane or xenon group. Significantly lower levels of troponin I could only be measured in the sham group (fig. 2).

Chronic Effects (T4)

No animals died between day 1 and day 28. The body weight gain and linear growth of the tibia were not significantly different between groups (average increases, respectively, 68 ± 4 g and 2.1 ± 0.1 mm). Compared to time point T3, at day 28 (T4) sAP and CO were significantly increased in all groups and thus returned to baseline values. HR remained unchanged. RS_{global} and RS_{ant}, on the contrary, were unchanged at day 28 compared to T3 and remain decreased compared to baseline (T0) in all infarct groups. At day 28, RS_{ant} was significantly higher only in the sham group (table 2). An overall increase in left ventricular end-diastolic diameter could be observed with significant lower values in the sham group (table 2).

The typical examples of pressure volume loops in figure 3A illustrate the differences in global cardiac function between the groups 4 weeks after ischemia. ANOVA indicated significant differences between groups, EDV, EF, and S_{PRSW} (fig. 3, B–D). *Post hoc* analysis showed that, compared to the control group, EDV was significantly lower after xenon anesthesia and in the sham group. EF and S_{PRSW} were significantly higher only in the xenon and sham groups.

Consistent with these differences, the normalized heart weight (to tibia length) was by far the highest in the control group (fig. 4A). The values measured in the isoflurane-treated animals and the sham group were significantly lower than those of the control group. Compared to the control group, the calculated remodeling index was significantly lower in the xenon group and sham-operated animals (fig. 4B). The thickness of the posterior wall was significantly different only between the control and sham groups (fig. 4C). No differences between groups for the cross-sectional areas of cardiomyocytes within the remote myocardium were found (fig. 4D).

The infarct size was approximately 50% of the initial hypo-/akinetic area, with no differences between the three infarct groups (fig. 5A). No effect on the amount of fibrosis and capillary density in the remote area could be observed (fig. 5, B and C). Expression of *caspase-3* was reduced after isoflurane or xenon anesthesia and reached comparable levels to that of the sham-operated animals (fig. 5D).

The ratio of α- and β-*MHC* was reduced in the control and isoflurane groups, whereas it remained nearly normal in the xenon group (fig. 6A). However, these effects just

Table 2. Hemodynamic and Echocardiographic Variables

	Control (n=12)	Isoflurane (n=12)	Xenon (n=12)	Sham (n=10)
HR, /min				
T0	309 ± 45	288 ± 40	278 ± 30	290 ± 44
T1$^{\$\$\$}$	283 ± 45	269 ± 41	229 ± 20	271 ± 30
T2$^{\$\$}$	319 ± 59	270 ± 49	274 ± 44	257 ± 27
T3$^{\$}$	335 ± 54	308 ± 32	315 ± 44	291 ± 26
T4	281 ± 72	293 ± 43	305 ± 49	322 ± 61
sAP, mmHg				
T0	125 ± 16	121 ± 19	119 ± 17	133 ± 27
T1$^{\$\$\$}$	118 ± 16	99 ± 12	107 ± 36	113 ± 29
T2$^{\$\$\$}$	111 ± 25	87 ± 11	104 ± 32	96 ± 14
T3$^{\$\$}$	123 ± 15	100 ± 19	109 ± 21	112 ± 19
T4$^{\$\$\$}$	119 ± 15	125 ± 18	133 ± 23	129 ± 19
CO, ml/min				
T0	165 ± 45	163 ± 29	167 ± 35	173 ± 37
T3$^{\$\$\$}$	131 ± 30	157 ± 30	145 ± 29	147 ± 29
T4$^{\$\$}$	167 ± 32	163 ± 38	172 ± 51	167 ± 52
LVEDd, mm				
T0	6.4 ± 1.0	6.8 ± 1.0	6.4 ± 1.4	6.6 ± 1.1
T3$^{\$\$}$	5.9 ± 0.8	6.1 ± 1.0	6.4 ± 1.4	5.8 ± 0.8
T4$^{\$\$\$}$	9.0 ± 1.0	9.1 ± 1.2	8.1 ± 1.4	6.9 ± 1.4***
RS_{global}, %				
T0	29 ± 14	31 ± 9	33 ± 8	32 ± 7
T3$^{\$\$\$}$	15 ± 13	21 ± 5	20 ± 8	23 ± 6
T4	22 ± 12	22 ± 6	20 ± 11	27 ± 12
RS_{ant}, %				
T0	32 ± 12	30 ± 7	29 ± 12	31 ± 7
T3$^{\$\$\$}$	7 ± 4	12 ± 6	16 ± 10*	21 ± 11**
T4	11 ± 9	11 ± 10	13 ± 12	32 ± 10***

Effect of anesthesia on heart rate (HR), systolic arterial pressure (sAP), cardiac output (CO), left ventricular end-diastolic diameter (LVEDd), and global and anterior radial strain (RS_{global}, RS_{ant}). (Values are means ± SD; contrast analysis *vs.* prior time point: $^{\$}P < 0.05$ $^{\$\$}P < 0.01$ $^{\$\$\$}P < 0.001$; Dunnett *post hoc* test *vs.* control: $^{*}P < 0.05$ $^{**}P < 0.01$ $^{***}P < 0.001$.)

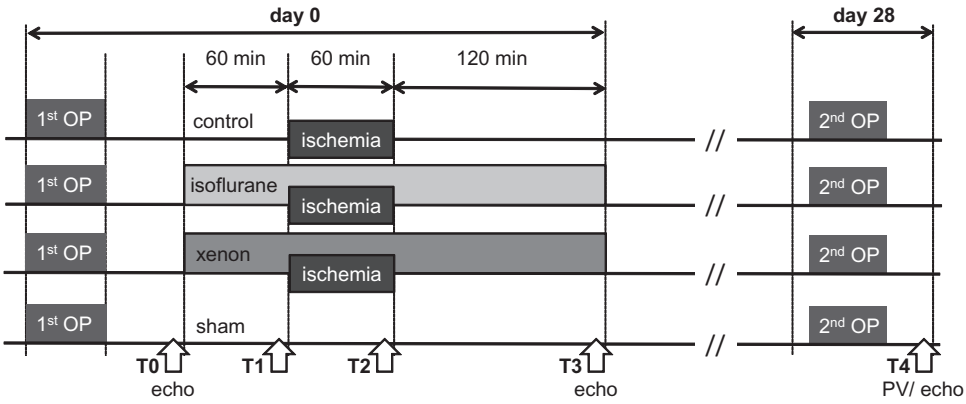

Fig. 1. Experimental protocol: animals were randomly assigned to the four study groups: control (s-ketamine), isoflurane, xenon, and sham. After instrumentation on day 0, (first OP) the rats were subjected to 60 minutes of myocardial ischemia and 120 minutes of reperfusion. After a follow-up of 28 days, conductance catheter measurements were performed following additional instrumentation (second OP). The *arrows* mark the measurement time points (T0–T4). Echocardiograms (echo) were acquired at TO, T3, and T4 and pressure volume loops (PV) at T4.

failed to reach statistical significance ($P = 0.054$). Anesthetic management influenced the expression of α-*skeletal actin*, with the highest values recorded in the control group (fig. 6B). The highest values of *periostin* expression were observed in the control and isoflurane groups, but significant differences between groups could be demonstrated (fig. 6C). Expression of *sarco/endoplasmic reticulum* Ca^{2+}*-ATPase* seemed to be reduced in the control group, but this effect did not reach a level of significance (fig. 6D).

Discussion

This investigation demonstrated for the first time the different functional and structural effects of isoflurane and xenon anesthesia on post-infarction cardiac remodeling. In the present model, 60 min of transient myocardial ischemia induced significant myocardial infarction, which led to progressive adverse remodeling after 4 weeks, with changes in structure, shape, and function. As expected, isoflurane and xenon reduced initial myocardial injury and hemodynamic compromise, whereas the extent of sequent adverse remodeling was different after 4 weeks. Decrease in remodeling index, impairment of cardiac function, and upregulation of fetal genes were impeded to a greater extent in xenon- than isoflurane-anesthetized animals at the administered concentrations.

Until now, only the acute effects of isoflurane and xenon on IR injury have been described. Comparative investigations demonstrated that the effects of the initial myocardial injury are reduced to the same extent after conditioning with either isoflurane or xenon.[14,18] Although xenon is less cardiodepressant than isoflurane in normal hearts,[19] differences in the hemodynamic responses to acute ischemia or afterload increase are the result of diverse actions on the vascular system and not myocardial contractility.[20,21] These observations could be confirmed by the current investigation, especially by the reduced impairment of regional contractility (RS_{ant}). Again, no differences in the effects of isoflurane or xenon on hemodynamics (HR, sAP, and CO), global function of the heart (RS_{global}), or the reduction of myocardial injury (troponin I) in the acute phase were found. Regional function as displayed by RS_{ant} seemed to be better after xenon anesthesia. Although this difference disappeared, relevant changes could be described 4 weeks after IR injury.

The processes involved in adverse cardiac remodeling after myocardial infarction include hypertrophy, necrosis,

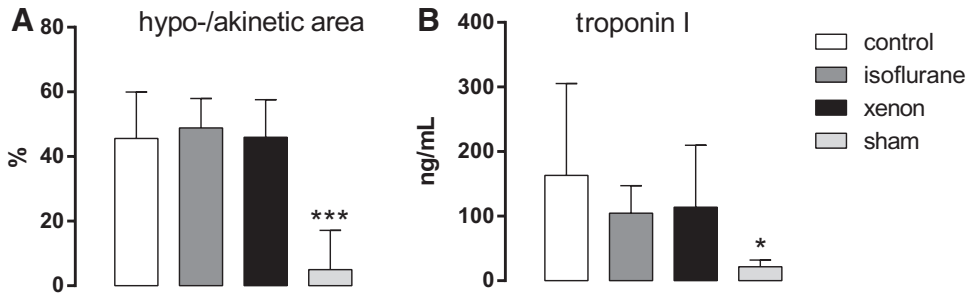

Fig. 2. Initial myocardial injury: effect of anesthesia on the size of hypo-/akinetic area determined by echocardiography (*A*) and serum concentrations of troponin I (*B*). (Values are means ± SD, * $P < 0.05$ and *** $P < 0.001$ *versus* control from ANOVA and *post hoc* test.)

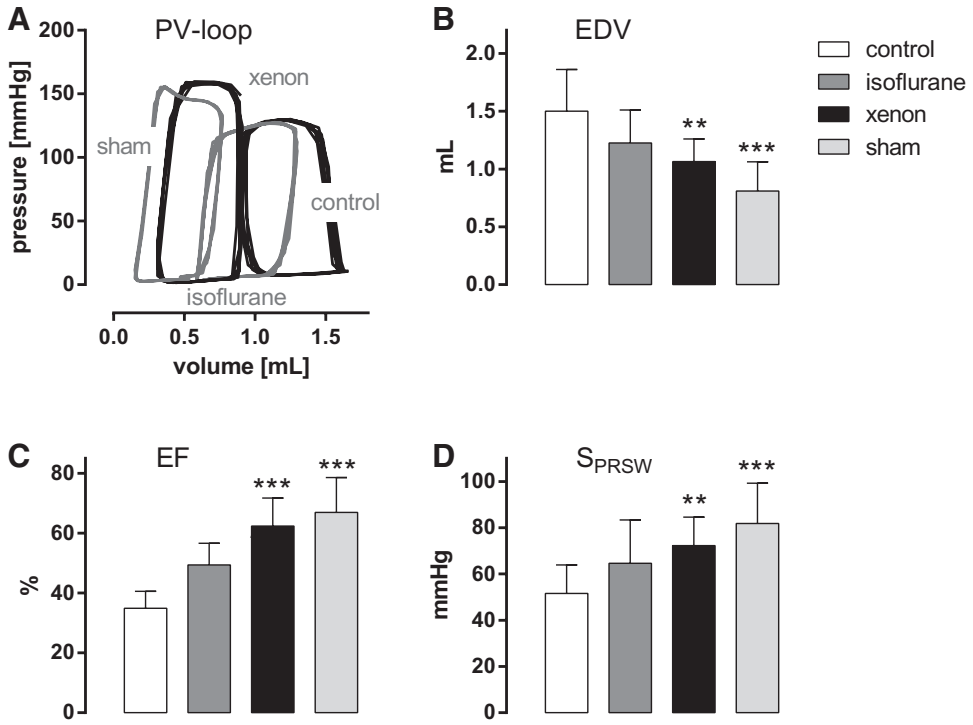

Fig. 3. Global cardiac function (T4): effect of anesthesia after 28 days on exemplary pressure volume (PV) loops (*A*), end-diastolic volume (EDV; *B*), ejection fraction (EF; *C*), and slope of the preload recruitable stroke work relation (S_{PRSW}; *D*) as measured by conductance catheter (values are means ± SD, ** $P < 0.01$ *** $P < 0.001$ *vs.* control from ANOVA and *post hoc* test).

apoptosis, fibrosis, angioneogenesis, and contractile dysfunction, primarily in the remote area and also the peri-infarct area, leading to progressive ventricular dilatation and deterioration of function.[22,23] Permanent coronary artery occlusion in the rat led to a 25% increase in heart weight, a 40–50% increase in ventricular volume/diameter, a 20% thinning of the posterior wall, and a 50–60% reduction of the EF after 4 weeks.[24–27] These results are consistent

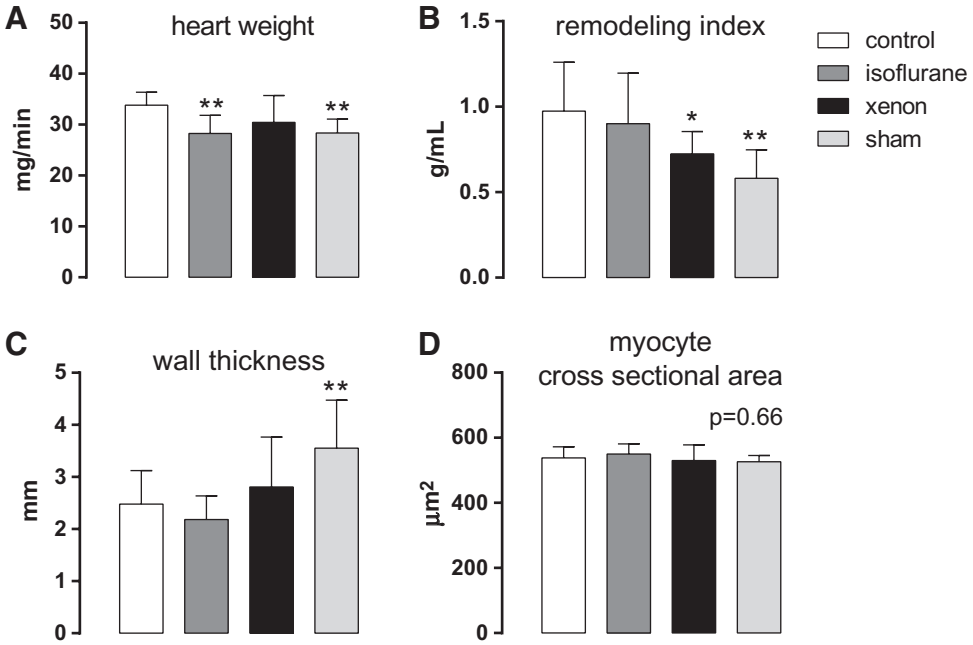

Fig. 4. Indices of myocardial hypertrophy (day 28): effect of anesthesia after 28 days on heart weight normalized to tibia length (*A*), remodeling index (*B*), thickness of the posterior wall (*C*), and cardiomyocyte cross-sectional area (*D*) (values are means ± SD, * $P < 0.05$ ** $P < 0.01$ *vs.* control from ANOVA and *post hoc* test).

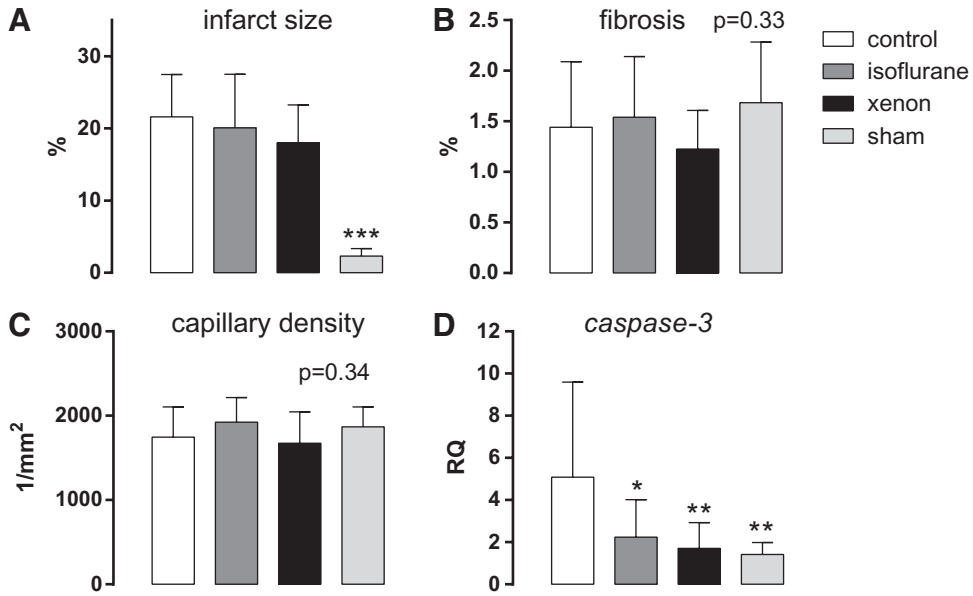

Fig. 5. Indices of myocardial remodeling (T4): effect of anesthesia after 28 days on infarct size (*A*), extent of fibrosis (*B*), capillary density (*C*), and relative quantity (RQ) of *caspase-3* expression (*D*) in the remote area (values are means ± SD, * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$ *vs.* control from ANOVA and *post hoc* test).

with the findings of this investigation and published data after transient ischemia and could be displayed changes in shape (left ventricular end-diastolic diameter, EDV, heart weight, wall thickness, and remodeling index) and function (EF, S_{PRSW}).[28–30] In contrast to the published data,[24,27] no significant increase in the myocyte cross-sectional area or fibrosis and no reduction in capillary density at a remote site were observed in untreated animals after IR-injury. As expected, the α/β-*MHC* ratio decreased and the expression of α-*skeletal actin* and *periostin* increased during the 4 weeks after IR injury, indicating adverse remodeling.[22,31] Downregulation of *sarco/endoplasmic reticulum* Ca^{2+}*-ATPase* did not reach a significant level,[32] whereas upregulation of the pro-apoptotic gene *caspase-3* was confirmed.[33]

Different therapies have been tested in animal models to limit post-infarction remodeling. Reperfusion within 60–120 min reduces ventricular dilation by 20% without reduction of the infarct size.[34] Treatment for 4 weeks with inhibitors of the angiotensin-converting enzyme, β1-receptor antagonists, and β2-agonists reduced heart weight by 30%, fibrosis by 40–50%, and dilatation by 10–20%, and it improved EF by 25%.[26,35–37]

Compared to these findings, xenon anesthesia inhibited ventricular dilation (EDV, remodeling index), functional deterioration (EF, S_{PRSW}), and expression of fetal genes (β-*MHC, periostin*) better than isoflurane, although infarct size and initial myocardial injury were comparable. The lower heart weight in combination with a thin ventricular wall and larger volume might indicate a greater loss of cardiomyocytes after isoflurane anesthesia. In contrast, the protective effects of xenon exceed the action of angiotensin-converting enzyme inhibitors and β1 receptor antagonists, with 30% less EDV and a retained EF of 62%, which did not differ from sham animals, while EF of untreated animals was 35%. Only the EF and S_{PRSW} as a load independent parameter of contractility were able to demonstrate functional differences between isoflurane, xenon, and control groups, whereas echocardiographic values failed (SR_{ant}, RS_{global}) 4 weeks after IR injury. Although comparative studies in rats between strain and pressure–volume loop analysis are missing, an echocardiogram should include all segments of the left ventricle to describe contractility after myocardial infarction.[38]

In summary, these differences between isoflurane and xenon surprisingly indicated that short-term therapy in the acute phase was able to prevent adverse cardiac remodeling, independent of the initial reduction of myocardial injury. However, at the concentrations administered, xenon appeared to be superior to isoflurane in prevention of ischemic damage and promotion of long-term recovery of contractile function.

The mechanisms can only be speculative. Whereas transcriptional changes of isoflurane after pre- or post-conditioning have been described in detail,[7] less is known about the effects of xenon. Differences in the activation of pro-survival and death-promoting signaling kinases were observed. Preconditioning was mediated, among others, by the activation of the mitogen-activated protein kinase, extracellular signal-regulated kinase, and Akt1 pathways.[39,40] Whereas xenon and isoflurane activated the phosphatidylinositide 3-kinase, Akt1, glycogen synthase kinase 3, extracellular signal-regulated kinase, and p38α, only isoflurane phosphorylated c-Jun N terminal kinases.[41,42] Activation of c-Jun N terminal kinases promotes apoptosis and pathologic ventricular dilation.[43] In contrast to isoflurane,

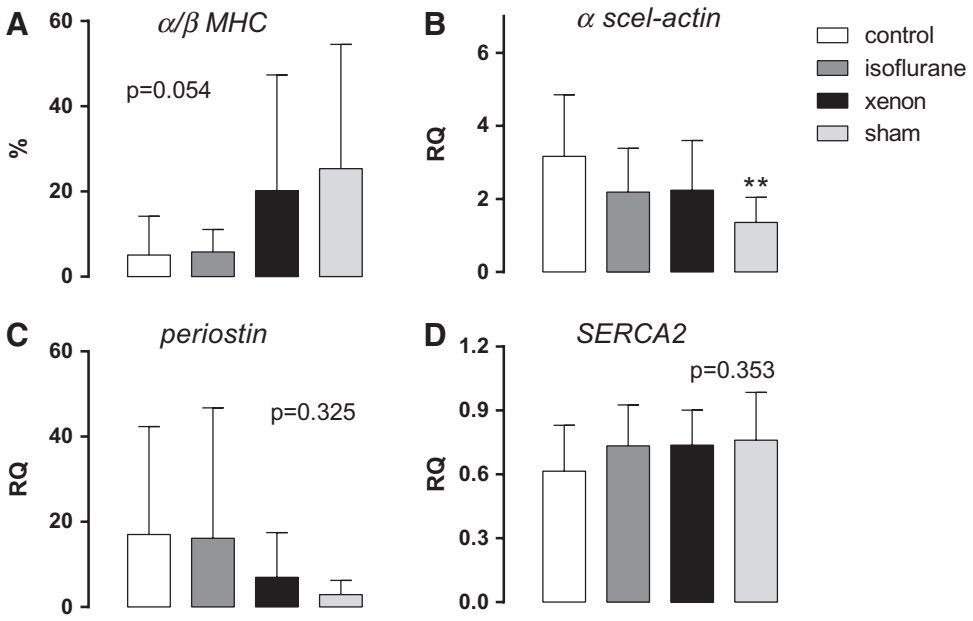

Fig. 6. Gene expression (day 28): effect of anesthesia after 28 days on the ratio of α- and β-*MHC* (*A*) and relative quantity (RQ) of α-*skeletal* (scel) actin (*B*), *periostin* (*C*), and *sarco/endoplasmic reticulum* Ca^{2+}*-ATPase* (SERCA2) (*D*) in the remote area (values are means ± SD, ** $P < 0.01$ *vs.* control from ANOVA and *post hoc* test).

xenon led to a translocation of heat shock protein 27 from the cytosolic fraction to the sarcomere fraction.[39] This has been shown to be relevant for the recovery of contractile function after IR injury.[44–46] It has also been demonstrated that heat shock protein 27 phosphorylation attenuates doxorubicin-induced dilative cardiomyopathy.[47] These findings require further comparative studies. Minor changes during the early phase might influence stress-mediated expansion of a non-infarcted myocardium (Grossman concept).[48]

As all animals received a substantial amount of s-ketamine and the cumulative dosage differs between groups, possible different interactions with isoflurane and xenon might influence the results. Binding sites for s-ketamine were described on cholinergic, adrenergic, ion channels (calcium, sodium), opiod and N-methyl d-aspartate receptors even in the heart.[49] Resulting direct or indirect sympathicomimetic properties (release of catecholamines and inhibition of reuptake) were opposed by a direct negative inotropic and N-methyl d-aspartate receptor 1-mediated negative chronotropic effect.[50] Although stimulation of opiod[51] and adrenergic receptors[52] before ischemia induced cardioprotection, s-ketamine does not affect ischemic preconditioning[53] or phosphorylation in IR injury of involved protein kinases.[39] Furthermore, no differences for HR, sAP, and anesthetic depth, which might influence IR injury, could be detected between groups. Thus, a relevant drug interaction between s-ketamine and isoflurane, respectively, xenon seemed to be unlikely.

It is noteworthy that in terms of anesthetic state, the corresponding MAC value of 0.6 vol% isoflurane was comparable in humans and rats (0.46 *vs.* 0.43) but was different for 70 vol% xenon (1.0 *vs.* 0.43). This suggests species-dependent different mechanism of action.[54] While the species-dependent effects of anesthetic gases on anesthetic depth and baroreceptor reflexes are described in detail, the sensitivity of the cardiomyocytes against them has not been compared yet. However, MAC comparisons have been the standard in anesthetic studies and led to comparable hemodynamic conditions, as in the current investigation. Whereas the extent of neuroprotection by xenon[54] and isoflurane[55] was dose dependent, the effect of isoflurane within the range of 0.25 and 1.75 MAC on the myocardium remained controversial. Reduction of myocardial infarct size by isoflurane seemed to be independent of concentration in the rat heart,[56] but dependent in dogs.[57] The mechanism of protection was dependent of dosage, as protein kinase Cε was phosphorylated only at low concentrations (0.4 MAC)[56] and was additionally influenced by coronary flow, especially in animals with preformed coronary collaterals.[57] Comparable results are missing for xenon, but 20 vol% of xenon was insufficient for post-conditioning in the rat heart.[58] Thus, it would be difficult to separate direct or indirect mediated effects of anesthetics on the heart, and further studies with various concentrations are needed.

Further limitations of this study include the low animal number per group and the short observational period. The effects on gene expression and cardiac function will be more pronounced after 3 months.[37,59] However, as the differences between isoflurane and xenon were not predictable, power calculations would have been difficult. Unfortunately, assessment of hemodynamics and echocardiograms was carried out unblinded from time point T0 to T3.

In conclusion, despite similar acute cardioprotective properties, xenon impeded adverse cardiac remodeling after IR injury more effectively than isoflurane, especially regarding the decrease in ventricular dilatation and the improvement of cardiac function.

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