The Influence of Age on Bupivacaine Cardiotoxicity

Marcio G. Kiuchi, MD,* Gisele Zapata-Sudo, MD, PhD,* Margarette M. Trachez, MD, PhD,† Douglas Ririe, MD,‡ and Roberto T. Sudo, MD, PhD*

BACKGROUND: The susceptibility of children and newborns to cardiotoxicity from racemic bupivacaine, RS(±)-bupivacaine, is controversial. Some studies indicate that newborns can sustain higher bupivacaine plasma levels than adults, without severe toxicity. In this study, we compared the influence of age on cardiotoxicity from RS(±)-bupivacaine and S(−)-bupivacaine in rats. The effects of these local anesthetics (LAs) on the regulation of intracellular Ca2+ concentrations in cardiac fibers were also investigated.

METHODS: The lethal dose was determined in ventilated male Wistar rats at 2, 4, 8, and 16 weeks of age by monitoring when cardiac electrical activity stopped after infusion of RS(±)-bupivacaine and S(−)-bupivacaine (4 mg · kg−1 · min−1). The effects on cardiac muscle contraction were investigated by in vitro measurement of papillary muscle twitches in the presence and absence of RS(±)-bupivacaine or S(−)-bupivacaine. Skinned ventricular fibers were used to investigate the intracellular effects on Ca2+ regulation induced by both LAs.

RESULTS: The lethal dose for RS(±)-bupivacaine and S(−)-bupivacaine in 2-week-old animals (4.5 ± 2.2 and 4.9 mg · kg−1, respectively) was higher than in 16-week-old animals (22.7 ± 1.3 and 22.0 ± 2.7 mg · kg−1, respectively). Papillary muscle twitches were reduced in a dose-dependent manner, with significant difference between young and adult hearts. In adults, the muscle twitches were reduced to 8.6% ± 0.8% of control by RS(±)-bupivacaine, and to 18.1% ± 2.7% of control by S(−)-bupivacaine (100 μM). S(−)-bupivacaine had a positive inotropic effect at <10 μM, but only in 2-week-old animals. In chemically skinned ventricular fibers, RS(±)-bupivacaine and S(−)-bupivacaine induced similar increases in Ca2+ release from the sarcoplasmic reticulum (SR) preactivated with caffeine (1 mM), and this effect was greater in younger rats than adults. In 16-week-old rats, caffeine-induced tension was 53.9% ± 1.7% of the maximal fiber response with RS(±)-bupivacaine, and 54.1% ± 3.2% with S(−)-bupivacaine. The caffeine response in 2-week-old rats was 81.1% ± 3.7% of the maximal response with RS(±)-bupivacaine, and 78.1% ± 4.5% with S(−)-bupivacaine. The Ca2+ sensitivity of contractile proteins was equally increased at both ages tested, with RS(±)-bupivacaine or S(−)-bupivacaine.

CONCLUSIONS: Differences in the mechanisms for regulating intracellular SR Ca2+ may contribute to the decreased susceptibility of young animals to cardiodepression induced by RS(±)-bupivacaine and S(−)-bupivacaine. (Anesth Analg 2011;112:574–80)

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METHODS
The Universidade Federal do Rio de Janeiro Animal Care and Use Committee approved the protocols used in this work. Animals were housed in a temperature, humidity, and 12-hour day/dark controlled room. Water and food were offered ad libitum.

Determination of RS(±)-Bupivacaine and S(−)-Bupivacaine LD
The LD was determined in male Wistar rats at 2, 4, 8, and 16 weeks of age. Under anesthesia with sodium pentobarbital (50 mg·kg⁻¹, intraperitoneally), a plastic tube was introduced into the trachea and connected to a Harvard Apparatus model 681 (Harvard Apparatus, Holliston, MA) for controlled pulmonary ventilation. The pattern of ventilation was adjusted to a volume of 15 mL·kg⁻¹ and 40 to 60 breaths per minute to maintain the pH, Po₂, and Pco₂ in the normal range. A pair of electrodes was fixed to the chest for electrocardiographic recording. RS(±)-bupivacaine or S(−)-bupivacaine was continuously infused via the external jugular vein at a dose of 4 mg·kg⁻¹·min⁻¹ through a micrometric Harvard pump (model 1100). This dose and rate of infusion were experimentally adjusted in preliminary experiments. The pump infusion was immediately stopped when absence of cardiac electrical activity, the variable used to indicate death, was observed. Amount of drug injected was calculated from the time and rate of infusion and converted to LD for each animal. LD was expressed as mean ± SEM for each LA. The estimated lethal free serum concentration was estimated considering a 90% protein binding to LA.

Preparation and Investigation of Papillary Muscle
Male Wistar rats at 2, 4, 8, and 16 weeks of age were killed under anesthesia with sodium pentobarbital (60 mg·kg⁻¹, intraperitoneally). Hearts were quickly removed and papillary muscles dissected, positioned in a vertical chamber filled with a solution of (in mM) NaCl, 130; KC1, 5; MgCl₂, 1; CaCl₂, 2.5; NaH₂PO₄, 0.5; NaHCO₃, 24; glucose, 5.6; pH 7.4, and prepared for isometric tension recording. The solution was continuously oxygenated with a carbonic mixture (95% O₂/5% CO₂) keeping temperature at 37°C ± 0.2°C. Each muscle was mounted between 2 hooks with one end attached to a fixed clamp and the other to a force transducer (model FT03; Grass Technologies) and the other to a micromanipulator (Narishige model 3, East Meadow, NY). Fascicles were stretched to 120% of resting length using a binocular stereomicroscope and exposed to solution R containing the detergent saponin (0.5% v/v; Merck Chemical Co., Darmstadt, Germany) over 5 minutes. This procedure created a sarcolemma lesion that permitted free access of solution without damaging the functionality of the contractile protein and SR. To investigate the direct effects of RS(±)-bupivacaine and isomers on the Ca²⁺ sensitivity of contractile proteins, SR membranes were further disrupted by 60 minutes of exposure to solution R containing the nonionic detergent octylphenoxypolyethoxyethanol (Tri-ton X-100, 1% v/v; Sigma Chemical Co., St. Louis, MO). Temperature was maintained at 22.0°C ± 0.5°C.

After the skinnning procedure, the maximal contractile response of the fibers was determined by exposure to a solution containing 15.85 µM Ca²⁺ (pCa 4.8), which was the amount of free Ca²⁺ required to induce the maximal contraction response (not shown). Fiber relaxation was induced by exposure to solution R during the Ca²⁺-activated contraction plateau. Isometric tension generated by the fibers was recorded on a chart recorder (Grass model 7400). After measuring the maximal contraction response, the SR was depleted of Ca²⁺ using 20 mM caffeine dissolved in solution R and the SR Ca²⁺ loading cycle induced by 3 minutes of exposure to pCa 6.8 (0.16 µM Ca²⁺) prepared by adding K₂EGTA and CaK₂EGTA to maintain a total EGTA concentration of 5 mM. Association constants for K₂EGTA/CaK₂EGTA ratios required to achieve the desired pCa were from Orentlicher et al. For other ligands, constants were from Fabiato and Fabiato. The efficiency of the SR Ca²⁺ loading procedure was evaluated by measuring the contractile response induced by caffeine (20 mM) in wash solution (solution R without EGTA).

To investigate the effect of RS(±)-bupivacaine and its isomer on Ca²⁺ release from the SR, after the loading cycle, each LA (0.001–10 mM) was added to solution R containing 1 mM caffeine, which was insufficient to promote complete release of stored Ca²⁺ from the SR. Thus, the tension amplitude was smaller than with 20 mM caffeine. Additional increases in the contraction amplitude were used to measure the effect of LA on Ca²⁺ release from the SR.

Effects on SR Ca²⁺ uptake were evaluated by repeating the loading cycle with pCa 7.4 over 1 minute in the presence or absence of RS(±)-bupivacaine or S(−)-bupivacaine. Data were presented as percentage of control twitches measured at the start of each experiment.

Cardiac Skinned Fiber Preparation
Fascicles approximately 0.30 mm in diameter and 1 to 2 mm long were excised from the subendocardium of the left ventricular wall from Wistar rats (2 and 16 weeks old) at room temperature in oxygenated (95% O₂ and 5% CO₂) nominally Ca²⁺-free buffered saline (in mM): NaCl, 130; KCl, 5; MgCl₂, 1; NaH₂O⁻, 0.5; NaHCO₃, 24; glucose, 5.6; pH 7.0. Fascicles were transferred to a 1-mL internal volume chamber filled with relaxation (R) solution (mM) (K propionate, 185; Mg acetate, 2.5; imidazole propionate, 10; KNa₂ATP, 5; and K₂-ethyleneglycol-bis[β-aminoethyl]ether]-N,N,N',N'-tetraacetic acid [EGTA], 5; pH 7.0) and the ends were attached to 2 hooks, one connected to a force transducer (model FT03; Grass Technologies) and the other to a micromanipulator (Narishige model 3, East Meadow, NY). Fascicles were stretched to 120% of resting length using a binocular stereomicroscope and exposed to solution R containing the detergent saponin (0.5% v/v; Merck Chemical Co., Darmstadt, Germany) over 5 minutes. This procedure created a sarcolemma lesion that permitted free access of solution without damaging the functionality of the contractile protein and SR. To investigate the direct effects of RS(±)-bupivacaine and isomers on the Ca²⁺ sensitivity of contractile proteins, SR membranes were further disrupted by 60 minutes of exposure to solution R containing the nonionic detergent octylphenoxypolyethoxyethanol (Tri-ton X-100, 1% v/v; Sigma Chemical Co., St. Louis, MO). Temperature was maintained at 22.0°C ± 0.5°C.

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concentration in the solution and the loading time. Procaine (40 mM) was added during loading to prevent Ca2+ leak. Tension response to caffeine (20 mM) was used as an indicator of Ca2+ accumulation.

Effects of RS(±)-bupivacaine and the isomer on Ca2+ sensitization of contractile proteins were studied in SR-disrupted preparations. The pCa versus isometric tension curves were performed in the absence (control) and presence of 5.0 mM RS(±)-bupivacaine or S(−)-bupivacaine. The pCa value that induced 50% of maximal tension (pCa50) was calculated using the Hill equation. Six experiments were performed for each LA for both the 2- and 16-week-old groups.

**Statistical Analysis**

All data were expressed as means ± SEM. Differences between LD means were evaluated by Student t test with statistical significance at P < 0.05. To analyze LA effects on papillary muscle isometric tension, one-way analysis of variance (ANOVA) was followed by the Dunnett test. ANOVA followed by Dunn’s method was used to compare responses between age groups. Tension amplitudes of skinned fibers were expressed as percentage of maximal fiber response. Comparison between RS(±)-bupivacaine and S(−)-bupivacaine on SR Ca2+ release was evaluated using ANOVA and the Bonferroni test for critical difference. The Kruskal-Wallis test followed by the Student-Newman-Keuls test was used to compare pCa50 values. Ca2+ concentration-response curves were fitted to the equation y = y_{max} \cdot \Ca^{2+n} (Ca^{2+n} + k_{0.5}), where y is the percentage of isometric tension, n the Hill coefficient, and k_{0.5} the Ca2+ concentration producing 50% of the maximal tension. Differences were considered significant at P < 0.05.

**RESULTS**

**Lethal Dose**

To determine the LD for mechanically ventilated rats, RS(±)-bupivacaine and S(−)-bupivacaine were infused at 4 mg·kg⁻¹·min⁻¹ until cardiac electrical activity stopped. As shown in Figure 1, the LD for RS(±)- and S(−)-bupivacaine was greater in 2-week-old rats than in older groups. The LD for RS(±)-bupivacaine in 2-week-old rats was 46.0 ± 5.2 mg·kg⁻¹, which was significantly higher (P < 0.01) than in 4-week-old rats (24.0 ± 3.4 mg·kg⁻¹), 8-week-old rats (23.3 ± 2.2 mg·kg⁻¹), or 16-week-old rats (22.7 ± 1.3 mg·kg⁻¹). For 2-week-old animals, the LD for infusion of S(−)-bupivacaine was 91.3 ± 4.9 mg·kg⁻¹, significantly higher (P < 0.01) than for animals at 4 weeks (24.0 ± 4.3 mg·kg⁻¹), 8 weeks (28.7 ± 3.2 mg·kg⁻¹), or 16 weeks (22.0 ± 2.7 mg·kg⁻¹). Also, in the 2-week-old group, the LD for S(−)-bupivacaine was higher (P < 0.01) than for RS(±)-bupivacaine.

**Effects of RS(±)-Bupivacaine and S(−)-Bupivacaine on Papillary Muscle Contraction**

Figure 2 shows the effects of RS(±)- and S(−)-bupivacaine (25 μM) on twitches from electrically stimulated papillary muscles of rats aged 2, 4, 8, and 16 weeks. At 2 weeks, twitches were not significantly decreased after exposure to RS(±)-bupivacaine (88.7% ± 3.4% control) or S(−)-bupivacaine (104.9% ± 5.6% control). However, they were significantly reduced in an age-dependent manner for both RS(±)- and S(−)-bupivacaine. The maximal depression effect was observed in 16-week-old animals with 28.2% ± 6.0% of the control for RS(±)-bupivacaine, and 53.4% ± 7.8% for S(−)-bupivacaine. Importantly, RS(±)-bupivacaine caused a more significant reduction (P < 0.05) in papillary muscle depression than S(−)-bupivacaine in 16-week-old rats (Fig. 2).

When papillary muscles of 2- and 16-week-old rats were exposed to increasing concentrations (2–100 μM) of RS(±)- and S(−)-bupivacaine, several different effects were observed (Fig. 3). Cardiac depression was more prominent in adults (16 weeks). In the presence of 10 μM RS(±)-bupivacaine, twitches in 2-week-old rats were 95.5% ± 2.1% of control, and in 16-week-old rats they were 35.1% ± 5.7% of the control (P < 0.01). In the adult group, the twitches were 8.6% ± 0.8% of control for 100 μM RS(±)-bupivacaine, and 18.1% ± 2.7% for S(−)-bupivacaine (Fig. 3). At the same concentration, RS(±)- and S(−)-bupivacaine reduced the twitches to 58.7% ± 2.9% and 56.0% ± 7.0% of the control in the young group (2 weeks old) (Fig. 3). Although not observed in adults, an interesting biphasic response to S(−)-bupivacaine occurred in 2-week-old rats with increased contractility at 5 to 10 μM (twitch amplitude at 10 μM was 119% ± 7% of control, P < 0.05), returning to control value at 20 μM and dose dependently decreasing with higher concentrations.
Effect of LA on Ca\textsuperscript{2+} Loading and Release from SR in Skinned Cardiac Muscle

A sustained and maximal contraction response was induced in skinned ventricular fibers by a high concentration of Ca\textsuperscript{2+} (pCa 4.8; 15.85 mM Ca\textsuperscript{2+}). All ordinate data in Figures 4 and 5 are expressed relative to maximal response.

Figure 4. Ca\textsuperscript{2+} release from the sarcoplasmic reticulum induced by caffeine (1 mM) in the absence or presence of RS(±)- and S(−)-bupivacaine (0.001–10 mM) in chemically denuded ventricular myocytes of rats 2 and 16 weeks old, previously loaded with pCa 6.8 during 3 minutes. Each point represents the mean ± SEM (n = 8) of percentage of maximal tension induced by exposure of the bundles to pCa 4.8. *P < 0.001 compared with 2-week-old rats.

Effect of LA on Ca\textsuperscript{2+} Loading and Release from SR in Skinned Cardiac Muscle

The effect of RS(±)-bupivacaine and the isomer on Ca\textsuperscript{2+} uptake from the SR was tested on ventricular skinned fibers.

Figure 3. Effect of RS(±)-bupivacaine (A) and S(−)-bupivacaine (B) in the twitches (percentage of control) of papillary muscle isolated from 2- and 16-week-old rats. The bars represents the mean ± SEM (n = 12). *P < 0.001 2 weeks versus 16 weeks.
loaded with pCa 7.4 solution (0.039 mM Ca\(^{2+}\)) for 1 minute in the absence or presence of the LAs at 0.1, 0.5, 1.0, or 5.0 mM. The accumulation of Ca\(^{2+}\) in the SR was evaluated by measuring the isometric tension after addition of caffeine (20 mM). No significant changes were observed in the caffeine-induced contraction when RS(\pm\)-bupivacaine or S(\(-\)-bupivacaine was present during SR loading in ventricle fibers from 2- or 16-week-old animals. This finding suggested that the SR Ca\(^{2+}\) loading procedure was not affected by RS(\pm\)- or S(\(-\)-bupivacaine at either age.

**Effect of LA on Ca\(^{2+}\) Sensitivity of Contractile Proteins in Skinned Cardiac Fibers**

Saponin-skinned ventricular fibers from 2- and 16-week-old rats were exposed to Triton X-100 for 60 minutes to disrupt SR membranes. To investigate the effects of LA on myofibril Ca\(^{2+}\) sensitivity, contraction was induced by increasing Ca\(^{2+}\) in the solution in the absence and presence of LA (5 mM). The Ca\(^{2+}\)-induced contractions were altered by RS(\pm\)- and S(\(-\)-bupivacaine in both 2- and 16-week-old rats, with both RS(\pm\)-bupivacaine and the isomer shifting the Ca\(^{2+}\)-response curves to the left (Fig. 5). In adult animals (16 weeks), the Ca\(^{2+}\) concentration that caused 50% of maximal response ([Ca\(^{2+}\)]\(_{50}\)) decreased from 1.62 ± 0.07 to 0.71 ± 0.03 μM with RS(\pm\)-bupivacaine (P < 0.01) and from 1.48 ± 0.02 to 0.66 ± 0.05 μM with S(\(-\)-bupivacaine (P < 0.01). The [Ca\(^{2+}\)]\(_{50}\) decreased from 1.58 ± 0.01 to 0.72 ± 0.03 μM with RS(\pm\)-bupivacaine (P < 0.01) and from 1.70 ± 0.01 to 0.71 ± 0.03 μM with S(\(-\)-bupivacaine (P < 0.01) in young animals (2 weeks) (Fig. 5). These results demonstrated that both LAs increased the Ca\(^{2+}\) sensitivity of contractile proteins, but this was not affected by age.

**DISCUSSION**

In this study, we investigated age dependency of toxicity induced by RS(\pm\)-bupivacaine and its S(\(-\)-isomer. A significant difference in LD was observed in young animals (2 weeks) compared with older animals (>4 weeks) for both RS(\pm\)- and S(\(-\)-bupivacaine. Two weeks of age seemed to be the limit for decreased susceptibility to the toxic effect of bupivacaine because no difference in LD was observed among animals older than 4 weeks. However, differences in susceptibility to the drugs were detected in the younger group. The LD in 2-week-old animals compared with older animals was approximately 4-fold higher for S(\(-\)-bupivacaine and approximately 2-fold higher for RS(\pm\)-bupivacaine. This is in agreement with reports that human neonates and children support a higher plasma concentration of bupivacaine after accidental IV administration.\(^{21,22}\) No data have been reported comparing toxicity between bupivacaine isomers in human neonates or children. A combination of several mechanisms of respiratory depression and cardiac toxicity may explain the decreased susceptibility of young animals to bupivacaine. Because our experiments to determine the LD were conducted in artificially ventilated and convulsion-controlled conditions, our results suggest that direct cardiac toxicity may be involved.

Experiments from papillary muscles with RS(\pm\)- and S(\(-\)-bupivacaine demonstrated a decrease in twitch amplitude that was age- and LA-concentration dependent. RS(\pm\)- and S(\(-\)-bupivacaine gave significantly different results, with S(\(-\)-bupivacaine showing less potency. Papillary muscles from 16-week-old animals were much more susceptible to depression induced by RS(\pm\)- and S(\(-\)-bupivacaine than muscles from 2-week-old animals (approximately 3 years old in humans). At < 10 μM (specifically 2.9 μg·mL\(^{-1}\)), which represents the clinical range, RS(\pm\)-bupivacaine did not cause any significant effects in 2-week-old animals. However, a >60% reduction in contraction was observed in 16-week-old animals. In this tissue, S(\(-\)-bupivacaine did not decrease Twitches, but increased them by approximately 20%, an observation that could be of clinical significance.

The cardiac depression induced by LAs could be related to the alterations in the regulation of intracellular Ca\(^{2+}\) concentration that gradually changes with age. In adults, the Ca\(^{2+}\) source needed for myofibril activation and cardiac contraction depends on a Ca\(^{2+}\)-induced–Ca\(^{2+}\)-release (CICR) process that is triggered by a small amount of Ca\(^{2+}\) entry to the cell via an L-type Ca\(^{2+}\) channel. In newborns, CICR is not so efficient, probably because of immaturity of the T tubule,\(^{31}\) lower density of RyR2,\(^{32}\) and decreased SERCA2 activity.\(^{33}\) The main sources responsible for cardiac contraction in newborns are Ca\(^{2+}\) influx via the L-type Ca\(^{2+}\) channel\(^{34-37}\) and Na\(^{+}/Ca\(^{2+}\) exchanger, which is reversely activated by a decreased intracellular Na\(^{+}\) concentration.\(^{38}\) There is no information concerning the effects of bupivacaine and its S(\(-\)-isomer on the L-type Ca\(^{2+}\) channel or in the Na\(^{+}]/Ca\(^{2+}\) exchanger in newborn rats. The negative inotropic effect observed with RS(\pm\)-bupivacaine could be explained by a decrease in Ca\(^{2+}\) influx into the cell, caused by direct interaction with L-type Ca\(^{2+}\) channels.\(^{12,13}\) Thus, we suggest that RS(\pm\)- and S(\(-\)-bupivacaine promote a more significant cardio-depressant effect in older animals because the intracellular Ca\(^{2+}\) concentration depends entirely on the activation of L-type Ca\(^{2+}\) channels, and these LAs produce a nonstereselective blockade.\(^{13}\) As a consequence, the CICR from intracellular stores could be impaired in these animals.

The LD varied from 20 to 90 mg·kg\(^{-1}\), which corresponded to a range of 61 to 277 μM. The free drug concentration would be approximately 6.1 μM (1.9 μg·mL\(^{-1}\)) to 27.7 μM (8.1 μg·mL\(^{-1}\)) considering the protein binding. However, because of several differences in young animals such as drug distribution, blood protein concentration, and rate of metabolism, the free drug concentration should be higher than expected. Pharmacokinetic data show that the lower plasma concentrations of albumin and α\(_1\)-acid glycoprotein in young animals and humans compared with adults\(^{38}\) may result in less RS(\pm\)- and S(\(-\)-bupivacaine binding. Consequently, higher serum levels of these substances are found in children after injection,\(^{39}\) which may contribute to more severe cardio-toxic effect in young patients. The immaturity of isoforms CYP3A4 and CYP1A2 of the cytochrome P450 in young patients may also contribute to the increased cardiotoxicity of RS(\pm\)- and S(\(-\)-bupivacaine in pediatric patients\(^{40}\) because of reduced intrinsic clearance.\(^{39}\) Nonetheless, despite factors that favor higher toxicity of RS(\pm\)- and S(\(-\)-bupivacaine in the young, our results did not show the predicted increase in cardiotoxicity in younger animals.
Tension recording in the chemically skinned ventricular myocytes technique demonstrated the effects of RS(±)- and S(−)-bupivacaine on intracellular Ca2+ handling at different ages. We investigated the influence of RS(±)- and S(−)-bupivacaine on Ca2+ release and uptake from the SR, and the effect on the Ca2+ sensitivity of myofibrils in the SR after disruption with Triton X-100. At 2 weeks of age, but not at 16 weeks, RS(±)- and S(−)-bupivacaine caused an increase in Ca2+ release from SR Ca2+-loaded myocytes preactivated by 1 mM caffeine in a concentration-dependent manner. This effect in young cardiac myocytes was seen at Ca2+ concentrations >10 µM, and the intracellular Ca2+ concentration may reach the mM range during tension development in physiological conditions. Thus, higher Ca2+ release may be a significant mechanism for cardiac protection against bupivacaine toxicity in young animals.

The mechanism of SR Ca2+ release is not clear. Several studies of adult animals suggest that RS(±)-bupivacaine may activate SR Ca2+ release in skeletal and cardiac cells. RS(±)-bupivacaine increases the likelihood of RyR1 channel opening in vesicles from rabbit skeletal muscle,11 and this effect was completely inhibited by procaine, an LA known to block SR Ca2+ release.32–44 Similar results were reported in skinned skeletal muscle fibers of rats, in which RS(±)-bupivacaine (1–15 mM) promoted SR Ca2+ release.45 RS(±)-bupivacaine also increases ryanodine binding in both skeletal and cardiac pig microsomes, suggesting activation of the RyR1 or RyR2.14 RS(±)-, S(−)-, and R(+)bupivacaine also promoted Ca2+ release from the SR trough RyR2 of cardiac myocytes and this effect was stereoselective, with S(−)-bupivacaine more effective than R(+)bupivacaine.15

An increase in SR Ca2+ uptake or a decrease in Ca2+ leakage could result in an additional mechanism that causes differences in the cardiac contractile response to bupivacaine. However, our data using skinned myocytes showed that RS(±)- or S(−)-bupivacaine during SR loading did not change the amplitude of caffeine-induced tension in either 2- or 16-week-old animals. Previous studies demonstrated that the diastolic Ca2+ concentration in spontaneously activated intact myocytes increased45 in the presence of RS(±)-, R(+)-, and S(−)-bupivacaine. These data suggest that bupivacaine may impair the mechanisms for removal of Ca2+ from the cytoplasm such as SERCA2, the Na+/Ca2+ exchanger, or the ATP-dependent Ca2+ pump, which are located in the sarcotylolem.46

The change in Ca2+ sensitivity of contractile proteins was investigated in skinned cardiac myocytes, in which the SR was completely destroyed. Exposure to RS(±)- and S(−)-bupivacaine shifted the pCa versus isotropic tension curve to the left in both 2- and 16-week-old animals, suggesting an increase in Ca2+ sensitivity of myofibrils. However, no difference was observed in the response to RS(±)- or to S(−)-bupivacaine. Thus, we concluded that the Ca2+ threshold for activation of myofibrils is reduced by RS(±)- and S(−)-bupivacaine, but this effect is not age dependent.

In conclusion, we demonstrated that the toxicity induced by RS(±)- and S(−)-bupivacaine in rats is age dependent. The LD for young animals (<2 weeks) is 2- to 4-fold higher than for 4-week-old or older animals. The decreased susceptibility of young animals to these LAs is probably related to differently regulated intracellular SR Ca2+ concentrations, but does not result from increasing the Ca2+ sensitivity of contractile protein. Thus, this study supported the hypothesis that young patients (younger than 4 years) are less susceptible to the systemic toxic effect of bupivacaine, especially to the S(−)-isomer.

DISCLOSURES
Name: Marcio G. Kiuchi, MD
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Attestation: Marcio G. Kiuchi has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
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