

Adding Bupivacaine to High-potassium Cardioplegia Improves Function and Reduces Cellular Damage of Rat Isolated Hearts after Prolonged, Cold Storage

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Background: Bupivacaine retards myocardial acidosis during ischemia. The authors measured function of rat isolated hearts after prolonged storage to determine whether bupivacaine improves cardiac protection compared with standard cardioplegia alone.

Methods: After measuring cardiac function on a Langendorff apparatus, hearts were perfused with cardioplegia alone (controls), cardioplegia containing 500 μ M bupivacaine, or cardioplegia containing 2 mM lidocaine; were stored at 4°C for 12 h; and were then reperfused. Heart rate and left ventricular developed pressures were measured for 60 min. Maximum positive rate of change in ventricular pressure, oxygen consumption, and lactate dehydrogenase release were also measured.

Results: All bupivacaine-treated, four of five lidocaine-treated, and no control hearts beat throughout the 60-min recovery period. Mean values of heart rate, left ventricular developed pressure, maximum positive rate of change in ventricular pressure, rate-pressure product, and efficiency in bupivacaine-treated hearts exceeded those of the control group ($P < 0.001$ at 60 min for all). Mean values of the lidocaine group were intermediate. Oxygen consumption of the control group exceeded the other groups early in recovery, but not at later times. Lactate dehydrogenase release from the bupivacaine group was less than that from the control group ($P < 0.001$) but did not differ from baseline.

Conclusions: Adding bupivacaine to a depolarizing cardioplegia solution reduces cell damage and improves cardiac function after prolonged storage. Metabolic inhibition may contribute to this phenomenon, which is not entirely explained by sodium channel blockade.

CARDIAC transplantation is the treatment of last resort for end-stage heart disease.¹ Limits to the acceptable time for preserving donor hearts in a no-flow state restrict the distance a donor heart can be transported after harvest and therefore also limit the number of potential recipients for a given heart.^{1,2} Methods to improve cardiac preservation after harvest have been a focus of intense scientific investigation for more than three de-

acades. Current mainstays of cardiac protection combine hypothermia and depolarizing (high-potassium) cardioplegia to reduce myocardial oxygen consumption. However, hyperpolarizing cardioplegia with adenosine, potassium channel openers, or local anesthetics may have advantages compared with standard depolarizing solutions because myocardial resting membrane potential is maintained at normal levels, reducing injurious transmembrane fluxes of sodium and calcium ions.³

Bupivacaine is a potent local anesthetic⁴ with a profile of cardiac toxicity that would seem to preclude its use for cardiac preservation. However, infusion of a triglyceride emulsion can rapidly reverse bupivacaine-induced asystole in both rats and dogs, suggesting that bupivacaine toxicity is not inherently irreversible.^{5,6} Bupivacaine also retards the onset and progression of myocardial acidosis during no-flow states *in vivo*,⁷ consistent with the finding that lipid reversal can restore normal hemodynamics even after prolonged "downtime." These observations suggest, contrary to its toxic profile, that bupivacaine might confer some degree of protection against ischemic myocardial damage. Here, we studied rat isolated hearts to ascertain whether addition of bupivacaine to a high-potassium storage solution and subsequent reversal with a lipid emulsion improves cardiac function after prolonged, cold storage.

Materials and Methods

Rats

Adult male Sprague-Dawley rats, weighing between 450 and 550 g (3–4 months old) were used in all experiments. All protocols were approved by the Animal Care Committee of the University of Illinois Office for Protection of Research Subjects and by the Institutional Animal Care and Use Committee of the Veterans Affairs Chicago Healthcare System (Chicago, Illinois).

Isolated Heart Systems

Rats were anesthetized by intraperitoneal injection of 60 mg/kg sodium pentobarbital (Abbott Labs, Abbott Park, IL), and after systemic heparinization, hearts were removed, cannulated through the ascending aorta, suspended from a Langendorff apparatus, and retrograde perfused at a constant pressure of 80 mmHg with Krebs Ringer's bicarbonate buffer (KRB) containing 100.00 mM NaCl, 4.74 mM KCl, 1.18 mM KH_2PO_4 , 1.18 mM MgSO_4 ,

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1.00 mM CaCl_2 , 25.00 mM NaHCO_3 , 11.50 mM glucose, 4.92 mM pyruvate, and 5.39 mM fumarate, pH 7.40 *via* roller pump. KRB perfusate was warmed in the Langendorff apparatus by countercurrent flow from a 37°C water bath, and the temperature of the KRB was continuously measured just above the heart and maintained at 37°C. The heart was suspended inside a glass cylinder warmed by the same countercurrent. KRB was also equilibrated with a 95%–5% mixture of oxygen and carbon dioxide by passage through a membrane oxygenator.

Storage and Recovery

Pressure data from a latex balloon in the left ventricle connected to a pressure transducer were recorded, archived, and analyzed by Powerlab Data Analysis System using Chart 5.2.1 (ADInstruments, Colorado Springs, CO). A catheter was placed in the pulmonary artery to sample outflow from the coronary circulation for determining partial pressure of oxygen. After 15 min of equilibration, the ventricular balloon was deflated (to left ventricular [LV] diastolic pressure of -10 mmHg) to prevent damage caused by diastolic contracture during storage. Hearts in the control group were arrested by injecting, over 30 s, 20 ml of a 37°C, high-potassium cardioplegia solution (Plegisol; Abbott Labs), containing 110.0 mEq/l sodium, 160.0 mEq/l chloride, 16.0 mEq/l potassium, 2.4 mEq/l calcium, and 32.0 mEq/l magnesium. Test hearts were treated similarly but with either 500 μM bupivacaine or 2 mM lidocaine (concentration equipotent to bupivacaine) added to the same cardioplegia solution. This concentration of bupivacaine was chosen because in isolated mitochondria it completely inhibits respiration supported by fatty acids, a possible mechanism of cardiac protection (see Discussion) without interfering with pyruvate-supported respiration. Therefore, the bupivacaine concentration is not high enough to impair substrate-independent components of oxidative phosphorylation such as electron transport or chemiosmotic force. There were five hearts in each group, and the assignment of each heart to one of the three treatment groups was randomized and the investigators were blinded to the assignment. Perfusion with control and test solutions rapidly induced asystole in all hearts. Hearts were then removed from the Langendorff apparatus, placed in a beaker containing chilled cardioplegia solution (without local anesthetic), and stored in a 4°C refrigerator for 12 h.

Hearts were gradually warmed during recovery by swirling the storage beaker in a 37°C water bath until the custodial cardioplegia solution reached 35°C (approximately 5 min). The hearts were then remounted onto the Langendorff apparatus and perfused at approximately 40 mmHg over 90 s with 20 ml of 37°C “reversal” solution. This solution was a mixture of 16 ml KRB, 4 ml University of Illinois Cardioplegia C (Central Admixture Phar-

macy Services, University of Illinois, Chicago, IL), and 1 ml soybean oil emulsion, 20% (Intralipid; Abbott Labs). The final composition of the “reversal” solution was 122.40 mM NaCl, 9.79 mM KCl, 0.80 mM CaCl_2 , 0.94 mM MgSO_4 , 0.94 mM KH_2PO_4 , 9.20 mM glucose, 3.94 mM pyruvate, 4.31 mM fumarate, 13.58 mM tromethamine, 11.20 mM glutamate, 11.20 mM aspartate, 0.64% dextrose, and a final triglyceride concentration of approximately 1% wt/vol. Glutamate, pyruvate, fumarate, and aspartate are metabolic intermediates added to replenish substrates depleted during storage and to support respiration. Lipid emulsion was included to reverse the cardiac depressant effects of bupivacaine and was injected in all hearts to control for possible positive or negative inotropic effects of triglycerides. Hearts were then perfused with 37°C KRB at 80 mmHg. After 15 min, the LV balloon was inflated to LV diastolic pressure of 10 mmHg to establish a unified baseline preload. Parameters of cardiac function were recorded at 15, 30, 45, and 60 min.

Metabolic and Functional Parameters

Heart rate, left ventricular developed pressure (LVdevP; = systolic pressure – diastolic pressure), maximum positive rate of change in LV pressure ($\text{dP}/\text{dt}_{\text{max}}$), and rate–pressure product (= heart rate \times LVdevP) were continuously monitored for 1 h after reperfusion on the Langendorff apparatus (for 15 min before increasing LV diastolic pressure and 45 min thereafter). The perfusate was sampled above the heart and from the pulmonary artery catheter to calculate oxygen consumption (= coronary flow \times 0.024 \times [arterial partial pressure of oxygen – venous partial pressure of oxygen]). Lactate dehydrogenase (LDH) activity was measured in the pulmonary artery effluent with a colorimetric enzyme assay (Cytotox96; Promega, Madison, WI) where optical density at 490 nm provides a quantitative measure of LDH activity after 30 min of incubation with an assay solution. To compensate for variation in LDH concentration due to differences in coronary perfusion, the optical density (OD; absorbance/ml) was multiplied by the perfusion rate (ml/min). Resulting rates of LDH release were then compared using an arbitrary unit, OD/min.

Statistical Analysis

All data sets were imported and analyzed in GraphPad Prism 4 (GraphPad Software, San Diego, CA). Rate–pressure product, $\text{dP}/\text{dt}_{\text{max}}$, LVdevP, heart rate, and oxygen consumption were analyzed for the three treatment groups at baseline with one-way analysis of variance (ANOVA). During the recovery phase, these parameters were analyzed over multiple time points using two-way ANOVA with a two-tailed Bonferroni posttest (α set at 0.05). LDH release was compared among the three groups at the end of the recovery period (60 min) by

Table 1. Baseline Values of Key Parameters for the Three Groups

	Control (n = 5)	Bupivacaine (n = 5)	Lidocaine (n = 5)
Heart rate, beats/min	265 ± 14	300 ± 6.3	267 ± 9.1
dP/dt _{max} , mmHg/s	2,080 ± 175	2,122 ± 105	2,288 ± 106
LVdevP, mmHg	105 ± 8.3	96.8 ± 3.1	116 ± 4.7
Rate–pressure product, mmHg · beats ⁻¹ · min ⁻¹	27,060 ± 1,159	31,010 ± 1,541	30,610 ± 1,412
Oxygen consumption, μl · min ⁻¹ · g ⁻¹	144 ± 20.2	158 ± 9.9	149 ± 5.4
Efficiency, mmHg · ml ⁻¹ · g	205 ± 34	198 ± 13	207 ± 17
LDH, OD/min	1.19 ± 0.03	1.23 ± 0.04	1.24 ± 0.03

All values are given as mean ± SEM. Baseline values for major parameters showed no significant differences among the three groups.

dP/dt_{max} = maximum value for the first derivative of left ventricular pressure; LDH = lactate dehydrogenase; LVdevP = left ventricular developed pressure (= systolic pressure – diastolic pressure); OD = optical density.

one-way ANOVA with Bonferroni multiple comparison posttest.

Results

Pilot studies in 12-h preservation were performed using hearts arrested with high potassium alone (n = 7) and hearts arrested with high potassium containing bupivacaine (n = 8). These hearts were electrically paced, and no metabolic parameters were measured. After 12 h of cold storage, only one control heart exhibited organized contractile activity when stimulated at 300 beats/min. Three of seven control hearts exhibited dysrhythmic, poorly organized contractions but did not pace with electrical stimulation, and three others showed no contractile recovery. After recovery from cold storage, six of the eight bupivacaine-treated hearts exhibited rhythmic, spontaneous contractions and were able to be electrically paced. The remaining two bupivacaine-treated hearts also exhibited rhythmic, spontaneous contractions but could not be electrically stimulated to the same rate. Bupivacaine-treated hearts demonstrated significantly better postrecovery contractility than control hearts when comparing rate–pressure products (data not shown). These studies and subsequent results were used to refine the current protocol, described in the Materials and Methods, where we added a second experimental group treated with equipotent concentrations of lidocaine, eliminated electrical pacing to study spontaneous rhythmicity, and examined metabolic parameters of cardiac function.

Baseline measures of cardiac performance, LDH release, and oxygen consumption were not different in the three experimental groups (table 1; n = 5 for all groups). However, 30 min after warming and reperfusion, differences in metrics of both categories became apparent. Three of five hearts in the control group never contracted, and all the control hearts stopped beating by the 60-min time point. All bupivacaine-treated hearts beat spontaneously by the 15-min time point, and all lidocaine-treated hearts were beating by 30 min. One lidocaine-treated heart stopped beating by the end of the

experiment; all hearts treated with bupivacaine beat throughout the observed times.

Functional Parameters

Heart rate (fig. 1A) and LVdevP (fig. 1B) were analyzed for the three groups at four time points (n = 5 for all groups). Significant differences in heart rate were found between the control and bupivacaine groups at 45 min

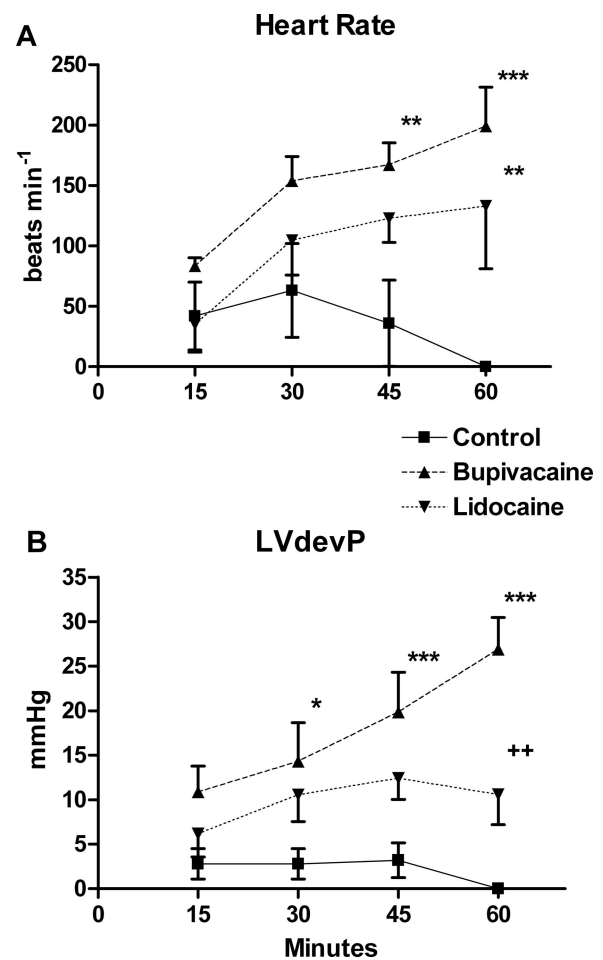


Fig. 1. (A) Heart rate (mean ± SEM) after reperfusion. (B) Left ventricular developed pressure (LVdevP) after reperfusion (mean ± SEM). * Significantly different from control. + Significantly different from bupivacaine-treated group. One symbol, $P < 0.05$; two symbols, $P < 0.01$; three symbols, $P < 0.001$.

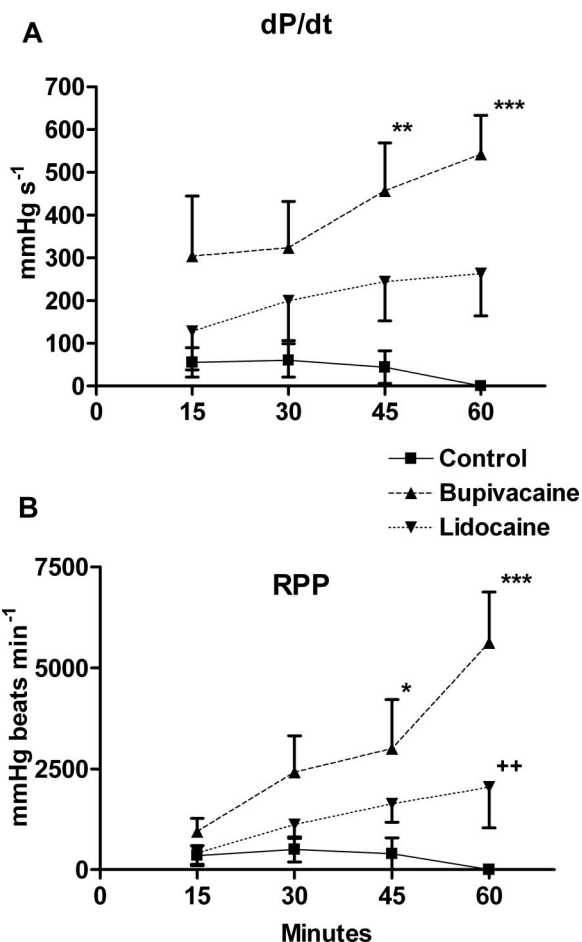


Fig. 2. (A) Maximum positive first derivative of left ventricular pressure (dP/dt; mean ± SEM) after reperfusion. (B) Rate-pressure product (RPP; mean ± SEM) after reperfusion. * Significantly different from control. + Significantly different from bupivacaine-treated group. One symbol, *P* < 0.05; two symbols, *P* < 0.01; three symbols, *P* < 0.001.

(*P* < 0.01) and 60 min (*P* < 0.001), whereas control heart rates differed from the lidocaine treatment group only at 60 min (*P* < 0.01). Heart rates did not differ at any point between bupivacaine and lidocaine treatment groups. Control values for LVdevP did not differ from those of the lidocaine group at any point. LVdevP differed in the bupivacaine-treated group from control values at all time points after the hearts were loaded to 10 mmHg diastolic pressure, 30 min (*P* < 0.05), 45 min (*P* < 0.001), and 60 min (*P* < 0.001). LVdevP was statistically different in lidocaine and bupivacaine groups at 60 min, where the bupivacaine group exhibited greater contractility than the lidocaine group (*P* < 0.01). The pattern was slightly different in another measure of contractility, dP/dt_{max}, where bupivacaine values were again greater than controls, but only at 45 min (*P* < 0.01) and 60 min (*P* < 0.001) (fig. 2A). Mean values of dP/dt_{max} for the lidocaine group were between those of the controls and bupivacaine groups but were not significantly different from either at any time.

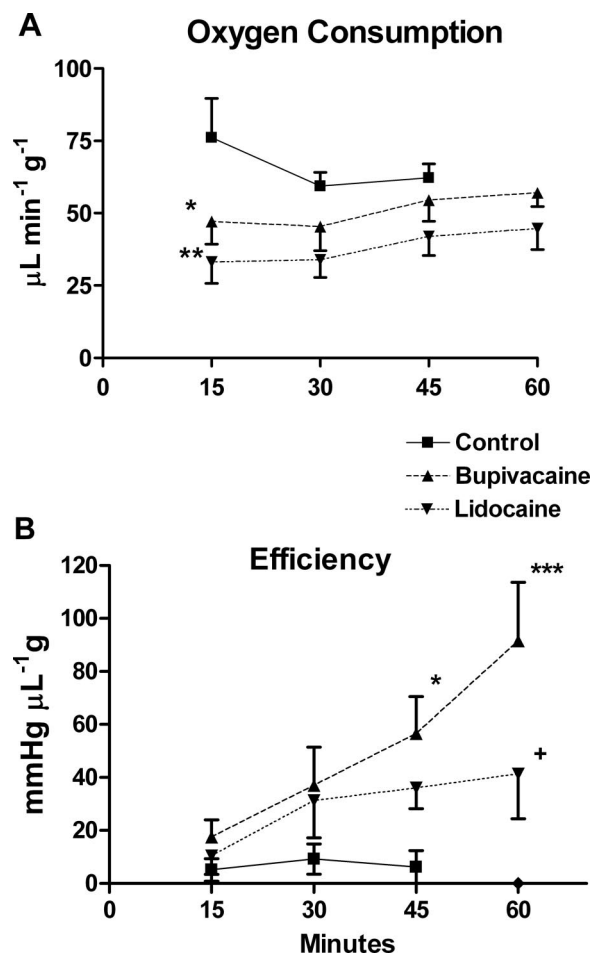


Fig. 3. (A) Oxygen consumption (mean ± SEM) after reperfusion. Oxygen consumption was not measured in control hearts at the 60-min time point. (B) Efficiency (mean ± SEM) after reperfusion. * Significantly different from control. + Significantly different from bupivacaine-treated group. One symbol, *P* < 0.05; two symbols, *P* < 0.01; three symbols, *P* < 0.001.

Figure 2B shows the plot of rate-pressure product for the three groups over the time course of the experiment. The bupivacaine- and lidocaine-treated groups showed increased rate-pressure product through the duration of the experiment. Differences in rate-pressure product were found between bupivacaine and control at 30 min (*P* < 0.05), 45 min (*P* < 0.001), and 60 min (*P* < 0.001). Rate-pressure product of control and lidocaine groups did not differ at any time point, whereas the two test groups differed only at 60 min (*P* < 0.01).

Metabolism

Oxygen consumption was measured after reperfusion (fig. 3A). Two-way ANOVA showed that, overall, treatment had a significant effect in oxygen consumption normalized to cardiac mass (*P* < 0.0002; *F* = 10.86). However, after Bonferroni posttests, the only significant differences among the three groups were at the first time point. Fifteen minutes after reperfusion and just before increasing LV volume, the control hearts demonstrated

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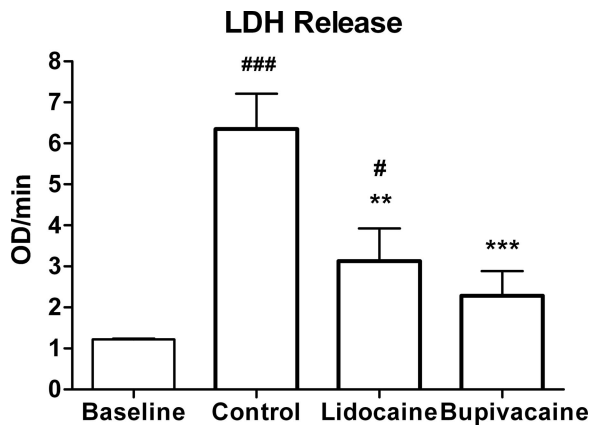


Fig. 4. Lactate dehydrogenase (LDH) release (mean \pm SEM) at baseline and after reperfusion. OD = optical density. * Significantly different from control. # Significantly different from baseline. One symbol, $P < 0.05$; two symbols, $P < 0.01$; three symbols, $P < 0.001$.

significantly higher rates of oxygen consumption normalized to cardiac mass than both the bupivacaine ($P < 0.05$) and lidocaine ($P < 0.01$) groups. At 15 min, the rates of oxygen consumption for the two treated groups were not significantly different, and at later time points, rates among the three groups were indistinguishable. Coronary flow rates did not differ among the three groups at any time point (data not shown).

The rate-pressure product can be normalized to the simultaneous rate of oxygen consumption to give a measure of cardiac efficiency, roughly work performed per volume of oxygen consumed per minute per gram of tissue. This metric is shown for the three groups after reperfusion in figure 3B. The greatest difference was found for control *versus* bupivacaine at 45 min ($P < 0.05$); comparisons were not made at 60 min because oxygen consumption was not measured in the control group at this point. Values in the control and lidocaine groups were not statistically different at any time, and efficiency in the bupivacaine group exceeded that of the lidocaine group only at 60 min ($P < 0.05$).

Cell Damage

Lactate dehydrogenase release was measured during the 15 min before preservation and for 45 min after reperfusion. Comparison by one-way ANOVA indicated that mean enzyme activity released in the 15 min before preservation (baseline) did not differ among the three groups (1.22 ± 0.07 OD/min; $n = 15$). LDH release for the 45 min after reperfusion was compared for each group with the mean baseline value (fig. 4). Treatment had a significant effect on the overall pattern of LDH release ($P < 0.0001$; $F = 23.7$). Bonferroni multiple comparison posttest indicated that the LDH released in the control group exceeded baseline values ($P < 0.001$). LDH released by the bupivacaine group after reperfusion was not statistically different from baseline values but

was significantly less than LDH released in the control group ($P < 0.001$) after reperfusion. LDH release in the lidocaine group after reperfusion was greater than baseline values ($P < 0.05$) and significantly less than that in the control group ($P < 0.01$) but not different from that in the bupivacaine group.

Discussion

We found that isolated rat hearts preserved with a solution containing bupivacaine exhibited improved contractile function and reduced cell damage after prolonged cold storage. However, the benefit provided by an equipotent concentration of lidocaine was significantly less, suggesting a mechanism independent of sodium channel blockade. A salutary metabolic effect is consistent with the observation that myocardial oxygen consumption immediately after reperfusion was highest in hearts of the control group (poorest recovery).

Use of local anesthetics in cardioplegia solutions has been studied for more than three decades, but the precise mechanism of their benefit remains elusive.^{8,9} The initial rationale was to arrest metabolism¹⁰ or “stabilize” membranes, and early studies indicate a reduced incidence of ventricular arrhythmias after cardioplegia containing either lidocaine or procaine.¹¹ Sodium channel blockade alone confers a preservative advantage, and tetrodotoxin, which induces polarized cardiac arrest, preserves cardiac function after ischemia and reperfusion.¹² Chambers¹³ postulated that cardiac arrest without loss of membrane potential reduces the harmful ionic perturbations seen in depolarizing cardioplegia, thereby decreasing the metabolic cost of correcting such changes, particularly increases in intracellular sodium and calcium content. He proposed the use of “hyperpolarizing” cardioplegia with adenosine, potassium channel openers, or local anesthetics over standard high-potassium depolarizing solutions. Building on this work, Dobson *et al.*^{14,15} and Canyon *et al.*¹⁶ showed in isolated rat hearts that arrest and perfusion with lidocaine plus adenosine preserved cardiac function better than cardioplegia with a high-potassium solution. Corvera *et al.*¹⁷ recently showed in dogs undergoing cardiopulmonary bypass that cardioplegia with warm or cold solutions of lidocaine and adenosine were equally effective as cold high-potassium solution in preventing myocardial injury. We confirmed this effect in the lidocaine-treated hearts, which exhibited less LDH release than controls; however, protection was limited to evidence of cell damage, because lidocaine-treated hearts were no different from controls in any parameter of contractility. The finding that bupivacaine-treated hearts functioned better over time than those preserved with an equipotent concentration of lidocaine suggests that factors other than sodium channel blockade may be in play.

Bupivacaine is a classic uncoupler of oxidative phosphorylation.^{18,19} Mitochondrial uncoupling is associated with preconditioning, a possible mechanism for preserving function after prolonged ischemia.^{20,21} However, this is not a likely explanation of our results because the concentration of bupivacaine required to induce uncoupling is approximately twofold to fourfold greater than those used in these experiments. Furthermore, we found that the rates of oxygen consumption in bupivacaine-treated hearts immediately after recovery were lower than those of the control group, which is consistent with reduced metabolism rather than the accelerated respiration that is typical of uncoupled respiration. Reduced oxygen consumption during the initial phase of reperfusion might explain, in part, the improved function of hearts treated with bupivacaine.

We previously observed that bupivacaine slows the onset and progression of myocardial acidosis during anaerobic metabolism,⁷ a finding consistent with reduced rates of adenosine triphosphate consumption. Bupivacaine also inhibits complex I of the electron transport chain^{22,23} and suppresses fatty acid-dependent cardiac respiration by inhibiting carnitine-acylcarnitine translocase, a key enzyme of mitochondrial fatty acid transport.²⁴ Fatty acid metabolism generates the preponderance of cardiac adenosine triphosphate under normal aerobic conditions but consumes considerable oxygen in the process. Metabolic modulation, particularly inhibition of fatty acid metabolism, is effective in treating myocardial ischemia.²⁵ This strategy might also conserve tissue energy stores when the predominant mode of adenosine triphosphate production presumably switches to anaerobic glycolysis. Bupivacaine-induced reductions in myocardial calcium transients and sensitivity²⁶ should further reduce contraction-dependent oxygen consumption.

Bupivacaine also possesses both antiinflammatory²⁷ and antioxidant²⁸ properties and protects against ischemic damage in different cells and experimental systems. For example, Lenfant *et al.*²⁹ showed that bupivacaine reduces damage to erythrocytes after an oxidative stress, and Niyama *et al.*³⁰ and Yamada *et al.*³¹ showed that bupivacaine protects CA1 neurons from ischemic damage.

Potential methods of reducing cell damage from ischemia and reperfusion injury include modulating metabolism to reduce oxygen consumption, improve scavenging and suppress the formation of highly reactive species, and enhance cytoprotective cell-signaling pathways.³² Our findings are restricted in scope to a proof of principle that adding bupivacaine to high-potassium cardioplegia improves myocardial preservation and provide a limited view of possible mechanisms of cell protection. Both cell death and oxygen consumption at the beginning of recovery are reduced in hearts treated with bupivacaine.

Bupivacaine use is not without potential problems. It inhibits diverse ionotropic and metabotropic^{33,34} cell-signaling pathways and potentially suppresses both myocardial conduction³⁵⁻³⁹ and contractility.^{38,40-42} Bupivacaine avidly binds the sodium channel for durations far greater than lidocaine, a less toxic local anesthetic.³⁷ This so-called "fast in-low out" binding could theoretically contravene its use in cardioplegia. Furthermore, Freysz *et al.* has shown that both hypothermia⁴³ and ischemia⁴⁴ increase myocardial sensitivity to bupivacaine toxicity. The same group also reported the obverse effect, namely that bupivacaine decreases the threshold to ischemia-induced ventricular fibrillation.⁴⁵ These findings suggest the possibility of additional problems with using bupivacaine in hearts subjected to prolonged, cold ischemia.

Summary

This study indicates a potential role for bupivacaine in cardiac preservation during no-flow states such as found during organ procurement and transport in advance of transplantation. We hypothesize that the combined effects of polarized arrest, reduced calcium activation, reduced tissue acidosis, and diminished oxygen consumption may attenuate myocardial ischemic and reperfusion injury. Additional studies are required to determine whether the salutary effects of bupivacaine reported here can translate to clinical applications where cardiac preservation is needed.

References

- Hunt SA: Current status of cardiac transplantation. *JAMA* 1998; 280:1692-8
- Massad MG: Current trends in heart transplantation. *Cardiology* 2004; 101: 79-92
- Chambers DJ: Polarization and myocardial protection. *Curr Opin Cardiol* 1999; 14:495-500
- Strichartz GR, Sanchez V, Arthur GR, Chafetz R, Martin D: Fundamental properties of local anesthetics: II. Measured octanol:buffer partition coefficients and pKa values of clinically used drugs. *Anesth Analg* 1990; 71:158-70
- Weinberg GL, VadeBoncouer T, Ramaraju GA, Garcia-Amaro MF, Cwik MJ: Pretreatment or resuscitation with a lipid infusion shifts the dose-response to bupivacaine-induced asystole in rats. *ANESTHESIOLOGY* 1998; 88:1071-5
- Weinberg G, Ripper R, Feinstein DL, Hoffman W: Lipid emulsion infusion rescues dogs from bupivacaine-induced cardiac toxicity. *Reg Anesth Pain Med* 2003; 28:198-202
- Weinberg G, Paisanthasan C, Feinstein D, Hoffman W: The effect of bupivacaine on myocardial tissue hypoxia and acidosis during ventricular fibrillation. *Anesth Analg* 2004; 98:790-5
- Tyers GF: Metabolic arrest of the ischemic heart. *Ann Thorac Surg* 1975; 20:91-4
- Doring V, Baumgarten HG, Pokar H, Gercken G: Metabolism and fine structure of the Mg++-procaine-arrested perfused heart. *Basic Res Cardiol* 1975; 70:186-97
- Bretschneider HJ, Hubner G, Knoll D, Lohr B, Nordbeck H, Spieckermann PG: Myocardial resistance and tolerance to ischemia: Physiological and biochemical basis. *J Cardiovasc Surg (Torino)* 1975; 16:241-60
- Sellevoold OF, Berg EM, Levang OW: Procaine is effective for minimizing postischemic ventricular fibrillation in cardiac surgery. *Anesth Analg* 1995; 81: 932-8
- Snabaitis AK, Shattock MJ, Chambers DJ: Comparison of polarized and depolarized arrest in the isolated rat heart for long-term preservation. *Circulation* 1997; 96:3148-56
- Chambers DJ: Mechanisms and alternative methods of achieving cardiac arrest. *Ann Thorac Surg* 2003; 75:S661-6
- Dobson GP, Jones MW: Adenosine and lidocaine: A new concept in

nondepolarizing surgical myocardial arrest, protection, and preservation. *J Thorac Cardiovasc Surg* 2004; 127:794-805

15. Dobson GP: Organ arrest, protection and preservation: Natural hibernation to cardiac surgery. *Comp Biochem Physiol B Biochem Mol Biol* 2004; 139:469-85

16. Canyon SJ, Dobson GP: Protection against ventricular arrhythmias and cardiac death using adenosine and lidocaine during regional ischemia in the *in vivo* rat. *Am J Physiol Heart Circ Physiol* 2004; 287:H1286-95

17. Corvera JS, Kin H, Dobson GP, Kerendi F, Halkos ME, Katzmark S, Payne CS, Zhao ZQ, Guyton RA, Vinten-Johansen J: Polarized arrest with warm or cold adenosine/lidocaine blood cardioplegia is equivalent to hypothermic potassium blood cardioplegia. *J Thorac Cardiovasc Surg* 2005; 129:599-606

18. Dabadie P, Bendriss P, Erny P, Mazat JP: Uncoupling effects of local anesthetics on rat liver mitochondria. *FEBS Lett* 1987; 226:77-82

19. Sun X, Garlid KD: On the mechanism by which bupivacaine conducts protons across the membranes of mitochondria and liposomes. *J Biol Chem* 1992; 267:19147-54

20. Minners J, van den Bos EJ, Yellon DM, Schwab H, Opie LH, Sack MN: Dinitrophenol, cyclosporin A, and trimetazidine modulate preconditioning in the isolated rat heart: Support for a mitochondrial role in cardioprotection. *Cardiovasc Res* 2000; 47:68-73

21. Rodrigo GC, Lawrence CL, Standen NB: Dinitrophenol pretreatment of rat ventricular myocytes protects against damage by metabolic inhibition and reperfusion. *J Mol Cell Cardiol* 2002; 34:555-69

22. Sztark F, Malgat M, Dabadie P, Mazat JP: Comparison of the effects of bupivacaine and ropivacaine on heart cell mitochondrial bioenergetics. *ANESTHESIOLOGY* 1998; 88:1340-9

23. Sztark F, Ouhabi R, Dabadie P, Mazat JP: Effects of the local anesthetic bupivacaine on mitochondrial energy metabolism: Change from uncoupling to decoupling depending on the respiration state. *Biochem Mol Biol Int* 1997; 43:997-1003

24. Weinberg GL, Palmer JW, VadeBoncouer TR, Zuechner MB, Edelman G, Hoppel CL: Bupivacaine inhibits acylcarnitine exchange in cardiac mitochondria. *ANESTHESIOLOGY* 2000; 92:523-8

25. Parang P, Singh B, Arora R: Metabolic modulators for chronic cardiac ischemia. *J Cardiovasc Pharmacol Ther* 2005; 10:217-23

26. Mio Y, Fukuda N, Kusakari Y, Amaki Y, Tanifuji Y, Kurihara S: Comparative effects of bupivacaine and ropivacaine on intracellular calcium transients and tension in ferret ventricular muscle. *ANESTHESIOLOGY* 2004; 101:888-94

27. De Iuliis A, Zanatta L, Vincenti E, Galzigna L: Differences of ropivacaine and bupivacaine relevant to antiinflammatory activity, platelet aggregation and antioxidant activity *in vitro*. *Farmaco* 2001; 56:153-7

28. Leduc C, Gentili ME, Estebe JP, Le Corre P, Moulinoux JP, Ecoffey C: The effect of local anesthetics and amitriptyline on peroxidation *in vivo* in an inflammatory rat model: Preliminary reports. *Anesth Analg* 2002; 95:992-6

29. Lenfant F, Lahet JJ, Volot F, Schafer I, Freysz M, Rochette L: Effects of bupivacaine on human erythrocytes submitted to stress and evidence for an

interaction between bupivacaine and flumazenil. *Br J Clin Pharmacol* 1999; 48:826-8

30. Niiyama S, Tanaka E, Yamamoto S, Yasumoto S, Kano T, Higashi H: Bupivacaine, but not tetracaine, protects against the *in vitro* ischemic insult of rat hippocampal CA1 neurons. *Neurosci Res* 2002; 42:231-41

31. Yamada A, Tanaka E, Niiyama S, Yamamoto S, Hamada M, Higashi H: Protective actions of various local anesthetics against the membrane dysfunction produced by *in vitro* ischemia in rat hippocampal CA1 neurons. *Neurosci Res* 2004; 50:291-8

32. Garcia-Dorado D: Myocardial reperfusion injury: A new view. *Cardiovasc Res* 2004; 61:363-4

33. Butterworth J, James RL, Grimes J: Structure-affinity relationships and stereospecificity of several homologous series of local anesthetics for the beta2-adrenergic receptor. *Anesth Analg* 1997; 85:336-42

34. Nietgen GW, Chan CK, Durieux ME: Inhibition of lysophosphatidate signaling by lidocaine and bupivacaine. *ANESTHESIOLOGY* 1997; 86:1112-9

35. Bozkurt P, Suzer O, Ekici E, Demirci O, Kaya G, Hacibekiroglu M: Effects of bupivacaine used with sevoflurane on the rhythm and contractility in the isolated rat heart. *Eur J Anaesthesiol* 2003; 20:199-204

36. Chang DH, Ladd LA, Copeland S, Iglesias MA, Plummer JL, Mather LE: Direct cardiac effects of intracoronary bupivacaine, levobupivacaine and ropivacaine in the sheep. *Br J Pharmacol* 2001; 132:649-58

37. Clarkson CW, Hondeghem LM: Mechanism for bupivacaine depression of cardiac conduction: Fast block of sodium channels during the action potential with slow recovery from block during diastole. *ANESTHESIOLOGY* 1985; 62:396-405

38. Simon L, Kariya N, Edouard A, Benhamou D, Mazoit JX: Effect of bupivacaine on the isolated rabbit heart: Developmental aspect on ventricular conduction and contractility. *ANESTHESIOLOGY* 2004; 101:937-44

39. Timour Q, Freysz M, Lang J, Beal JL, Lakhal M, Bertrix L, Faucon G: Electrophysiological study in the dog of the risk of cardiac toxicity of bupivacaine. *Arch Int Pharmacodyn Ther* 1987; 287:65-77

40. Heavner JE: Cardiac toxicity of local anesthetics in the intact isolated heart model: A review. *Reg Anesth Pain Med* 2002; 27:545-55

41. Groban L, Deal DD, Vernon JC, James RL, Butterworth J: Cardiac resuscitation after incremental overdosage with lidocaine, bupivacaine, levobupivacaine, and ropivacaine in anesthetized dogs. *Anesth Analg* 2001; 92:37-43

42. Lynch C, 3rd: Depression of myocardial contractility *in vitro* by bupivacaine, etidocaine, and lidocaine. *Anesth Analg* 1986; 65:551-9

43. Freysz M, Timour Q, Mazze RI, Bertrix L, Cohen S, Samii K, Faucon G: Potentiation by mild hypothermia of ventricular conduction disturbances and reentrant arrhythmias induced by bupivacaine in dogs. *ANESTHESIOLOGY* 1989; 70:799-804

44. Freysz M, Timour Q, Bertrix L, Loufoua-Moundanga J, Omar S, Faucon G: Enhancement by ischemia of the risk of cardiac disorders, especially fibrillation, in regional anesthesia with bupivacaine. *Acta Anaesthesiol Scand* 1993; 37:350-6

45. Freysz M, Timour Q, Bertrix L, Loufoua J, Aupetit JF, Faucon G: Bupivacaine hastens the ischemia-induced decrease of the electrical ventricular fibrillation threshold. *Anesth Analg* 1995; 80:657-63