Myocardial Accumulation of Bupivacaine and Ropivacaine Is Associated with Reversible Effects on Mitochondria and Reduced Myocardial Function

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BACKGROUND: Mechanisms of local anesthetic cardiac toxicity are still not completely understood. In this study, we analyzed whether concentrations of local anesthetics found in clinical toxicity affect myocardial mitochondrial structure and oxygen consumption.  

METHODS: Guinea pig isolated heart Langendorff preparations were exposed to bupivacaine (3.0 and 7.5 μg/mL) and ropivacaine (3.6 and 9.0 μg/mL) for 10 minutes. Heart rate, systolic blood pressure, the first derivative of left ventricular pressure (+dP/dt), electrocardiogram, and coronary flow were recorded. The local anesthetic tissue concentration was measured either immediately after local anesthetic exposure, or after 20- and 60-minute washout periods. In addition, electron microscopy of myocardial mitochondria was performed using a scoring system for structural damage of mitochondria. Cardiomyocyte cell culture was incubated with bupivacaine, and oxygen consumption ratio, extracellular acidification, and relative amounts of PGC-1α mRNA, a regulator of cellular energy metabolism, were determined.  

RESULTS: Bupivacaine and ropivacaine induced reversible PR interval and QRS prolongation, and left ventricular pressure and +dP/dt reduction. Myocardial tissue concentration of local anesthetics was 3-fold the arterial concentration. Mitochondria showed a significant concentration-dependent morphological swelling after local anesthetic application. These changes were reversed by a 20-minute washout period for ropivacaine and by a 60-minute washout for bupivacaine. Bupivacaine reduced mitochondrial oxygen consumption and increased PGC-1α expression in neonatal cardiomyocyte cell cultures, whereas fatty acid metabolism remained unaffected.  

CONCLUSIONS: Bupivacaine and ropivacaine accumulate in the myocardium. Reversible local anesthetic-induced mitochondrial swelling occurs at concentrations that induce a negative inotropic effect. Bupivacaine reduces cellular metabolism, whereas this reduction is reversible by fatty acids. Interaction with mitochondria may contribute to the negative inotropic effect of local anesthetics. (Anesth Analg 2013;116:83–92)
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An isolated perfused, nonrecirculating Langendorff guinea pig heart preparation was used for this study. The investigation was performed in accordance with the Guide for the Care and Use of Laboratory Animals issued by the United States National Institutes of Health and was approved by the local government authority (24D-9168.24-1-2006-22). All isolated heart experiments were conducted with guinea pigs (16–22 weeks old; weighing 215–230 g) purchased from Charles River (Sulzfeld, Germany). The animals were intraperitoneally heparinized (1000 U/kg) to prevent the formation of intracoronary microthrombi and were anesthetized with 150 mg/kg intraperitoneal thiopental (Byk Gulden, Germany). Hearts were rapidly excised, weighed, and then perfusion was performed in a retrograde manner via the aorta at a constant perfusion pressure of 65 mm Hg with a modified Krebs-Henseleit buffer (KHB in mmol/L): NaCl 116, KCl 4.56, MgSO₄ 2.24, KH₂PO₄ 1.18, NaHCO₃ 25.0, glucose 8.27, pyruvate 2.0, and CaCl₂ 2.52 as described previously. The solution was continuously bubbled with 95% oxygen and 5% carbon dioxide and pH was maintained at 7.35 ± 0.03. Arterial and effluent perfusate PO₂ and PCO₂ (sampled via the inflow line or via a catheter placed in the pulmonary artery, respectively) were measured regularly (AVL 990; Medical Instruments, Bad Homburg, Germany). All elements of the perfusion apparatus were water-jacketed and maintained at 37°C. Left ventricular systolic pressure (LVP) and its first derivative +dP/dt were continuously measured with a balloon catheter inserted into the left ventricle (Gould Inc., Statham Instruments, Oxnard, CA) via a cut in the mitral valve. Diastolic left ventricular pressure was adjusted to 5 mm Hg. Coronary flow and coronary perfusion pressure were continuously measured by an in-line flowprobe (Transonic Flowprobe; Transonic Systems Inc., New York, NY) and a pressure transducer (Gould Nicolet, Erlensee, Germany) attached to the perfusion cannula 2 cm above the orifice of the coronary vessels. Hemodynamic variables and derivatives (heart rate HR, LVP, +dP/dt, coronary flow) and electrocardiogram data (PR, QRS, QTc) were continuously sampled and documented by a software system (PoNeMah, P3 plus Version 4; Gould LDS Test and Measurement LLC, Valley View, OH). Biventricular pacing was performed using an HSE Stimulator P (Hugo Sachs Elektronik, March, Germany) with a 2-millisecond/2-V amplitude. Vo₂ was calculated from the arterial-venous partial pressure difference (avDo₂), Bunsen absorption coefficient (αO₂ = 0.0316 μL × mL buffer⁻¹ × mm Hg⁻¹), and coronary flow according to the Fick principle as follows: Vo₂ μL/min × g = avDo₂ mm Hg × αO₂ μL/mL × mm Hg × flow mL/min × g. All infused compounds were administered through a stainless-steel cannula placed into the aortic inflow line proximal to the flowprobe (Precidor; Infors AG, Basel, Switzerland). The experimental protocol was started when LVP, +dP/dt, and HR had reached stable baseline values, i.e., 20 minutes after artificial perfusion had been commenced.

**METHODS**

**Preparation of Isolated Hearts**

Pilot experiments were performed with 2 isolated guinea pig hearts to determine the local anesthetic concentration that led to a stable 50% decrease in LVP and +dP/dt over 10 minutes. This concentration was 3.0 μg/mL (10.4 μM) for bupivacaine and 3.6 μg/mL (13.1 μM) for ropivacaine. These concentrations were set as the low concentration for further experiments. The high concentration was set as 2.5-fold of the low concentration: 7.5 μg/mL (26 μM) for the high bupivacaine concentration and 9.0 μg/mL (32.8 μM) for the high ropivacaine concentration.

The hearts were constantly paced at 250 bpm and were randomized by lot to 1 of 5 groups (5 hearts per group) with either a local anesthetic in a low or high concentration in KHB or KHB alone for 10 minutes. The same protocol was used in a further 8 groups, except for an additional 20-minute (w₀₂₂) or 60-minute (w₀₂₃) washout period with KHB after the local anesthetic infusion (5 hearts per group). At the end of each protocol, heart tissue samples were collected from 4 sites of the left ventricle and 100 mg of heart tissue was weighed for further investigations.

**Subcellular Fractionation**

Subcellular fractionation of the Langendorff perfused hearts was performed according to previously reported procedures with some modifications. In brief, hearts were uncoupled from the perfusion system and both ventricles were minced into 1- to 2-mm pieces and homogenized with 3 to 4 strokes at 600 rpm using a glass-Teflon Potter-Elvehjem tissue grinder (Sücht homogen plus®; Schütt Labortechnik GmbH, Göttingen, Germany). The resulting homogenate was separated by filtration through double-layered gauze and centrifuged at 700g for 10 minutes at 4°C. After centrifugation, mitochondria, crude nuclei, intact cells, and gross debris were pelleted, whereas the cytoplasm with microsomes was found in the supernatant.
Local Anesthetic Concentration Measurement
A liquid chromatography–tandem mass spectrometric (LC-MS-MS) method with a rapid and simple sample preparation was developed and validated for the detection of bupivacaine and ropivacaine as described previously.10 The mass spectrometer was operated in the multiple reaction monitoring mode. A simple protein precipitation was sufficient for sample preparation, as described previously.11 In brief, the LC-MS-MS system used was a Quattro micro (Waters, Milford, MA) equipped with an electrospray interface. The chromatographic separation was performed on a Synergy 4-μm Polar-RP 80A, 150 x 2 mm column. A mobile phase gradient was applied with a mixture of acetonitrile, ammonium acetate in water, and formic acid. Retention times for bupivacaine were 4.6 minutes and 4.2 minutes for ropivacaine. A good linear response rate was found from 1 to 200 ng/mL. Higher concentrated samples were diluted.

Transmission Electron Microscopy
A left ventricular sample of each heart measuring approximately 3 mm³ was fixed in 0.1 M sodium cacodylate buffer (pH 7.4) containing 2.5% glutaraldehyde and stored overnight at 4°C. The tissue was postfixed with 1% osmium tetroxide (pH 7.4) in 0.1 M cacodylate buffer for 1 hour at 4°C; dehydration was performed with ethanol and ethanol-epon. The whole mounts were then epon-embedded overnight at 4°C, and epon-filled flat embedding molds were subsequently polymerized for 48 hours at 60°C. Ultrathin sections (60–80 nm) were cut at room temperature with a Reichert Ultracut S microtome (Leica Microsystems, Wetzlar, Germany) and mounted on nickel grids. Sections were then contrasted with 0.4% lead citrate and 3% uranyl acetate and viewed using an EM 906 electron microscope (Carl Zeiss, Oberkochen, Germany). All buffers, fixatives, and embedding materials for electron microscopy were purchased from Serva (Heidelberg, Germany).

Qualitative Morphological Scoring
We evaluated high-resolution black and white electron photomicrographs of cardiac mitochondria using the following ultrastructural injury scoring system from 0 to 5, first described by Joshi et al.:10 0 = normal appearance, 1 = swelling of endoplasmic reticulum, minimal mitochondrial swelling, 2 = mild mitochondrial swelling, 3 = moderate or focal high-amplitude swelling, 4 = diffuse high-amplitude swelling, disruption of crystal membrane integrity, and 5 = high-amplitude swelling with some mitochondrial flocculent densities and/or calcifications. Six photomicrographs of each heart sample depicting between 5 and 10 mitochondria each were evaluated. This scoring was performed by an experimenter blinded to the groups with extensive experience in morphological scoring and electron microscopy (LK).

Mouse Cardiomyocyte Isolation
Pregnant NMRI mice were purchased from Janvier (St Berthevin, France). Maintenance and animal experimentation were in accordance with the Swiss Federal Veterinary Office (BVET) guidelines. Neonatal mouse (NMRI) ventricles were in accordance with the Swiss Federal Veterinary Office (BVET) guidelines. Neonatal mouse (NMRI) ventricles were obtained from pregnant NMRI mice purchased from Janvier (St Berthevin, France). Maintenance and animal experimentation were in accordance with the Swiss Federal Veterinary Office (BVET) guidelines. Neonatal mouse (NMRI) ventricles were obtained from pregnant NMRI mice purchased from Janvier (St Berthevin, France). Maintenance and animal experimentation were in accordance with the Swiss Federal Veterinary Office (BVET) guidelines. Neonatal mouse (NMRI) ventricles were obtained from pregnant NMRI mice purchased from Janvier (St Berthevin, France). Maintenance and animal experimentation were in accordance with the Swiss Federal Veterinary Office (BVET) guidelines. Neonatal mouse (NMRI) ventricles were obtained from pregnant NMRI mice purchased from Janvier (St Berthevin, France). Maintenance and animal experimentation were in accordance with the Swiss Federal Veterinary Office (BVET) guidelines. Neonatal mouse (NMRI) ventricles were obtained from pregnant NMRI mice purchased from Janvier (St Berthevin, France). Maintenance and animal experimentation were in accordance with the Swiss Federal Veterinary Office (BVET) guidelines. Neonatal mouse (NMRI) ventricles were obtained from pregnant NMRI mice purchased from Janvier (St Berthevin, France). Maintenance and animal experimentation were in accordance with the Swiss Federal Veterinary Office (BVET) guidelines. Neonatal mouse (NMRI) ventricles were obtained from pregnant NMRI mice purchased from Janvier (St Berthevin, France). Maintenance and animal experimentation were in accordance with the Swiss Federal Veterinary Office (BVET) guidelines. Neonatal mouse (NMRI) ventricles were obtained from pregnant NMRI mice purchased from Janvier (St Berthevin, France).

Oxygen Consumption Rate and Extracellular Acidification Rate
Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured using a Seahorse Bioscience XF24 analyzer in 24-well plates at 37°C, with correction for positional temperature variations adjusted from 4 empty wells evenly distributed within the plate. Neonatal mouse cardiomyocytes were seeded at 50,000 cells/well 3 days before the analysis. Growth medium was changed to unbuffered Krebs-Henseleit assay medium (mmol/L): NaCl 111, KCl 4.7, MgSO₄ 2, Na₂HPO₄ 1.2, glucose 2.5 adjusted to a pH of 7.4 with 1 N NaOH, 1 hour before starting the experiment. After baseline measurements of OCR and ECAR, either 5 or 10 μM (1.5 μg/mL or 2.9 μg/mL) bupivacaine or 0.1% dimethylsulfoxide (1 μL/1 mL) as a solvent control was added and OCR and ECAR were plotted. Finally, palmitic and oleic acid were added in a concentration of 0.6 mM. Each experimental condition was performed on 10 biological replicates as recommended by the manufacturer (Seahorse Bioscience).

Quantitative Reverse Transcriptase Polymerase Chain Reaction
Cultured neonatal mouse cardiomyocytes were treated with 100 μM bupivacaine for 6 hours just before lysis for RNA isolation. RNA was isolated with an RNA-Isolation Kit (Macherey-Nagel, Düren, Germany) and cDNA generated using a Reverse Transcription Kit (GE, Munich, Germany) and random hexamers (Fermentas). Quantitative polymerase chain reactions (qPCR) were run on a Roche LightCycler 480 (Roche, Grenzach-Wyhlen, Germany) using the following cycling conditions: 5 minutes of denaturation at 95°C was followed by amplification of 40 cycles—10 seconds at 95°C, 20 seconds at 60°C, and 20 seconds at 72°C, then maintained for 7 minutes at 72°C.

The relative gene expression levels were calculated using the comparative Cₐ method and are reported as ratios to those of the housekeeping gene hypoxanthine-guanine phosphoribosyltransferase (Hprt). The sequences from quantitative real-time PCR primers used are as follows: Hprt (Fwd) TCA GTC AAC GGG GGA CAT AAA/(Rev) GGG GCT GTA CTG CTT AAC CAG; PGC-1α (Fwd) GGATTGAAGTGGTGTAGCGAC/GCTCATTGTTGTA and (Rev) CTGGTTGGA (Rev).

Statistical Analysis
All data are presented as mean ± SEM unless otherwise indicated. For between-group comparisons of hemodynamic variables, values from 0 to 10 minutes were averaged.
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and used as baseline values. Statistical analyses were performed using SPSS software for MS Windows (release 11.0; SPSS Inc., Chicago, IL) and SigmaStat (release 3.0; Systat Software Inc., San Jose, CA). All variables were tested for normal distribution with the Kolmogorov-Smirnov test. Analysis of variance for repeated measures and Bonferroni post hoc adjustment were performed for parametric data. The results of the mitochondrial ultrastructural injury scoring were analyzed with Kruskal-Wallis analysis of variance on ranks with post hoc Dunn procedure for nonparametric data. Intergroup significances were calculated using the Student t test for unpaired data. A P value <0.05 indicated statistical significance.

RESULTS

Baseline Isolated Heart Hemodynamics
Coronary flow was 12 ± 2.9 mL/min, systolic pressure 100.9 ± 16.5 mm Hg, and +dP/dt 1837.5 ± 278.9 mm Hg/s. Perfusion oxygenation values and pH were similar for all experimental groups at baseline. Baseline Vo2 was 207.2 ± 12.6 μL/min × g, PR interval 60 ± 20 milliseonds, QRS complex duration 30 ± 7 milliseconds and QTc 279.2 ± 41 milliseconds.

Effects of Local Anesthetics on Isolated Heart Hemodynamics
Bupivacaine and ropivacaine had a negative inotropic effect. The addition of both bupivacaine and ropivacaine at the low as well as at the high concentration led to a significant decrease of +dP/dt and LVP (Fig. 1, A and B; P < 0.05). The PR interval was significantly prolonged in the 2 high local anesthetic concentration groups (Fig. 2A; P < 0.05). QRS complex duration was significantly prolonged by bupivacaine and ropivacaine in comparison to controls at both the low and high concentration (Fig 2B; P < 0.05), whereas QTc remained unaffected over the whole experiment (data not shown). Neither the low nor the high local anesthetic concentration significantly influenced coronary flow (data not shown). However, both local anesthetics led to a significant decrease in Vo2 as shown in Figure 3 (P < 0.05).

Local Anesthetic Tissue Concentration
No local anesthetics were detected in the arterial (influent) samples of control hearts. Measurement of arterial and tissue local anesthetic concentrations after 10 minutes of local anesthetic infusion showed that the tissue concentration was always significantly higher than the arterial concentration (Fig 4; P < 0.05 for comparison between arterial and tissue concentrations). Arterial versus tissue concentrations in the low bupivacaine group were 3.14 ± 0.18 μg/mL versus 18.54 ± 5.98 μg/g, and 7.20 ± 0.8 μg/mL versus 33.5 ± 7.1 μg/g in the high bupivacaine group. In the low ropivacaine group, concentrations were 3.62 ± 0.20 μg/mL versus 17.5 ± 1.4 μg/g, and 9.69 ± 3.21 μg/mL versus 28.66 ± 9.09 μg/g in the high group.

Mitochondrial Effects of Local Anesthetics
The evaluation of the electron microscopy images of the cardiac mitochondria after isolated heart perfusion showed that a 10-minute perfusion with local anesthetics led to significant mitochondrial structural changes compared with the control group. This was reflected in a higher mitochondrial damage score (Fig. 5 exemplary electron microscopy images; Fig. 6 mitochondrial score). Significantly higher scores were assigned to samples treated with high local anesthetic concentrations compared with low local anesthetic concentrations, whereas no difference was found between treatment with ropivacaine and bupivacaine before washout (P < 0.05).

Reversibility of Mitochondrial Effects
In the 20- and 60-minute local anesthetic washout groups +dP/dt, LVP, PR interval and QRS complex duration, and Vo2 recovered to baseline values as shown in Figure 1, A and B; Figure 2, A and B; and Figure 3. Neither bupivacaine nor ropivacaine was detectable in the inflow samples or the myocardial tissue after the washout period. Electron microscopy evaluation showed a significantly increased mitochondrial score in the 2 bupivacaine groups in comparison to controls and both ropivacaine groups after a 20-minute washout (Figs. 5 and 6; P < 0.05). After a 60-minute washout
period, mitochondrial morphology was comparable between the bupivacaine and control groups (Figs. 5 and 6).

**In Vitro OCR and ECAR**

In vitro noninvasive metabolic readout of neonatal mouse cardiomyocytes showed that the addition of bupivacaine at concentrations of 5 and 10 μM resulted in a dose-dependent reduction of the OCR (Figs. 7 and 8). ECAR was similarly reduced by the addition of bupivacaine (Fig. 8). The in vitro respiratory response to fatty acids was unchanged by bupivacaine (Fig. 9).

**PGC-1α**

Incubation of neonatal mouse ventricle cardiomyocytes with 100 μM bupivacaine for 6 hours led to a 2-fold increase in PGC-1α mRNA compared with controls (Fig. 10; P < 0.001).

**DISCUSSION**

The main novel finding of this work is that concentrations of bupivacaine and ropivacaine that lead to a depression of myocardial function in the isolated guinea pig heart also induce changes in mitochondrial morphology. These morphological changes are reversible after local anesthetic washout, with a longer persistence for bupivacaine than for ropivacaine. We could show that a 10-minute infusion leads to an approximately 3-fold accumulation of local anesthetics in cardiomyocytes. In vitro mitochondrial VO₂ is reduced by bupivacaine, but is reversed by fatty acids. In addition, bupivacaine is associated with an increase in PGC-1α in neonatal mouse cardiomyocytes.

In our experiments, both bupivacaine and ropivacaine at a low as well as a high concentration decreased LVP and +dP/dt. The extent of this negative inotropic effect was comparable to results of previous studies using this same
Negative inotropy has been considered to be a result of inhibition of the ryanodine receptor of sarcoplasmic reticulum or even contractile apparatus inhibition. In addition, it has been speculated that the inhibition of mitochondrial processes may also contribute to negative inotropy. Tanz et al. examined the effect of bupivacaine in an isolated heart model and described a reduction in myocardial $V_{O_2}$, yet did not pace hearts to control for the concomitant reduction in HR induced by local anesthetics.

Mitochondria have traditionally been considered for their role in cellular oxygen phosphorylation and ATP synthesis. In recent years it has become clear that mitochondria are also closely involved in cellular calcium homeostasis and reactive oxygen species–mediated processes. Cellular stressors, i.e., ischemia, lead to an intracellular calcium overload. When this stimulus is accompanied by oxidative stress and ATP depletion, a transient opening of a nonspecific permeability transition pore (PTP) in the inner mitochondrial membrane is induced. This PTP opening leads to matrix swelling and in the end to rupture of the outer membrane, releasing apoptotic factors (e.g., cytochrome $c$) into the cytosol. In isolated rat skeletal muscle mitochondria, bupivacaine-induced PTP opening was found to be the relevant mitochondrial membrane depolarizing mechanism.

In our study, electron microscopy of isolated heart myocardium samples at the end of a 10-minute local anesthetic infusion period showed a significant mitochondrial swelling compared with mitochondria from control hearts. The extent of mitochondrial swelling was also significantly increased in the high- versus the low-concentration local anesthetic groups. This effect therefore seems to be concentration dependent.

In several previous studies, mitochondria were isolated from the myocardium and then exposed to local anesthetics. Inhibitory effects, predominantly on complex I electron transport, were found at local anesthetic concentrations between 0.5 and 1 mM. Other experiments on the effects of local anesthetics on mitochondria of fibroblasts in cell culture demonstrated that bupivacaine leads to a collapse of mitochondrial transmembrane potential at buffer concentrations of 2.5 mM and to a 50% decrease in $V_{O_2}$ at 1.5 mM.

The concentrations of local anesthetics used in our isolated heart study were comparable to clinical toxic concentrations, far below the concentrations used in the isolated mitochondria experiments. One possible explanation for this discrepancy between in vitro and in vivo concentrations is that highly lipophilic local anesthetics accumulate.

Figure 5. Exemplary electron microscopy images of cardiac mitochondria of each of the 13 experimental groups (wo = washout, bupi low = 3.0 μg/mL, bupi high = 7.5 μg/mL, ropi low = 3.6 μg/mL, and ropi high = 9.0 μg/mL, scale = 0.5 μm). White arrows = focal high-amplitude matrix swelling.
in cardiac myocytes, resulting in far higher intracellular concentrations, as hypothesized for peripheral muscle tissue.\textsuperscript{25} We measured the cytosolic local anesthetic concentration after a 10-minute infusion to test this hypothesis. Indeed, the tissue concentration was approximately 3-fold of the extracellular concentration, but nevertheless still 20 to 50 times lower than the millimolar concentrations used in isolated mitochondrial experiments.

In further experimental groups, we examined the effect of an additional 20- and 60-minute washout period after local anesthetic infusion. Hemodynamic variables returned to baseline values, indicating that negative inotropy was reversed. Measurement of the tissue concentrations revealed that local anesthetics were washed out of the myocardium. Myocardial washout has been described by Mazoit et al.\textsuperscript{26} in isolated rabbit hearts.

Figure 6. Mitochondrial damage score: each graph depicts the mitochondrial damage score (0 = no swelling to 5 = maximum swelling) distribution in percent of n evaluated mitochondria in each of the experimental groups (bupi low = 3.0 μg/mL, bupi high = 7.5 μg/mL, ropi low = 3.6 μg/mL, and ropi high = 9.0 μg/mL). wo = washout, time-matched controls (TMC) with 10 or 30 minutes of perfusion with Krebs-Henseleit buffer (white). Bupivacaine perfusion for 10 minutes with and without washout at the low and high concentration (gray). Ropivacaine perfusion for 10 minutes with and without washout at the low and high concentration (black).
showing that $T_{1/2}$ for bupivacaine was 11.5 minutes. In the electron microscopy evaluation, mitochondrial swelling after a 20-minute washout persisted only in the bupivacaine groups, not in the ropivacaine-treated isolated hearts. After a 60-minute washout, bupivacaine-treated hearts were similar to controls. Differences between bupivacaine and ropivacaine have been found for effects on myocardial conductance delay, extent of negative inotropy, and for isolated mitochondrial toxicity.

The baseline in vitro $V_{O_2}$ rate in isolated cardiomyocytes was reduced by the addition of bupivacaine. Interestingly, extracellular acidification as a sign of lactic acidosis resulting from anaerobic metabolism was not increased in cells treated with local anesthetics. This indicates that overall cellular metabolism and not only mitochondrial function is reduced by bupivacaine. Even more importantly, this overall reduction was easily and equally reversed by the addition of fatty acids to the cell supernatant. Maximum respiratory activity was comparable in cells with and without bupivacaine, showing that mitochondrial fatty acids metabolism is unimpaired. This parallels the positive inotropic effects of a lipid infusion we have previously seen in isolated hearts and underlines the positive effects of lipids in cases of myocardial toxicity of local anesthetics.

The reduction in contractile function and cell metabolism elicited by local anesthetics is accompanied by changes in regulators of cellular energy metabolism. PGC-1α is a transcriptional coactivator that regulates genes involved in energy metabolism and directly senses a multitude of signals of metabolic and neurohumoral conditions, e.g., ADP/ATP levels via adenosine monophosphate–activated protein kinase. PGC-1α is induced by ischemia and ATP wasting. In vitro incubation of cardiomyocytes with bupivacaine induced a 2-fold increase in PGC-1α, indicating that bupivacaine has an effect at the regulatory level of cellular energy metabolism.
In summary, we compared the effects of 2 negative inotropic concentrations of bupivacaine or ropivacaine on mitochondrial swelling in isolated guinea pig hearts. Mitochondrial swelling was concentration dependent and could be reversed by a 20-minute washout period for ropivacaine, and by a 60-minute washout period for bupivacaine. Bupivacaine and ropivacaine accumulated in the myocardium but did not reach concentrations previously needed in in vitro experiments to induce mitochondrial inhibitory effects. Overall cellular metabolism was reduced by bupivacaine, whereas this inhibition could be reversed by fatty acids.

DISCLOSURES
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Attestation: Nicole Hiller has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files.
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