CONTRACTION KINETICS OF RED MUSCLE IN SCUP: MECHANISM FOR VARIATION IN RELAXATION RATE ALONG THE LENGTH OF THE FISH

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Summary

We studied possible mechanisms for the twofold difference in red muscle relaxation times between the posterior (207.2 ms) and anterior (98.4 ms) musculature of scup Stenotomus chrysops, which has been shown to have a large effect on power generation during swimming. This difference was not due to contamination of the anterior bundles with faster fiber types, as histological examination showed that all bundles contained more than 98.9 % red fibers. Further, maximum velocities of shortening (V_{max}) at 20°C were nearly identical, 5.37 ML s^{-1} (where ML is muscle length) for the anterior musculature and 5.47 ML s^{-1} for the posterior musculature, suggesting that the difference in relaxation times was not due to a difference in the crossbridge detachment rates associated with different myosin isoforms. The possibility of differences in the Ca²⁺ pumping rate influencing relaxation rate was explored using cyclopiazonic acid (CPA), a sarcoplasmic reticulum (SR) Ca²⁺-ATPase inhibitor. The concentration of CPA could be adjusted to slow the relaxation rate of an anterior muscle to that of a posterior muscle. However, SDS gels showed no difference in the intensity of SR Ca²⁺-ATPase protein bands between muscle positions. These results suggest that differences in the Ca²⁺ pumping could account for the observed difference in relaxation rate, but do not support the simplest hypothesis that the difference in relaxation rates is due to differences in numbers of Ca²⁺ pumps. Other possible mechanisms for this difference are explored.

Key words: scup, Stenotomus chrysops, contraction kinetics, relaxation rate, Ca²⁺-ATPase, muscle.

Introduction

Maximum velocity of shortening (V_{max}) is the major determinant of a muscle’s intrinsic power-generating capability during shortening contractions (Rome et al. 1988). However, during oscillatory contractions, the rate of activation and particularly the rate of relaxation are also important determinants of power production (Marsh, 1990; Woledge, 1992; Josephson, 1993; Rome and Swank, 1992). Different muscle fiber types are designed to operate at different oscillatory frequencies. Not unreasonably, as the frequency of operation increases, the V_{max} and relaxation rate increase in concert (Rome et al. 1996). This fact makes it difficult to assess the importance of relaxation rate alone on power production and to test hypotheses concerning the importance of relaxation rate in muscular system design (Marsh, 1990; Josephson, 1993; Johnson et al. 1993). Therefore, it is of interest to consider how a muscle’s ability to generate power would be affected by independently changing its relaxation rate, and how, from a mechanistic viewpoint, relaxation rate is altered during evolution.

Relaxation rates in different skeletal muscle fiber types can vary by as much as 50-fold in the same animal (Rome et al. 1996), but relaxation rate (as well as other properties) is generally thought to be constant within a fiber type of a given species. Recently, it was found that cod (Gadus morhua) anterior white muscle has faster relaxation characteristics than posterior white muscle (Davies and Johnston, 1995). The anterior muscle also has a 2.4-fold faster V_{max} as well as a twofold faster activation rate.

A possible case of relaxation rate changing independently of V_{max} is the slow-twitch red muscle of scup. In scup, red muscle relaxation rate increases systematically moving from the posterior to the anterior myotomes, resulting in a twofold difference in relaxation rate between the ANT-1 and POST positions (which are 29 % and 70 %, respectively, down the length of the fish; Rome et al. 1993). Previous studies suggest that all scup red muscle, irrespective of position, has the same activation rate and therefore perhaps the same V_{max} (Rome and Swank, 1992; Rome et al. 1993). This suggests that the only difference between the ANT-1 and POST muscles may be in relaxation rate. This difference is functionally important as it...
has a marked effect on the performance of the muscles during locomotion. For instance, when isolated muscle is driven using *in vivo* stimulus and length change conditions measured from the ANT-1 position, ANT-1 muscle generates considerable power whereas POST muscle relaxes too slowly to generate any net power at all (Rome et al. 1993). Thus, if the ANT-1 muscle did not have a faster relaxation rate, then it too would be unable to generate power.

Relaxation rate is thought to be set by a combination of the time course of occupancy of troponin sites by Ca$^{2+}$ and the kinetics of crossbridge detachment (Baylor et al. 1983; Gillis, 1985; Rome et al. 1996). In this study, we tried to determine which class of mechanism is responsible for the difference in relaxation rates. We first excluded two simple explanations for the relaxation rate difference by showing that the ANT-1 muscle was not contaminated with a faster fiber type and that the POST muscle did not have a larger series elastic component which might slow relaxation. We then measured $V_{\text{max}}$ to ascertain whether relaxation rate changed independently of $V_{\text{max}}$ and, more mechanistically, whether a change in crossbridge kinetics underlies this difference in relaxation rate. We also used cyclopiazonic acid (CPA), an SR Ca$^{2+}$-ATPase inhibitor, to determine whether a change in the time course of troponin occupancy could be responsible for this difference. CPA slows Ca$^{2+}$ pumping and therefore should prolong the time course of troponin occupancy. Thus, if it were responsible for the different relaxation rates, we should be able to slow the relaxation rate of ANT-1 muscle to that of POST muscle using CPA. Finally, by using SDS gels to compare the amount of Ca$^{2+}$-ATPase protein at the ANT-1 and POST positions, we tested the hypothesis that the faster relaxation rate of ANT-1 muscle is due to a greater number of SR Ca$^{2+}$ pumps.

**Materials and methods**

**Muscle preparation**

Scup (*Stenotomus chrysops* L.), 19–23 cm long, were caught at Woods Hole, MA, USA, and kept in a saltwater flow-through tank at 10 °C. Fish were acclimated for 6 weeks prior to use. Scup were killed by a blow to the head and pithed. Red muscle bundles were dissected as in Rome and Swank (1992). The bundles were taken from two specific longitudinal positions referred to as ANT-1 or POST (which are 29% and 70%, respectively, down the length of the fish; Rome et al. 1993). Bundles were removed 2–3 cm above the mid-line and were composed of approximately 125 fibers. The thickness, width and length of the bundle were estimated by photographing it from two aspects through a dissecting microscope. Because of the large amount of connective tissue, the measured volume was multiplied by 43% to obtain fiber volume (Rome et al. 1992b). Bundle mass was calculated assuming a density of 1.05 g cm$^{-3}$ for muscle.

**Solutions**

Muscle bundles were bathed in a physiological saline containing (in mmol l$^{-1}$): 132 NaCl, 2.6 KCl, 2.7 CaCl$_2$, 10 imidazole, 10 sodium pyruvate and 1 MgCl$_2$, pH 7.7 at 15 °C (based on Altringham and Johnston, 1990). A stock solution of 100 μmol l$^{-1}$ CPA was made by dissolving 5 mg of CPA (Sigma) in 1.98 ml of dimethyl sulfoxide (DMSO) and diluting in saline. Therefore, a working concentration of 1.6μmol l$^{-1}$ CPA had 0.08 % DMSO in the saline. Control experiments revealed that this concentration of DMSO in the saline had no apparent effect on the muscle.

**Muscle mechanics preparations**

The apparatus used for the muscle mechanics experiments and the mounting of the fiber bundle to the apparatus have been described previously (Rome and Swank, 1992). All mechanics experiments were performed at 20 °C. The stimulus pulse duration and voltage giving maximum twitch force were determined (a pulse duration of 1 ms was generally used). For tetani, a stimulus frequency of 200 Hz generated maximal force (Rome and Swank, 1992). The optimal length of the muscle was determined by generating a force–length curve. The starting length of the bundle for force-clamp experiments (force–velocity curves) was set slightly beyond the plateau of the force–length curve to allow most of muscle shortening to occur on the plateau. Resting force was 2.3±0.2 % (means ± S.E.M.) of active force for ANT-1 bundles and 3.7±0.6 % for POST bundles at this length. This was not a statistically significant difference (t-test; $P=0.07$).

**Measurements of relaxation and activation times**

Relaxation and activation times were measured before each force–velocity or CPA experiment by recording an isometric twitch and tetanus (duration 125 ms). Time of activation ($T_{a,10-90}$) was the period from 10% to 90% of maximum force generation. Relaxation was measured in three different ways: from 90% to 10% of maximum force ($T_{r,90-10}$), from 95% to 80% of maximum force ($T_{r,95-80}$), and as the time from the last stimulus pulse to 10% of maximum force ($T_{r,\text{last}}$). All values are reported as the mean ± S.E.M.

**$V_{\text{max}}$ and $W_{\text{max}}$ measurements**

$V_{\text{max}}$ for both ANT-1 and POST bundles was determined in order to assess potential differences in myosin crossbridge detachment rates ($V_{\text{max}}$ is believed to reflect crossbridge detachment rate; Huxley, 1957). For each bundle, the velocity of shortening was determined at 12 different loads (ranging from 2% of $P_0$ to 80% of $P_0$, where $P_0$ is isometric force) using the force-clamp method (Julian et al. 1986). The data were fitted with the Hill equation not constrained to pass through $P/P_0=1$ (Rome et al. 1992b). $V_{\text{max}}$ was estimated by extrapolating the force–velocity curve to zero load. Power ($W_{\text{max}}$) is the product of the force and velocity taken from the force–velocity curve.

**Series elastic component measurements**

In theory, a large compliance in series with the contractile component will slow the rise and fall of force during activation and relaxation. Therefore, we tried to determine whether a
larger series compliance could explain the slower relaxation rate in the POST muscle. Each bundle was given five quick releases to determine the series elastic component (stress–strain curve). The muscle was first tetanized then quickly shortened (<1 ms) with a release length between 0.5% and 6% of the muscle’s length. The force immediately after each release was plotted against the step size. A regression line was fitted to all the points except those for which the force following release reached zero. The minimum step size required to reduce the force to zero (i.e. the intercept of the regression line with the length axis) was taken as the series elastic component.

Oscillatory power

Work loops were performed to measure the effect of the different relaxation times on cyclical power production. An oscillation frequency of 4.7 Hz was used, which has been measured from scup swimming steadily at 50 cm s⁻¹ (Rome et al. 1993) and is within the range of frequencies (4.5–5.5 Hz) at which scup red muscle produces maximal power at 20 °C (Rome and Swank, 1992). Stimulus phase, strain and stimulus duration were −48°, 8%, and 80 ms, respectively, where a phase of 0° corresponds to the stimulus starting at maximal muscle length (Rome and Swank, 1992). These particular conditions were chosen because they reveal how the slower relaxation rate of POST muscle can limit power generation. These conditions allow sufficient time for the force in the fast-relaxing ANT-1 muscle to return to resting levels during lengthening, but the force in the slow-relaxing POST muscle is unable to return to resting levels (or returns only briefly).

Fiber type identification

Following mechanics experiments, muscle bundles were removed from the apparatus, soaked in Trypan Blue for 1 h, embedded in gelatin (15 g in 100 ml of saline) at resting length, and frozen in liquid N₂ (Rome and Swank, 1992). These particular times varied significantly with position. As subsequently verified in a larger data set, these differences were not statistically significant. Therefore, only relaxation times varied significantly with position.

Relaxation kinetics in the presence of CPA

To determine how sensitive muscle relaxation was to a change in Ca²⁺ transient duration, the effect of CPA was measured on a different set of muscle bundles. The optimal stimulation conditions and muscle length for force production were determined as in the force–velocity experiments. Tetanic force was then measured. CPA-containing saline was then substituted in the bath, and the muscle was stimulated every 4 min for 1 h to allow the new relaxation rate to stabilize (Du et al. 1994). Tetani were then repeated.

Ca²⁺-ATPase protein measurements

SDS–polyacrylamide gels were loaded with semipurified SR vesicles from ANT-1 and POST red muscle (O’Brien et al. 1993) and unpurified whole-muscle homogenates. Total protein content of the lanes was equalized prior to running the gel using a modified Lowry assay (Bio-Rad). Typically, 60 µl of 1 mg ml⁻¹ sample was loaded. A 3% stacking and a 6% separating gel of 1.5 mm thickness were used. After staining with Coomassie Brilliant Blue, the gel was analyzed using a densitometer and related software (Masterscan, Analytics). t-tests were used for statistical analysis unless otherwise noted.

Results

Isometric relaxation and activation times

The POST red muscle had a twofold slower relaxation rate than the ANT-1 muscle (see Fig. 3A; Table 1). Tetanic measurements revealed that the relaxation time of POST muscle was twofold longer than that of ANT-1, while the relaxation time of twitches was approximately 2.5-fold longer in the POST muscle (P<0.001) (Table 1). By contrast, tetanus and twitch activation times showed only slight differences (approximately 15%). Tetanic activation times tended to be longer in ANT-1 muscle bundles compared with POST, but twitch activation times tended to be shorter in ANT-1 bundles. As subsequently verified in a larger data set, these differences were not statistically significant. Therefore, only relaxation times varied significantly with position.

<table>
<thead>
<tr>
<th></th>
<th>ANT-1</th>
<th>POST</th>
<th>T&lt;sub&gt;r,90–10&lt;/sub&gt;</th>
<th>T&lt;sub&gt;r,95–80&lt;/sub&gt;</th>
<th>T&lt;sub&gt;r,Last&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Twitch</td>
<td>33.9±1.8</td>
<td>80.5±5.8</td>
<td>16.8±0.8</td>
<td>165.3±8.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>37.7±0.9</td>
<td>212.2±9.8</td>
<td>44.5±3.6</td>
<td>330.0±9.9</td>
<td></td>
</tr>
<tr>
<td>Tetanus</td>
<td>54.9±1.7</td>
<td>98.4±3.2</td>
<td>30.5±1.2</td>
<td>145.6±4.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>46.5±2.7</td>
<td>207.2±4.8</td>
<td>51.4±1.3</td>
<td>271.3±7.2</td>
<td></td>
</tr>
<tr>
<td>CPA tetanus</td>
<td>51.6±1.6</td>
<td>201.4±10.6</td>
<td>49.7±3.4</td>
<td>271.2±7.6</td>
<td></td>
</tr>
</tbody>
</table>

Activation time was measured from 10% to 90% of maximum tetanic force (T<sub>r,10–90</sub>). Relaxation time was measured from 90% to 10% of maximum tetanic force (T<sub>r,90–10</sub>), 95% to 80% of maximum tetanic force (T<sub>r,95–80</sub>) and from the last stimulus to 10% of maximum tetanic force (T<sub>r,Last</sub>).

Values are means ± s.e.m. (N=6).
At least 98.9% of the live fibers in the experimental muscle bundles from the ANT-1 and POST regions were the red muscle fiber type (Fig. 1A). Only one of seven ANT-1 bundles examined contained any live pink fibers, representing 0.95% of the total live area. Two out of six POST muscle bundles had a few pink fibers (see arrowheads in Fig. 1B), representing 0.6% and 1.1% of the total live area. During dissection, remnants of white fibers remained attached to the bundles. Dark staining of these white fibers with Trypan Blue confirmed that they were dead during the mechanics experiments. Therefore, differences in relaxation rate between ANT-1 and POST muscle cannot be attributed to differences in fiber type composition.

**Series elastic component**

The series elastic components for muscle from the ANT-1 position (4.4±0.20%; N=6) and the POST position (3.9±0.18%;...
N=6) were not significantly different (P=0.08) and thus do not explain the difference in relaxation rates. The tendency towards a larger series elastic component in the ANT-1 muscle would, if anything, produce a slower relaxation rate compared with the POST position – the opposite of what is observed. Finally, the tendency towards a larger series elastic component in the ANT-1 position (13%) is explained by a tendency towards a higher maximum isometric force (14%) (Table 2). This means that the series compliances (Δstrain/Δforce) were nearly identical in the ANT-1 and POST positions.

Steady-state measurements: \( V_{\text{max}} \) and \( W_{\text{max}} \)

The \( V_{\text{max}} \) of ANT-1 muscle was nearly identical to that of POST muscle (P=0.79) (Table 2; Fig. 2). In addition, the other force–velocity curve constants (\( a/P_0^* \) and \( P_0^* \)) were also nearly identical, as were maximum steady-state power values (\( W_{\text{max}} \)) (P=0.90) (Table 2; Fig. 2B). Further, isometric force production was not statistically different at the two positions (P=0.55) (Table 2).

Oscillatory power

More power was produced by ANT-1 muscle bundles than by POST bundles when measured using the work loop technique (Fig. 3D). The mean power output from ANT-1 bundles was 1.5-fold higher than that of POST bundles using identical length change and stimulus conditions (specified in Materials and methods) (Table 2). As seen in Fig. 3D, the major difference between work loops measured from ANT-1 and POST muscle bundles was that the POST muscle shows a slower relaxation rate after stimulation ends, which results in the force never returning to resting level (force=0).

CPA inhibition of SR \( Ca^{2+} \)-ATPase

We found that by increasing the concentration of CPA from 0.5\( \mu \)mol l\(^{-1} \) (at which it first affected relaxation), we could progressively slow the rate of relaxation of the bundle until, at a concentration of approximately 50\( \mu \)mol l\(^{-1} \), the muscle did not relax at all. Using only a modest concentration of CPA (1.6\( \mu \)mol l\(^{-1} \)), we were able to slow the isometric relaxation time of ANT-1 muscle to that of normal POST muscle. Further, the tension in the CPA-modified ANT-1 muscle fell with a nearly identical time course over most of the force range as in

### Table 2. \( V_{\text{max}} \) of ANT-1 and POST muscle measured using the force-clamp technique

<table>
<thead>
<tr>
<th></th>
<th>( V_{\text{max}} ) (ML s(^{-1} ))</th>
<th>( a/P_0^* )</th>
<th>( P_0^* )</th>
<th>Force (kN m(^{-2} ))</th>
<th>( W_{\text{max}} ) (W kg(^{-1} ))</th>
<th>Power (W kg(^{-1} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANT-1</td>
<td>5.37±0.27</td>
<td>0.21±0.02</td>
<td>1.53±0.07</td>
<td>168±20.5</td>
<td>122±16.3</td>
<td>46.7±3.4†</td>
</tr>
<tr>
<td>POST</td>
<td>5.47±0.21</td>
<td>0.23±0.02</td>
<td>1.66±0.12</td>
<td>147±17.9</td>
<td>125±13.7</td>
<td>30.9±4.1</td>
</tr>
</tbody>
</table>

ML is muscle length.

\( a/P_0^* \) is the Hill constant. \( P_0^* \) is the extrapolated load at zero velocity of the Hill curve. Force is maximum isometric force. \( W_{\text{max}} \) is the maximum steady-state power measured using the force-clamp technique. Power is oscillatory power measured using the specific work loop conditions outlined in Materials and methods.

Values are means ± S.E.M. N=6 for all measurements except POST power, where N=7.

†Signifies statistical difference between ANT-1 and POST.

Fig. 2. Mean force–velocity and power–velocity curves from ANT-1 and POST muscle bundles. (A) The curves were constructed using the Hill equation from the mean values of \( V_{\text{max}} \), \( a/P_0^* \) and \( P_0^* \) (see Table 2 for definitions) for ANT-1 and POST bundles (Julian et al. 1986; Rome and Sosnicki, 1990). The curves from 0 to 0.8\( P_0 \) were not constrained to intercept the force axis at \( P/P_0 =1 \) as unconstrained Hill fits result in more accurate \( V_{\text{max}} \) values (Julian et al. 1986). Between loads of 0.8\( P_0 \) and \( P_0 \), a spline curve (broken line) was used which did pass through \( P/P_0 =1 \). (B) Power curves constructed by multiplying the force and velocity values from A. A spline curve (broken line) was used to interpolate the curves from loads of 0.8\( P_0 \) to \( P_0 \).
1.6 relaxation, two control experiments demonstrated that the POST muscle (Fig. 4; Table 1). Despite the large effect on relaxation, two control experiments demonstrated that 1.6 μmol l⁻¹ CPA had no effect on V_max, and six experiments showed that this level of CPA had no effect on isometric force or activation time (Table 1).

![Graph showing mechanical properties of ANT-1 and POST muscles](image)

**Discussion**

We have previously found a difference in relaxation rate between the ANT-1 and POST red myotomal muscle (Rome et al. 1993) and we now show that other mechanical properties of the muscle, such as V_max, isometric force and activation time are identical. By contrast, the white muscle of cod Gadus morhua (Davies and Johnston, 1995) and saithe Pollachius virens (Altringham et al. 1993) also relaxes faster in the

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**Ca²⁺-ATPase protein measurements**

Polyacrylamide gel electrophoresis of crude red muscle extracts revealed a protein which was identified as the SR Ca²⁺-ATPase. The protein was identified by running semipurified scup red muscle SR vesicles in lanes adjacent to the crude red muscle extracts (Fig. 5A). The Ca²⁺-ATPase constitutes 60 % of total SR vesicle protein (Meissner et al. 1996; Maruyama et al. 1989), and no other protein in the SR besides the Ca²⁺-ATPase protein has a molecular mass close to 100 kDa (MacLennan et al. 1987). Densitometry scans of lanes loaded with ANT-1 and POST crude homogenates showed that there was no difference in the magnitude of the peak for the Ca²⁺-ATPase protein between ANT-1 and POST muscle (Fig. 5B). The ratio of the ANT-1 to POST values was 0.99±0.08 (mean ± s.e.m., N=4, P=0.86). Internal controls for the densitometry scan (the optical densities of most other protein bands were nearly identical) showed that the lanes had been loaded with the same amount of total protein.
antior than in the posterior position, but in these species activation time and/or $V_{\text{max}}$ appear to change in proportion to relaxation rate. This suggests that the changes in relaxation rate are partially (if not totally) due to changes in crossbridge kinetics. The red muscle of scup is the only instance yet observed in vertebrates where relaxation rate appears to change independently of crossbridge kinetics within a single fiber type.

**Mechanism for different relaxation rates**

The difference in relaxation rates in scup red muscle was not due to fiber heterogeneity in the experimental bundles or to differences in series elasticity. Although live pink fibers were found in a few instances (representing 1% or less of cross-sectional area), the majority of muscle bundles examined from both the POST and ANT-1 positions contained only red fibers. Therefore, the cause of the difference in relaxation times must be within the red muscle fibers themselves.

The rate of relaxation of a muscle fiber is determined by a complex interaction between several molecular components. During and following SR Ca$^{2+}$ release, the SR Ca$^{2+}$-ATPase pumps Ca$^{2+}$ into the SR. Parvalbumin (a soluble Ca$^{2+}$-binding protein), if present, may also play a role in relaxation by binding myoplasmic Ca$^{2+}$. When [Ca$^{2+}$] falls below approximately 10$^{-5}$ mol l$^{-1}$ (depending on the Ca$^{2+}$ sensitivity of troponin), Ca$^{2+}$ unbinds from troponin and further crossbridge attachment to actin is blocked by thin filament inactivation. However, for force to drop, cross bridges must...
detach. Thus, the rate of muscle relaxation is set by two major steps: (1) the time course of occupancy of troponin sites by Ca²⁺ and (2) the kinetics of crossbridge detachment. The time course of troponin occupancy is in turn determined by the kinetics of Ca²⁺ sequestration and the kinetics of Ca²⁺ unbinding from troponin. Rome et al. (1996) found that the kinetics of all these rates is increased in concert in muscles ranging from the slow-twitch red muscle to the superfast swimbladder muscle in toadfish (Opsanus tau).

To assess whether the crossbridge detachment rate is the cause of differences in relaxation rate, we measured V_{max}. It seems unlikely that myosin isoforms with different detachment rates could be present without also affecting V_{max} or activation rate (which is set primarily by the sum of the isometric crossbridge attachment and detachment rate constants) (Brenner, 1988). We found that activation rate and V_{max}, as well as the shapes of the force–velocity curves and the power–velocity relationships (Fig. 3B,C), were the same in bundles from the ANT-1 and POST muscle. This suggests that functionally different myosin isoforms are not present and thus did not contribute to the different relaxation time courses.

Therefore, it seems likely that the difference in relaxation rates is due to a difference in the time course of troponin occupancy and, more specifically, to a difference in Ca²⁺ transient duration. The force traces in Fig. 3A support this hypothesis. Given the likely similarity between the crossbridge kinetics, the higher twitch force recorded in the POST than in the ANT-1 muscle is consistent with a longer troponin occupancy in the POST muscle. Similarly, the longer plateau of maximal tetanic force in POST muscle for the same stimulus duration (see also Fig. 4) is also consistent with [Ca²⁺] remaining above the troponin-saturating level for longer following the final stimulus in this muscle.

We tried to obtain additional support for the hypothesis that Ca²⁺ transient duration underlies differences in relaxation by altering the duration of the Ca²⁺ transient. The most powerful approach would be pharmacologically to speed up the time course of Ca²⁺ reuptake by the POST muscle to determine whether the POST muscle can be made to relax as quickly as the ANT-1 muscle. If so, this would show that the Ca²⁺ transient duration was limiting the rate of relaxation in the POST muscle and thus is the likely difference between ANT-1 and POST muscles. An approach using Ca²⁺ chelators which might permit this type of experiment has been recently reported (Johnson and Jiang, 1996).

We used an opposite approach, that is slowing the rate of relaxation using CPA. CPA, which is a highly specific and dose-dependent blocker of the SR Ca²⁺-ATPase (Seidler et al. 1989; Goeger and Riley, 1989), has been observed to slow the return of [Ca²⁺] to resting level in frog muscle fibers (S. M. Baylor, personal communication). By slowing the rate of Ca²⁺ pumping using CPA, we were able to slow the isometric relaxation rate of ANT-1 to that of POST muscle bundles. This shows that changing the Ca²⁺ transient duration is a potential means of changing relaxation rate in scup red muscle.

This approach, however, has an important limitation. The ability to slow relaxation rate using CPA does not prove that the Ca²⁺ transient duration is limiting the rate of relaxation in the POST muscle. This point is illustrated by considering the effects of CPA on two models of the mechanism underlying the difference in relaxation rates of the ANT-1 and POST muscles. In model 1, the crossbridge kinetics of the ANT-1 and POST muscle are identical and relatively fast, and the relaxation rate is set by the time course of the Ca²⁺ transient (i.e. [Ca²⁺] falls in approximately 100 ms in the ANT-1 muscle and approximately 200 ms in the POST muscle). In model 2, the Ca²⁺ transients are identical and short (e.g. approximately 10 ms) in the two muscle positions, and the difference in relaxation rate reflects a difference in crossbridge kinetics (i.e. fast in the ANT-1 and slower in the POST muscle). Although the addition of a given concentration of CPA to the ANT-1 muscle will slow the relaxation rate in both models, differences in the responses allow us to differentiate between them.

Two observations suggest that the CPA results are consistent with model 1. First, a twofold slowing of the relaxation rate is achieved with a very low CPA concentration (1.6 μmol l⁻¹). In model 1 (where crossbridge detachment kinetics is assumed to be rapid compared with Ca²⁺ and troponin kinetics), a twofold slowing of the relaxation rate would require an approximately twofold slowing of the Ca²⁺ transient. By contrast, in model 2, a twofold change in relaxation rate would require an approximately 10-fold slowing of the Ca²⁺ transient (e.g. slowing the decay in the [Ca²⁺] from 10 to 110 ms). S. M. Baylor (personal communication) found that a CPA concentration of 10 μmol l⁻¹ slowed the Ca²⁺ transient in frog fibers by at most two- to threefold, and thus it is unlikely that a concentration of 1.6 μmol l⁻¹ (close to the threshold of 0.5 μmol l⁻¹ and well below the saturating level of 50 μmol l⁻¹) could cause a 10-fold change in Ca²⁺ transient duration.

Second, over most of the force range, the time course of relaxation of the CPA-modified ANT-1 muscle is nearly identical to that of the POST muscle (Fig. 4; Table 1). This result is consistent with model 1 and excludes model 2, as it is not possible to obtain the same time course of relaxation if crossbridge kinetics alone were responsible for the difference between ANT-1 and POST muscle. For example, muscle force should start to decline only after [Ca²⁺] falls below that required to saturate troponin. If the ANT-1 and POST muscle had exactly the same Ca²⁺ transient (model 2), they would be expected to have the same duration of maximum force generation for a given stimulus duration. Further, because the exposure to CPA prolongs the time that [Ca²⁺] remains above the saturating level, if model 2 were true, the addition of CPA to slow the relaxation rate of the ANT-1 muscle would be expected to prolong the duration of maximum force generation of the ANT-1 muscle beyond that of the POST muscle. However, neither of these two predictions occurs. As described above, the duration of maximum force is shorter in the unmodified ANT-1 than in the POST muscle, and CPA-modified ANT-1 muscle has precisely the same maximum force duration as POST muscle (Table 1; Fig. 4). These results exclude model 2 and are consistent with the differences in...
relaxation rate being due to differences in the Ca^{2+} transient duration (model 1).

It should be noted that, at low forces, the force records diverge. We have interpreted the result in the following way: at low [Ca^{2+}], the Ca^{2+}-sequestering capability of ANT-1 muscle in the presence of CPA is slower than that of the POST muscle. As [Ca^{2+}] drops, the CPA-inhibited pumps may become proportionally less effective, as suggested by the results of Seidler et al. (1989).

Stronger evidence for model 1 would be obtained by measuring the Ca^{2+} transient (see below). Importantly, the results from another species in which the Ca^{2+} transient has been measured suggest that model 1 may be generally applicable to fish red muscle. Recent experiments on the red muscle of toadfish demonstrate that the reduction in force paralleled the fall in [Ca^{2+}], and that [Ca^{2+}] stays above the threshold for force generation for nearly the whole duration of the twitch. Thus, in toadfish red muscle, the Ca^{2+} transient duration sets the relaxation rate (Rome et al. 1996).

The simplest hypothesis for how the Ca^{2+} transient could be varied is that the faster relaxation rate in the ANT-1 muscle is due to it having more Ca^{2+} pumps than the POST muscle. This hypothesis was not supported by SDS–polyacrylamide gel electrophoresis. Although the volume density of SR Ca^{2+} pumps is known to be correlated to Ca^{2+} transient duration and varies markedly in different types of muscle (Ferguson and Franzini-Armstrong, 1988; Appelt et al. 1991), the gels in our experiments gave no indication that POST muscle might have less Ca^{2+}-ATPase protein. A difference in pump number smaller than that detectable by the gels (<25% difference) would not seem to be enough to change the relaxation rate by twofold.

Another possible cause of a difference in the Ca^{2+} transient duration and hence in relaxation rate is differential parvalbumin (PARV) concentrations. PARV content has been shown to correlate with changes in relaxation rate (Hou et al. 1991; Muntener et al. 1995). Although it is known to exist in the fast-twitch muscle of carp and other teleosts (Zawadowska and Supikko, 1992), whether PARV is present in fish red muscle has been controversial (Zawadowska and Supikova, 1992; Gerday et al. 1979). Preliminary experiments with scup red muscle have not supported the presence of parvalbumin. If PARV is contributing to relaxation, relaxation rate should decrease after PARV becomes saturated with Ca^{2+} (e.g. after long periods of stimulation). This effect is expected to be more dramatic in the presence of the SR Ca^{2+}-pump inhibitor 2,5-di-((tert-butyl)-1,4-benzoquinone (TBQ), which makes binding to parvalbumin, if present, the dominant mechanism for Ca^{2+} sequestration (Jiang et al. 1996). In preliminary experiments in the presence of subsaturating TBQ, we found that in short tetani relaxation rate was markedly slowed. However, when tetanic stimulation duration was increased to 1 s, at which PARV would be expected to be at least partially saturated, no further slowing of relaxation was observed. Finally, a saturable protein would not seem to be a useful relaxation mechanism for a muscle type that is used in a repetitive fashion for long periods (Rome et al. 1992a).

If parvalbumin is absent, and both ANT-1 and POST muscle have the same number of SR Ca^{2+} pumps, how could the Ca^{2+} transient be much faster in the ANT-1 than in the POST muscle? Because the same amount of Ca^{2+} must be removed from troponin in each case, the pumps in the ANT-1 muscle must be pumping faster than those in the POST muscle. It should be noted that the rate at which the SR Ca^{2+}-ATPase pumps Ca^{2+} is not constant, rather it depends on [Ca^{2+}]. Above a certain [Ca^{2+}], (approximately 3 μmol l^-1), pumping rate will be maximal, below this [Ca^{2+}], pumping rates will be lower (Lytton et al. 1992). In theory, if the troponin of the ANT-1 muscle had a lower affinity than that of the POST muscle, the ANT-1 muscle could have a much faster Ca^{2+} transient, shorter troponin occupancy and faster relaxation. If the troponin affinities were in the range where Ca^{2+} pumping rates were submaximal, then the lower-affinity troponin in the ANT-1 muscle would unload Ca^{2+} at a higher [Ca^{2+}], where the pumping rate is rapid. By contrast, in the POST muscle, the Ca^{2+} would unload at a lower [Ca^{2+}], where the pumping rate could be much slower. Because the same amount of Ca^{2+} has to be pumped in both cases, it would be pumped faster in the ANT-1 muscle.

To determine whether this mechanism underlies the faster relaxation rate in ANT-1 muscle, it is necessary not only to measure the force–pCa relationships (an indicator of troponin affinity) but also the pump rate versus [Ca^{2+}] relationships and the Ca^{2+} transients. By developing methods for making measurements from a single fiber within a small bundle, the first Ca^{2+} transients from fish muscle have recently been recorded (Rome et al. 1996). However, because of the small diameter of the fibers and the large volume of connective tissue (Zhang et al. 1996), substantial technical refinements will be required to obtain these measurements on scup red muscle.

Importance of relaxation rate to oscillatory power production

The importance of relaxation to muscle function has been the subject of much recent investigation since Josephson (1985) pioneered the work loop technique in synchronous muscle. Relaxation rate has been hypothesized to be a determinant of contractile frequency in lizards (Marsh, 1990; Johnson et al. 1993) and fish (Altringham and Johnston, 1990; Woleged, 1992) and to influence the power-generating capabilities of cyclically locomoting animals (Marsh, 1988; Moon et al. 1991; Rome and Swank, 1992; Rome et al. 1993; Johnson et al. 1994; for a review, see Josephson, 1993). In all these cases, however, V_{max} and/or activation rate have changed along with relaxation rate. Hence, scup red muscle provides a unique opportunity to study how relaxation rate affects power production in the absence of confounding differences in crossbridge kinetics.

An excellent example of how a slower relaxation rate can limit power output is shown in Fig. 3. The ANT-1 and POST muscle bundles produce similar levels of power when measured under steady-state conditions. However, when measured using the work loop technique under identical conditions, power is
1.5-fold greater in the ANT-1 muscle, due solely to the twofold difference in relaxation rate between the two positions. To a certain extent, stimulation conditions can be altered to compensate for the different relaxation rates. For example, under ‘optimized’ conditions, the stimulation duty cycle is shorter in the POST than the ANT-1 muscle (0.27 versus 0.39). Even so, the difference in relaxation rates still plays an important role as the ANT-1 muscle still generates 1.25-fold higher oscillatory power than the POST muscle (Rome et al. 1993).

It has been suggested that scup have a faster relaxation rate in the anterior musculature to enable the generation of higher levels of power for swimming (Rome et al. 1993). Measurements of scup swimming at 80 cm s\(^{-1}\) at 20°C revealed that the ANT-1 muscle undergoes a strain of only 1.6% and has a long stimulus duty cycle, 46%, while the POST muscle has a longer strain, 5.7%, and a shorter stimulus duty cycle, 26%. The longer stimulus duty cycle in the ANT-1 muscle means that there is less time for relaxation before the muscle is relengthened. Furthermore, the small strain decreases shortening deactivation which, in fish muscle, speeds relaxation rate during shortening (Josephson, 1989; Altringham and Johnston, 1990; Rome and Swank, 1992). Therefore, a faster intrinsic relaxation rate is needed in the ANT-1 muscle to compensate for the short strain and long stimulus duration that it undergoes.

In conclusion, this alteration of relaxation rate, apparently due to a change in the time course of troponin occupancy, represents an effective, but subtle, way to adjust the capabilities of a muscle for different functions in the whole animal. Elucidation of the mechanism for different relaxation rates will further help us to understand how molecular variations in muscle components relate to contraction characteristics and whole-animal locomotory performance.

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Contraction kinetics of red muscle in scup 1307