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Differential Effects of Bupivacaine on Intracellular Ca^{2+} Regulation

Potential Mechanisms of Its Myotoxicity

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Background: **Bupivacaine produces skeletal muscle damage in clinical concentrations. It has been suggested that this may be caused by an increased intracellular level of [Ca^{2+}]. Therefore, the aim of this study was to investigate direct intracellular effects of bupivacaine on Ca^{2+} release from the sarcoplasmic reticulum (SR), on Ca^{2+} uptake into the SR, and on Ca^{2+} sensitivity of the contractile proteins.**

Methods: **Saponin skinned muscle fibers from the extensor digitorum longus muscle of BALB/c mice were examined according to a standardized procedure described previously. For the assessment of effects on Ca^{2+} uptake and release from the SR, bupivacaine was added to the loading solution and the release solution, respectively. Force transients and force decays were monitored, and the position of the curve relating relative isometric force *versus* free [Ca^{2+}] was evaluated in the presence or absence of bupivacaine.**

Results: **Bupivacaine induces Ca^{2+} release from the SR. In addition, the Ca^{2+} loading procedure is suppressed, resulting in smaller caffeine-induced force transients after loading in the presence of bupivacaine. The decay of caffeine-induced force transients is reduced by bupivacaine, and it also shifts [Ca^{2+}]–force relation toward lower [Ca^{2+}].**

Conclusions: **These data reveal that bupivacaine does not only induce Ca^{2+} release from the SR, but also inhibits Ca^{2+} uptake by the SR, which is mainly regulated by SR Ca^{2+} adenosine triphosphatase activity. It also has a Ca^{2+}-sensitizing effect on the contractile proteins. These mechanisms result in increased intracellular [Ca^{2+}] concentrations and may thus contribute to its pronounced skeletal muscle toxicity.**

SKELETAL muscle toxicity induced by local anesthetics was reported first in 1959,[1] and has been described in several studies and case reports since then.[2–11] All tested local anesthetic agents are myotoxic, whereby procaine and tetracaine produce the least and bupivacaine the most severe muscle injury.[4,12] Intramuscular injections of local anesthetics result in reversible myonecrosis, and the extent of muscle damage is dose-dependent, worsening with serial or continuous administration.[4,11,13,14] The histologic pattern and the time course of skeletal muscle injury appear uniform: approximately 5 min after intramuscular injection, hypercontracted myofibrills are evident, followed by lytic degeneration of striated muscle sarcoplasmic reticulum (SR) and by myocyte edema and necrosis over the next 1–2 days.[2,4,5,13] Myoblasts, basal laminae, and connective tissue elements remain intact, which ensures muscular regeneration within 3–4 weeks.[4,10,13]

Subcellular pathomechanisms of local anesthetic myotoxicity are still not understood in detail. Increased intracellular [Ca^{2+}] concentrations appear to be the most important element in myocyte injury, since denervation, inhibition of sarcolemmal Na^+ channels, and direct toxic effects on myofibrills have been excluded as sites of action.[15,16] Two observations confirm pathologic Ca^{2+} efflux from the SR as major etiologic mechanism: (1) local anesthetic myotoxicity can be "imitated" by caffeine; and (2) myoblasts without intracellular membrane systems for Ca^{2+} sequestration are not affected by local anesthetics.[8,12,15–19] This Ca^{2+} efflux can be the result of either a specific action of local anesthetics on Ca^{2+} release channels (ryanodine receptors [RyR]) or a nonspecific increase in Ca^{2+} permeability of SR membranes. The latter has been shown for many local anesthetics, and the extent of Ca^{2+} efflux depends on agent specific lipid solubility.[12,19]

Recently, Komai and Lokuta examined the interactions of bupivacaine with the SR Ca^{2+} release channel of mammalian skeletal muscles.[12,19,20] The investigators found that bupivacaine in clinical concentrations enhances RyR activity, and suggested that this specific effect on the RyR together with a nonspecific action on SR membranes significantly increases Ca^{2+} permeability and may thus contribute to the pronounced myotoxicity of bupivacaine.[4,12]

Therefore, the current study was conducted to obtain further information about these findings, using the well-established model of skinned murine skeletal muscle fibers.[21–24] In particular, we wanted to elucidate the specific effects of bupivacaine on intracellular Ca^{2+} regulation of the SR in striated muscle cells and on Ca^{2+} sensitivity of contractile proteins.

Materials and Methods

Preparation of the Fiber Bundle

After we obtained Institutional Animal Care Approval of the University of Heidelberg, BALB/c mice were ex-

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Table 1. Experimental Bathing Solutions

	LRS	HRS	HAS	LS
ATP (mM)	8	8	8	8
Creatine phosphate (mM)	10	10	10	10
Creatine kinase (U/l)	150	150	150	150
HEPES (mM)	30	30	30	30
EGTA (mM)	0.3	30	30	30
HDTA (mM)	29.7	—	—	—
Ca^{2+} (μM)	—	—	24.9	0.4
Mg^{2+} (mM)	0.4	0.4	0.4	0.4
Na^{+} (mM)	36	36	36	36
K^{+} (mM)	66	66	66	66

LRS = low relaxing solution; HRS = high relaxing solution; HAS = high activating solution; LS = loading solution; ATP = adenosine triphosphate; EGTA = ethylene-glycol-bis(β-aminoethylether)-N,N,N′,N′-tetraacetic acid; HDTA = 1,6-diaminohexane-N,N,N′,N′-tetraacetic acid.

posed to carbon dioxide for 3–4 min before being killed in deep anesthesia by cervical dislocation. The fast-twitch extensor digitorum longus muscle was dissected and stored in paraffin oil at 4°C. Subsequently, a fiber bundle consisting of one to three single muscle cells (diameter between 70 and 150 μm) was isolated and glued to a force transducer recording system (based on AE 801; SensoNor A.S., Horten, Norway) and to an micrometer adjustable metal pin.[21–23] Analyzing the diffraction pattern of a helium–neon laser beam, the sarcomere length was adjusted to 2.6 μm.[21,22]

Experimental Solutions

The ionic concentrations of the different basic experimental solutions are summarized in table 1.[21–23] All solutions were adjusted to pH 7.0 and contained 60 mM HEPES buffer, 8 mM adenosine triphosphate (ATP), and an ATP-regenerating system consisting of 10 mM creatine phosphate and 150 U/l creatine kinase. In addition, all solutions contained 66 mM K^{+}, 36 mM Na^{+}, and 0.4 mM Mg^{2+} (free ion concentrations).

The high activating solution (24.9 μM Ca^{2+}) and the high relaxing solution (without Ca^{2+}) included 30 mM EGTA with a high affinity to Ca^{2+} for "clamping" the free Ca^{2+} concentration. In contrast, the low relaxing solution (without Ca^{2+}) included 0.3 mM EGTA, but 29.7 mM HDTA with a very low affinity to Ca^{2+} ions. Free ionic concentrations and the ionic strength of all solutions (157 mM at pH 7.00 and 23.0°C) were calculated using the REACT program by Smith and Miller.[25]

The skinning solution was obtained by adding saponin (50 μg/ml) to the low relaxing solution and the loading solution (0.41 μM Ca^{2+}) by mixing equal volumes of high activating solution and high relaxing solution. The release solutions consisted of low relaxing solution with 10 mM caffeine, with 1 mM, 5 mM, 10 mM, and 15 mM bupivacaine (bupivacaine HCl; Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany), and with both caffeine and bupivacaine, respectively.

To assess the $[Ca^{2+}]$–force relation, high activating solution and high relaxing solution were appropriately mixed to obtain six different concentration levels (0.41, 0.62, 0.96, 1.62, 3.39, and 24.9 μM) of free $[Ca^{2+}]$. These measurements were conducted without any addition to the solutions, with caffeine, with bupivacaine, and with both caffeine and bupivacaine, respectively.

Experimental Protocol

All measurements were conducted at four different concentrations of bupivacaine (1, 5, 10, and 15 mM), and six fiber bundle preparations were examined at each concentration level.

At the beginning of each experiment, the muscle fiber preparation was placed in the skinning solution for 5 min. After the skinning procedure, the fiber was dipped for 5 s into the release solution to deload the SR, for 5 s into the high relaxing solution, and for 2 min into the low relaxing solution for equilibration. Then the fibers' SR were loaded with Ca^{2+} for 1 min in the loading solution, and the preparations were subsequently dipped into the high relaxing solution for 5 s before transferring them again into the low relaxing solution for 2 min. In the following, the fibers were exposed to the release solution, and the force transient induced by 10 mM caffeine was recorded for at least 100 s. Finally, the maximal force transient was measured in the high activating solution at 24.9 μM Ca^{2+}. As saponin skinning may reduce the Ca^{2+} loading capacity of the SR and to control the permeabilization procedure of the fibers, the initial caffeine release had to be at least 30% of maximal force, otherwise the preparation was discarded.

To investigate the ability of bupivacaine to induce Ca^{2+} release from the SR, the Ca^{2+} loaded fibers were exposed to a "modified" release solution containing bupivacaine but no caffeine. Subsequently, we recorded the force transients induced by both caffeine and bupivacaine in named concentrations. In the next step, bupivacaine was added to the loading solution, and force transients were measured after transferring the preparations into release solutions containing caffeine and the combination of both caffeine and bupivacaine, respectively. Finally, control measurements were performed in the absence of bupivacaine to demonstrate the return of baseline function.

At the end of our experimental protocol, individual $[Ca^{2+}]$–force relations were assessed for each fiber at different Ca^{2+} concentrations without any addition, and in the presence of caffeine, bupivacaine, and both caffeine and bupivacaine, respectively. Table 2 summarizes the experimental protocol.

Data Analyses

To analyze our data, all force transients were normalized to the corresponding maximum force at 24.9 μM Ca^{2+}. To assess and quantify force decay, the remaining force transient 60 s after the initial force peak was measured and normalized to the latter.

Table 2. Experimental Protocol

	Ca^{2+} Loading	Release
1. Control	LS	RS
2.	LS	LRS + bupivacaine (1/5/10/15 mM)
3.	LS	RS + bupivacaine (1/5/10/15 mM)
4.	LS + bupivacaine (1/5/10/15 mM)	RS
5.	LS + bupivacaine (1/5/10/15 mM)	RS + bupivacaine (1/5/10/15 mM)
6. Control	LS	RS
7. $[Ca^{2+}]$/force relation	0.03–24.9 μM Ca^{2+}	
8. $[Ca^{2+}]$/force relation	0.03–24.9 μM Ca^{2+} + caffeine (10 mM)	
9. $[Ca^{2+}]$/force relation	0.03–24.9 μM Ca^{2+} + bupivacaine (1/5/10/15 mM)	
10. $[Ca^{2+}]$/force relation	0.03–24.9 μM Ca^{2+} + caffeine (10 mM) + bupivacaine (1/5/10/15 mM)	

LS = loading solution; RS = release solution; LRS = low relaxing solution.

$[Ca^{2+}]$–force relations representing the individual Ca^{2+} sensitivity of the contractile apparatus were quantified by fitting a Hill curve to the measured data points.[26] Each curve is characterized by the Hill coefficient (n) as an indicator of maximal steepness of the sigmoidal graph, and the pCa_{50} value (negative decadic logarithm of $[Ca^{2+}]$ at which isometric force is half maximal) as an indicator of Ca^{2+} sensitivity of the contractile apparatus.

For each fiber, the characteristic Hill coefficient and pCa_{50} values were used to mathematically transform force transients into free myofibrillar Ca^{2+} transients according to an algorithm described by Uttenweiler *et al.*[24]

Statisical Analyses

For statistical analysis, we applied the Kolmogorov-Smirnov test to confirm normal distribution for each group, one-way analysis of variance and the Bonferroni *t* test. P values ≤ 0.05 were considered significant. All data are presented as mean ± SD.

Results

Ca^{2+} Release

In the absence of caffeine, bupivacaine itself in concentrations of 5, 10, and 15 mM induced Ca^{2+} isometric force transients in a dose-dependent manner, with relative peak values of 0.02 ± 0.01, 0.47 ± 0.10, and 0.66 ± 0.08, respectively. In contrast, no effects were seen in the presence of 1 mM bupivacaine (fig. 1).

In comparison to control transients induced by 10 mM caffeine (0.90 ± 0.06), the force transients induced by both caffeine and bupivacaine (1 mM: 0.89 ± 0.09; 5 mM: 0.94 ± 0.04; 10 mM: 0.90 ± 0.05; 15 mM: 0.88 ± 0.10) did not change significantly (data not graphically shown).

Ca^{2+} (Re)uptake and Loading Procedure

The presence of bupivacaine in all tested concentration levels during the loading procedure decreased subsequent caffeine induced force transients from 0.90 ± 0.04 (control) to 0.74 ± 0.13, 0.52 ± 0.06, 0.36 ± 0.26, and 0.16 ± 0.15, respectively (fig. 2A). Analogously, the calculated free myofibrillar Ca^{2+} concentrations were significantly reduced from 5.30 ± 0.72 μM (control) to 3.46 ± 1.16, 2.42 ± 0.84, 1.89 ± 1.59, and 1.27 ± 1.23 μM, respectively (fig. 2B).

The addition of 5, 10, and 15 mM bupivacaine to the release solution significantly increased the remaining force transients 60 s after the initial force peak to 72.2 ± 4.1%, 70.0 ± 17.2%, and 70.1 ± 14.5%, respectively (control, 42.4 ± 22.7%). However, this effect on the remaining force transients was not seen after adding 1 mM bupivacaine to the release solution (fig. 2C). Figure 2D shows the corresponding remaining $[Ca^{2+}]$ transients that were calculated using the specific Hill coefficient and pCa_{50} values of the fibers assessed in the presence of bupivacaine and caffeine.

After these experiments, another control measurement was performed to exclude long-term effects of bupivacaine after the washout. There was no difference between these force transients and the transients assessed at the beginning of our protocol (0.90 ± 0.06 *vs.* 0.88 ± 0.09; $P > 0.05$; data not graphically shown).

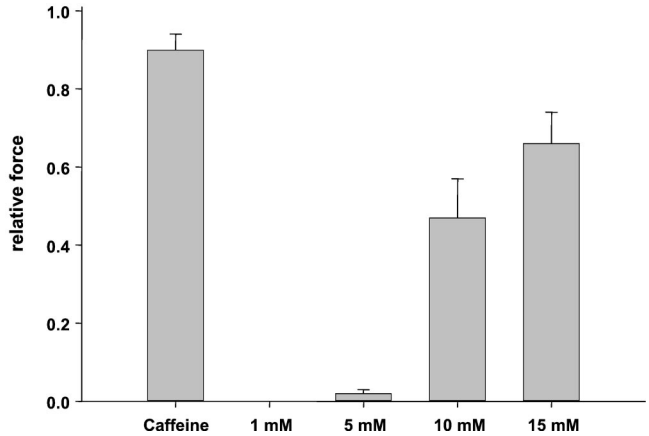
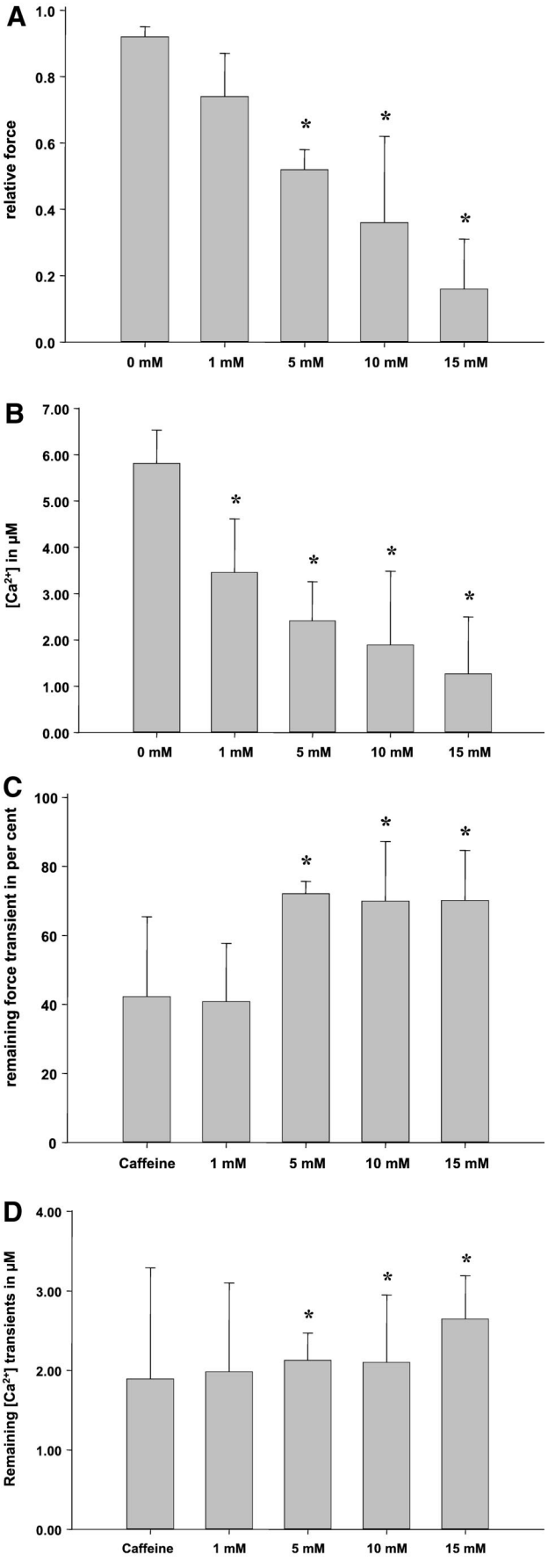


Fig. 1. Mean peak force induced by 10 mM caffeine (control) and 1, 5, 10, and 15 mM bupivacaine. All force transients are normalized to maximum force transient at 24.9 μM Ca^{2+}.



Ca^{2+} Sensitivity

In comparison to control measurements (5.56 ± 0.16), bupivacaine caused an increase in pCa_{50} values to 5.65 ± 1.11, 5.76 ± 0.05, 5.82 ± 0.06, and 5.78 ± 0.04, respectively. Corresponding measurements conducted in the presence of both caffeine and bupivacaine did not differ significantly from these values (fig. 3B).

With respect to the Hill coefficients, only 15 mM bupivacaine significantly affected the maximum steepness of the $p[Ca^{2+}]$–force curve and reduced n from 2.56 ± 0.68 (control) to 1.88 ± 0.13. Again, there was no significant difference between these values and those assessed in the presence of both bupivacaine and caffeine (fig. 3A).

To summarize our findings, a representative recording of bupivacaine (5 mM)-induced effects on Ca^{2+} regulation in a single fiber bundle is shown in figure 4A. Bupivacaine itself induced a small isometric force transient (plot 2) but did not alter caffeine-induced peak force (plot 3; plot 1 = control). In addition, it reduced the peak transients after being present during the loading procedure (plots 4 and 5) and decreased force decay being present in the release solution (plots 3 and 5).

Figure 4B shows individual $p[Ca^{2+}]$–force curves assessed in the same fiber preparation in the presence or absence of bupivacaine and caffeine, respectively. The leftward shifting of the Hill curve caused by bupivacaine indicates a Ca^{2+}-sensitizing effect of this agent. In contrast, the respective addition of 10 mM caffeine did not cause any significant changes in the shape and position of these curves.

Discussion

The pronounced myotoxicity of bupivacaine has been confirmed in multiple studies and case reports, and even animal models have been established using this agent as myotoxin to induce skeletal muscle fiber degeneration.[1–9,11–16,27–31] As major pathomechanism, Benoit *et al.* revealed the increase in intracellular $[Ca^{2+}]$ concentrations caused by alterations of intracellular Ca^{2+} homeostasis, but until today further details were still missing.[8,15] Therefore, the current study was conducted in an attempt to examine bupivacaine-induced effects on Ca^{2+} regulation in skinned fiber preparations

Fig. 2. (*A*) Mean force transients induced by 10 mM caffeine after loading in the presence of 1, 5, 10, and 15 mM bupivacaine. *$P < 0.05$ compared with transient induced after loading in the absence of bupivacaine. (*B*) Conversion of force transients (*A*) into corresponding $[Ca^{2+}]$ transients, based on individual $[Ca^{2+}]$–force relation of each fiber. *$P < 0.05$ in comparison to control $[Ca^{2+}]$ transients after loading in the absence of bupivacaine. (*C*) Remaining force transients assessed in the presence of 1, 5, 10, and 15 mM bupivacaine 60 s after the initial caffeine induced force peak and normalized to the latter. *$P < 0.05$ compared with remaining caffeine-induced transients in the absence of bupivacaine. (*D*) Conversion of force transients (*C*) into corresponding $[Ca^{2+}]$ transients. *$P < 0.05$ compared with remaining $[Ca^{2+}]$ transients in the absence of bupivacaine.

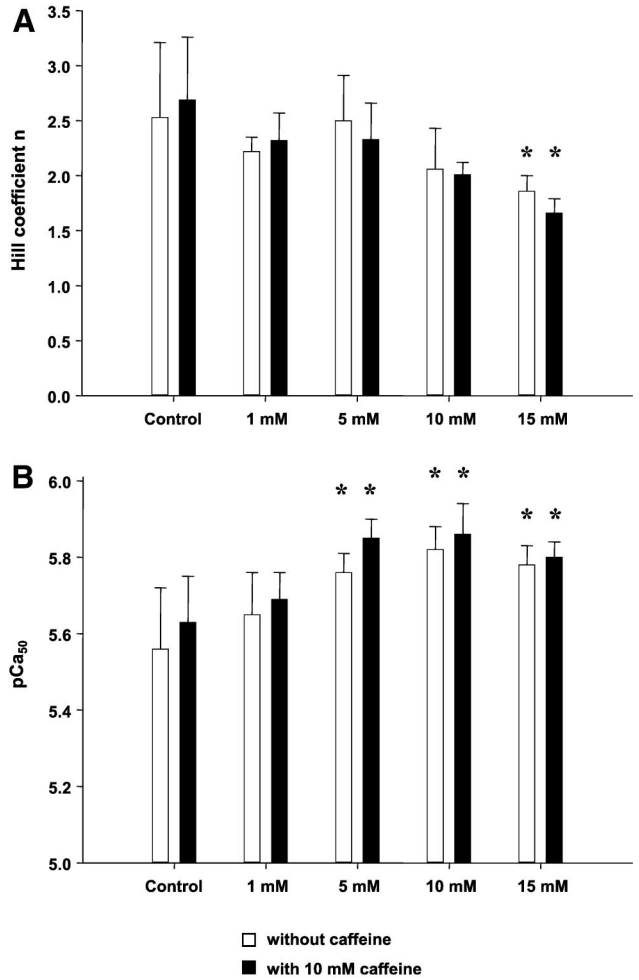


Fig. 3. (*A*) Hill coefficients (n) assessed in the presence of 1, 5, 10, and 15 mM bupivacaine with or without 10 mM caffeine, respectively. **P* < 0.05 compared with control values in the absence of bupivacaine with or without caffeine. (*B*) pCa_{50} values assessed in the presence of 1, 5, 10, and 15 mM bupivacaine with or without 10 mM caffeine, respectively. **P* < 0.05 compared with control values in the absence of bupivacaine with or without caffeine.

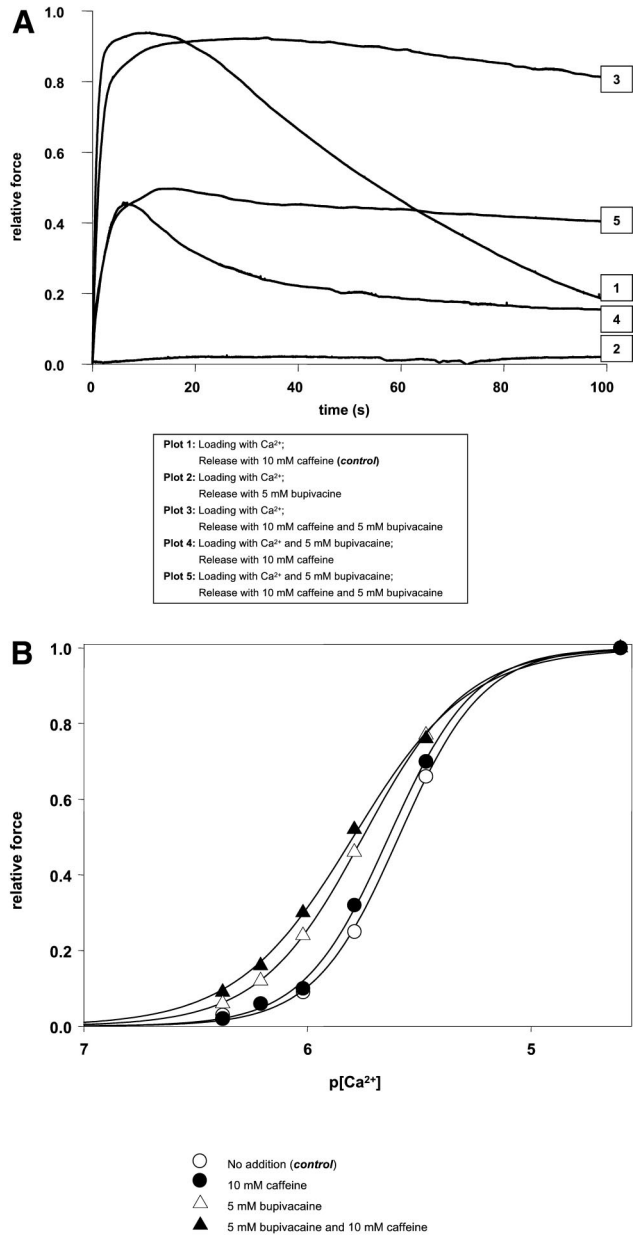


Fig. 4. (*A*) Representative recording of the effects of 5 mM bupivacaine on Ca^{2+}-induced force transients of a single fiber bundle, according to the experimental protocol shown in table 2. All transients are normalized to maximum force at 24.9 μM Ca^{2+}. (*B*) Individually fitted Hill curves showing the effects of 5 mM bupivacaine on p[Ca^{2+}]–force relations of the same muscle fiber preparation at experimental conditions named in table 2.

of mammalian skeletal muscle. In summary, we found out that this local anesthetic induces Ca^{2+} release from the SR and simultaneously inhibits Ca^{2+} reuptake into the SR, resulting in persistently elevated intracellular [Ca^{2+}] concentrations.

The Ca^{2+} homeostasis in myocytes is mainly regulated by the SR and depends on an intact sarcolemma to mark off the interstitial environment.[32,33] Local anesthetics as amphophilic agents are able to penetrate membrane systems and alter sarcolemmal Ca^{2+} permeability.[34] In addition, bupivacaine specifically reduces the activity of sarcolemmal ouabain-sensitive Na^+–K^+–ATPase and of Na^+–Ca^{2+} exchange, but only little is known about possible intracellular interactions of this agent.[19]

To obtain direct information about the effects of bupivacaine on intracellular Ca^{2+} regulation by the SR and on Ca^{2+} sensitivity of the contractile proteins, we chose the methodologic approach of chemically skinned mammalian muscle fibers.[35] In this model, isometric force transients can be measured and mathematically transformed into corresponding free intracellular [Ca^{2+}] transients.[21–26] Several studies on intracellular Ca^{2+} distribution using fluorescence microscopy and digital image analysis have shown that, because of the fast Ca^{2+} binding kinetics of troponin C, the time course of caffeine-induced force transients occurs under quasi–steady state conditions for intracellular [Ca^{2+}] concentrations and force.[23,35,36] Nevertheless, intracellular [Ca^{2+}] is not di-

rectly determined but only interferred with mechanical responses, which has to be mentioned as a methodologic limitation of the used technique.

The effects of 1, 5, 10, and 15 mM bupivacaine were tested to mimic direct exposure of the myocytes to this agent in clinically relevant concentrations (15 mM = 0.5% bupivacaine solution).[8,12,19]

As bupivacaine induced Ca^{2+} efflux from the SR in our model, three possible pathophysiological mechanisms must be discussed to explain this observation: efflux through gated channels, pump mediated pathways, and an increased diffusion rate from the SR caused by an elevated Ca^{2+} permeability.[19] Referring to our experimental conditions, pump-mediated efflux by reversal of Ca^{2+}–ATPases can be excluded.[19,37,38] With regard to the time course of the force transients, efflux through gated channels seems to be the major mechanism of Ca^{2+} release in our model, as the kinetics of transmembraneous diffusion are known to be markedly slower.[34–38]

This hypothesis is confirmed by results of Komai and Lokuta, who recently quantified direct interactions of bupivacaine with the Ca^{2+} release channel RyR.[12] They demonstrated that bupivacaine had a biphasic effect on [^{3}H] ryanodine binding, enhancing it at 5 mM and inhibiting it at 10 mM. In contrast to their results, we recorded a dose-dependent increase in Ca^{2+} release from the SR for a concentration range up to 15 mM bupivacaine. This obvious contradiction may be resolved by the fact that in the aforementioned study, the investigators used SR vesicles enriched with ryanodine receptors as unique membrane protein in their *in vitro* setting. Using skinned fiber preparations, we were able to monitor bupivacaine effects on both the intact SR and the contractile apparatus.

Theoretically, the dose-dependent increase in force transients could be a result of changes in Ca^{2+} sensitivity of the contractile apparatus, but our calculations postulating a constant Ca^{2+} release, and using corresponding Hill coefficient and pCa_{50} values, show that this mechanism is not supposed to be the explanation.

Nevertheless, there was no increase in caffeine-induced force peaks in the presence of bupivacaine. Here, the specific characteristics of the p[Ca^{2+}]–force relation may explain these findings. The force peaks of our caffeine-induced control measurements regularly exceeded 85% of maximum isometric force. Because of the sigmoidal shape of the p[Ca^{2+}]–force curve, the sensitivity of our method is not high enough in this range to detect smaller additional increases in free [Ca^{2+}], and we can only exclude marked further release effects during these experimental conditions.[24,26]

Makabe *et al.* examined the contribution of the SR Ca^{2+}–ATPase to caffeine-induced Ca^{2+} transients in murine skinned muscle fibers.[23] They could show that competitive inhibition of these transport proteins with cyclopiazonic acid increased the size and the duration of Ca^{2+} transients after caffeine induced release. As bupivacaine significantly reduced the decay of force transients, too, we analogously presume that this agent also inhibits SR Ca^{2+}-transport ATPases.

This hypothesis is confirmed by further experimental results. Having loaded the SR in the presence of bupivacaine, we recorded significantly decreased caffeine-induced force and Ca^{2+} transients. Physiologically, Ca^{2+}–ATPases transport Ca^{2+} ions from the sarcoplasma back into the SR during relaxation.[34,35] Exposing the fiber bundle preparations to the loading solution, we can mimic this ATP consumptive process. Consequently, a decreased subsequent Ca^{2+} release—as seen in our experiments after "loading" in the presence of bupivacaine—indicates an inhibition of Ca^{2+} (re)uptake of the SR. Although bupivacaine may inhibit the mitochondrial function and reduce energy supply, this mechanism can be excluded to be responsible for reduced Ca^{2+} uptake as all experiments were conducted with an overshoot of ATP.[39,40]

In our experimental approach, force decay depends on Ca^{2+} reuptake and diffusion of Ca^{2+} ions in the "third compartment."[23,24] In the presence of bupivacaine, force and [Ca^{2+}] concentrations remained significantly larger 60 s after the initial peak. This also points to an inhibition of Ca^{2+} reuptake, as changes in diffusion kinetics are not probable.

In parallel, Makabe *et al.* described similar changes in the kinetics of these transients after specific inhibition of SR Ca^{2+}–ATPases.[23] Furthermore, Takahashi recently examined the effects of bupivacaine on SR vesicles after injection in rabbit masseter muscles.[19] He also demonstrated that bupivacaine inhibits SR Ca^{2+}–ATPases and potently reduces the efficiency of Ca^{2+} uptake into the vesicles.

Thus, our data show for the first time that bupivacaine in clinically relevant concentrations induces Ca^{2+} release from the SR and simultaneously inhibits Ca^{2+} reuptake in skeletal muscle fibers. Because of these synergistic effects, intracellular concentrations of free Ca^{2+} remain elevated in the presence of this local anesthetic. In addition, bupivacaine sensitizes the contractile apparatus to free [Ca^{2+}].

In a recent pilot study, we examined the effects of tetracaine on intracellular Ca^{2+} homeostasis in skeletal muscle cells.[41] Tetracaine in clinically relevant concentrations inhibits RyR activity and does not interact with SR Ca^{2+}–ATPases. In consequence, tetracaine in opposition to bupivacaine does not induce a permanent and excessive increase in intracellular [Ca^{2+}], which is supposed to be a reason for its moderate rate of myotoxicity.

In conclusion, we think that the differential intracellular effects of bupivacaine seen in our model essentially contribute to the outstanding rate of its myotoxicity. They also may explain the evidence of hypercontracted myofibrills immediately after intramuscular injection of bupivacaine, which is known to be an early histologic sign of myonecrosis.[4,8]

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