Interaction of Bupivacaine and Tetracaine with the Sarcoplasmic Reticulum Ca\(^{2+}\) Release Channel of Skeletal and Cardiac Muscles

Hirochika Komai, Ph.D.,* Andrew J. Lokuta, Ph.D.†

Background: Although various local anesthetics can cause histologic damage to skeletal muscle when injected intramuscularly, bupivacaine appears to have an exceptionally high rate of myotoxicity. Research has suggested that an effect of bupivacaine on sarcoplasmic reticulum Ca\(^{2+}\) release is involved in its myotoxicity, but direct evidence is lacking. Furthermore, it is not known whether the toxicity depends on the unique chemical characteristics of bupivacaine and whether the toxicity is found only in skeletal muscle.

Methods: The authors studied the effects of bupivacaine and the similarly lipid-soluble local anesthetic, tetracaine, on the Ca\(^{2+}\) release channel–ryanodine receptor of sarcoplasmic reticulum in swine skeletal and cardiac muscle. \(^{3}\)H]ryanodine binding was used to measure the activity of the Ca\(^{2+}\) release channel–ryanodine receptors in microsomes of both muscles.

Results: Bupivacaine enhanced (by two times at 5 ms) and inhibited (66% inhibition at 10 ms) \(^{3}\)H]ryanodine binding to skeletal muscle microsomes. In contrast, only inhibitory effects were observed with cardiac microsomes (about 3 ms for half-maximal inhibition). Tetracaine, which inhibits \(^{3}\)H]ryanodine binding to skeletal muscle microsomes, also inhibited \(^{3}\)H]ryanodine binding to cardiac muscle microsomes (half-maximal inhibition at 99 ms).

Conclusions: Bupivacaine's ability to enhance Ca\(^{2+}\) release channel–ryanodine receptor activity of skeletal muscle sarcoplasmic reticulum most likely contributes to the myotoxicity of this local anesthetic. Thus, the pronounced myotoxicity of bupivacaine may be the result of this specific effect on Ca\(^{2+}\) release channel–ryanodine receptor superimposed on a non-specific action on lipid bilayers to increase the Ca\(^{2+}\) permeability of sarcoplasmic reticulum membranes, an effect shared by all local anesthetics. The specific action of tetracaine to inhibit Ca\(^{2+}\) release channel–ryanodine receptor activity may in part counterbalance the nonspecific action, resulting in moderate myotoxicity. (Key words: pH; \(\beta,\gamma\)-methyleneadenosine 5-triphosphate.)

LOCAL anesthetics have been known to cause histologic changes in skeletal muscles.1 Through experimental intramuscular injection, local anesthetic myotoxicity has been shown for many local anesthetics.2 The efflux of Ca\(^{2+}\) from the sarcoplasmic reticulum (SR) appears to be a key element in myotoxicity.1

In the current study, we obtained more direct evidence of the effects of bupivacaine on SR Ca\(^{2+}\) release channel–ryanodine receptor (RyR) by measuring the effects of bupivacaine on \(^{3}\)H]ryanodine binding to microsomes obtained from swine skeletal muscles. Because only the open conformational state of RyR binds ryanodine, \(^{3}\)H]ryanodine binding can be used as an index of SR Ca\(^{2+}\) release channel activity.3

We compared the effects of bupivacaine with those of tetracaine, because both local anesthetics have a similar octanol–aqueous buffer distribution coefficient.4 Thus, different local anesthetic effects on RyR function may be attributed to differences in their specific interactions with RyRs, rather than to nonspecific effects on lipid bilayers.

The effect of bupivacaine on RyR function is not known, although the stimulative effects of lidocaine and prilocaine on \(^{3}\)H]ryanodine binding have been reported.5 In contrast, tetracaine (an ester local anesthetic) decreases \(^{3}\)H]ryanodine binding to the skeletal muscle RyR6 and decreases the open probability of both skeletal muscle RyR6 and cardiac RyR.7 These effects of tetracaine are similar to those of another ester local anesthetic, procaine.5,6,8 Benzocaine and dibucaine are other local anesthetics found by Shoshan-Barmatz and Zchut5.
to inhibit [3H]ryanodine binding. With isolated rabbit skeletal muscle SR, Volpe et al.\(^2\) reported values of 2.66 mM bupivacaine and 0.49 mM tetracaine for the half-maximal inhibition of spontaneous Ca\(^{2+}\) release, indicating that the effects of these local anesthetics are different despite their similar lipid solubility.

It is not known whether local anesthetic effects on RyRs also depend on which RyR isoform is being examined. Furthermore, local anesthetic block of nerve conduction involves diffusion through the nerve sheath by the neutral form of the anesthetic, whereas the cationic form is the species that interacts with the cell surface receptor.\(^5\) Whether the same is true in myotoxicity is not known. Finally, adenosine triphosphate (ATP) decreases the degree of tetracaine (or dibucaine) inhibition of [3H]ryanodine binding but does not interfere with stimulation by lidocaine.\(^5\) It is not known whether the effects of bupivacaine on [3H]ryanodine binding are influenced by ATP. Accordingly, we compared the effects of bupivacaine and tetracaine on skeletal muscle RyR with those on cardiac muscle RyR at different pH levels and in the presence and absence of a nonhydrolysable analog of ATP, \(\beta,\gamma\text{-methyleneadenosine 5'-triphosphate (AMP-PCP).}\)

### Materials and Methods

Microsomes enriched in SR were prepared as described before.\(^1\) The hind limb skeletal muscles and the ventricles of the heart were obtained from adult pigs that were killed according to a protocol approved by the Animal Care and Use Committee of the University of Wisconsin. The freshly dissected tissue was minced into small pieces and homogenized using a blender (for 2 min at high speed) in five volumes of an isolation medium. The medium contained 154 mM NaCl, 10 mM Tris-Maleate (pH, 6.8), 2 \(\mu\text{M}\) leupeptin, 100 \(\mu\text{M}\) phenylmethylsulfonyl fluoride, and 500 \(\mu\text{M}\) benzamidine. The homogenate still contained small lumps of tissue, which were dispersed using a Brinkmann Polytron probe (Westbury, NY) three times for 15 s each at setting 2. The homogenate was centrifuged at 4,000g for 20 min. The supernatant was filtered through three layers of gauze and centrifuged at 8,000g for 20 min. The resulting supernatant was filtered through gauze and centrifuged at 40,000g for 30 min. The 40,000g pellet was suspended again in the isolation medium containing 0.3 \(\mu\text{M}\) sucrose and kept frozen (\(-70^\circ\text{C}\)) in small aliquots until it was used. The protein concentration was determined according to the Bradford method using bovine serum albumin as the standard. In each experiment, two to four microsome preparations obtained from different pigs were used.

The effects of local anesthetics on [3H]ryanodine binding were measured according to a published method.\(^1\) Except for the measurements of local anesthetic effects at different pH levels, the medium contained 0.2 mM KCl, 30 mM 3-(N-morpholino) propanesulfonic acid (pH 7.2), 10 \(\mu\text{M}\) CaCl\(_2\), 7 \(\mu\text{M}\) [3H]ryanodine, and 0.6 mg microsomal protein/ml. When the measurements were taken at different pH levels, 3-(N-morpholino) propanesulfonic acid in the previously described medium was replaced by the same concentration of piperazine-N,N-bis[2-ethanesulfonic acid] (pH, 6.5), HEPES (pH, 7.5), and by Tris-HCl (pH, 8.0 and 8.5). 3-(N-morpholino) propanesulfonic acid was used for the medium at pH 7.0. When AMP-PCP was used, the same concentration of MgCl\(_2\) was also included, and an appropriate concentration of CaCl\(_2\), calculated according to the method of Fabiato,\(^2\) was added to obtain a free Ca\(^{2+}\) concentration of 10 \(\mu\text{M}\). Specific binding was calculated by subtracting nonspecific binding obtained in the presence of an excess (100 \(\mu\text{M}\)) of nonlabeled ryanodine from the total binding. Duplicate samples of 0.1 ml were incubated for 90 min at 36°C and filtered onto glass fiber filters (Whatman GF/C) and washed twice with 5 ml distilled water using a Brandel M-24R cell harvester (Gaithersburg, MD). The radioactivity was measured using a Beckman LS-5000 TD counter (Palo Alto, CA).

Curve fitting was performed using MacCurveFit (Kevin Raner Software, Mt. Waverley, Victoria, Australia) with the Quasi-Newton algorithm. Bupivacaine hydrochloride, tetracaine hydrochloride, leupeptin, phenylmethylsulfonyl fluoride, benzamidine, HEPES, 3-(N-morpholino) propanesulfonic acid, piperazine-N,N-bis[2-ethanesulfonic acid], Tris, and AMP-PCP were obtained from Sigma Chemical Company (St. Louis, MO). [3H]ryanodine was obtained from Du Pont New England Nuclear (Wilmington, DE).

### Statistical Analysis

Data are expressed as the mean \pm SD (n = 4). The significance of the effects of different concentrations of bupivacaine was determined by repeated measures analysis of variance followed by Dunnett’s t test. The same method was used for the pH dependence of the effects of bupivacaine and tetracaine. Paired t tests were used...
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![Graph showing concentration-dependent effects of bupivacaine on [3H]ryanodine binding.](image)

Fig. 1. The concentration-dependent effects of bupivacaine on [3H]ryanodine binding. \( P < 0.05 \) compared with corresponding control.

for the effect of a single concentration of AMP-PCP. \( P < 0.05 \) was considered significant. The closeness of curve fitting was indicated by \( r^2 \).

**Results**

**Effects of Bupivacaine on [3H]Ryanodine Binding to Skeletal and Cardiac Muscle RyR**

Bupivacaine had biphasic effects on [3H]ryanodine binding to skeletal muscle microsomes, enhancing [3H]ryanodine binding about two times at 5 mM and inhibiting it by 66% at 10 mM (fig. 1). In contrast, bupivacaine had only inhibitory effects on [3H]ryanodine binding to cardiac microsomes (fig. 1). The concentration of bupivacaine for half-maximal inhibition of [3H]ryanodine binding to cardiac microsomes was approximately 3 mM. The enhancement observed with skeletal muscle microsomes was expected from the chemical similarity of bupivacaine to lidocaine, QX-314, and prilocaine, all of which enhance [3H]ryanodine binding to skeletal muscle RyR.

**pH Dependence of the Effects of Bupivacaine on [3H]Ryanodine Binding**

To determine if the cationic form of bupivacaine is the active species that modulates [3H]ryanodine binding, we measured the effects of 5 mM bupivacaine at different pH levels. From the pK value of 8.10 for bupivacaine at 36°C, the calculated percentage in the cationic form is 98% at pH 6.5, 93% at pH 7.0, 80% at pH 7.5, 56% at pH 8.0, and 28% at pH 8.5. Consistent with the known effect of pH on RyR activity, control [3H]ryanodine binding values obtained with skeletal muscle microsomes were 13 ± 1 fmoles/mg at pH 6.5 and 325 ± 36 fmoles/mg at pH 8.5; and with cardiac microsomes they were 49 ± 10 fmoles/mg at pH 6.5 and 129 ± 8 at pH 8.5. No simple pH dependence was observed with skeletal muscle microsomes. The enhancement of [3H]ryanodine binding was relatively independent of pH except that there was no enhancement at pH 6.5 (fig. 2). Bupivacaine inhibited [3H]ryanodine binding to cardiac microsomes more strongly at low pH than at high pH levels (fig. 2). The extent of inhibition at pH 8.5 was less than that at pH levels of 8.0 and less \( (P < 0.05) \). The results suggest that the cationic form is the active modulatory species.

**Influence of Adenine Nucleotide on the Effects of Bupivacaine on [3H]Ryanodine Binding**

The characteristic concentration-dependent effects of bupivacaine on [3H]ryanodine binding to skeletal and cardiac muscle microsomes, as seen in figure 1, were preserved in the presence of 1 mM AMP-PCP. In the absence of bupivacaine, 1 mM AMP-PCP increased [3H]ryanodine binding to skeletal microsomes (from 126 ± 65 to 290 ± 145 fmoles/mg, a 2.3-fold increase; \( P < 0.05 \)) and had a marginal effect \( (P = 0.05) \) on...
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Fig. 3. The effects of bupivacaine on \(^{[3]H}\)ryanodine binding in the absence and presence of 1 mM \(\beta,\gamma\)-methyleneadenosine 5'-triphosphate (AMP-PCP). (A) Skeletal muscle microsomes. (B) Cardiac microsomes. \(^{[3]H}\)ryanodine binding in the presence of bupivacaine was expressed as a percentage of the corresponding control value (no bupivacaine). \(^*\) \(P = 0.05\) (A) and \(P < 0.05\) (B). +AMP-PCP vs. corresponding NO AMP-PCP in the presence of the same concentration of bupivacaine.

\(^{[3]H}\)ryanodine binding to cardiac microsomes (101 ± 41 fmoles/mg in the absence of AMP-PCP and 152 ± 50 fmoles/mg in the presence of AMP-PCP). The effect of adenine nucleotide is more pronounced with skeletal muscle RyR than with cardiac RyR.13 The dose-dependent effects of bupivacaine expressed as a percentage of control in the presence and absence of AMP-PCP were nearly superimposable (fig. 3). However, there were small but significant effects of AMP-PCP, such as less inhibition by 1 mM bupivacaine to cardiac microsomes and less enhancement by 5 mM bupivacaine to skeletal microsomes (fig. 3).

* Tetracline Inhibition of \(^{[3]H}\)Ryanodine Binding

Figure 4 shows a representative dose-dependent effect of tetracline on \(^{[3]H}\)ryanodine binding to skeletal muscle microsomes and cardiac microsomes determined under the conditions of the current study. The data were fit to a Hill equation of the form

\[
\text{\(^{[3]H}\)ryanodine binding (\% of control)} = \frac{100}{1 + ([\text{Tetracline}] / \text{Ki})^n}
\]

where Ki is the tetracline concentration for half-maximal inhibition and n is the Hill coefficient. From four determinations, we obtained \(\text{Ki} = 35 \pm 8 \mu\text{M}\text{ and }n = 1.5 \pm 0.2\) for skeletal muscle microsomes and \(\text{Ki} = 99 \pm 7 \mu\text{M}\text{ and }n = 1.5 \pm 0.1\) for cardiac microsomes. The Ki value we obtained for pig skeletal muscle microsomes is considerably less than the value reported by Shoshan-Barmatz and Zchut\(^7\) obtained with rabbit skeletal muscle RyR using 20 nM rather than 7 nM \(^{[3]H}\)ryanodine in the assay. The values of Hill coefficients we obtained are larger than 1 but not as large as the values of about 2 reported from the single channel studies.\(^6,7\)

Fig. 4. Concentration dependence of tetracline inhibition of \(^{[3]H}\)ryanodine binding. \(^{[3]H}\)ryanodine binding in the presence of tetracline was expressed as a percentage of the corresponding control value. Control values were skeletal, 55 fmoles/mg; cardiac, 89 fmoles/mg. From curve fitting: with skeletal muscle microsomes, \(\text{Ki} = 43 \mu\text{M}, n = 1.7\) \((r^2 = 0.999600)\); with cardiac microsomes, \(\text{Ki} = 90 \mu\text{M}, n = 1.6\) \((r^2 = 0.99235)\).
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![Graph](image)

Fig. 5. The pH dependence of tetracaine inhibition of $[^3]H$ryanodine binding. $[^3]H$ryanodine binding in the presence of tetracaine was expressed as a percentage of the corresponding control value at each pH. $P < 0.05$ compared with the corresponding value at pH 8.5.

pH Dependence of Tetracaine Inhibition of $[^3]H$Ryanodine Binding

The small effect of pH on tetracaine inhibition reported by Shoshan-Barmatz and Zchut may be caused in part by their use of a high concentration (0.5 mM) of tetracaine. Based on the dose–response relationships (Fig. 4), we used 50 μM tetracaine for skeletal muscle microsomes and 200 μM for cardiac microsomes to determine the pH dependence of tetracaine inhibition. From the pK value of 8.38 for tetracaine at 36°C, the calculated percentage in the cationic form is 99% at pH 6.5, 96% at pH 7.0, 88% at pH 7.5, 71% at pH 8.0, and 43% at pH 8.5. Control $[^3]H$ryanodine binding values with skeletal muscle microsomes were $12 \pm 4$ fmoles/mg at pH 6.5 and $293 \pm 54$ fmoles/mg at pH 8.5; and with cardiac microsomes they were $49 \pm 19$ fmoles/mg at pH 6.5 and $149 \pm 16$ fmoles/mg at pH 8.5. The inhibitory effect of tetracaine was more pronounced in acidic solution for both skeletal muscle and cardiac microsomes (Fig. 5). With skeletal muscle microsomes, the extent of inhibition at pH 8.5 was less than at pH 7.0 and 6.5 ($P < 0.05$). With cardiac microsomes, the extent of inhibition at pH 8.5 was less than at pH levels of 8.0 or less ($P < 0.05$). These results suggest that the cationic form of tetracaine is the active modulatory species that inhibits $[^3]H$ryanodine binding to skeletal and cardiac microsomes.

Influence of Adenine Nucleotide on Tetracaine Inhibition of $[^3]H$Ryanodine Binding

To clarify the nature of the interaction between tetracaine and adenine nucleotides reported by Shoshan-Barmatz and Zchut, we measured the effects of tetracaine on $[^3]H$ryanodine binding in the presence and absence of 1 mM AMP-PCP. In the absence of tetracaine, 1 mM AMP-PCP increased $[^3]H$ryanodine binding to skeletal muscle microsomes (from 79 ± 17 fmoles/mg to 387 ± 194 fmoles/mg, a 4.9-fold increase in this determination; $P < 0.05$) and to cardiac microsomes (from 57 ± 10 fmoles/mg to 228 ± 57 fmoles/mg, a 3.9-fold increase in this determination; $P < 0.05$).

![Graph](image)

Fig. 6. The effects of β,γ-methyladenosine 5-triphosphate (AMP-PCP) on tetracaine inhibition of $[^3]H$ryanodine binding. (A) Skeletal muscle microsomes. (B) Cardiac microsomes. $[^3]H$ryanodine binding in the presence of tetracaine was expressed as a percentage of the corresponding control value (no tetracaine). $P < 0.05$, AMP-PCP compared with corresponding NO AMP-PCP in the presence of the same concentration of tetracaine.

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Fig. 7. Plots of the reciprocal of [3H]yanodine binding versus tetracaine concentration. (A) Skeletal muscle microsomes. (B) Cardiac microsomes. The data are the same as in figure 4, and a Hill coefficient n = 1 was used.

Microsomes (from 129 ± fmol/mg to 218 ± 50 fmol/mg, a 1.7-fold increase, P < 0.05). The presence of AMP-PCP altered tetracaine’s dose-response relationship with cardiac microsomes but not with skeletal muscle microsomes (fig. 6). The effect of AMP-PCP on the Ki (concentration of tetracaine for half-maximal inhibition) was determined as follows. By rearranging the Hill equation,

\[
\frac{1}{[3H]yanodine binding \text{ expressed as fraction of control}} = 1 + \frac{1}{Ki} \times [\text{Tetracaine}]^n
\]

From this equation, Ki is the reciprocal of the slope of the plot of \(\frac{1}{[3H]yanodine binding \text{ expressed as fraction of control}}\) versus [Tetracaine]. Figure 7 shows such plots obtained with the data of figure 6. The Ki values obtained from the slopes in figure 7 are as follows: with skeletal muscle microsomes, 42 µM in the absence of AMP-PCP and 45 µM in the presence of AMP-PCP; and with cardiac microsomes, 106 µM in the absence of AMP-PCP and 357 µM in the presence of AMP-PCP. The calculated Ki values in the absence of AMP-PCP are similar to those obtained in separate experiments (described previously). Although 1 mM AMP-PCP had little effect on the dissociation constant of tetracaine for skeletal muscle microsomes, it is possible that there are some interactions between tetracaine binding and AMP-PCP binding, because we observed that the extent of inhibition by 100 µM tetracaine was decreased (P < 0.05) from 88 ± 4% to 54 ± 11% in the presence of 3 mM AMP-PCP.

Discussion

The observed enhancement of [3H]yanodine binding to skeletal muscle microsomes induced by bupivacaine is consistent with the notion that an increase in RyR channel activity may contribute to the myotoxicity of bupivacaine. In a case report and review of local anesthetic myotoxicity, Hogan et al. considered the possible direct action of local anesthetics on RyR. Shoshan-Barmatz and Zchut showed that amidine local anesthetics, lidocaine, QX-314 (a quaternary ammonium derivative of lidocaine), and prilocaine enhance skeletal muscle RyR activity as determined by measurements of [3H]yanodine binding. Their results suggested that bupivacaine (also an amide local anesthetic) may enhance [3H]yanodine binding. Using skinned fibers from frog semitendinosus muscle, Saida and Suzuki reported that prilocaine may induce SR Ca\(^{2+}\) release through a depolarization-like action. On the other hand, Pike et al. found that tetracaine enhancement of Ca\(^{2+}\) efflux from the SR of skinned skeletal muscles was not blocked by ruthenium red, an inhibitor of RyR. Thus, this effect of tetracaine does not appear to involve RyR.

Previously, measurements of [3H]yanodine binding have been used in determining the effects of inhalational anesthetics on cardiac SR Ca\(^{2+}\) release channels. In another situation relevant to anesthesiology, [3H]yanodine binding was used to characterize differences in SR Ca\(^{2+}\) release channel functions from skeletal muscle of healthy and malignant hyperthermia-susceptible patients. A solution of 0.125% bupivacaine hydrochloride contains 3.8 mM bupivacaine. Therefore, the concentrations
of the local anesthetic used in the current study are relevant to those reached clinically at the injection sites. The enhancing effect of bupivacaine was unique to skeletal muscle microsomes, because we found only inhibition with cardiac microsomes. Thus, the effect of bupivacaine depends on RyR isoforms. The skeletal muscle RyR (rYr1) and the cardiac RyR (rYr2) are different in many respects, including their manner of activation in excitation-contraction coupling. Thus, rYr1 can be activated directly by depolarization of the skeletal muscle, whereas rYr2 activation requires Ca$^{2+}$ influx across the sarcolemma (calcium-induced calcium release).

Skeletal muscle contraction is activated by Ca$^{2+}$ release from the SR, and an increase in [H]ryanodine binding correlates with an increase in contraction. Ikai et al. have reported the effects of bupivacaine on skeletal muscle contracture. These authors showed that bupivacaine causes contracture of skinned rat muscles (extensor digitorum longus and soleus), suggesting that bupivacaine enhances SR Ca$^{2+}$ release. More recently, Takahashi used isolated SR of the rabbit masseter muscle and suggested that bupivacaine enhances SR Ca$^{2+}$ release.

Concerning the mechanism of action of local anesthetics, Bianchi suggested that the cationic form of local anesthetics inhibits SR Ca$^{2+}$ release and the neutral form enhances SR Ca$^{2+}$ release by a caffeine-like action. Shooshan-Barmatz and Zchut proposed that local anesthetics such as lidocaine and QX-314 with a short carbonyl-to-amine distance enhance [H]ryanodine binding, whereas those with a long carbonyl-to-amine distance such as tetracaine inhibit it. On the other hand, Xu et al. proposed that QX-314 blocks skeletal muscle SR Ca$^{2+}$ release channel by entering the channel, whereas tetracaine inhibits SR Ca$^{2+}$ release channel through an allosteric mechanism. Our finding that bupivacaine enhanced [H]ryanodine binding to skeletal muscle microsomes but inhibited [H]ryanodine binding to cardiac microsomes (figs. 1-3) indicates that whether a local anesthetic with a short carbonyl-to-amine distance enhances or inhibits [H]ryanodine binding really depends on the RyR isoform being studied. Bupivacaine had a complex action on skeletal muscle RyR, because it showed stimulatory and inhibitory effects (fig. 1). Previously, dibucaine was shown to have the dual effects on [H]ryanodine binding to skeletal muscle microsomes. Further studies are necessary to elucidate the mechanism of action of bupivacaine on cardiac RyR. All local anesthetics may possess potentially stimulative and inhibitory effects, and which effect predominates depends on the concentration, pH, and isoforms of RyR.

The pH dependence of bupivacaine inhibition of [H]ryanodine binding to cardiac microsomes (fig. 2) suggests that the cationic form of the local anesthetic inhibits cardiac RyR activity. Our data did not indicate that the cationic form of bupivacaine is the active species that enhances [H]ryanodine binding to skeletal muscle microsomes. Nevertheless, we consider it likely that the cationic form of bupivacaine is the active form, because enhancement of [H]ryanodine binding is observed with QX-314, which is permanently cationic. We speculate that protonation of a group on the RyR with a pK in the range of 6 or 7 may prevent enhancement of [H]ryanodine binding, accounting for the observed lack of enhancement by 98% cationic bupivacaine at pH 6.5. Conflicting reports exist concerning the effect of pH on tetracaine inhibition of RyR activity. Bianchi reported that the cationic form of tetracaine is the species that competitively inhibits skeletal muscle contracture induced by caffeine, an activator of RyR. On the other hand, Xu et al. found that 100 µM tetracaine nearly doubled the half-time of Ca$^{2+}$ efflux from skeletal muscle SR vesicles at pH levels of 7.8 and 6.8. From these results, those authors reported that the efficacy of tetracaine to inhibit SR Ca$^{2+}$ release was not appreciably altered by a 10-fold change in the concentration of the uncharged form of tetracaine (20% at pH 7.8 and 2% at pH 6.8). In those experiments, the concentration of the cationic form of tetracaine changed only by 1.25 times (80% at pH 7.8 to 98% at pH 6.8). Shoshan-Barmatz and Zchut reported that tetracaine inhibition of [H]ryanodine binding to skeletal muscle RyR decreased from approximately 98% to 77% by a shift in pH from 6.1 to 8.5. Strong inhibition caused by the high concentration of tetracaine (0.3 m) used by those authors may account in part for the small decrease in the magnitude of inhibition. Our results suggest that the cationic form and not the neutral form is the active species of tetracaine that inhibits [H]ryanodine binding to cardiac or skeletal muscle microsomes.

Understanding the interactions between local anesthetics and adenosine nucleotides on RyRs is important, partly because millimolar ATP is a normal constituent of muscle cells. Local anesthetic effects that diminish in the presence of millimolar concentrations of adenosine nucleotide may not be important in intact muscles. O'Brien et al. found that 100 µM tetracaine inhibits [H]ryanodine binding to fish skeletal muscle microsomes but not to cardiac microsomes in the presence of 2.5 mM AMP-PCP.

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With skeletal muscle RyR, Shoshan-Barmatz and Zchut\(^5\) showed that ATP decreases the degree of inhibition of \(^{[3]H}\)ryanodine binding by tetracaine and dibucaine but does not interfere with stimulation by lidocaine. Not all the local anesthetics seem to interact with nucleotides and, furthermore, the nature of the interactions appear to depend on the isoforms of RyR. Our finding that the effects of bupivacaine on \(^{[3]H}\)ryanodine binding to skeletal muscle microsomes and to cardiac muscle microsomes were minimally affected by 1 mM AMP-PCP suggests that there is little interaction between bupivacaine binding and adenine nucleotide binding. This is consistent with the report that ATP does not affect lidocaine stimulation of \(^{[3]H}\)ryanodine binding by skeletal muscle RyR.\(^5\) We cannot, however, exclude the possibility that higher concentrations of AMP-PCP might alter the dose-dependent effects of bupivacaine. The observed increase in the Ki value of tetracaine induced by AMP-PCP with cardiac microsomes suggests that tetracaine binding is competitive with adenine nucleotide binding, or that adenine nucleotide binding allosterically alters tetracaine binding. In this regard, the results are similar to those O’Brien et al.\(^2\) obtained with fish RyRs. Although the dissociation constant of tetracaine for skeletal muscle microsomes was unaffected by 1 mM AMP-PCP, it is possible that some interactions occur between the tetracaine binding site and adenine nucleotide binding site, because we observed that the extent of inhibition by 100 \(\mu\)M tetracaine was decreased by 3 mM AMP-PCP. Also, that Shoshan-Barmatz and Zchut\(^5\) reported tetracaine inhibition of photoaffinity labeling of skeletal muscle RyR by \([\alpha-\beta^2P]Benzoyl ATP\) Except for the effect of tetracaine on cardiac RyR, it is likely that the effects of bupivacaine and tetracaine we observed in the absence of AMP-PCP persist in intact muscle containing ATP.

In conclusion, the results of the current study indicate that, in addition to the chemical structure of local anesthetics as suggested by Shoshan-Barmatz and Zchut,\(^5\) the isoforms of RyR are important in determining whether a local anesthetic enhances or inhibits \(^{[3]H}\)ryanodine binding. The bupivacaine enhancement of \(^{[3]H}\)ryanodine binding to skeletal muscle microsomes is a direct demonstration of the effect of this local anesthetic on RyR, as suggested by others.\(^1\),\(^2\),\(^21\) Myotoxicity resulting from a nonspecific action on lipid bilayers to increase the Ca\(^{2+}\) permeability of SR membranes is likely most common to various local anesthetics. We speculate that the specific action of bupivacaine to enhance skeletal muscle SR Ca\(^{2+}\) release contributes to the massive damage\(^2\) of skeletal muscle caused by this local anesthetic. In this regard, the specific action of tetracaine to inhibit RyR activity may partly counterbalance its nonspecific action, resulting in moderate muscle damage.\(^2\)

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