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Effect of bupivacaine enantiomers on Ca^{2+} release from sarcoplasmic reticulum in skeletal muscle

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Abstract

Local anesthetics affect intracellular Ca^{2+} movement in the myocyte. The use of isomers may help to reveal specific mechanisms of action, such as receptor mediation. In the present study, we used skinned fibers from mammalian skeletal muscle to test whether bupivacaine enantiomers had different effects on Ca^{2+} release and uptake by the sarcoplasmic reticulum, and on the Ca^{2+} sensitivity of the contractile system. Ca^{2+}-induced Ca^{2+} release was enhanced by S-bupivacaine 1∼3 mM, but inhibited by R-bupivacaine 3 mM, remaining unaffected at lower doses. These enantiomers inhibited Ca^{2+} uptake to different degrees, with R-bupivacaine having a stronger effect. Ca^{2+} sensitivity of the contractile system was equally enhanced by R- and S-bupivacaine. These findings might help to explain the myoplasmic Ca^{2+} elevation induced by bupivacaine. The observed stereoselectivity suggests effects on specific proteins, the ryanodine Ry_1 receptor and the Ca^{2+}-ATPase pump, rather than non-specific increase in Ca^{2+} permeability.

Keywords: Ca^{2+}-induced Ca^{2+} release; Bupivacaine enantiomers; Stereoselectivity; Ryanodine receptor; Skeletal muscle; Skinned fiber

1. Introduction

Local anesthetics have diverse effects on Ca^{2+}-related functions in myocytes determined by their chemical properties and by assay conditions (Volpe et al., 1983). Specifically, these agents are known to affect Ca^{2+} release and uptake by the sarcoplasmic reticulum, as well as the sensitivity of the contractile system to Ca^{2+} (Endo, 1977).

Of the local anesthetics, which induce reversible skeletal muscle damage by increasing the concentration of cytosolic Ca^{2+}, bupivacaine has the strongest myotoxic effect (Nonaka et al., 1983; Hogan et al., 1994; Irwin et al., 2002). In the presence of bupivacaine, mature skeletal muscle fibers undergo necrosis, while myoblasts, which lack Ca^{2+}-sequestrating organelles, remain unaffected (Saito and Nonaka, 1994). This process can be replicated by the application of caffeine (Hogan et al., 1994; Takahashi, 1994). Therefore, a pathologic Ca^{2+} efflux from the sarcoplasmic reticulum has been regarded as a key factor in the pathogenesis of local anesthetic myotoxicity, through specific interactions with the Ca^{2+} release channel-ryanodine Ry_1 receptor combined with a non-specific increase in the Ca^{2+} permeability of the sarcoplasmic reticulum membrane.

Komai and Lokuta (1999) demonstrated that bupivacaine exerts a biphasic effect on the Ca^{2+}-release channel ryanodine Ry_1 receptor in skeletal muscle microsomes, enhancing ^{3}H-ryanodine binding at 5 mM and inhibiting it at 10 mM. Recently, Zink et al. (2002) found that bupivacaine not only affects Ca^{2+} release from the sarcoplasmic reticulum, but also inhibits its reuptake, which is mainly regulated by the activity of the Ca^{2+}-ATPase pump located in the sarcoplasmic reticulum membrane.

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In the aforementioned studies, bupivacaine was used in the form of 1:1 racemic mixture of the levorotatory S- and dextrorotatory R-optically active enantiomers. Recently, a solution of the pure S-isomer (levobupivacaine) has been developed for clinical use, which demonstrates apparently similar potency for sensory nerve block, but reduced cardiovascular and central nervous system toxicity (Vladimirov et al., 2000; Foster and Markham, 2000). In addition to the clinical advantages promised by their potential ability to decrease the incidence of adverse effects, single enantiomers of chiral anesthetic drugs are attracting the interest of researchers as a means to study specific receptor-mediated responses. Although stereospecific effects of bupivacaine on the atrioventricular conduction have been described (Graf et al., 1997; Gonzalez et al., 2002), there have been no reports to date on the effects of single bupivacaine enantiomers on Ca^{2+}-related functions in skeletal muscle. The present study, using skinned fibers from guinea pig skeletal muscle, was conducted to investigate the effects of S- and R-bupivacaine on Ca^{2+}-induced Ca^{2+} release from sarcoplasmic reticulum, Ca^{2+} uptake into sarcoplasmic reticulum and the Ca^{2+} sensitivity of the contractile system.

2. Materials and methods

2.1. Biopsies and skinned fibers preparation

These experiments were conducted in accordance with the Experimental Animals Regulations set forth in the Japanese Law Concerning the Protection and Control of Animals (Law No.105, October 1, 1973) and the Animal Care and Use Guidelines of the Experimental Animal Center of Toho University. Hartley strain male guinea pigs (~500 g) used in the experiments were killed by cervical dislocation. Following sacrifice, the fast twitch muscle extensor digitorum longus was dissected and immediately immersed in a relaxing solution (Table 1). Excess connective tissue was removed manually under a light stereomicroscope. In the chemical skinning process, the entire specimen was soaked for 30 min in a relaxing solution containing 50 μg/ml saponin, which acts to preserve the sarcoplasmic reticulum membrane (Endo and Iino, 1980). Fibers in which the Ca^{2+} sensitivity of the contractile system was to be tested, were soaked in a more concentrated skinning solution (200 μg/ml), which disrupted the sarcoplasmic reticulum membrane. This strategy eliminates the possibility of Ca^{2+} release from intracellular storage sources, thereby ensuring that muscle contraction occurs solely in response to Ca^{2+} added from external sources (i.e., experimental solutions; Endo and Iino, 1980). Individual skinned muscle fibers of 2~3 mm-length were manually isolated under a light stereomicroscope.

2.2. Experimental setup

The ends of a single skinned fiber were tied with silk thread to tungsten needles (Fig. 1). One needle was glued to a strain-gauge transducer (AE-801 Transducer Element; Aksjeselskapet Mikro-Elektrinokk, Norway) and the other to a manipulator, which was equipped with an assembly of micrometric gears and screws used to adjust the fiber's position and its resting tension. The fiber was stretched to approximately 1.2 times its resting length. The experimental solutions were poured into 15 bare aluminum wells of 0.2 ml each engraved on a plate. The transducer's signal was stabilized using a Wheatstone-bridge circuit, and amplified through a strain meter (DSA-606 B Strain Amp; NMB Minebea, Japan). The tension/time curve of isometric contraction was plotted by a pen recorder on ruled paper (U-228 Pantos Unicorder; Nippon Denshi Kagaku, Japan).

2.3. Experimental solutions and chemicals

The variation in composition of the experimental solutions through the experiments is depicted schematically in Fig. 1 and Table 1. In all solutions, pH was buffered by 20 mM piperazine-*N*-*N*'-bis (2-ethanesulfonic acid) (PIPES) and brought to 7.0 at 20 °C by titration of KOH. Ionic strength was maintained at 0.22 M by adjusting the concentration of potassium methanesulfonate. The solutions used for Ca^{2+} loading and for activation of Ca^{2+}-induced Ca^{2+} release contained 10 mM ethylene glycol-bis (β-aminoethyl ether)-*N*,*N*,*N*',*N*'-tetraacetic acid (EGTA). The desired free Ca^{2+} concentration was achieved by adding calcium methanesulfonate, assuming a Ca-EGTA apparent association constant of 5×10^5 M^{-1} (Harafuji and Ogawa, 1980). The Mg-ATP stability constant was assumed to be 10^4 M^{-1} (Kikuchi et al., 1987). KOH, methanesulfonic acid, MgO, $CaCO^+$, EGTA and saponin were purchased from Wako Pure Chemical Industries (Tokyo, Japan); procaine and anhydrous caffeine from Sigma Aldrich (St. Louis Missouri, USA); PIPES from Merck GmbH (Haar, Germany) and $ATPNa_2$ from Roche Diagnostics GmbH (Mannheim, Germany). S- and R-bupivacaine were generously donated by Maruishi Pharmaceutical (Tokyo, Japan).

2.4. Ca^{2+}-induced Ca^{2+} release experiment (Fig. 1; Table 1A)

The rate of Ca^{2+}-induced Ca^{2+} release was determined by methods previously described by Endo (1977) and Ohta et al. (1989). Briefly, the sarcoplasmic reticulum was loaded with a given amount of Ca^{2+} by incubating the fibers in a 2×10^{-7} M Ca^{2+}-buffered solution for 2 min. Ca^{2+}-induced Ca^{2+} release was stimulated by exposing the preloaded fibers to increasing concentrations of Ca^{2+} for specific periods (Table 1B). Thereafter, the amount of Ca^{2+} remaining in the sarcoplasmic reticulum was determined

Table 1
Sequence of the experiments and composition of the solutions

A

Step	Name	Purpose	Time (s)	Mg^{2+}	ATP-$MgSO_4$	Ca^{2+}	EGTA	Others
1	G_2	Relaxation	30	1.5	3.5		2	
2	Loading	SR[a]Ca^{2+} uptake	120	1.5	3.5	2×10^{-7}	10	
3	G_{10}	Ca^{2+} wash out, uptake stop	30	1.5	3.5		10	
4	G_2r[b]	ATP wash out	90	1.5			2	
5	G_2r Mgφ	Mg^{2+} wash out	60				2	
6	CICR	Ca^{2+} release stimulation	*t*	0.15		Table 1B	10	
7	Stop	Ca^{2+} release stop	30	10			10	Procaine 10
8	G_2	ATP restoration	30	1.5	3.5		2	
9	Pre-assay	Pre-challenge conditioning	30	1.5	3.5		0.1	Procaine 5
10	Assay	SR Ca^{2+} depletion	∞	0.1	1		0.1	Caffeine 50

B

Activating solution	Ca^{2+} (M)	*t*(s)
G10*r* Mg^{2+} 0.15	0	120
pCa^{2+} 6.5	3×10^{-7}	120
pCa^{2+} 6.0	10^{-6}	90
pCa^{2+} 5.5	3×10^{-6}	30
pCa^{2+} 5.0	10^{-5}	20

(A) Solutions used for Ca^{2+}-induce Ca^{2+} release and Ca^{2+} uptake experiments. The complete sequence was performed to evaluate Ca^{2+}-induce Ca^{2+} release, whereas steps 4–7 were bypassed in Ca^{2+} uptake experiment. Concentrations are expressed in mM. (B) Composition of solutions used for triggering the Ca^{2+}-induced Ca^{2+} release with their specific stimulation times. All activating solutions contained 0.15 mM Mg^{2+}.

[a] Sarcoplasmic reticulum.

[b] "*r*" Refers to rigor that is produced in the absence of ATP (see Fig. 1).

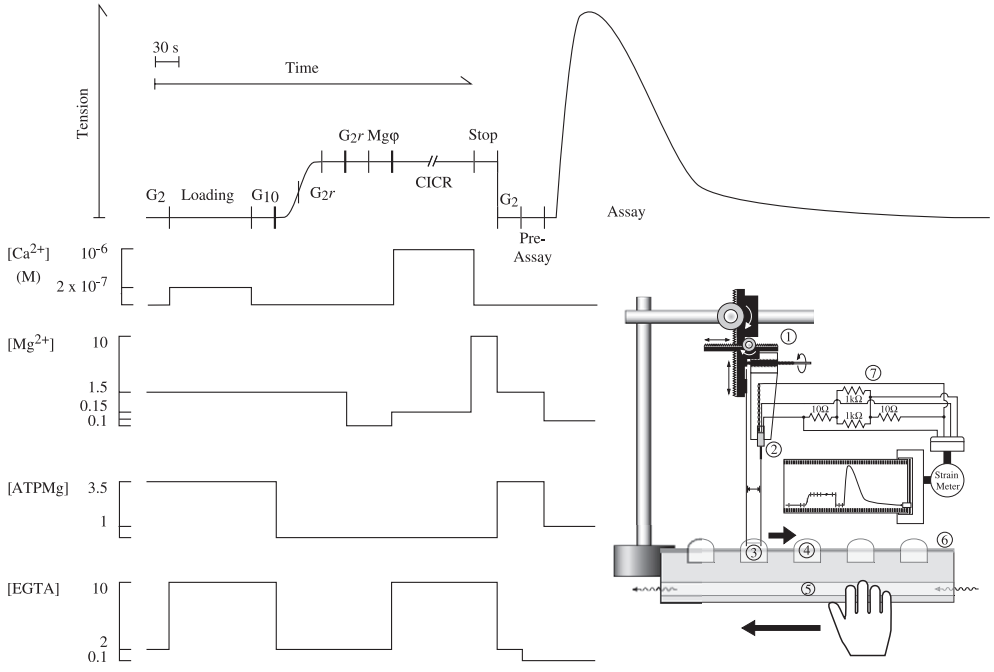

Fig. 1. Modified Endo's technique for determination of Ca^{2+}-induced Ca^{2+} release rate. The upper part of the figure represents tension recordings from the Ca^{2+}-induced Ca^{2+} release experiment. The composition of solutions used in each step is schematically depicted at bottom left. With the exception of [Ca^{2+}], all concentrations are expressed in mM. Rigor occurs in the absence of ATP. After restoring ATP, muscle contraction was elicited by 50 mM caffeine challenge, which depletes the sarcoplasmic reticulum Ca^{2+} content. Paper speed was set to maximum on the pen recorder during the assay phase, in order to integrate the tension/time curve with greater accuracy. A scheme of the experimental setup is shown at the bottom right: 1, manipulator; 2, transducer; 3, skinned fiber; 4, experimental solutions; 5, circulating water at 20 °C; 6, silicone gel layer; 7, Wheatstone bridge. The plate was spread with silicone jelly to increase surface tension, causing the solution's menisci to protrude above the surface, making it possible to transfer the skinned fiber across the set of solutions by simply swift-sliding the plate.

after a 50 mM caffeine challenge by measuring the resulting contraction of the skinned fiber, considering that the tension/time integral of the caffeine-induced contracture is proportional to the amount of released Ca^{2+}. Preparations of either S-bupivacaine or R-bupivacaine 1~3 mM were added only to the Ca^{2+}-release activating solutions. A Ca^{2+}-free relaxing solution was applied for 120 s instead of giving the Ca^{2+} release stimulation to evaluate Ca^{2+} leakage through the sarcoplasmic reticulum membrane.

2.5. Calculation of Ca^{2+}-induced Ca^{2+} release rate

Five Ca^{2+}-induced Ca^{2+} release runs were inserted between control runs in which Ca^{2+} release was omitted. The tension/time integral of the caffeine-induced contracture of each run was normalized to interpolated values from those of the control runs, linear run down being assumed. The normalized tension/time integral (S) decreased exponentially as the stimulation time increased (data not shown), as described before (Ohta et al., 1989). A standard stimulation time t for each activating solution was chosen (Table 1B) to give that the elicited contracture after Ca^{2+}-induced Ca^{2+} release stimulation was 30~70% that of control runs. The Ca^{2+}-induced Ca^{2+} release rate was calculated as $E=-(\ln S)/t$, since Ca^{2+} release follows first-order kinetics. To avoid the possibility of variance in results due to differences in individual tissue samples, the Ca^{2+} release test was performed on the same skinned fiber for each releasing solution, so the fiber served as its own control.

2.6. Ca^{2+} uptake experiment

The Ca^{2+} uptake experiment was done by simply incubating the skinned fibers in various concentrations of Ca^{2+} for various periods, and the amount of loaded Ca^{2+} was determined by the caffeine challenge. The relative amount of loaded Ca^{2+} was expressed as the ratio to the standard loading ($[Ca^{2+}]=2\times10^{-7}$ M for 120 s), that is known as a submaximal loading of the sarcoplasmic reticulum (Ohta et al., 1989). R- and S-bupivacaine 1 mM were added separately to the loading solutions to determine their effects on Ca^{2+} uptake by the sarcoplasmic reticulum.

2.7. Ca^{2+} sensitivity of the contractile system

After skinning with 200 μg/ml saponin to destroy the membrane of the sarcoplasmic reticulum, muscle contraction was elicited by transferring the preparation iteratively from the relaxing medium to solutions at increasing Ca^{2+} concentrations (3×10^{-5}~10^{-7} M) containing 0.15 mM Mg^{2+}, 3.5 mM ATP-Mg and 10 mM EGTA. The fiber was returned to the relaxing solution after peak tension was reached. The relative peak tension values for each Ca^{2+} solution were calculated. S- and R-bupivacaine 1 mM were added to separate Ca^{2+}-containing solutions to evaluate their effects on the Ca^{2+} sensitivity of the contractile system.

2.8. Statistical analysis

Statistical significance was assessed by multi-way analysis of variance. Sample size for each group varied from $n=5$~8. Least significant difference, Tukey's and Newmann–Keuls' tests were performed for post-hoc multiple comparisons using the harmonic mean for groups of different size (Hassard, 1991; Lane, 2003). In most contexts, statistical significance was defined as $P<0.05$; otherwise $P<0.01$, when specified. All results are expressed as mean ± S.E.M.

3. Results

3.1. Ca^{2+}-induced Ca^{2+} release from sarcoplasmic reticulum

Ca^{2+}-induced Ca^{2+} release rate was significantly faster in the presence of 3 mM S-bupivacaine than under the control condition (Fig. 2). At that drug concentration, Ca^{2+}-induced Ca^{2+} release rate increased from 1.8 ± 0.3, 4.9 ± 0.4, 11 ± 0.7 and 24.7 ± 4.01 $s^{-1}\times10^{-3}$ ($n=7$) to 7.2 ± 0.3, 19.2 ± 0.8, 35.02 ± 1.9, 43.1 ± 2.8 $s^{-1}\times10^{-3}$ ($n=6$), respectively ($P<0.01$). The magnitude of the enhancement was dependent on both the drug dosage and the Ca^{2+} concentration used to stimulate Ca^{2+} release. No significant inhibition of Ca^{2+}-induced Ca^{2+} release could be detected when 1 mM S-bupivacaine was administered at $[Ca^{2+}]=3\times10^{-7}$ mM (3.6 ± 0.4 $s^{-1}\times10^{-3}$; $P>0.05$).

In contrast, $[Ca^{2+}]$-dependent inhibition of Ca^{2+}-induced Ca^{2+} release was observed on the application of R-bupivacaine; however, this effect was evident only at high Ca^{2+} concentrations ($>3\times10^{-6}$ M). No significant Ca^{2+} leakage through the sarcoplasmic reticulum membrane was detected, either in the presence or in the absence of drug.

3.2. Ca^{2+} uptake into sarcoplasmic reticulum

The time course of Ca^{2+} uptake into the sarcoplasmic reticulum was evaluated using loading solutions of various Ca^{2+} concentrations in the absence of drug, and in solutions containing either 1 mM R- or S-bupivacaine (Fig. 3). Both enantiomers inhibited Ca^{2+} uptake by the sarcoplasmic reticulum. This effect was also stereoselective, in the sense that the inhibition of Ca^{2+} uptake was significantly more pronounced for R-bupivacaine than for S-bupivacaine when loading with 3.1×10^{-8} M Ca^{2+} for 120 s (0.09 ± 0.02 versus 0.24 ± 0.03; $n=6$, $P<0.01$), and with 2×10^{-7} M Ca^{2+} for 15 and 30 s (0.32 ± 0.05 versus 0.56 ± 0.07, and 0.59 ± 0.03 versus 0.79 ± 0.05, respectively; $n=6$, $P<0.01$). When loading was performed with 10^{-6} M

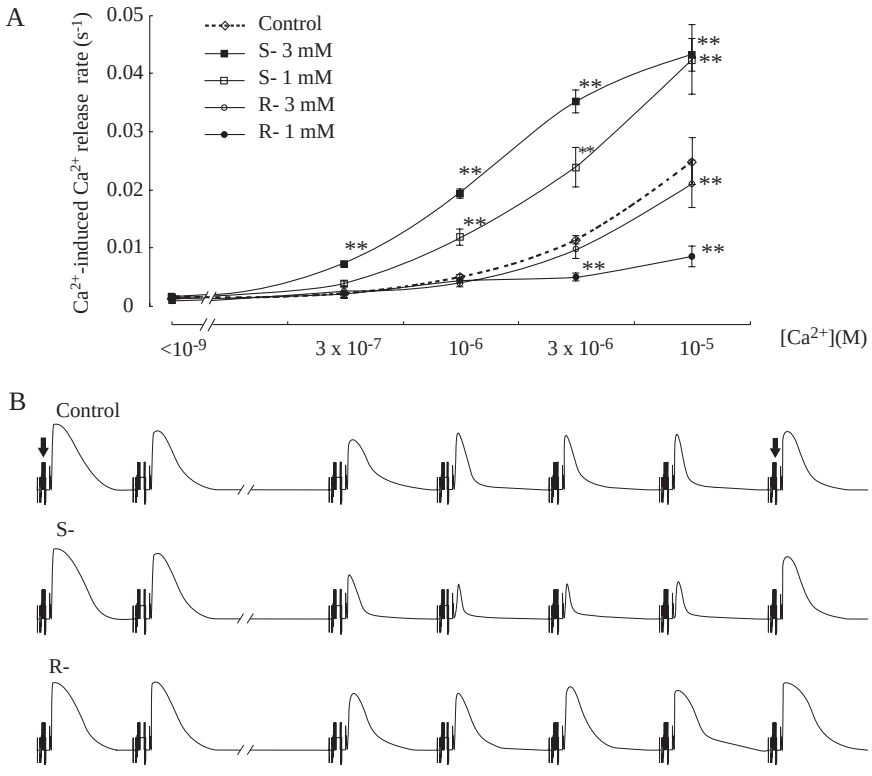

Fig. 2. Distinct effects of R- and S- bupivacaine enantiomers on Ca^{2+}-induced Ca^{2+} release. (A) Symbols and vertical segments represent the mean and S.E.M., respectively. The dashed line corresponds to control curve (i.e., absence of drug). The points at $[Ca^{2+}] < 10^{-9}$ M represent non-specific Ca^{2+} release. $^{*}P<0.05$, $^{**}P<0.01$. (B) Representative tension/time plots were aligned with the abscissas on the upper chart corresponding to Ca^{2+} concentrations at which Ca^{2+} release was stimulated. The enhancement of Ca^{2+}-induced Ca^{2+} release in the presence of S-bupivacaine is registered as a decrease in the area of caffeine-induced contracture (i.e., decrease in the amount of Ca^{2+} remaining in the sarcoplasmic reticulum after the stimulation of Ca^{2+} release). Dark arrows indicate the flanking control runs.

Ca^{2+}, however, the inhibitory effect of R-bupivacaine diminished as the loading time increased, and disappeared at 120 s (1.27 ± 0.09 [$n=6$] versus 1.28 ± 0.09 [$n=7$] for R-bupivacaine and control, respectively; $P>0.05$), while the inhibitory effect of S-bupivacaine persisted irrespective of loading time (1.07 ± 0.01; $n=6$, $P<0.01$). It has been suggested that a high Ca^{2+} concentration may counteract the inhibitory effect of drugs on Ca^{2+} uptake, while Ca^{2+}-induced Ca^{2+} release is simultaneously activated due to the prolonged stimulation time (Ohta et al., 1989). Thus, the persistent decrease in calcium loading in the sarcoplasmic reticulum might be the result of the stimulatory effect of S-bupivacaine on Ca^{2+}-induced Ca^{2+} release, as the highest observed Ca^{2+} load in the sarcoplasmic reticulum was reached in the presence of R-bupivacaine, which has a weaker effect on Ca^{2+}-induced Ca^{2+} release. Consequently, this phenomenon was observed neither in the initial uptake phase nor when loading was performed using low Ca^{2+} concentrations.

3.3. Ca^{2+} sensitivity of the contractile system

When applied at 1 mM, both bupivacaine enantiomers increased the sensitivity of the contractile system to Ca^{2+}, shifting the curve of Ca^{2+} concentration versus relative tension to the left (Fig. 4). Thus, higher tension values were achieved at lower Ca^{2+} concentrations in comparison to the control curve. Peak tension was reached at significantly lower Ca^{2+} concentrations (about 2×10^{-6} M) in the

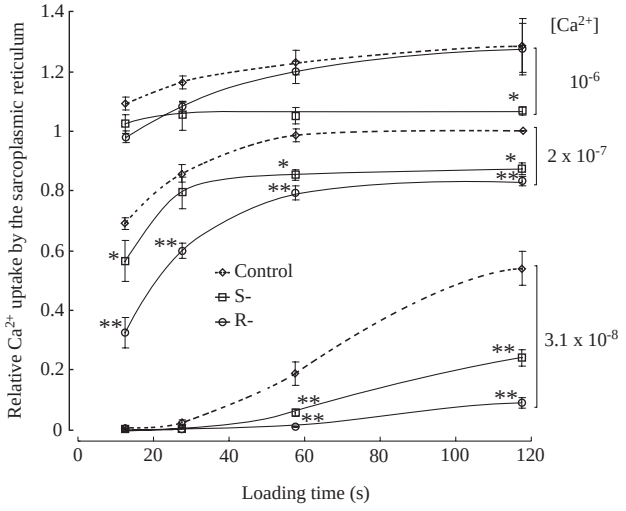

Fig. 3. Time course of Ca^{2+} uptake by the sarcoplasmic reticulum. Bupivacaine enantiomers are identified as R- and S-. Symbols and vertical segments represent the mean and S.E.M., respectively. $^{*}P<0.05$, $^{**}P<0.01$ with respect to control.

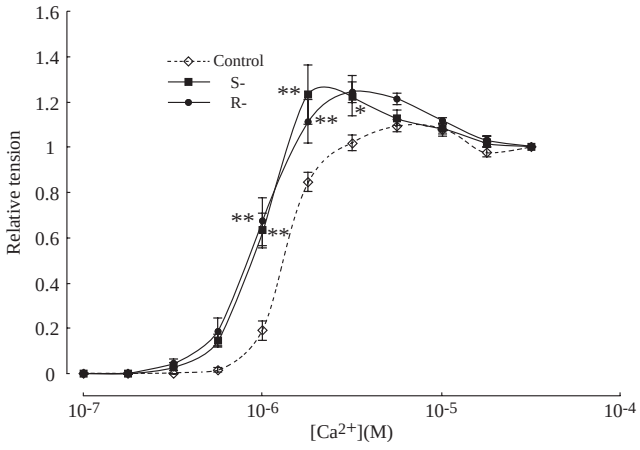

Fig. 4. Effect of bupivacaine enantiomers on the Ca^{2+} sensitivity of the muscle contractile system. Curves for bupivacaine enantiomers are identified as R- and S-. Symbols and vertical segments represent the mean and S.E.M., respectively ($n=6\sim8$). *$P<0.05$, **$P<0.01$ with respect to control.

presence of either of the enantiomers than in the absence of drug (10^{-5} M). There was no significant difference in the relative peak tension values compared to control.

4. Discussion

Studies of stereoselectivity can provide evidences of specific receptor-mediated processes (Nau and Strichartz, 2002; Gibb, 2003). The stereoselective effect of bupivacaine enantiomers on Ca^{2+}-induced Ca^{2+} release demonstrated in our experiment is consistent with the notion that the elevation of myoplasmic Ca^{2+} induced by bupivacaine is mediated by a chirally specific drug-receptor interaction, rather than by a non-specific increase in the membrane permeability of the sarcoplasmic reticulum. Ca^{2+}-induced Ca^{2+} release in skeletal muscle is mediated by the ryanodine Ry_1 receptor (Coronado et al., 1994).

Zink et al. (2002) reported that bupivacaine itself induces Ca^{2+} efflux from the sarcoplasmic reticulum in chemically skinned muscle fibers, and prevents Ca^{2+} reuptake as well, probably through the inhibition of Ca^{2+}-ATPase at the sarcoplasmic reticulum. The authors demonstrated a dose-dependent increase in Ca^{2+} release for concentrations of up to 15 mM, as well as the inhibition of Ca^{2+} reuptake by the sarcoplasmic reticulum, and sensitization of the contractile apparatus to free Ca^{2+}. Although a gated channel was suggested as the most probable target site, their experimental setup did not allow the elucidation of whether the increase in myoplasmic Ca^{2+} concentration was produced by a non-specific increase in the permeability of the sarcoplasmic reticulum to Ca^{2+}, or by the direct action of bupivacaine on the Ca^{2+} release channel. In a case series review, Hogan et al. (1994) considered the possible direct actions of local anesthetics on the ryanodine receptor. Komai and Lokuta (1999) demonstrated that bupivacaine exerts a biphasic effect on the ryanodine Ry_1 receptor in skeletal muscle microsomes, enhancing ^{3}H-ryanodine binding at 5 mM and inhibiting it at 10 mM. Due to the relatively short Ca^{2+} release stimulation periods, the sensitivity of our test was not high enough to detect a significant Ca^{2+} release on the application of bupivacaine enantiomers in the absence of Ca^{2+} (i.e., Ca^{2+} leakage), as reported by Ikai et al. (1984). Nevertheless, we demonstrated strong stereoselectivity in the Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum modulated by bupivacaine enantiomers, which had significantly different effects when applied at the same concentration.

Although the muscle contraction in the Ca^{2+}-induced Ca^{2+} release experiment was elicited in the absence of the enantiomers, which were only applied during the stimulation of Ca^{2+} release, the apparent inhibitory effects on Ca^{2+}-induced Ca^{2+} release (increase in force transients-Fig. 2B) may have resulted from a residual increase in the Ca^{2+} sensitivity of the contractile system. This would mean that the dose-dependent enhancement of Ca^{2+}-induced Ca^{2+} release by S-bupivacaine may actually be more pronounced, as it was inferred from the observed decrease in force transients with respect to control. The same reasoning might also be applied to the interpretation of the reduction of Ca^{2+} uptake in the presence of bupivacaine enantiomers, which was also inferred from a decrease in force transients.

Makabe et al. (1996) showed that competitive inhibition of Ca^{2+}-ATPase increased the magnitude of Ca^{2+} transients after caffeine-induced Ca^{2+} release. Zink et al. (2002) also reported a significant reduction in the decay of caffeine-induced force transients after loading the sarcoplasmic reticulum in the presence of racemic bupivacaine. They attributed this effect to a decrease in Ca^{2+} re-uptake into the sarcoplasmic reticulum, most likely due to inhibition of Ca^{2+}-ATPase. The pattern of force decay in their results was not indicative of permeability changes in the sarcoplasmic reticulum membrane, and a reduction in energy supply due to bupivacaine-induced inhibition of mitochondrial function was ruled out as the source of reduced Ca^{2+} uptake by conducting the experiments with an overshoot of ATP. Using an analogous experimental setup, we evidenced a significant decrease in the caffeine-induced force transients after loading in the presence of bupivacaine enantiomers. This effect was stereoselective, suggesting that bupivacaine affects Ca^{2+} reuptake by inhibiting Ca^{2+}-ATPase (Makabe et al., 1996; Zink et al., 2002), which transports Ca^{2+} from the cytoplasm back into the sarcoplasmic reticulum during relaxation. Takahashi (1994) demonstrated that racemic bupivacaine inhibits Ca^{2+}-ATPase and reduces Ca^{2+} uptake into sarcoplasmic reticulum vesicles. S-bupivacaine is likely to have a stronger influence in the increase of myoplasmic Ca^{2+} than the R-enantiomer, as the former stimulates Ca^{2+}-induced Ca^{2+} release and concomitantly inhibits Ca^{2+} uptake, while the latter seems to antagonize or at least to have a milder effect on Ca^{2+}-induced Ca^{2+} release.

We conclude that, in the sarcoplasmic reticulum of mammal skeletal muscle, the response of Ca^{2+}-induced Ca^{2+} release and of Ca^{2+} uptake to bupivacaine enantiomers is stereoselective, while the increase in the sensitivity to free Ca^{2+} of the muscle contractile system is not.

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