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Metabolic context affects hemodynamic response to bupivacaine in the isolated rat heart

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Abstract

Previous studies have demonstrated that the local anesthetic bupivacaine selectively inhibits oxidative metabolism of fatty acids in isolated cardiac mitochondria. In the present investigation, we compare the development of bupivacaine cardiotoxicity during fatty acid and carbohydrate metabolism. Hearts from adult male Sprague-Dawley rats were excised and retrograde perfused with a solution containing fatty acid (oleate or octanoate) or carbohydrate substrates for cardiac metabolism. An infusion of bupivacaine was initiated and sustained until asystole, after which full cardiac recovery was allowed. During fatty acid metabolism, substantially lower bupivacaine doses induced both arrhythmia (60.4 ± 11.5 μg oleate and 106.8 ± 14.8 octanoate versus 153.4 ± 21.4 carbohydrate; $P < 0.05$) and asystole (121.0 ± 30.1 μg and 171.5 ± 20.2 versus 344.7 ± 34.6; $P < 0.001$). Dose–response analysis revealed significantly increased sensitivity to bupivacaine toxicity during fatty acid metabolism, indicated by lower V50 doses for both heart rate (70.6 ± 5.6 μg oleate and 122.3 ± 6.2 octanoate versus 152.6 ± 8.6) and rate-pressure product (63.4 ± 5.1 μg and 133.7 ± 7.9 versus 165.1 ± 12.2). Time to recovery following bupivacaine exposure was elevated in the fatty acid group (24.3 ± 2.0 s versus 15.8 ± 3.1; $P < 0.04$). Fatty acid metabolism was shown to predispose the isolated heart to bupivacaine toxicity, confirming that the local anesthetic exerts specific effects on lipid processes in cardiomyocytes.

Keywords: Bupivacaine; Cardiotoxicity; Fatty acid metabolism; Local anesthesia; Isolated heart; Metabolic modulation

1. Introduction

Bupivacaine is a potent local anesthetic commonly used in hospitals and outpatient care centers as a standard for performing neural blockade. This popularity persists despite a well-known cardiotoxic profile which can cause rapid cardiovascular collapse upon entering the circulation. Severe systemic reactions to local anesthetics are comparatively rare clinical events, but standard resuscitation protocols are often inadequate and are increasingly complemented by novel techniques such as lipid emulsion infusion [1]. Multiple mechanisms underlying bupivacaine's severe cardiotoxicity have been implicated, including the inhibition of sodium [2], calcium [3], and potassium [4] ion channels, alteration of beta-adrenergic transduction pathways [5], and direct interference with complex I of the respiratory chain [6]. Bupivacaine has been shown to inhibit the

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activity of carnitine-acylcarnitine translocase (CACT) in isolated cardiac mitochondria [7]. CACT is carnitine porter–antiporter in the inner mitochondrial membrane that is required for the transfer of fatty acids into the mitochondrial matrix for oxidative metabolism. Fatty acids serve during normal aerobic metabolism as the chief substrate to meet the high energetic demands of the heart [8], implying that deficits in such metabolism caused by local anesthetic compounds could cause significant reduction in cardiac function. Carbohydrates can serve as an alternative substrate for myocardial oxidative and non-oxidative metabolism when fatty acid utilization is suppressed. The present study sought to compare the effects of fatty acid and carbohydrate substrates on the development and progression of bupivacaine cardiotoxicity in the isolated rat heart.

2. Methods

All protocols were approved by the Animal Care Committee of the University of Illinois and by the Institutional Animal Care and Use Committee of the VA Chicago Healthcare System (Chicago, IL). Adult male Sprague-Dawley rats weighing between 450 and 550 g were anesthetized through intra-peritoneal injection of 60 mg/kg sodium pentobarbital (Abbott Labs, Abbott Park, IL). Following systemic heparinization, hearts were excised, cannulated through the ascending aorta and suspended from a Langendorff apparatus. Retrograde perfusion was initiated at 16 mL/min via roller pump. Perfusate was comprised of Krebs Ringers bicarbonate buffer (KRB) warmed to 37 °C and equilibrated with a 95%/5% mixture of oxygen and carbon dioxide to a pH of 7.40. The final perfusate contained 100.00 mM NaCl, 4.74 mM KCl, 1.18 mM KH_2PO_4, 1.18 mM $MgSO_4$, 1.00 mM $CaCl_2$, and 25.00 mM $NaHCO_3$.

Intraventricular pressure measurements were transduced from a latex balloon placed in the left ventricle. The pressure and temperature of the inflowing perfusate were also monitored. Data was archived using a Powerlab data acquisition system (ADInstruments, Colorado Springs, CO) connected to a Pentium IV desktop computer running Windows XP. Chart data analysis software (ADInstruments) was used to extract parameters of cardiac function, including heart rate (HR), left ventricular developed pressure (LVdevP = systolic–diastolic pressure), and rate-pressure product (RPP = HR X LVdevP).

During each experiment, solutions of oleate (net concentration in perfusate 1.2 mM), octanoate and malate (1.2 and 4.95 mM, respectively), or carbohydrates (11.50 mM glucose, 4.92 mM pyruvate, and 5.39 mM fumarate) were added to the perfusate to provide a substrate for cardiac metabolism. Oleate was formulated in solution of 40% bovine serum albumin to permit solubilization into the aqueous phase. Following determination of baseline hemodynamic parameters, a continuous infusion of 5.0 mM bupivacaine was added to the perfusate to achieve a final concentration of 30 μM. The bupivacaine infusion was continued until the development of asystole or a sustained RPP below 10% baseline was reached, at which point the bupivacaine infusion was stopped. Time until spontaneous recovery of cardiac activity was determined in octanoate and carbohydrate trials. No therapy was administered during the recovery period.

2.1. Statistical analysis

Extracted data of cardiac function were imported into Graphpad Prism (GraphPad Software, San Diego, California) for statistical analysis. Cumulative doses to arrhythmia and asystole for all groups were compared using one-way analysis of variance (ANOVA) with Bonferroni's multiple comparison tests. Time to recovery data was assessed with an unpaired two-tail *t*-test. HR and RPP versus cumulative dose data, normalized to baseline performance in each trial prior to bupivacaine infusion, were evaluated using two-way ANOVA tests with repeated measures. Normalized data were also fit to Boltzmann-sigmoidal regression curves to evaluate dose–response and determine V50 values, representing cumulative drug exposure required for 50% reduction of HR or RPP. Statistical significance was taken as $P < 0.05$. R^2 values for regressions ranged from 0.775 to 0.829. Data are plotted mean ± S.E.M.

3. Results

All hearts demonstrated rapid recovery of rate, rhythm and LVdevP once the anesthetic infusion was stopped, and multiple experiments were performed on each heart with no major degradation in baseline physiological performance. No significant variance in baseline physiology (HR or RPP) was present between study groups (data not shown).

Hearts using carbohydrate substrate exhibited consistently lower sensitivity to bupivacaine toxicity than those using fatty acids. Prior to onset of asystole, a period of arrhythmias developed in each heart, marked by extrasystoles and an abrupt-onset bradycardia. There was also a rise in developed ventricular pressure indicative of post-extrasystolic potentiation [9], concurrent with missed beats or other rhythm irregularity (Fig. 1). Hearts utilizing octanoate or oleate experienced arrhythmia significantly faster after initiating