Anesthesiology 2004; 101:888–94

Comparative Effects of Bupivacaine and Ropivacaine on Intracellular Calcium Transients and Tension in Ferret Ventricular Muscle

Yasushi Mio, M.D., Norio Fukuda, Ph.D.,† Yoichiro Kusakari, M.D., Ph.D.,‡ Yoshikiyo Amaki, M.D.,§
Yasumasa Tanifuji, M.D.,§ Satoshi Kurihara, M.D., Ph.D.‖*

Background: **Recent evidence suggests that ropivacaine exerts markedly less cardiotoxicity compared with bupivacaine; however, the mechanisms are not fully understood at the molecular level.**

Methods: **Isolated ferret ventricular papillary muscles were microinjected with the Ca^{2+}-binding photoprotein aequorin, and intracellular Ca^{2+} transients and tension were simultaneously measured during twitch in the absence and presence of bupivacaine or ropivacaine.**

Results: **Bupivacaine and ropivacaine (10, 30, and 100 μM) reduced peak systolic $[Ca^{2+}]_i$ and tension in a concentration-dependent manner. The effects were significantly greater for bupivacaine, particularly on tension (approximately twofold). The percentage reduction of tension was linearly correlated with that of $[Ca^{2+}]_i$ for both anesthetics, with the slope of the relationship being ≈1.0 for ropivacaine and ≈1.3 for bupivacaine (slope difference, $P < 0.05$), suggesting that the cardiodepressant effect of ropivacaine results predominantly from inhibition of Ca^{2+} transients, whereas bupivacaine suppresses Ca^{2+} transients and the reaction beyond Ca^{2+} transients, *i.e.*, myofibrillar activation, as well. BAY K 8644, a Ca^{2+} channel opener, abolished the inhibitory effects of ropivacaine on Ca^{2+} transients and tension, whereas BAY K 8644 only partially inhibited the effects of bupivacaine, particularly the effects on tension.**

Conclusion: **The cardiodepressant effect of bupivacaine is approximately twofold greater than that of ropivacaine. Bupivacaine suppresses Ca^{2+} transients more markedly than does ropivacaine and reduces myofibrillar activation, which may at least in part underlie the greater inhibitory effect of bupivacaine on cardiac contractions. These results suggest that ropivacaine has a more favorable profile as a local anesthetic in the clinical settings.**

IT is well known that bupivacaine, a commonly used long-lasting local anesthetic, markedly suppresses contractile performance of cardiac muscle[1–3] and induces arrhythmia,[1,4,5] which may result in cardiac arrest on accidental rapid intravenous injection.[6] Ropivacaine was developed to provide a local anesthetic with fewer cardiotoxic effects than bupivacaine.[7,8] Recent evidence suggests that bupivacaine and ropivacaine show similar sensory blockade potencies[9–11] and that ropivacaine exerts less cardiodepression than bupivacaine.[12–14] It has been reported that, in cardiac muscle, ropivacaine has weaker blocking effects on sarcolemmal Na^+ channels[15] and less interference with mitochondrial energy metabolism,[16] giving rise to less cardiodepression compared with bupivacaine. However, considering the multiple inhibitory actions of bupivacaine on cardiac contractions that have been reported hitherto, including the inhibition of Ca^{2+} release[17,18] and Ca^{2+} sequestration[19] of the sarcoplasmic reticulum (SR) and decreases in myofibrillar activation (*i.e.*, activation of actomyosin molecules at a certain $[Ca^{2+}]_i$ that assemble into myofilaments),[20] it is unlikely that the above mechanisms fully account for the differences in cardiodepressant effects between bupivacaine and ropivacaine. Therefore, further studies that focus on the effects of the local anesthetics on intracellular Ca^{2+} transients and myofibrillar activation are warranted.

In the current study, we compared the effects of bupivacaine and ropivacaine on Ca^{2+} transients and myofibrillar activation in isolated ferret ventricular muscle. Results showed that bupivacaine suppressed twitch contraction approximately twofold greater than ropivacaine, which results from greater inhibitory effects on Ca^{2+} transients and from decreases in myofibrillar activation.

Materials and Methods

Muscle Preparations

All experiments conducted in the current study strictly conformed to the *Guiding Principles for the Care and Use of Animals* approved by the Council of the Physiologic Society of Japan, Tokyo, Japan. Male ferrets (body weight, 800–1200 g) were anesthetized with pentobarbital sodium (100 mg/kg intraperitoneal administration). Then, the hearts were quickly removed and perfused through the aorta with the normal Tyrode solution (135 mM Na^+, 5 mM K^+, 2 mM Ca^{2+}, 1 mM Mg^{2+}, 102 mM Cl^-, 20 mM HCO_3^-, 1 mM HPO_4^{2-}, 1 mM SO_4^{2-}, 20 mM acetate, 10 mM glucose, 5 U/l insulin, and pH 7.35 at 30 ± 0.5°C when equilibrated with 5% CO_2-95% O_2). Pap-

---

* Staff Anesthesiologist, Department of Anesthesiology, The Jikei University School of Medicine, Tokyo, Japan. † Staff Physiologist, Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, Washington. ‡ Staff Physiologist, Department of Physiology (II), § Professor, Department of Anesthesiology, ‖ Professor, Department of Physiology (II), The Jikei University School of Medicine, Tokyo, Japan.

Received from the Department of Anesthesiology and the Department of Physiology (II), The Jikei University School of Medicine, Tokyo, Japan and the Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, Washington. Submitted for publication January 23, 2004. Accepted for publication May 24, 2004. Supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan, Tokyo, Japan (to Dr. Kurihara), by the American Heart Association (Northwest Affiliate), Seattle, Washington (to Dr. Fukuda), by the Japan Heart Foundation, Tokyo, Japan (to Dr. Fukuda) and by the Uehara Memorial Foundation, Tokyo, Japan (to Dr. Fukuda). AstraZeneca, Cheshire, UK provided the ropivacaine for this study. Yasushi Mio and Norio Fukuda contributed equally to this work.

Address reprint requests to Dr. Mio: Department of Anesthesiology, The Jikei University School of Medicine, 3-25-8 Nishishinbashi, Minato-ku, Tokyo 105-8461, Japan. Address electronic mail to: mio@jikei.ac.jp. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

illary muscles with a suitable size (length, 3.0–4.5 mm; diameter, 0.5–1.0 mm) were dissected from the right ventricle. Extreme care was taken during dissection to avoid overstretching of the muscle.

Experimental Procedures

Experiments were carried out according to our previously reported procedure.[21–23] Briefly, the papillary muscle was mounted horizontally in an experimental chamber perfused with the normal Tyrode solution, where one end of the muscle was connected to a tension transducer (BG-10; Kulite Semiconductor Products, Leonia, NJ) and the other to a motor (JCCX-101A; General Scanning, Watertown, MA). A pair of platinum electrodes was placed parallel to the muscle for electrical stimulation. The muscle was stimulated with a square pulse at 1.2-fold threshold with 5 ms duration at 0.2 Hz. The muscle was slowly stretched from the slack length to the length at which developed tension reached the maximum (L_{max}). The diameter of the preparation was measured to obtain tension per cross-sectional area at L_{max} (in mN/mm^2).

After twitch tension had been stabilized, the stimulation was interrupted and the Ca^{2+}-sensitive photoprotein aequorin was microinjected into 50–100 superficial cells of the muscle. Aequorin was purchased from John R. Blinks, M.D., Ph.D. (Professor Emeritus, Friday Harbor Laboratories, University of Washington, Friday Harbor, WA). Aequorin signals were detected with a photomultiplier (EMI 9789 A; Ruislip, UK) and converted to $[Ca^{2+}]_i$ using an *in vitro* calibration curve:

$$I/I_{max} = [(1 + K_R\,[Ca^{2+}])/(1 + K_{TR} + K_R\,[Ca^{2+}])]^n$$

where I indicates aequorin light intensity and I_{max} indicates peak light intensity at the saturating $[Ca^{2+}]_i$ (pCa, 4.5). The latter was calculated from the total light obtained by quickly destroying the cell membrane in the HEPES-Tyrode solution containing 1% (vol/vol) polyethylene glycol mono-p-isooctylphenyl ether (Triton X-100; Nacalai Tesque, Kyoto, Japan) at the end of the experiments. The composition of the HEPES-Tyrode solution was as follows: 128 mM Na^+, 5 mM K^+, 2 mM Ca^{2+}, 1 mM Mg^{2+}, 117 mM Cl^-, 1 mM SO_4^{2-}, 20 mM acetate, 5 mM HEPES, 10 mM glucose, 5 U/l insulin, and pH 7.40 (adjusted with NaOH) at 30 ± 0.5°C when equilibrated with 100% O_2. The constants used in the current study were as follows: n, 3.14; K_R, 4,025,000; K_{TR}, 114.6.[24,25]

After injection of aequorin, the solution was changed to the HEPES-Tyrode solution and experiments were performed. We measured the following parameters in the absence and presence of 10, 30, and 100 μM bupivacaine (Sigma, St. Louis, MO) or ropivacaine (gift from AstraZeneca, Cheshire, UK) during twitch: the peak values of systolic $[Ca^{2+}]_i$ and tension, the time for aequorin light to reach its peak from the onset of stimulus (time to peak light), the time for aequorin light to decay from 75 to 25% of the peak (decay time, DT), the time for tension measured from the onset of stimulus to the peak (time to peak tension), and the time for tension to decrease from the peak to 50% (relaxation time). We confirmed *in vitro* that bupivacaine or ropivacaine at concentrations up to 100 μM did not affect the aequorin light signals in the presence of various concentrations of free Ca^{2+} (pCa, 9.0–4.5) (data not shown). For both Ca^{2+} transients and tension, sixty-four records of the signals were averaged to improve the signal-to-noise ratio.

In a different set of experiments, we tested the effects of 0.6 μM BAY K 8644 (Sigma, St. Louis, MO) on bupivacaine or ropivacaine (100 μM)-induced decreases in Ca^{2+} transients and tension. Previous studies suggest that BAY K 8644 dramatically increases the open probability of sarcolemmal Ca^{2+} channels in cardiac muscle, resulting in marked increases in systolic $[Ca^{2+}]_i$ and developed tension.[26] In this series of experiments, we measured the peak values of Ca^{2+} transients and tension.

Statistical Analysis

Concentration-dependent effects of the anesthetic were assessed using one-way analysis of variance with repeated measures and the Scheffé *post hoc* test, and unpaired Student t test was employed to compare the effects of bupivacaine and ropivacaine. The least-square method was used for correlations between the anesthetic-induced reduction of peak systolic $[Ca^{2+}]_i$ and that of tension. Paired Student t test was used in the experiments with BAY K 8644. Statistical significance was verified at $P < 0.05$ in all cases. All data were expressed as mean ± SD, with "n" representing the number of muscles.

Results

Figure 1 shows typical chart recordings for changes in $[Ca^{2+}]_i$ and tension during twitch in the absence (control) and presence of 100 μM bupivacaine (A) or ropivacaine (B). Under the control condition without an anesthetic, the values of peak systolic $[Ca^{2+}]_i$ and tension were similar to those previously reported by us.[21–23] Bupivacaine suppressed Ca^{2+} transients and tension more markedly than ropivacaine (note that the difference in the inhibitory effect was particularly noticeable on tension).

Figures 2A and B summarize the concentration-dependent inhibitory effects of the anesthetics on peak systolic $[Ca^{2+}]_i$ and tension. Bupivacaine significantly decreased $[Ca^{2+}]_i$ at 30 and 100 μM and tension at 10–100 μM in a concentration-dependent manner (fig. 2A). Ropivacaine significantly decreased $[Ca^{2+}]_i$ and tension at 30 and 100 μM in a concentration-dependent manner (fig. 2B). Conversion of absolute values of $[Ca^{2+}]_i$ into percentage changes in $[Ca^{2+}]_i$ revealed that the inhibitory effect on $[Ca^{2+}]_i$ was significantly greater for bupivacaine at 30

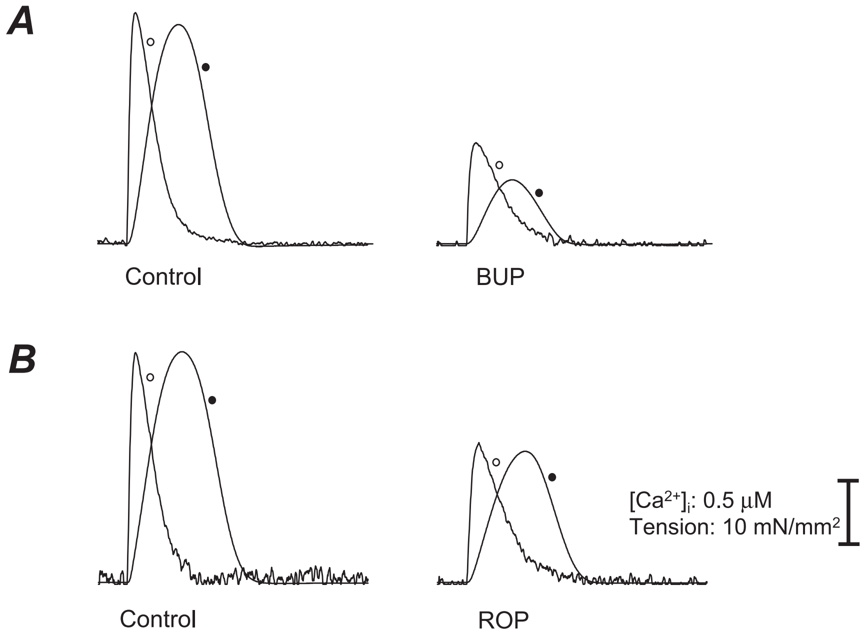

Fig. 1. Typical chart recordings of changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and tension during twitch contraction in ferret ventricular papillary muscle in the absence (control) and presence of 100 μM bupivacaine (*A*) or ropivacaine (*B*). *BUP* = bupivacaine; *ROP* = ropivacaine. It is clearly seen that bupivacaine suppresses $[Ca^{2+}]_i$ and tension more dramatically than ropivacaine (compare A and B). Data of 64 signals were averaged for (*A*) and (*B*). *Open circles* =CaT; *closed circles* = tension.

and 100 μM (fig. 2C). Likewise, the inhibitory effect on tension was significantly greater with bupivacaine at all concentrations used (fig. 2D). We plotted the percentage reduction of $[Ca^{2+}]_i$ *versus* that of tension with the anesthetics and found that a significant linear relationship was present between the parameters for both bupivacaine and ropivacaine (fig. 2E). The slopes were significantly different ($P < 0.05$), with ≈1.0 and ≈1.3 for ropivacaine and bupivacaine, respectively.

Table 1 summarizes the effects of bupivacaine and ropivacaine on the time courses of Ca^{2+} transients and tension. The local anesthetics prolonged time to peak light and DT, and the effects were statistically significant with 100 μM bupivacaine for both parameters. Time to peak tension tended to be shortened with bupivacaine or ropivacaine. Relaxation time tended to decrease with bupivacaine or ropivacaine, similar to the case when tension is decreased with decreases in the extracellular Ca^{2+} concentration.[21] However, the changes of time to peak tension and relaxation time did not reach statistical significance.

Figure 3 shows the influence of BAY K 8644 on the inhibitory effects of 100 μM bupivacaine (A) or ropivacaine (B) on peak systolic $[Ca^{2+}]_i$ and tension. BAY K 8644 only partially suppressed the inhibitory effects of bupivacaine, whereas it almost completely abolished those of ropivacaine. It should be stressed that the recovery of tension with BAY K 8644 was smaller in magnitude than that of $[Ca^{2+}]_i$ in the presence of bupivacaine.

Discussion

In the current study, we demonstrated in insolated ferret ventricular muscle that bupivacaine and ropivacaine suppressed Ca^{2+} transients and tension during twitch, with the effects being more pronounced with bupivacaine, particularly on tension. We discuss the current results focusing on the effects of the local anesthetics on intracellular Ca^{2+} regulation and myofibrillar activation.

Earlier studies with guinea pig atrial[3] and rat ventricular muscles[20] suggest that bupivacaine at a concentration of 10 μM decreases peak twitch tension by ≈30%. Although a different animal species was used in the current study under experimental conditions different than these previous studies, our findings on bupivacaine are in reasonable agreement with those of the previous studies (*i.e.*, ≈20% decrease) (figs. 2A and D). Taking into account the results of figure 2D (tension), it is reasonable to conclude that bupivacaine exerts a cardiodepressant effect approximately twofold greater than ropivacaine in ferret ventricular muscle.

Previous findings suggest that the acute inhibitory effects of bupivacaine on Ca^{2+} transients in cardiac muscle are primarily attributable to its blocking effects on sarcolemmal Na^+ and Ca^{2+} channels.[27–30] It is therefore reasonable to assume that the blocking effects of bupivacaine on cardiac Ca^{2+} channels, as well as on Na^+ channels,[15] are stronger than those of ropivacaine and that this may at least in part be an underlying factor in its greater inhibitory effect on Ca^{2+} transients and, therefore, on tension (fig. 2). It is also known that bupivacaine exerts a cardiodepressant effect *via* inhibition on the SR function (*i.e.*, inhibition of Ca^{2+} release or Ca^{2+} sequestration).[17–19] Here we discuss the possible effects of bupivacaine and ropivacaine on the SR function.

The aequorin method is a powerful technique to determine the time courses of Ca^{2+} transients during twitch (which primarily reflects the SR function) and has

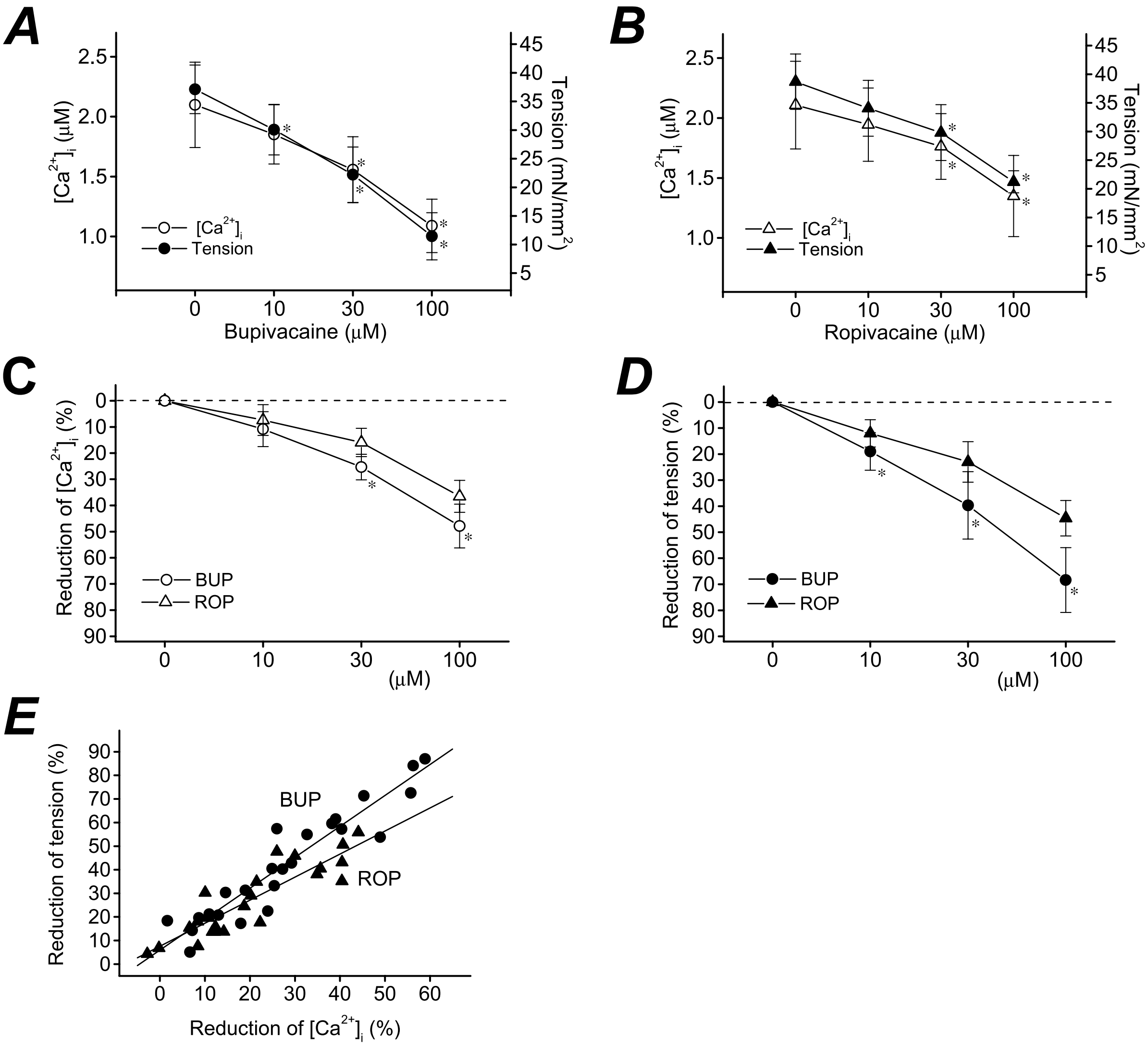

Fig. 2. Concentration-dependent effects of bupivacaine or ropivacaine on peak systolic $[Ca^{2+}]_i$ and tension. (*A*) Effects of bupivacaine. 0 μM = no bupivacaine. *$P < 0.05$ for $[Ca^{2+}]_i$: 0 μM *versus* 30 μM, 0 μM *versus* 100 μM, 10 μM *versus* 100 μM, 30 μM *versus* 100 μM; *$P < 0.05$ for tension: 0 μM *versus* 10 μM, 0 μM *versus* 30 μM, 0 μM *versus* 100 μM, 10 μM *versus* 30 μM, 10 μM *versus* 100 μM, 30 μM *versus* 100 μM; n = 8. (*B*) Effects of ropivacaine. 0 μM = no ropivacaine. *$P < 0.05$ for $[Ca^{2+}]_i$: 0 μM *versus* 30 μM, 0 μM *versus* 100 μM, 10 μM *versus* 100 μM; *$P < 0.05$ for tension: 0 μM *versus* 30 μM, 0 μM *versus* 100 μM, 10 μM *versus* 100 μM, 30 μM *versus* 100 μM; n = 8. (*C*) Comparison of the inhibitory effects of bupivacaine and ropivacaine on peak systolic $[Ca^{2+}]_i$. Data are taken from (*A*) and (*B*) and shown as percentage changes in $[Ca^{2+}]_i$ (normalized with respect to control). *BUP* = bupivacaine; *ROP* = ropivacaine. $P < 0.05$ compared with the data for ROP. (*D*) Same as in (*C*) for peak systolic tension. *$P < 0.05$ compared with the data for ROP. (*E*) Relations between the reduction of tension and that of $[Ca^{2+}]_i$ with bupivacaine and ropivacaine. Data obtained in (*C*) and (*D*) are used. *BUP (circles)*, Y = 1.31X + 5.95 (R = 0.94, $P < 0.01$); *ROP (triangles)*, Y = 0.99 X + 6.70 (R = 0.89, $P < 0.01$). The slopes for BUP and ROP are significantly different ($P < 0.05$).

been widely used to investigate pharmacological actions of various drugs, including anesthetics.[31,32] In the current study, time to peak light was prolonged with bupivacaine or ropivacaine and the effect was statistically significant with 100 μM bupivacaine (table 1). This suggests that the local anesthetics, especially bupivacaine, may directly inhibit the SR Ca^{2+} release channels,[18] resulting in slowing of the rise in $[Ca^{2+}]_i$ upon twitch. Also, both bupivacaine and ropivacaine prolonged DT, and the effect was statistically significant with 100 μM bupivacaine. It is well established that DT is under the strong influence of Ca^{2+} removal by the SR Ca^{2+} pump. Takahashi *et al.*[19] reported that bupivacaine reduces Ca^{2+} uptake by the SR Ca^{2+} pump in rabbit masseter muscle. Although higher concentrations were used on different preparations in their study, it is reasonable to assume that the prolonged DT observed with bupivacaine or ropivacaine in the current experiments results from inhibition of the SR Ca^{2+} pump. Further studies are needed to establish the effects of bupivacaine and ropivacaine on the SR function and the resultant changes in the time courses of Ca^{2+} transients; however, considering the significant effects of bupivacaine on time to peak light and DT, it is reasonable to conclude that the overall

Table 1. Summary of the Effects of Bupivacaine and Ropivacaine on Time Courses of Ca^{2+} Transients and Tension

	TPL (ms)		TPT (ms)		DT (ms)		RT (ms)	
	BUP	ROP	BUP	ROP	BUP	ROP	BUP	ROP
Control	38 ± 5.0	37 ± 3.8	188 ± 12	189 ± 16	44 ± 2.2	46 ± 3.4	109 ± 17	116 ± 16
10 μM	40 ± 4.1	37 ± 4.7	188 ± 16	186 ± 18	44 ± 3.7	46 ± 5.1	109 ± 14	113 ± 15
30 μM	41 ± 6.2	38 ± 3.5	188 ± 16	185 ± 20	47 ± 4.8	47 ± 3.8	110 ± 11	113 ± 13
100 μM	46 ± 5.1*	40 ± 3.2	181 ± 12	183 ± 21	52 ± 5.6*	49 ± 3.9	105 ± 10	112 ± 13

Values are mean ± SD.

BUP = bupivacaine; Control = no bupivacaine or ropivacaine; DT = the time for aequorin light to decay from 75 to 25% of the peak; ROP = ropivacaine; RT = the time for tension to decrease from the peak to 50%; TPL = the time for aequorin light to reach its peak from the onset of stimulus; TPT = the time for tension measured from the onset of stimulus to the peak.

* $P < 0.05$ compared with "Control"; n = 8.

effects on the SR function may be substantially greater with bupivacaine than with ropivacaine.

Our regression analysis revealed that there exists a linear relationship between the percentage reduction of peak systolic $[Ca^{2+}]_i$ and that of tension for both bupivacaine and ropivacaine, and that the slope of the linear regression line was significantly steeper for bupivacaine (fig. 2E). We previously found in ferret ventricular muscle that when the extracellular Ca^{2+} concentration was decreased to decrease systolic $[Ca^{2+}]_i$ ($[Ca^{2+}]_i$ varied between ≈1.5 and ≈2.5 μM) the slope of the linear regression line became ≈1.0 (data analyzed from Komukai *et al.*[21]). Therefore, it is reasonable to assume that the cardiodepressant effect of an anesthetic predominantly results from inhibition of intracellular Ca^{2+} regulation when the value of the linear regression line is 1.0, whereas the effect involves inhibition of myofibrillar activation (*i.e.*, the reaction beyond Ca^{2+} regulation), as well as that of Ca^{2+} transients, when the value is greater than 1.0. Recently, we reported that bupivacaine markedly reduces myofibrillar activation *via* inhibition on the actomyosin interaction in rat ventricular muscle.[20] The expression profiles of contractile proteins may vary in rat and ferret (*e.g.*, V1 and V3 myosin heavy chains are expressed in rat and ferret, respectively);[33,34] however, it is likely that bupivacaine suppresses actomyosin interaction in ferret ventricular muscle as well. Here, care should be taken in the interpretation of figure 2E, as the slope of the linear relationship is an approximate measurement of Ca^{2+} sensitivity of tension, which may be affected, to some extent, in a condition where the rise of $[Ca^{2+}]_i$ is vastly altered. Further studies are awaited to establish the Ca^{2+} sensitivity measurement with isolated cardiac preparations during physiologic twitch where the effects of bupivacaine and ropivacaine can be examined in a more systematic manner.

BAY K 8644 abolished the inhibitory effects of ropivacaine on Ca^{2+} transients and tension, but its effect was partial for bupivacaine, particularly on tension (fig. 3). Therefore, as reported by Hirota *et al.*,[35] the blocking effect of bupivacaine on sarcolemmal Ca^{2+} channels in rat cerebrocortical membranes may be more pronounced than that of ropivacaine in ferret ventricular muscle as well. It is also possible that the reduction of Ca^{2+} entry through sarcolemmal Ca^{2+} channels *via* inhibition on Na^+ channels is more pronounced with bupivacaine than ropivacaine as a result of bupivacaine's stronger blocking effect on Na^+ channels.[15] In any case, the current work suggests that the inhibitory effect of bupivacaine on Ca^{2+} transients *via* reduction of Ca^{2+}

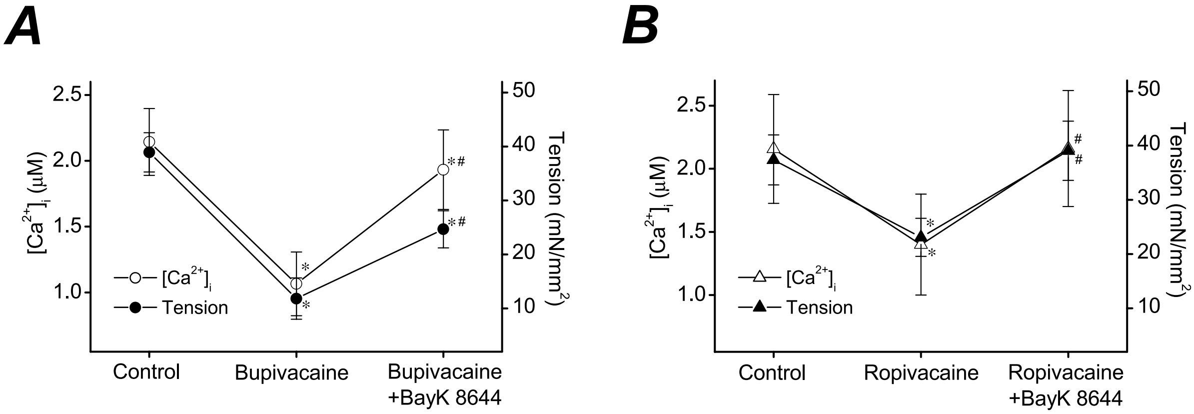

Fig. 3. Effects of 0.6 μM BAY K 8644 on decreases in peak systolic $[Ca^{2+}]_i$ and tension induced by 100 μM bupivacaine (*A*) or ropivacaine (*B*). *Control* = in the absence of an anesthetic and BAY K 8644. (*A*) Effects of BAY K 8644 on bupivacaine-induced changes in $[Ca^{2+}]_i$ and tension. *$P < 0.05$ compared with control; #$P < 0.05$ compared with bupivacaine; n = 6. (*B*) Effects of BAY K 8644 on ropivacaine-induced changes in $[Ca^{2+}]_i$ and tension. *$P < 0.05$ compared with control; #$P < 0.05$ compared with ropivacaine; n = 6.

entry is more marked than that of ropivacaine. Here, it should be pointed out that the remaining inhibitory effect of bupivacaine (after the addition of BAY K 8644) was clearly greater on tension (≈60%) than on Ca^{2+} transients (≈15%). This appears to support the above notion that, unlike ropivacaine, bupivacaine exerts a cardiodepressant effect *via* inhibition of myofibrillar activation as well as *via* inhibition of Ca^{2+} transients.

Here it is worth noting that local anesthetics such as bupivacaine may modulate contractile function differently in cardiac and skeletal muscle. In fact, Zink *et al.*[36] reported that there are major functional differences in the effects of local anesthetics on the SR function as well as on myofibrillar activation. First, the authors found that bupivacaine shows skeletal muscle toxicity *via* increases in myoplasmic Ca^{2+} concentrations by enhancing Ca^{2+} release from the SR and *via* inhibition of Ca^{2+} uptake by the SR Ca^{2+} pump. In contrast, the current results, as well as the results of others,[17,18] suggest that bupivacaine inhibits Ca^{2+} release as well as Ca^{2+} uptake (table 1). The differences in the effect of local anesthetics on Ca^{2+} release from the SR may be attributable to differential expression profiles of ryanodine receptors in cardiac (type 2 dominant) and skeletal muscle (type 1 dominant).[37] Second, the authors reported that bupivacaine increases Ca^{2+} sensitivity of tension in skeletal muscle, opposite to its effect on cardiac muscle.[20] The molecular mechanisms for this discrepancy are unknown, however, differential expression profiles of contractile proteins that can potentially affect myofibrillar activation (*i.e.*, myosin heavy and light chains, troponin, tropomyosin), and hence resultant differential actions of local anesthetics on myofibrillar activation may be involved. Clearly, further studies are needed to clarify the differences in the local anesthetic actions on Ca^{2+} release from the SR and on myofibrillar activation in cardiac and skeletal muscle.

Graf *et al.*[38] reported that bupivacaine at lower concentrations (*i.e.*, 1–5 μM) than those used in the current study (10 μM and higher) significantly depresses cardiac contractions in guinea pig whole heart. As discussed above, bupivacaine shows similar potencies on isolated cardiac muscle preparations in guinea pig and ferret; species difference unlikely accounts for the difference in the depressant effect of bupivacaine. Instead, we consider that accumulation of bupivacaine inside cardiomyocytes *via* coronary vessels may at least in part be involved in its greater effect reported by Graf *et al.*[38] If this is the case, the current experiments, conducted with isolated muscle preparations, may underestimate the cardiodepressant effect of bupivacaine and, probably, of ropivacaine compared with the isolated heart and *in vivo* situations. Nevertheless, considering the fact that at the upper range of clinically relevant concentrations (*i.e.*, 10 μM), the cardiodepression of ropivacaine is significantly less than that of bupivacaine (fig. 2D) and that similar doses are used for both compounds in the clinical settings[10,11,39] (*i.e.*, plasma concentration, ≈3-≈10 μM),[40,41] ropivacaine may exert less of a cardiodepressant effect in humans and may be less likely to cause cardiac arrest on accidental rapid intravenous injection; hence it may have a favorable profile as a local anesthetic.

In conclusion, the cardiodepressant effect of bupivacaine is approximately twofold greater than that of ropivacaine in ferret ventricular muscle. This is because bupivacaine exerts more marked inhibitory effects on Ca^{2+} transients and reduces myofibrillar activation. The greater inhibitory effects on Ca^{2+} transients of bupivacaine may result from greater inhibition of the SR function, as well as from stronger blocking effects on sarcolemmal Na^{+} or Ca^{2+} channels. These results provide evidence that ropivacaine may possess a more favorable profile than bupivacaine as a local anesthetic in the clinical settings.

The authors thank Masato Konishi, M.D., Ph.D., Professor, Department of Physiology, Tokyo Medical University, Tokyo, Japan for critical reading of the manuscript and Naoko Tomizawa, Technician, Department of Physiology (II), The Jikei University School of Medicine, Tokyo, Japan for technical assistance.

References

1. Tanz RD, Heskett T, Loehning RW, Fairfax CA: Comparative cardiotoxicity of bupivacaine and lidocaine in the isolated perfused mammalian heart. Anesth Analg 1984; 63:549–56
2. Buffington CW: The magnitude and duration of direct myocardial depression following intracoronary local anesthetics: A comparison of lidocaine and bupivacaine. Anesthesiology 1989; 70:280–7
3. Herzig S, Ruhnke L, Wulf H: Functional interaction between local anaesthetics and calcium antagonists in guineapig myocardium: 1. Cardiodepressant effects in isolated organs. Br J Anaesth 1994; 73:357–63
4. Wheeler DM, Bradley EL, Woods WT Jr: The electrophysiologic actions of lidocaine and bupivacaine in the isolated, perfused canine heart. Anesthesiology 1988; 68:201–12
5. de la Coussaye JE, Brugada J, Allessie MA: Electrophysiologic and arrhythmogenic effects of bupivacaine: A study with high-resolution ventricular epicardial mapping in rabbit hearts. Anesthesiology 1992; 77:132–41
6. Albright GA: Cardiac arrest following regional anesthesia with etidocaine or bupivacaine. Anesthesiology 1979; 51:285–7
7. Morrison SG, Dominguez JJ, Frascarolo P, Reiz S: A comparison of the electrocardiographic cardiotoxic effects of racemic bupivacaine, levobupivacaine, and ropivacaine in anesthetized swine. Anesth Analg 2000; 90:1308–14
8. Mather LE, Chang DH: Cardiotoxicity with modern local anaesthetics: Is there a safer choice? Drugs 2001; 61:333–42
9. Bader AM, Datta S, Flanagan H, Covino BG Comparison of bupivacaine- and ropivacaine-induced conduction blockade in the isolated rabbit vagus nerve. Anesth Analg 1989; 68:724–7
10. Zaric D, Nydahl PA, Philipson L, Samuelsson L, Heierson A, Axelsson K: The effect of continuous lumbar epidural infusion of ropivacaine (0.1%, 0.2%, and 0.3%) and 0.25% bupivacaine on sensory and motor block in volunteers: A double-blind study. Reg Anesth 1996; 21:14–25
11. Casati A, Fanelli G, Magistris L, Beccaria P, Berti M, Torri G: Minimum local anesthetic volume blocking the femoral nerve in 50% of cases: A double-blinded comparison between 0.5% ropivacaine and 0.5% bupivacaine. Anesth Analg 2001; 92:205–8
12. McClure JH: Ropivacaine. Br J Anaesth 1996; 76:300–7
13. Lefrant JY, de La Coussaye JE, Ripart J, Muller L, Lalourcey L, Peray PA, Mazoit X, Sassine A, Eledjam JJ: The comparative electrophysiologic and hemodynamic effects of a large dose of ropivacaine and bupivacaine in anesthetized and ventilated piglets. Anesth Analg 2001; 93:1598–605
14. Aya AG, de la Coussaye JE, Robert E, Ripart J, Cuvillon P, Mazoit JX, Jeannes P, Fabbro-Peray P, Eledjam JJ: Comparison of the effects of racemic bupivacaine, levobupivacaine, and ropivacaine on ventricular conduction, refractoriness, and wavelength: An epicardial mapping study. Anesthesiology 2002; 96:642–50
15. Arlock P: Actions of three local anaesthetics: lidocaine, bupivacaine and ropivacaine on guinea pig papillary muscle sodium channels (Vmax). Pharmacol Toxicol 1988; 63:96–104

16. 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

17. Lynch C III: Depression of myocardial contractility in vitro by bupivacaine, etidocaine, and lidocaine. Anesth Analg 1986; 65:551–9

18. Komai H, Lokuta AJ: Interaction of bupivacaine and tetracaine with the sarcoplasmic reticulum Ca^{2+} release channel of skeletal and cardiac muscles. Anesthesiology 1999; 90:835–43

19. Takahashi S: Local anaesthetic bupivacaine alters function of sarcoplasmic reticulum and sarcolemmal vesicles from rabbit masseter muscle. Pharmacol Toxicol 1994; 75:119–28

20. Mio Y, Fukuda N, Kusakari Y, Tanifuji Y, Kurihara S: Bupivacaine attenuates contractility by decreasing sensitivity of myofilaments to Ca^{2+} in rat ventricular muscle. Anesthesiology 2002; 97:1168–77

21. Komukai K, Kurihara S: Effect of developed tension on the time courses of Ca^{2+} transients and tension in twitch contraction in ferret myocardium. Pflügers Arch 1996; 32:384–90

22. Komukai K, Ishikawa T, Kurihara S: Effects of acidosis on Ca^{2+} sensitivity of contractile elements in intact ferret myocardium. Am J Physiol 1998; 274: H147–54

23. Ishikawa T, Kajiwara H, Kurihara S: Modulation of Ca^{2+} transient decay by tension and Ca^{2+} removal in hyperthyroid myocardium. Am J Physiol 1999; 276:H289–99

24. Allen DG, Blinks JR, Prendergast FG: Aequorin luminescence: Relation of light emission to calcium concentration—a calcium-independent component. Science 1977; 195:996–8

25. Okazaki O, Suda N, Hongo K, Konishi M, Kurihara S: Modulation of Ca^{2+} transients and contractile properties by beta-adrenoceptor stimulation in ferret ventricular muscles. J Physiol (Lond) 1990; 423:221–40

26. Schramm M, Thomas G, Towart R, Franckowiak G: Novel dihydropyridines with positive inotropic action through activation of Ca^{2+} channels. Nature 1983; 303:535–7

27. 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

28. Wulf H, Gödicke J, Herzig S: Functional interaction between local anaesthetics and calcium antagonists in guinea pig myocardium: 2. Electrophysiological studies with bupivacaine and nifedipine. Br J Anaesth 1994; 73:364–70

29. Valenzuela C, Snyders DJ, Bennett PB, Tamargo J, Hondeghem LM: Stereoselective block of cardiac sodium channels by bupivacaine in guinea pig ventricular myocytes. Circulation 1995; 92:3014–24

30. Rossner KL, Freese KJ: Bupivacaine inhibition of L-type calcium current in ventricular cardiomyocytes of hamster. Anesthesiology 1997; 87:926–34

31. Bartunek AE, Housmans PR: Effects of sevoflurane on the intracellular Ca^{2+} transient in ferret cardiac muscle. Anesthesiology 2000; 93:1500–8

32. Hannon JD, Cody MJ, Housmans PR: Effects of isoflurane on intracellular calcium and myocardial crossbridge kinetics in tetanized papillary muscles. Anesthesiology 2001; 94:856–61

33. Takeda N, Nakamura I, Hatanaka T, Ohkubo T, Nagano M: Myocardial mechanical and myosin isoenzyme alterations in streptozotocin-diabetic rats. Jpn Heart J 1988; 29:455–63

34. MacKinnon R, Gwathmey JK, Allen PD, Briggs GM, Morgan JP: Modulation by the thyroid state of intracellular calcium and contractility in ferret ventricular muscle. Circ Res 1988; 63:1080–9

35. Hirota K, Browne T, Appadu BL, Lambert DG: Do local anaesthetics interact with dihydropyridine binding sites on neuronal L-type Ca^{2+} channels? Br J Anaesth 1997; 78:185–8

36. Zink W, Graf BM, Sinner B, Martin E, Fink RH, Kunst G: Differential effects of bupivacaine on intracellular Ca^{2+} regulation: Potential mechanisms of its myotoxicity. Anesthesiology 2002; 97:710–6

37. Sutko JL, Airey JA: Ryanodine receptor Ca^{2+} release channels: Does diversity in form equal diversity in function? Physiol Rev 1996; 76:1027–71

38. Graf BM, Abraham I, Eberbach N, Kunst G, Stowe DF, Martin E: Differences in cardiotoxicity of bupivacaine and ropivacaine are the result of physicochemical and stereoselective properties. Anesthesiology 2002; 96:1427–34

39. Owen MD, Thomas JA, Smith T, Harris LC, D'Angelo R: Ropivacaine 0.075% and bupivacaine 0.075% with fentanyl 2 microg/mL are equivalent for labor epidural analgesia. Anesth Analg 2002; 94:179–83

40. Dauphin A, Gupta RN, Young JE, Morton WD: Serum bupivacaine concentrations during continuous extrapleural infusion. Can J Anaesth 1997; 44: 367–70

41. Erichsen CJ, Sjövall J, Kehlet H, Hedlund C, Arvidsson T: Pharmacokinetics and analgesic effect of ropivacaine during continuous epidural infusion for postoperative pain relief. Anesthesiology 1996; 84:834–42