Force-velocity characteristics for calcium-activated mammalian slow-twitch and fast-twitch skeletal fibers from the guinea pig

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ABSTRACT  Twitch fiber bundles from a slow (soleus) and a fast (extensor digitorum longus) mammalian muscle after mild chemical treatment were activated with calcium and relaxed in calcium-free solution. Like the electrically activated whole muscles, the force-velocity relationship was such that, at each relative load, the steady speed of shortening for the fast fibers was about two times greater than that for the slow twitch fibers. The duration of pre-steady motion in the two types of fibers was the same. The data provide direct evidence that the difference in the shortening characteristics of the two types of fibers is due to differences in their cross-bridge properties.

Slow and fast mammalian skeletal muscles are distinguished in physiological experiments by differences in the properties of their isotonic and isometric contractions (1-4). The study of isotonic shortening shows that in a given species a slow muscle has a lower velocity of shortening, at a particular load relative to the isometric tension, than does a fast muscle, and its maximum velocity of shortening is about half of that for the fast muscle (5). Because the force-velocity relationship for amphibian as well as mammalian skeletal muscle fibers is sensitive to the ionic milieu of the contractile proteins (6-8), the difference in Vmax between slow and fast muscles can be explained by two possibilities: (i) the composition of the intracellular solutes is different in the two muscles, or (ii) there are inherent differences in the properties of the contractile machinery. Recent biochemical experiments (9, 10) showed that the actin-activated ATPase activity of the myosins from these two muscles are different under identical test conditions which indicates that the two contractile proteins have different biochemical properties. Barany's (11) hypothesis that the Vmax and the ATPase (adenosinetriphosphatase, ATP phosphohydrolyase, EC 3.6.1.3) are coupled (and indeed there is qualitative correlation between these parameters, see ref. 9, but compare with ref. 10), can be construed as indirect evidence that the differences in the force-velocity relationships of the slow and the fast mammalian muscles reflect underlying modification in the properties of interaction between actin and myosin.

The present study provides direct evidence for this correlation. The data show that the differences in the isometric contractions of the two muscles are definitely not due to possible differences in ionic strength, in Mg or ATP concentrations, or in their activation processes. This was made possible by the recent finding that a simple modification of the glycinerinating procedure, as originally described by Szent-Györgyi (ref. 12; see also ref. 6), allowed the rabbit psoas to be repeatedly activated by calcium and relaxed in calcium-free solution (ref. 13; manuscript in preparation). This activation-relaxation cycle could be repeated many times without increase in the resting tension. A similar chemical treatment of guinea pig soleus and extensor digitorum longus (EDL) muscles yields fibers that are also activated by calcium.

MATERIALS AND METHODS

Fibers from soleus and EDL from guinea pig were used in this study since these muscles have been used previously as representative of the slow-twitch and fast-twitch muscles respectively in physiological and biochemical experiments in a number of animals (14-16). The animal was sacrificed by a blow on the head and placed in an ice bucket. Immediately thereafter the leg muscles were exposed and continuously bathed with a thin layer of cold physiological salt solution of composition: 121 mM NaCl; 5 mM KCl; 22.5 mM NaHCO3; 1.2 mM NaH2PO4; 2.5 mM CaCl2; 1.2 mM MgCl2; and 5.6 mM glucose (17). The solution had been previously saturated with 95% O2 and 5% CO2 and the pH was close to 7.4. Large bundles of approximately 1 mm width and 1-2 cm length were excised from each of the two muscles. These were gently blotted and transferred on glass cover slips to cold mineral oil (Saybolt viscosity 125/135, Fisher Scientific Co., USA). The bundles could be left in the cold oil for at least an hour without alteration in the physiological properties measured in the present study. Under a dissection microscope, small bundles of 4 to 12 fibers in segments 0.5-1 cm long were very carefully isolated from each of the large bundles with fine stainless steel needles. A segment was tied to stainless steel attachment rods on a force transducer and on a displacement lever as described earlier (18). Care was taken to avoid twisting of individual fibers when tying the bundle. The mounted segment was transferred to a relaxing solution consisting of 140 mM KCl, 5 mM ATP, 1 mM MgCl2, 10 mMimidazole, and 5 mM ethylene glycol-bis-(beta-aminethyly ether)-N,N'-tetraacetic acid (EGTA). The contracting solution contained (CaEGTA + EGTA) instead of EGTA alone. The relative amounts of EGTA and CaEGTA in the contracting solution were calculated by using the stability constant for CaEGTA as 10^6 M^-1 at pH 7.0 so that pCa was 5. The pH of both the relaxing and the contracting solutions was adjusted to 7.0 at 0°C. The solutions were contained in anodized aluminum chambers that were thermoelectrically cooled. The sarcomere length was set near 2.3 μm as indicated by a helium-neon laser beam of 1 mm diameter (Spectra-Physics model 133, Spectra Physics, Inc., Mountain View, Calif.; λ = 6328 Å).

The segments were made permeable in a solution containing 3.4 M glycerol, 105 mM KCl, 3.7 mM ATP, 7.5 mM imidazole, 3.7 mM EGTA, and 0.75 mM MgCl2 as follows. The mounted segment was transferred from the relaxing solution to the glycerol-containing solution at 10°C. This incubation lasted 10 min after which the segment was returned to the relaxing solution at the same temperature. It was left in the relaxing solution for additional 10 min. At the completion of this treatment, the segment was found to be permeable to solutes in the

Abbreviations: EDL, extensor digitorum longus; EGTA, ethylene glycol-bis-(beta-aminethyly ether)-N,N'-tetraacetic acid; P0, isometric tension; pCa, minus the log of free calcium concentration.
At the treated guinea-pig to After the solution bathing in solution cycling, to amount of the two 1

FIG. 1. Isometric activation with calcium of the chemically treated guinea-pig soleus (slow twitch) and EDL (fast twitch) fiber bundles. After the fibers were made permeable as described in the text, they were transferred to a chamber containing relaxing solution. At the arrows marked C, the bundles were transferred to the contracting solution (pCa = 5). At the arrows marked R, the fibers were returned to the relaxing solution. The temperature was maintained at 10°C throughout the experiments.

bathing solution as tested by transferring it to the contracting solution and measuring the isometric tension (P0) developed.

In the experiments described below, each segment was carried through five to six contraction-relaxation cycles. The P0 for the last contraction was within 20% of that in the first contraction. In the few cases where P0 fell more than 20% during cycling, the experiments were discarded.

RESULTS
Fig. 1 shows the isometric activation with calcium (10^-5 M) of the two types of fibers. In each bundle, the tension was maximal within 60 sec. The tension developed by these fibers was sensitive to temperature, lowering the temperature reduced the amount of tension at the fixed pCa used. The plateau reached with P0 = 5 was not changed on transferring the fibers to another solution at the same temperature but with higher free calcium.

Fig. 2 shows the isotonic displacement responses of calcium activated slow and fast fibers to quick application of three different load steps. In each case, the fibers were initially activated under isometric conditions. After the force became steady, the loading for the coming isotonic phase was adjusted and muscle was released so that it could shorten against the load. The displacement response of the mammalian fibers consists of a pre-steady phase followed by a steady phase. During the steady phase, the velocity of shortening is fairly constant. In the top panels of Fig. 2, the steady speeds are back extrapolated by sloping dashed lines and their intersection with the instantaneous displacement in the records are marked by arrows. The pre-steady motion is seen as oscillating around the dashed lines and the part of oscillation before it crosses over the sloping line consists of three phases (18–20). First, there is quick initial shortening that occurs simultaneously with the application of the force step at time t0. This is followed by a phase during which the velocity of shortening is faster than the steady speed. During the third phase shortening speed is slower than steady speed. The null time, τ, of the pre-steady motion is the time elapsed between t0 and the arrow marking the end of the third phase of the transient. Amplitude of the pre-steady motion is the amount of fluctuation from the dashed line.

Duration of pre-steady motion of the fast and slow fibers
Quantitative comparison of duration of the pre-steady motion in the two types of fibers is made by measuring the null times of the isotonic velocity transients from the type of records shown in Fig. 2. These measurements for the EDL fibers in Fig. 2, indicate that the null times at relative loads of 0.46 and 0.69 are 20 and 33 msec, respectively. This parameter for the soleus fibers has values of 22 msec and 30 msec for comparable relative loads. The results from this and three other experiments are summarized in Table 1 and show that null times of the pre-steady motion in the two types of fibers are quite similar.

Steady speeds
Comparison of steady speeds of the fast and the slow fibers at three values of relative loads, in Fig. 2, shows that fibers from the EDL muscle are about twice as fast as fibers from the soleus muscle. In Fig. 3, force velocity relationships are plotted for pooled results from four bundles and the results show that the speeds of the two types of fibers differ by about a factor of two for the entire range of relative loads. The estimated value of Vmax for the guinea pig EDL at 10° is about 0.5 L0/sec, where L0 indicates the length of the activated fiber bundle. The Vmax for the slow fibers is estimated to be 0.2 L0/sec. The Vmax at 6° for the EDL fibers from two guinea pigs, estimated as above, was found to be 0.25 L0/sec.

DISCUSSION
A major finding of the present study is that the calcium-activated mammalian fibers from the guinea-pig EDL and soleus muscles differed in their contraction speeds by a factor of about two. This is the most direct evidence that the differences in the isotonic displacement behavior between the slow and the fast mammalian muscles originates in the contractile machinery. Because the isotonic contraction properties of the chemically treated fibers from a slow and a fast muscle of the guinea-pig were examined after equilibration in bathing media of identical composition, the study shows that the difference in the in vitro

Table 1. Transient null times (τ) with varying relative loads PRef = (P/P0)

<table>
<thead>
<tr>
<th>Experiment*</th>
<th>PRef</th>
<th>τF(msec)</th>
<th>PRef</th>
<th>τS(msec)</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>0.30</td>
<td>14</td>
<td>0.45</td>
<td>22</td>
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<td>0.78</td>
<td>43</td>
<td>0.67</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>0.69</td>
<td>18</td>
<td>0.58</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>0.60</td>
<td>24</td>
<td>0.71</td>
<td>33</td>
</tr>
</tbody>
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PRef indicates load relative to the isometric tension; P refers to the actual load; τ indicates null time.
* All experiments were performed at 10°C.
FIG. 2. The quick displacement response of a fast (A) and a slow (B) fiber bundle to three different load steps (P/P₀). The two bundles in this experiment were obtained from the same animal. In each panel, the top trace indicates the length of the fibers, the middle indicates the force developed, and the bottom trace shows the zero force. The mean steady isometric force before the application of the load step was 92 mg in A and 43 mg in B. The length scale is given as Å per one-half sarcomere. At the end of the quick shortening that occurs simultaneously with the application of the load steps, there are initially fast matching oscillations in both the displacement (top) traces and the force (middle) traces. These damped oscillations originate in the equipment and last 10–15 msec. In addition, there are slower oscillations in the displacement traces and these last 50–100 msec. These slow transients are thought to arise in the cross-bridge mechanism (18–20). The sloping dashed lines in the top panels back extrapolate the speeds of shortening at the end of the cross-bridge transients.

properties of the two muscles can not be due to possible differences in their internal milieu. The direct activation of the contractile proteins with calcium also rules out any possible contribution of the external membranes and of the sarcoplasmic reticulum on the shortening properties of these muscles.

The contractile force of skeletal muscle is generated by the cross bridges that form between the actin and myosin filaments. During shortening, the two sets of filaments slide past each other and the dependence of shortening velocity on load as revealed by the force-velocity relationship imposes constraints on the kinetic properties of the cross bridge turnover (23). The data presented here show that some of these kinetic properties must be different between the slow and the fast mammalian muscle fibers.

Comparison with other studies

Earlier studies have shown that contraction speeds of the intact slow- and fast-twitch muscles also differed by a factor of about two (5). The studies with the electrically stimulated intact cells are at 37°, whereas the present experiments with the calcium-activated fibers were made at 10°. To compare the actual numbers for shortening speed of each individual muscle from
FIG. 3. Force-velocity relationships for slow and fast muscles in four different fiber bundles at 10\(^\circ\). The lengths of the fibers varied between 1 and 3 mm. The points were measured from records as described for Fig. 2. The solid lines are calculated from Hill's relation (21-22):

\[
\frac{P}{P_0} = \frac{1 - V/V_{\text{max}}}{1 + (P_0/a) \cdot (V/V_{\text{max}})}
\]

with \((P_0/a) = 4\) as is found also for frog muscle at 0\(^\circ\); \(V_{\text{max}} = 0.5\) Lo/sec for the EDL and 0.2 Lo/sec for the soleus.

the two studies, a value of \(Q_{10}^*\) is needed. By assuming a value for \(Q_{10}\) as four, the \(V_{\text{max}}\) of shortening at 37\(^\circ\) is extrapolated from the measured velocities at 10\(^\circ\) for the calcium-activated fibers, to be 25 Lo/sec for the fast fibers and 10 Lo/sec for the slow fibers. The sarcomere length corresponding to L0 at which the fibers were activated was 2.3 \(\mu\)m/sec so the \(V_{\text{max}}\) is 58 \(\mu\)m/sec for the guinea pig EDL and 23 \(\mu\)m/sec for the soleus. These values are close to those estimated for the electrically activated whole fast and slow muscles from some small mammals (14). It is interesting to note that, the \(Q_{10}\) value needed to make the above comparison is close to the \(Q_{10}\) value obtained for Mg\(^{2+}\)-dependent actin-heavy meromyosin ATPases at saturating actin concentrations (24). Also, comparison of estimated unloaded shortening velocities at 10 and 6\(^\circ\) for the guinea-pig EDL from the measured force-velocity relationships gave a similar value for the \(Q_{10}\) in this short temperature range.

Pre-steady kinetics

The transient phase that precedes the steady velocity of shortening at each relative load gives additional information regarding the cross-bridge mechanisms of force generation in the skeletal muscle (25, 26). A quantitative analysis of these isotonic velocity transients was made in the present study by measuring the null times for the two types of mammalian muscles. As in earlier studies, null times vary with relative loads, being shorter for smaller values of relative loads. At 10\(^\circ\), for comparable relative loads, the null times for the frog skinned muscles were the same as those for the frog skinned fibers at 5\(^\circ\). This parameter has a high \(Q_{10}\) in all preparations studied so far (refs. 13, 18, and 19; also unpublished observations on the guinea pig EDL), which is indirect evidence that, under the same experimental conditions, the isotonic velocity transients in the chemically-skinned mammalian fibers are of much longer duration than those in the frog skinned fibers. This shows that there are differences between the cross-bridge properties of the mammalian muscles and of the frog muscle in aspects that determine the duration of the pre-steady motion.

The duration of the transients was found to be similar in the fast and the slow mammalian fibers. This means that the relevant cross-bridge parameters underlying these properties of the velocity transients are similar in the two types of mammalian fibers.

Further analysis of the transients, from higher resolution records, is required to characterize differences in the cross-bridge parameters of the slow and the fast muscles. The data presented here allowed measurements of the null times. An additional quantitative evaluation of the transients is possible by measuring the amplitude of the velocity transients (19, 27, 28). Also, it appears that the Z-lines in the slow fibers are about twice as wide as those in the fast fibers (29-32). Because it is of interest to know if the Z-line structure makes a significant contribution to the overall compliance of the skeletal muscle (33), measured as the quick displacement to drop the force of activated muscle fibers from \(P_0\) to 0 (19, 21), it would be worthwhile to make precise compliance measurements on the two types of fibers.

Concluding remarks

A chemical treatment procedure is described for disrupting the cell membranes of mammalian fiber bundles from a slow-twitch and a fast-twitch muscle of the guinea pig. The difference in the isotonic contraction properties of the calcium-activated fibers from the two types of muscles is the same as that between the shortening speeds of the electrically activated intact slow and fast mammalian muscles. This is evidence that these differences originate within the cross-bridge mechanism of the slow and the fast muscles. Also, because the procedure of chemically “skinning” the guinea-pig fibers is similar to that
used earlier on the rabbit psoas, it may be that a mild glycerol treatment is widely applicable for preparing mammalian fiber bundles that can be repeatedly activated by calcium.

Dr. Richard Podolsky provided the initial stimulus for this study and I am grateful to him for his continual support. Dr. John Buck and Dr. Evan Eisenberg also gave constant encouragement.