Carnitine Deficiency Increases Susceptibility to Bupivacaine-induced Cardiotoxicity in Rats

Gail K. Wong, M.B.B.S., F.A.N.Z.C.A.,* Mark W. Crawford, M.B.B.S., F.R.C.P.C.†

ABSTRACT

Background: Anecdotal reports suggest that carnitine deficiency increases susceptibility to bupivacaine-induced cardiotoxicity. Bupivacaine inhibits lipid-based respiration in myocardial mitochondria *via* inhibition of acylcarnitine exchange in rats. The authors hypothesized that carnitine deficiency increases susceptibility to bupivacaine-induced asystole in rats and that acute repletion with L-carnitine reverses this effect.

Methods: Thirty male Sprague-Dawley rats were assigned to three groups. Rats assigned to the L-carnitine–deficient and L-carnitine–replete groups received subcutaneous D-carnitine on the 10 d before the experiment to induce L-carnitine deficiency. Control rats received an equal volume of subcutaneous normal saline. The rats were anesthetized and mechanically ventilated. Bupivacaine was infused intravenously at a rate of 2.0 $mg \cdot kg^{-1} \cdot min^{-1}$ until asystole occurred. The L-carnitine–replete group received intravenous L-carnitine 100 $mg \cdot kg^{-1}$ immediately before bupivacaine infusion. At asystole, blood was sampled to measure bupivacaine concentration. The primary outcome was time to asystole.

Results: L-carnitine deficiency significantly decreased survival duration ($P < 0.0001$). Time to bupivacaine-induced asystole decreased by 22% ($P < 0.05$) in the L-carnitine–deficient group (847 s [787–898]) (median [interquartile range]) compared with controls (1,082 s [969–1,427]). Intravenous administration of L-carnitine completely reversed the reduction in time to asystole. At asystole, the median plasma bupivacaine concentration in the L-carnitine–deficient group was 38% ($P < 0.05$) less than that in control animals. Plasma bupivacaine concentration was similar in L-carnitine–replete and control animals.

Conclusions: Carnitine deficiency increased sensitivity to bupivacaine-induced asystole, an effect that was reversed completely by L-carnitine repletion. This study suggests that carnitine deficiency may predispose to bupivacaine-induced cardiotoxicity. L-carnitine may have a protective role against bupivacaine cardiotoxicity.

What We Already Know about This Topic

- Anecdotal reports suggest that L-carnitine deficiency increases susceptibility to bupivacaine-induced ventricular arrhythmias

What This Article Tells Us That Is New

- In rats, carnitine deficiency increases susceptibility to bupivacaine-induced cardiotoxicity, and this was reversed with L-carnitine supplementation

BUPIVACAINE-INDUCED cardiotoxicity is a rare but potentially devastating complication of regional anesthesia. The incidence of systemic toxicity from local anesthetic use is estimated to be 7.5–20 per 10,000 regional blocks in adults, but the incidence of bupivacaine cardiotoxicity is difficult to estimate and unknown.[1] Bupivacaine-induced cardiac arrest frequently is a consequence of inadvertent intravascular injection or drug overdose resulting in toxic plasma concentrations; however, cardiac arrests have occurred at seemingly nontoxic doses.[2–4]

Anecdotal reports suggest that L-carnitine deficiency increases susceptibility to bupivacaine-induced ventricular arrhythmias. We have reported an intraoperative cardiac arrest after administration of bupivacaine in a child who was later found to have L-carnitine deficiency.[3] The plasma concentration of bupivacaine at the time of the arrest was found to be less than the toxic range. In addition, a case report published by Weinberg *et al.* describes severe ventricular arrhythmias after subcutaneous administration of a small dose of bupivacaine in a patient with known L-carnitine deficiency.[4]

L-carnitine is an essential cofactor in the transfer of long-chain fatty acids into the mitochondrial matrix for β-oxidation.[5] The cardiac mitochondrion is more than 70% dependent on fatty acids for energy. Studies have demonstrated that bupivacaine inhibits fatty acid oxidation in rat myocardial mitochondria and that toxicity is related to impaired

* Assistant Professor and Staff Anesthesiologist, † Associate Professor and Anesthesiologist-in-Chief, Department of Anesthesia and Pain Medicine, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada.

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Address correspondence to Dr. Wong: Department of Anesthesia and Pain Medicine, The Hospital for Sick Children, University of Toronto, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8. gail.wong@sickkids.ca. Anesthesiology 2011; 114:1417–24

mitochondrial function.[6–10] Whether existing L-carnitine deficiency further reduces the availability of fatty acid substrate in the mitochondria and potentially sensitizes the myocyte to bupivacaine-induced toxicity is unclear. We tested the hypothesis that L-carnitine deficiency increases susceptibility to bupivacaine-induced asystole in rats and that acute repletion with L-carnitine reverses this effect.

Materials and Methods

Approval for the study was obtained from the Animal Care Committee of the Hospital for Sick Children, Toronto, Canada. Thirty adult, pathogen-free, male Sprague-Dawley rats (Charles River Breeding Laboratories, Saint-Constant, Quebec, Canada) weighing between 300 and 400 g were studied. The rats were assigned to one of three groups: control (n = 10), L-carnitine–deficient (n = 10), and L-carnitine–replete (n = 10) groups. They were housed in a controlled environment with a 12-h light-dark cycle for 10 d before the experiments and had free access to a standard nonpurified diet (Prolab RHM 1000 LabDiet, PMI Nutrition International, Brentwood, MO) and tap water. They were not denied food on the day of the experiments.

Induction of L-carnitine Deficiency

We induced L-carnitine deficiency using D-carnitine 500 mg/kg (Sigma–Aldrich, St. Louis, MO) administered subcutaneously for 10 d before the experiment.[11–14] Anhydrous D-carnitine was reconstituted with sterile water to a concentration of 250 mg · ml^{-1} immediately before subcutaneous injection. Ten control rats received the same volume of daily subcutaneous normal saline.

Experimental Protocol

Anesthesia was induced using 2% isoflurane in oxygen in an induction chamber. The trachea was intubated *via* tracheostomy. The lungs were mechanically ventilated with isoflurane in oxygen using a rodent ventilator (Harvard Apparatus, Saint-Laurent, Quebec, Canada) set to deliver a tidal volume of 10 ml · kg^{-1} body weight and a respiratory rate of 65 breaths/min. Ventilation was adjusted to maintain normocapnia ($PaCO_2$ 35–45 mmHg) as determined by arterial blood gas analysis (ABL 700 Series blood gas analyzer, Radiometer, Copenhagen, Denmark).

A catheter (24-gauge Angiocath; BD, Franklin Lakes, NJ) was inserted into the tail vein for drug administration and into the right carotid artery for arterial blood pressure monitoring and blood sampling. Lead 2 of the electrocardiograph was monitored (Biopac Data Acquisition System; Harvard Apparatus, Holliston, MA) using needle electrodes inserted subcutaneously in three limbs. Rectal temperature was monitored using a thermistor probe and maintained at 36.5–37.5°C using an under-body heating pad. Arterial blood pressure, temperature, and hemoglobin oxygen saturation were continuously recorded, as was electrocardiographic monitoring. Arterial blood (0.5 ml) was sampled for blood gas analysis and measurement of L-carnitine concentration.

Immediately after preparation, rats in the L-carnitine–replete group received 100 mg · kg^{-1} intravenous L-carnitine (Sigma–Aldrich). L-carnitine was reconstituted with sterile water to a concentration of 250 mg · ml^{-1} immediately before injection. The administered volumes ranged from 0.13 to 0.16 ml. The dose of L-carnitine was based on that used in previous animal studies[15–17] and is consistent with the recommended dose used clinically in the treatment of hepatotoxicity or symptomatic hyperammonemia resulting from acute valproate toxicity in children.[18,19]

Twenty minutes after surgical preparation, baseline heart rate and mean arterial blood pressure (MAP) were recorded. An intravenous infusion of bupivacaine, 0.5% (AstraZeneca, Mississauga, Ontario, Canada), was administered to all groups *via* the tail vein catheter at a rate of 2.0 mg · kg^{-1} · min^{-1} using a calibrated electronic infusion pump (Alaris System Syringe Module; Cardinal Health, Vaughan, Ontario, Canada). The infusion rate of bupivacaine was based on a previous study demonstrating the development of asystole at 17–20 min after the start of infusion in normal rats.[20] Bupivacaine was infused until asystole occurred. Asystole was defined as the absence of recognizable electrocardiograph activity for 10 s after the last systole. Arterial blood (0.1 ml) was sampled for plasma bupivacaine concentration at the time of asystole. In the L-carnitine–replete group, blood was sampled at asystole to measure the serum L-carnitine concentration.

The primary outcome was time to asystole. Secondary outcomes included times to 20% increase in the PR and QRS intervals, first arrhythmia, and 50% reductions in heart rate and MAP. PR intervals were measured on the electrocardiograph from the beginning of P waves till the beginning of the corresponding QRS complexes. QRS intervals were measured from the beginning to the end of QRS complexes. First arrhythmia was defined as the first abnormal electrocardiograph complex that was accompanied by an abnormal systole on the arterial blood pressure trace.

Measurement of Bupivacaine and L-carnitine Concentrations

The bupivacaine concentration was measured using the 4000 Q-Trap Tandem Mass Spectrometer (Applied Biosystems, Foster City, CA) in accordance with a technique developed at our institution.[21] Separation of bupivacaine and delivery of the sample to the mass spectrometer was accomplished by high-performance liquid chromatography followed by electrospray ionization. The mass spectrometer is a hybrid instrument consisting of a triple quadrupole system and an ion trap system, which uses collision-induced dissociation to produce a product ion from a precursor ion. The system monitors for a specific ion pair based on the mass-to-charge ratio, which for bupivacaine is 289.3/140.2. The detector monitors the ion current, amplifies it, and transmits the signal to the data system, where it is recorded in the form of a

Table 1. Baseline Data

Variable	Control Group (n = 10)	L-carnitine–deficient Group (n = 10)	L-carnitine–replete Group (n = 10)
Body weight (g)	360 ± 53	342 ± 32	334 ± 26
pH	7.43 ± 0.03	7.43 ± 0.01	7.44 ± 0.03
$Paco_2$ (mmHg)	40.6 ± 2.1	39.3 ± 2.9	38.3 ± 1.9
Pao_2 (mmHg)	357 ± 77.9	355 ± 66.7	381 ± 59.7
K^+ (mM)	4.2 ± 0.5	4.4 ± 0.6	4.4 ± 0.5
HCO_3^- (mM)	26.5 ± 1.1	25.5 ± 2.2	25.7 ± 1.1
Ca^{2+} (mM)	1.29 ± 0.04	1.29 ± 0.02	1.26 ± 0.06
Lactate (mM)	2.2 ± 0.5	2.3 ± 0.9	2.5 ± 0.5
Heart rate (beats/min)	339 ± 34	349 ± 25	389 ± 38*
MAP (mmHg)	90 ± 15	85 ± 14	127 ± 23†

Data are mean ± SD.

* $P < 0.05$ compared with control and L-carnitine–deficient groups. † $P < 0.001$ compared with control and L-carnitine–deficient groups.

MAP = mean arterial pressure.

mass spectrum. Quantification of bupivacaine is based on analyzing a six-point calibration curve (0–150 mg/l). An internal standard (prilocaine, mass to charge ratio 221.2/86.1) is included with each calibration, quality control, or animal sample.

L-carnitine concentration was determined by spectrophotometric enzymatic assay using a Roche Cobas Mira analyzer (Roche Diagnostics, Nutley, NJ). The coefficient of variation was 4–12%. The method is based on the reaction catalyzed by the enzyme carnitine acetyltransferase acting on L-carnitine. The resulting free coenzyme A is combined with 5,5′-dithiobis-2-nitrobenzioc acid forming a phenolate ion that is spectrometrically measured at 405 nm.[22]

Statistical Analysis

Data are presented as mean ± SD or median [interquartile range] as appropriate. One-way analysis of variance or the Kruskal-Wallis test was used for between-group comparisons as appropriate. *Post hoc* Sidak or Dunn's multiple comparison tests were used for pairwise comparisons. Kaplan–Meier analysis and the log-rank test with Bonferroni adjustment were used for analysis of survival duration. $P < 0.05$ was considered statistically significant unless dictated by Bonferroni correction. All statistical analyses were performed using Stata version 10.0 (Statacorp, College Station, TX) or GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA).

Results

Baseline Data

Body weight, baseline arterial blood gases, and metabolic variables did not differ significantly among groups (table 1). There were no observed differences in physical appearance or activity level among groups. Baseline hemodynamic variables did not differ significantly in control and L-carnitine–deficient groups. After acute repletion of L-carnitine, the baseline heart rate increased by 14% compared with control ($P < 0.05$). Similarly, baseline MAP increased by 41% ($P < 0.001$) compared with control after acute repletion of L-carnitine.

Primary Outcome

Time to bupivacaine-induced asystole decreased by 22% ($P < 0.05$) in the L-carnitine–deficient group (847 s [787–898]) (median [interquartile range]) compared with controls (1,082 s [969–1,427]) (fig. 1). Intravenous administration of L-carnitine in the L-carnitine–replete group completely reversed the reduction in time to asystole observed in the L-carnitine–deficient group (fig. 1). In the L-carnitine–replete group, time to asystole was 35% greater ($P < 0.001$) than in the L-carnitine–deficient group (1,310 s [1,265–1,737] *vs.* 847 s [787–898]) (fig. 1). Kaplan–Meier curves showing the percentage of animals still alive at time points during the experiments are presented in figure 2. Analysis of

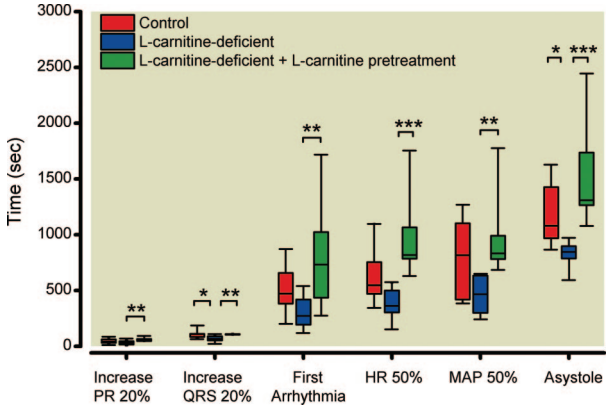

Fig. 1. Times to defined electrocardiographic and hemodynamic endpoints during bupivacaine infusion in control (*red bars*; n = 10), L-carnitine–deficient (*blue bars*; n = 10) and L-carnitine–replete (*green bars*; n = 10) rats. Data are median, interquartile range, range. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Increase PR 20% = 20% increase in PR interval; Increase QRS 20% = 20% increase in QRS interval; HR 50% = 50% decrease in heart rate; MAP 50% = 50% decrease in mean arterial blood pressure.

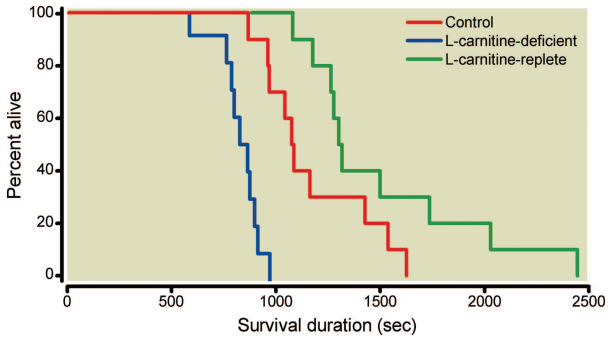

Fig. 2. Kaplan–Meier curves demonstrating percentage of animals surviving during bupivacaine infusion. Duration of survival was significantly shorter in the L-carnitine–deficient group compared with the control and L-carnitine–replete groups. $P < 0.0001$.

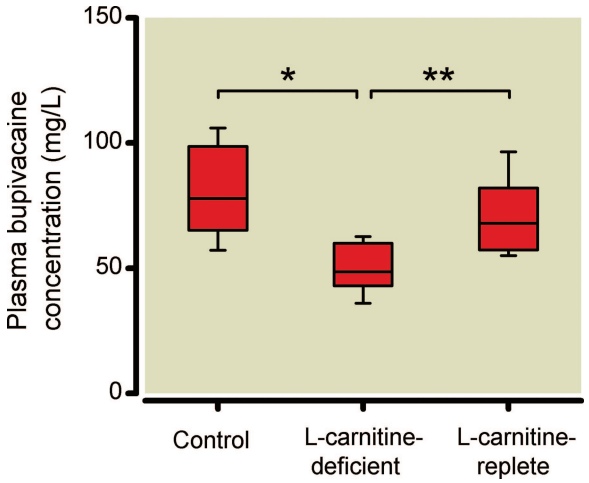

Fig. 3. Plasma bupivacaine concentration at time of asystole in control (n = 10), L-carnitine–deficient (n = 10), and L-carnitine–replete (n = 10) rats. Data are median, interquartile range, range. * $P < 0.05$, ** $P < 0.01$.

the curves using the log-rank test with Bonferroni adjustment indicated a significant difference in survival duration between the L-carnitine–deficient group compared with the control and L-carnitine–replete groups ($P < 0.0001$). There was a trend toward increased survival duration in the L-carnitine–replete group compared with controls, although the difference did not achieve statistical significance (fig. 2). The coefficient of variation in time to asystole in the L-carnitine–replete group was greater than in the control group (fig. 1), which may explain this lack of statistical significance.

Secondary Outcomes

Time to QRS complex widening was decreased by 37% ($P < 0.05$) in the L-carnitine–deficient group compared with controls (fig. 1). Time to first arrhythmia and 50% reduction in heart rate and MAP also were decreased in the L-carnitine–deficient group compared with controls, although these differences did not reach statistical significance (fig. 1). No significant difference was found in time to 20% PR interval prolongation.

L-carnitine repletion completely reversed the reductions in secondary outcomes seen in the L-carnitine–deficient group. Times to 20% prolongation of the PR interval and QRS complex were increased in the L-carnitine–replete group by 63% ($P < 0.01$) and 52% ($P < 0.01$), respectively, compared with the L-carnitine–deficient group (fig. 1). Time to first arrhythmia in the L-carnitine–replete group was more than double ($P < 0.01$) that in the L-carnitine–deficient group (fig. 1). Likewise, times to 50% reductions in heart rate and MAP in the L-carnitine–replete group were approximately double ($P < 0.001$ and $P < 0.01$, respectively) those in the L-carnitine–deficient group (fig. 1).

Serum L-carnitine Concentration

Ten days of subcutaneous D-carnitine administration decreased mean serum L-carnitine concentration by 57% in the L-carnitine–deficient group (25.7 ± 3.4 μM) compared with controls (59.7 ± 7.5 μM) ($P < 0.001$). Serum L-carnitine concentration in the L-carnitine–replete group was 27.4 ± 3.6 μM before intravenous administration of L-carnitine 100 mg · kg^{-1}. After intravenous carnitine administration, serum L-carnitine concentration in the L-carnitine–replete group was 1,221.7 ± 201.1 μM, which is consistent with the serum concentration measured in humans after bolus intravenous administration of L-carnitine 100 mg · kg^{-1}.[23]

Plasma Bupivacaine Concentration

At the time of asystole, the median [interquartile range] plasma bupivacaine concentration in the L-carnitine–deficient group (48.6 [42.5–60.2] mg/L) was 38% less than that in the control group (77.9 [64.5–100.0] mg/L) ($P < 0.05$) (fig. 3). The median plasma bupivacaine concentration in the L-carnitine–replete group (68.0 [56.7–83.4] mg/L) did not differ significantly from controls at the time of asystole (fig. 3). In keeping with the shorter time to asystole, the median dose of bupivacaine administered at the time of asystole in L-carnitine–deficient rats (28.2 [26.0–30.0] mg/kg) was significantly less than that in controls (36.1 [32.2–48.5] mg/kg) ($P < 0.05$). The median dose of bupivacaine in the L-carnitine–replete group (43.0 [40.7–52.0] mg/kg) was 20% greater than that in controls, although this difference did not achieve statistical significance (fig. 4).

Discussion

Carnitine is a naturally occurring amino acid derivative synthesized endogenously in the kidneys and liver from lysine and methionine and derived from dietary sources. The transfer of long-chain fatty acids into the mitochondria for β-oxidation is a carnitine-dependent process[5] (fig. 5). Our results demonstrate that carnitine deficiency increased sensitivity to bupivacaine-induced asystole and QRS prolongation and that acute L-carnitine repletion completely reverses these effects. Asystole oc-

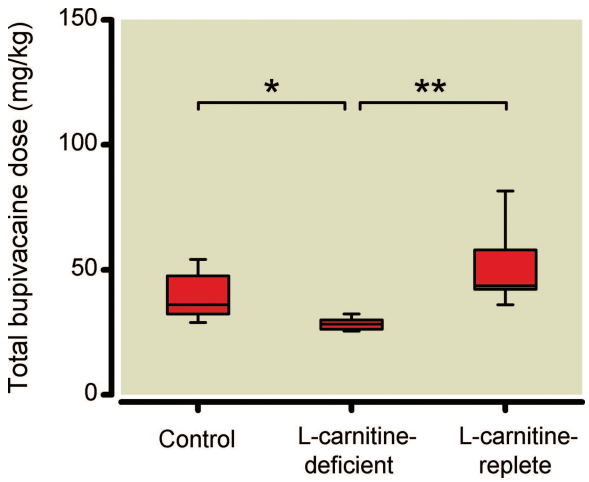

Fig. 4. Total bupivacaine dose (mg/kg) at time of asystole in control (n = 10), L-carnitine–deficient (n = 10), and L-carnitine–replete (n = 10) rats. Data are median, interquartile range, range. * $P < 0.05$, ** $P < 0.01$.

curred at a lower plasma concentration of bupivacaine in the L-carnitine–deficient group than in control and replete animals, suggesting that the reduced time to asystole was a pharmacodynamic, rather than pharmacokinetic, effect.

Whereas times to primary and secondary endpoints differed among groups, the observed progression of electrocardiograph and hemodynamic changes during infusion of bupivacaine was similar. Animals in all groups demonstrated predictable and systematic progression from normal sinus rhythm to PR and QRS interval prolongation before the occurrence of the first arrhythmia. Sustained bigeminy or trigeminy followed the onset of arrhythmia and was accompanied by the onset of broad complex bradycardia and hypotension. Reductions in heart rate beyond 50% were followed by decreases in MAP of 50% or more. Before asystole, heart rate had decreased by at least 90% from baseline.

The incidence of carnitine deficiency in the general population is unknown; however, carnitine deficiency is known to occur in diverse clinical scenarios. Primary carnitine deficiency is caused by a deficiency in the plasma membrane carnitine transporter, which results in urinary carnitine wasting. The incidence of primary carnitine deficiency in North America is unknown; in Australia and Japan, it occurs in approximately 1 in 37,000–100,000 newborns.[24–27]

Secondary carnitine deficiency may be congenital or acquired. Congenital deficiency is caused by metabolic disorders, including fatty acid oxidation disorders, organic

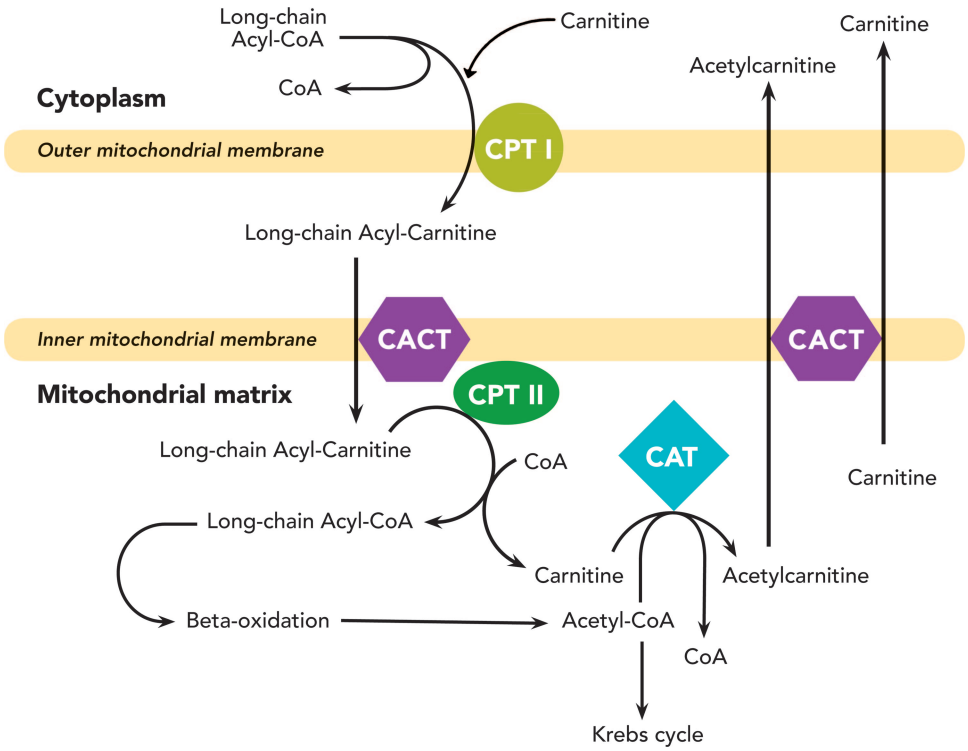

Fig. 5. Fatty acid transport into the mitochondrial matrix. The acyl group of long-chain acyl-coenzyme A (CoA), the active form of long-chain fatty acids, is transferred to carnitine *via* carnitine palmitoyltransferase I (CPT I), forming acylcarnitine and generating free coenzyme A. Acylcarnitine is transported into the mitochondrial matrix *via* carnitine-acylcarnitine translocase (CACT). Carnitine palmitoyltransferase II (CPT II) regenerates free carnitine by catalyzing the transfer of the acyl group from carnitine to coenzyme A in the mitochondrial matrix. Carnitine is transported back to the cytoplasm *via* carnitine-acylcarnitine translocase as free carnitine or acetyl carnitine after acetylation *via* carnitine acetyl transferase (CAT). Bupivacaine strongly inhibits carnitine-acylcarnitine translocase, thereby preventing the entry of long-chain acylcarnitine into the mitochondrial matrix and reducing the return of carnitine into the cytoplasm.

acidemias, urea cycle defects, and disorders of the mitochondrial respiratory chain. These conditions may cause carnitine depletion *via* the formation of acylcarnitines, which are excreted in the urine and inhibit carnitine uptake at the level of the carnitine transporter in renal cells.[25] Acquired causes include cirrhosis and chronic renal failure, which impair the biosynthesis of carnitine, and malabsorption syndromes and dietary factors. Iatrogenic causes include the use of certain drugs, including zidovudine and valproic acid. Valproylcarnitine, a metabolite of the latter, reduces renal tubular reabsorption of free carnitine and acylcarnitine and decreases endogenous synthesis of carnitine by inhibition of the enzyme butyrobetaine hydroxylase.[19,24,28–30]

Whereas local anesthetic nerve conduction block is classically explained by neuronal sodium channel inhibition, the mechanisms of myocardial and neurologic toxicity are poorly understood. Myocardial toxicity manifests clinically as conduction blockade and negative inotropy resulting in arrhythmia, decreased cardiac output, and progression to asystole.[31,32] These effects have been attributed to blockade of sodium, potassium, and calcium channels[33–38] and impairment of cellular energy metabolism *via* uncoupling of oxidative phosphorylation.[7–9] Weinberg *et al.* measured oxygen consumption using lipid and nonlipid substrates with varying concentrations of lidocaine, bupivacaine, etidocaine, and ropivacaine in rats. They found that the local anesthetics inhibited lipid-based respiration in myocardial mitochondria *via* inhibition of carnitine-acylcarnitine translocase. The extent of inhibition paralleled the clinically observed cardiotoxicity. Thus, bupivacaine was found to be a potent inhibitor of carnitine-dependent respiration, whereas ropivacaine and lidocaine were intermediate and weak inhibitors, respectively.[6]

This finding from Weinberg's study implies that bupivacaine reduces the availability of fatty acids for β-oxidation in myocardial mitochondria by specific inhibition of the enzyme systems involved in the carnitine shuttle. The resulting loss of energy substrate potentially could contribute to the cardiotoxicity observed clinically. L-carnitine deficiency may further enhance mitochondrial substrate deprivation and increase susceptibility to local anesthetic cardiotoxicity. The observed reversal of this effect by L-carnitine repletion supports this hypothesis and suggests that L-carnitine may have a protective role against local anesthetic cardiotoxicity. The apparent efficacy of lipid emulsion in local anesthetic cardiotoxicity[39] also supports the hypothesis that energy substrate deprivation contributes to bupivacaine cardiotoxicity. Although the mechanism of action of lipid emulsion in this setting remains undetermined, one postulated mechanism is a direct myocardial metabolic effect.[39]

Although a chronotropic effect of L-carnitine has not been described in the literature, positive inotropy has been demonstrated in animal studies.[17,40] L-carnitine also reduces electrocardiographic and metabolic evidence of myocardial injury secondary to ischemia and increases the threshold of ischemia-induced ventricular fibrillation in the intact canine heart.[41] Myocardial protection by L-carnitine or its analog, in other ischemic or cardiomyopathic animal models has been demonstrated in studies by Kawasaki *et al.* and Ferrari *et al.*[42,43] Long-term administration of L-carnitine or propionyl-L-carnitine has been demonstrated to improve mechanical function in the ischemic or failing myocardium.[42–44] The mechanism underlying these effects of L-carnitine is undetermined but may be related to the role of L-carnitine as the requisite carrier of long-chain acyl groups into the mitochondrial matrix and the stimulation of fatty acid oxidation and oxidative phosphorylation in myocardial mitochondria.[45,46]

We used a model of carnitine deficiency that has been demonstrated to reduce total myocardial L-carnitine concentration by 61% in Wistar rats.[14] Paulson and Shug demonstrated that total serum L-carnitine concentration decreased by 21% after 750 mg/kg intraperitoneal D-carnitine (the physiologically inactive isomer of carnitine) was administered daily for 4 days in Sprague-Dawley rats, compared with the 57% reduction observed in the current study after 10 days. In addition, these investigators reported that total L-carnitine concentration in skeletal and myocardial muscle was reduced by 52% and 65%, respectively.[11] The mechanism of depletion of L-carnitine by D-carnitine is multifactorial and includes cellular membrane exchange of L-carnitine with extracellular D-carnitine, inhibition of carnitine transferase, and competitive inhibition of renal tubular L-carnitine reuptake, resulting in increased urinary loss of L-carnitine.[11–13]

Our study has several limitations. We used isoflurane for maintenance of anesthesia despite evidence that isoflurane attenuates bupivacaine-induced arrhythmia in rats[47]; however, given that isoflurane was applied equally to all groups, our comparative findings are valid. For the purposes of observing and recording the onset of specific endpoints, we chose to administer bupivacaine by intravenous infusion, rather than bolus injection, which would be similar to most clinical scenarios of local anesthetic toxicity. Infusion of bupivacaine could have increased the dose required to achieve toxicity. Moreover, quantifying free bupivacaine concentration in fresh plasma would more accurately reflect the pharmacodynamically active component; however, we measured total plasma bupivacaine concentrations given that the blood specimens were frozen and analyzed in batches. We have demonstrated that our model of L-carnitine deficiency resulted in a 57% decrease in serum L-carnitine concentration, but we did not measure tissue L-carnitine concentration, although other investigators have reported depletion of L-carnitine in cardiac and skeletal muscle.[11,14] The model used also evaluates acute, but not chronic, L-carnitine deficiency, which is likely the more prevalent clinical scenario, and the situation in which carnitine-deficiency–induced dilated or hypertrophic cardiomyopathy might contribute to susceptibility to fatal arrhythmogenesis.[48,49] Zaugg *et al.* have demonstrated that the hearts of rats that were chronically depleted of L-carnitine developed systolic dysfunction and

reduced contractile reserve,[50] which may contribute to increased sensitivity to cardiotoxicity. Finally, the addition of dose–response repletion studies would have strengthened a potential causal relationship between acute L-carnitine repletion and decreased susceptibility to bupivacaine-induced cardiotoxicity.

In conclusion, our data demonstrate that carnitine deficiency predisposes to bupivacaine-induced cardiotoxicity in rats and that acute L-carnitine repletion completely reverses this effect. These findings support *in vitro* evidence demonstrating bupivacaine-induced inhibition of lipid substrate oxidation in cardiac mitochondria *via* the carnitine system. Of clinical relevance, our findings suggest that susceptibility to local anesthetic-induced cardiotoxicity might vary from patient to patient, depending on biochemical and/or metabolic factors, and imply that regional anesthesia should be used with caution in patients with suspected or known L-carnitine deficiency because the cardiotoxic dose of local anesthetic may be significantly decreased in such individuals. In addition, an important clinical implication of our findings is that L-carnitine may be a valuable addition to the management of resuscitation from local anesthetic cardiotoxicity.

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