Cross bridges account for only 20% of total ATP consumption during submaximal isometric contraction in mouse fast-twitch skeletal muscle

Shi-Jin Zhang, Daniel C. Andersson, Marie E. Sandström, Håkan Westerblad, and Abram Katz

Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

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Zhang, Shi-Jin, Daniel C. Andersson, Marie E. Sandström, Håkan Westerblad, and Abram Katz. Cross bridges account for only 20% of total ATP consumption during submaximal isometric contraction in mouse fast-twitch skeletal muscle. Am J Physiol Cell Physiol 291: C147–C154, 2006. First published February 15, 2006; doi:10.1152/ajpcell.00578.2005.—It is generally believed that cross bridges account for >50% of the total ATP consumed by skeletal muscle during contraction. We investigated the effect of N-benzyl-p-toluene sulphonamide (BTS), an inhibitor of myosin ATPase, on muscle force production and energy metabolism under near-physiological conditions (50-Hz stimulation frequency at 30°C results in 35% of maximal force). Extensor digitorum longus muscles from mice were isolated and stimulated to perform continuous isometric tetanic contractions. Metabolites of energy metabolism were analyzed with fluorometric techniques. ATP turnover was estimated from the changes in phosphocreatine (PCr), ATP, and lactate (ΔATP ∼ ΔPCr + [1.5 lactate]). During contractions (2–10 s), BTS decreased force production to ∼5% of control. Under these conditions, BTS inhibited ATP turnover by only 18–25%. ATP turnover decreased markedly and similarly with and without BTS as the duration of contraction progressed. In conclusion, cross bridges (i.e., actomyosin ATPase) account for only a small fraction (∼20%) of the ATP consumption during contraction in mouse fast-twitch skeletal muscle under near-physiological conditions, suggesting that ion pumping is the major energy-consuming process.

ATPase; high-energy phosphates; lactate

DURING MUSCLE CONTRACTION, 50–80% of the total ATP consumption is thought to be accounted for by actomyosin ATPase, with the remainder accounted for by ion pumping (1, 7, 12, 23, 25, 30, 31, 37, 38, 41). Such estimates are usually derived from experiments performed at low temperatures (0–20°C) and under conditions that elicit maximal force. In intact muscle preparations, estimates of cross-bridge ATP utilization have often been obtained with the stretch technique (14, 31, 36). The principle of this technique is that at muscle lengths greater than the optimal length, the degree of actomyosin interaction is decreased, and, hence, less force is produced. A relationship is plotted between heat production (or ATP turnover) and tension at different muscle lengths. The line defining the relationship is extrapolated to zero tension, which then allows the partitioning of heat production into tension-dependent (actomyosin ATPase) and tension-independent [sarcoplasmic reticulum (SR) Ca2+-ATPase and Na+-K+-ATPase] components (12, 13). Thus the method is indirect. An alternative approach involves the use of agents that inhibit cross-bridge formation, such as 2,3-butanedione monoxime (BDM) (16, 37). In intact muscle preparations, use of BDM is complicated because it can inhibit ryanolide receptors (40) and, consequently, SR Ca2+ release, as well as interfere with other aspects of Ca2+ handling (4, 9).

Recently, N-benzyl-p-toluene sulphonamide (BTS) was identified as a highly specific inhibitor of myosin II ATPase that does not interfere with skeletal muscle Ca2+ handling (3, 5, 29, 35, 46). We have therefore used BTS to assess cross-bridge ATP consumption during isometric contraction in intact muscle preparations under near-physiological conditions (stimulation frequency of 50–70 Hz, 30°C). Under these conditions, cross-bridge ATP consumption in mouse fast-twitch muscle accounts for only ∼20% of the total ATP consumption.

METHODS

Materials. BTS was obtained from Sigma. All other reagents were from either Sigma or Boehringer Mannheim.

Male NMRI strain mice (∼30 g) and Wistar rats (∼100 g) were housed at room temperature with 12:12-h light-dark cycle. Food and water were ingested ad libitum. Animals were euthanized by rapid cervical dislocation and thereafter extensor digitorum longus (EDL) and soleus muscles were isolated. The Stockholm North local ethical committee approved all animal care procedures.

Mounting, solution, and stimulation. Stainless steel hooks were tied with nylon thread to the muscle tendons. The muscles were then transferred to a stimulation chamber (World Precision Instruments) and mounted in between a force transducer and an adjustable holder. The chamber temperature was set at 30°C with a water-jacketed circulation bath in all but one of the experiments, which was performed at 15°C. We measured the temperature of mouse leg muscles in situ to be 35.1 ± 0.3°C (n = 8), but chose to perform most of our experiments at 30°C. This is because in preliminary experiments, we found that muscle force production decreased by ∼10% in 20 min, whereas at 30°C, force production remained stable (see below). The muscle was bathed in a Tyrode solution of the following composition (in mM): 121 NaCl, 5 KCl, 1.8 CaCl2, 0.4 NaH2PO4, 0.5 MgCl2, 24 NaHCO3, 0.1 EDTA, 5.5 glucose, and 0.1% fetal calf serum. The solution was continuously gassed with 5% CO2-95% O2, which results in a pH of 7.4. Muscles were stimulated with current pulses (0.5-ms duration; ∼150% of the voltage required for maximum force response) via plate electrodes lying parallel to the fibers. The muscle was set to the length where maximum tetanic force was produced. Thereafter, a small volume of a BTS stock solution (in DMSO) was added to the chamber, yielding a final concentration of 25 μM; contralateral control muscles received an equivalent volume of DMSO (final concentration = 0.05% vol/vol).

In all experiments, muscles were incubated for 60 min in the absence or presence of BTS before initiation of stimulations. In one series of experiments, muscles were frozen in liquid N2 after the 60-min incubation period (nonstimulated). In another series, following the 60-min incubation period, muscles were stimulated to perform isometric contractions (EDL, 300-ms tetanic duration and soleus,
600-ms tetanic duration at 1-min intervals) to generate force-frequency curves.

Different stimulation protocols were used before the stimulated muscles were frozen. First, EDL muscles were stimulated to perform continuous tetanic contractions (50 Hz, tetanic duration of 2–10 s) in the presence of 2 mM NaCN (NaCN). Second, fatigue was induced by repeated tetani: 70 Hz, 300-ms tetanic duration, 1 train/2 s for a total of 100 tetani in soleus in the absence or presence of NaCN. Third, fatigue was induced by repeated tetani: 70 Hz, 300-ms tetanic duration, 1 train/2 s for a total of 50 tetani in EDL; 50 Hz, 600-ms tetanic duration, 1 train/2 s for a total of 100 tetani in soleus in the absence or presence of 2 mM NaCN. NaCN was added 5 min before onset of stimulation to eliminate mitochondrial O2 utilization (19). CN-mediated inhibition of O2 consumption in isolated human and mouse skeletal muscle preparations occurs with K values that range from 5 to 10 μM (21, 45) and is complete at a concentration of 1 mM (28). Therefore, it is likely that mitochondrial respiration was fully inhibited by 2 mM NaCN in the present study. Immediately after the last stimulation pulse, the chamber was lowered (<1 s), leaving the muscle suspended in the air between the force transducer and the adjustable holder. The muscle was then detached and frozen in liquid N2 (<5 s). With this procedure the metabolite values in the frozen muscle represent those at the end of contraction because lactate release from the muscle is stopped, whereas phosphocreatine (PCr) resynthesis, an O2-dependent process (34), is prevented by the NaCN.

Analysis. Force signals were sampled on-line and stored in a desktop computer for subsequent analysis. The muscles were stored in liquid N2 until they were freeze dried. The hooks were removed and the muscles were dissected free of the nonmuscle constituents and powdered. The powder was thoroughly mixed and aliquoted.

For analysis of glycogen, aliquots of powder were digested with hot 1 N KOH and hydrolyzed enzymatically to free glucose. Glucose was then analyzed enzymatically with a fluorometric technique (26). For analysis of metabolites, ice-cold 0.5 M perchloric acid was added to aliquots of muscle powder. The extract was kept in an ice bath for 15 min while being agitated with a vortex mixer and then centrifuged (10,000 g). The supernatant was neutralized with 2.2 M KHCO3 and again centrifuged. The latter supernatant was assayed for PCr, Cr, ATP, Pi, glucose 6-P, lactate, and malate with enzymatic techniques [changes in NAD(P)H] adapted for fluorometry (26). In some experiments, lactate was also analyzed in the medium. To adjust for variability in solid nonmuscle constituents, metabolite values were divided by the sum of PCr + Cr (total Cr) and then multiplied by the mean total creatine content for the whole material. BTS did not significantly affect total creatine under any condition (data not shown). Total creatine averaged 90 ± 2 to 107 ± 3 μmol/g dry wt in EDL in the different series of experiments, and 60 ± 4 and 71 ± 2 for mouse and rat soleus, respectively.

ATP turnover was calculated from the following equation: \(-2ΔATP = ΔPCR + (1.5Δlactate)\), as we have used earlier (18–20), where Δ refers to the mean contraction value minus the mean basal value. The same basal values (see Table 1) were used for all ATP turnover calculations at 30°C. With the use of this formula, it is assumed that 1) glycogen is the sole source for lactate production (hence 1.5 mol ATP per mol of lactate produced) during short-term intense contractions; 2) the decreases in ATP are associated with stoichiometric increases in inosine 5′-monophosphate in isolated muscle EDL muscle (19), thereby justifying the term \(-2ΔATP\); and 3) there is no ATP production from oxidative phosphorylation.

Statistics. Values are given as means ± SE, unless indicated otherwise. Statistically significant (P < 0.05) differences were determined with the paired t-test.

RESULTS

Effects of BTS on force production in fast- and slow-twitch muscles. In preliminary experiments it was found that a concentration of 25 μM BTS and an incubation time of 1 h were required to obtain maximal and stable effects on force production at 30°C (data not shown). In the EDL muscle, BTS inhibited force production to <5% of control at frequencies up to 70 Hz (Fig. 1A). At higher frequencies, the effect of BTS was slightly less but still very marked. This is consistent with the observation of the high specificity of BTS for skeletal muscle myosin II ATPase (5), which is highly expressed in EDL muscle of NMRI mice (~80% MHC IIb; ~20% MHC IIa).

Table 1. Effect of BTS on metabolites in mouse EDL muscle in basal state

<table>
<thead>
<tr>
<th></th>
<th>PCr</th>
<th>Cr</th>
<th>Pi</th>
<th>ATP</th>
<th>Glycogen</th>
<th>G6P</th>
<th>Lactate</th>
<th>Malate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>70.2 ± 1.3</td>
<td>21.7 ± 1.3</td>
<td>16.0 ± 0.3</td>
<td>23.8 ± 0.5</td>
<td>78.3 ± 8.2</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td>BTS</td>
<td>74.0 ± 0.7*</td>
<td>17.3 ± 0.7*</td>
<td>16.8 ± 1.6</td>
<td>23.8 ± 0.6</td>
<td>75.3 ± 5.6</td>
<td>0.3 ± 0.1</td>
<td>0.4 ± 0.2</td>
<td>0.10 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5–6 muscles. Values are given in μmol/g dry wt, with the exception of glycogen (in μmol glucosyl units/g dry wt). PCr, phosphocreatine; G6P, glucose 6-P; BTS, N-benzyl-p-toluene sulfonamide. Muscles were incubated for 60 min at 30°C in the absence (Con) or presence of 25 μM BTS, frozen, and analyzed. *P < 0.01 vs. Con by paired t-test.

Fig. 1. Effect of N-benzyl-p-toluene sulfonamide (BTS) on force production in isolated skeletal muscle. Muscles were stimulated at 30°C, as described in METHODS. Shown are data for mouse extensor digitorum longus (EDL) (A), mouse soleus (C), and rat soleus (E) muscles, respectively, before (●) and after (○) 60-min exposure to 25 μM BTS. Data for the contralateral mouse EDL (B), mouse soleus (D), and rat soleus (F) muscles, respectively, are shown before (●) and after (○) 60-min exposure to diluent (DMSO). Values are means ± SE for 6 muscles.
In contrast, the effect of BTS was markedly diminished, but still significant in mouse soleus muscle (Fig. 1C). We reasoned that the large residual effect of BTS was the consequence of a significant proportion (~40%) of fast-twitch fibers in soleus muscle of NMRI mice (27). To examine this possibility, additional experiments were performed on rat soleus muscle, which contains relatively few fast-twitch fibers (10–20%) (39). Indeed, the BTS effect was markedly diminished and in general BTS inhibited <20% of force in rat soleus muscles (Fig. 1E).

In EDL muscle, BTS markedly abolished force production during a series of 50 repeated tetani. The force × time integral in BTS-treated muscles amounted to only 3.5 ± 0.4% of that in control (n = 6). In the mouse soleus, the force × time integral in BTS-treated muscles amounted to 54 ± 6% (n = 3) of that in control; the corresponding value for the rat soleus was 101 ± 12% (n = 4). These results were consistent with those obtained during single tetani (Fig. 1). All subsequent experiments were performed only on mouse EDL muscles.

Continuous tetani with NaCN at 15°C. We first employed conditions traditionally used to distinguish between relative cross-bridge and SR-Ca2+ ATP utilization (low temperature and high force). Accordingly, muscles were studied at 15°C and stimulated at 50 Hz, which resulted in near-maximal tetanic force (24, 32). Muscles were incubated for 60 min in the absence or presence of BTS and were then either frozen (basal) or stimulated continuously for 2 s. Representative force recordings are presented in Fig. 2. In contrast to the almost complete inhibition of force production by BTS at 30°C, BTS inhibited the force-time integral by 77.9 ± 3.6% (n = 6) at 15°C. BTS had no significant effect on high-energy phosphates or lactate in the basal state (Fig. 3). After contraction, there was an attenuated increase in lactate and decrease in PCr in the BTS-treated muscles, whereas ATP remained stable during contraction in both groups. ATP turnover averaged 28.3 µmol/g dry wt during control and 16.6 during BTS. Correcting for residual force, cross bridges accounted for 53% of the total ATP consumption during the 2-s contraction at 15°C [(28.3 – 16.6)/28.3] × [100/77.9] × 100%. Thus BTS yielded results that were comparable to those obtained with other techniques under similar experimental conditions.

Continuous tetani with NaCN at 30°C. Next, we examined the effects of BTS on ATP turnover under more physiological conditions. We stimulated muscles at 30°C and at 50 Hz, because in living rodents EDL muscles are usually activated at about this frequency (11). In addition, this frequency allowed for maintenance of nearly constant force during prolonged tetani. At this frequency, ~35% of maximal force is obtained (Fig. 1, A and B). Muscles were treated as at 15°C, with the exception that in addition to the 2-s continuous contraction, 5-
Table 2. Effect of BTS on metabolites in mouse EDL muscle after continuous tetanic contractions

<table>
<thead>
<tr>
<th>Contraction Time</th>
<th>PCr</th>
<th>Cr</th>
<th>ATP</th>
<th>P&lt;sub&gt;i&lt;/sub&gt;</th>
<th>G6P</th>
<th>Lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 s</td>
<td>43.4±3.0</td>
<td>56.6±3.0</td>
<td>25.7±0.4</td>
<td>35.4±2.0</td>
<td>4.8±0.2</td>
<td>20.9±2.6</td>
</tr>
<tr>
<td>BTS</td>
<td>54.4±2.4†</td>
<td>45.6±2.4‡</td>
<td>27.2±0.4</td>
<td>27.1±2.6*</td>
<td>3.9±0.5</td>
<td>16.5±2.2†</td>
</tr>
<tr>
<td>5 s</td>
<td>28.5±2.6</td>
<td>71.4±2.6</td>
<td>22.9±0.3</td>
<td>49.5±5.7</td>
<td>11.5±1.1</td>
<td>34.0±2.3</td>
</tr>
<tr>
<td>BTS</td>
<td>37.4±2.7†</td>
<td>62.5±2.7†</td>
<td>23.6±1.0</td>
<td>36.6±1.8*</td>
<td>7.4±0.8*</td>
<td>26.5±1.9‡</td>
</tr>
<tr>
<td>10 s</td>
<td>21.5±0.7</td>
<td>78.5±0.7</td>
<td>13.6±0.9</td>
<td>52.6±1.4</td>
<td>10.6±1.3</td>
<td>40.3±1.2</td>
</tr>
<tr>
<td>BTS</td>
<td>24.4±1.4</td>
<td>75.6±1.4</td>
<td>23.4±1.1‡</td>
<td>41.8±1.5†</td>
<td>11.7±0.7</td>
<td>37.6±2.5</td>
</tr>
</tbody>
</table>

Values are means ± SE for 6–7 muscles, and are given in µmol/g dry wt. EDL, extensor digitorum longus. Muscles were incubated for 55 min at 30°C in the absence (Con) or presence of 25 µM BTS. At 55 min, NaCN was added to the medium, bathing both groups of muscles and yielding a final concentration of 0.3 mM. At 60 min, muscles were stimulated at 50 Hz for the indicated durations and immediately frozen. Lactate release was not measured because it is insignificant during such short-term contractions (19). *P < 0.05; †P < 0.01; ‡P < 0.001 vs. Con by paired t-test.
lactate), ATP turnover averaged 261 and 195 μmol/g dry wt in control and BTS, respectively, and, hence, cross bridges accounted for only 25% of the total ATP consumption during repeated tetani. Thus the differential effects of BTS on ATP turnover and force × time integral during repeated tetani were in agreement with those observed during continuous tetani (Fig. 5). Of note, recent studies (8) used BTS to examine the role of actomyosin interactions during repeated contractions in activation of mitogen-activated protein kinases in isolated mouse EDL muscle. It was assumed that BTS resulted in substantial decreases in the rates of ATP consumption. The present results indicate that this assumption may not be justified.

**DISCUSSION**

It is generally accepted that actomyosin ATPase accounts for 50–80% of the ATP consumed during muscle contraction during conditions of maximal force generation. The present results indicate that a much smaller fraction of the total ATP consumption during conditions of maximal force generation. The present results indicate that this assumption may not be justified.

**Actomyosin and SR ATP consumption.** Under all conditions studied at 30°C, cross bridges accounted for only ~20% of total ATP consumption during isometric contractions. Inherent in this conclusion is the assumption that 1) mitochondrial ATP production was fully suppressed by NaCN, and 2) BTS inhibited myosin ATPase to the extent that it inhibited force and that it did not affect other energy-consuming processes. That mitochondrial ATP production was abolished by NaCN in the present study is suggested by earlier findings in isolated muscle preparations (21, 28, 45). Moreover, NaCN abolished the contraction-mediated increase in malate, a marker of oxidative metabolism, in the present study.

While other studies (5) have addressed the issue of BTS specificity on purified enzymes, potential nonspecific effects always remain a concern when working with intact muscle preparations. It has previously been shown that BTS inhibits actin-activated purified myosin ATPase with an IC50 of 3–5 μM (5, 35). Moreover, in skinned muscle fibers, BTS inhibited force and actomyosin ATPase activity to similar extents and in the presence of 25 μM BTS virtually all force and actomyosin ATPase activity were abolished (<5% of control) (46). Therefore, one may conclude that because force production was inhibited by ~95% in the EDL muscle, actomyosin ATPase was inhibited to a similar extent.

**Table 3. Effect of BTS on ATP turnover in mouse EDL muscle**

<table>
<thead>
<tr>
<th>Tetanic Time</th>
<th>−2ΔATP</th>
<th>−ΔPCr</th>
<th>+1.5Δlactate</th>
<th>ATP Turnover</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmol/g dry wt</td>
<td>μmol/g dry wt/s</td>
<td>mM/s</td>
<td></td>
</tr>
<tr>
<td>2 s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>0</td>
<td>26.8</td>
<td>31.1</td>
<td>57.9</td>
</tr>
<tr>
<td>BTS</td>
<td>0</td>
<td>19.6</td>
<td>24.2</td>
<td>43.8</td>
</tr>
<tr>
<td>5 s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>1.8</td>
<td>41.7</td>
<td>50.7</td>
<td>94.2</td>
</tr>
<tr>
<td>BTS</td>
<td>0.4</td>
<td>36.6</td>
<td>39.2</td>
<td>76.2</td>
</tr>
<tr>
<td>10 s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>20.4</td>
<td>48.7</td>
<td>60.2</td>
<td>129.3</td>
</tr>
<tr>
<td>BTS</td>
<td>0.8</td>
<td>49.6</td>
<td>55.8</td>
<td>106.2</td>
</tr>
</tbody>
</table>

Values are calculated from the mean values shown in Tables 1 and 2. ΔATP, ΔPCr, and Δlactate values are measured as μmol/g dry wt of ATP turnover = −2ΔATP − ΔPCr + (1.5Δlactate), where Δ denotes the mean contraction value minus the mean basal value. A value of 0 assumes no increase in ATP during a 2 s tetanus (see text). In the far right column, ATP turnover is also expressed in mM/s, assuming 2.05 liter intracellular water per kg dry wt (33).

**Fig. 5.** Comparative effects of BTS on ATP turnover and force × time integral from mouse EDL muscle during continuous (2–10 s) and repeated contractions (50 tetani). Values are means (ATP turnover; open bars) or means ± SE (force × time integral; solid bars) and are expressed as %control, which is set to 100%.

**Fig. 6.** Effect of BTS on force production of mouse EDL muscles during 50 repeated contractions in the presence of Na+ cyanide (NaCN). After incubation in the absence (●) or presence (○) of 25 μM BTS, muscles were stimulated to perform isometric contractions (70 Hz, tetanic duration = 300 ms, 1 train/2 s). Values are means ± SE for 6 muscles.
The other major ATP consuming enzymes in contracting skeletal muscle are considered to be SR Ca\(^{2+}\)-ATPase and Na\(^{+}\)-K\(^{+}\)-ATPase (12, 23). In intact frog skeletal muscle fibers, BTS abolished twitch force but had no effect on the shape of the Ca\(^{2+}\) transient (5). Other studies (3, 29) have also demonstrated that BTS does not affect the shape of Ca\(^{2+}\) transients in intact rat and mouse fast-twitch muscle fibers during isometric tetanic contractions. Furthermore, 25 \(\mu\)M BTS had no effect on SR Ca\(^{2+}\)-ATPase activity in skinned muscle fibers (46). These data suggest that BTS did not affect Ca\(^{2+}\) release or uptake by the SR in the present study. The extent to which BTS may affect Na\(^{+}\)-K\(^{+}\)-ATPase is not clear. At \(\approx 100 \mu\)M BTS, the refractory period for repetitive stimulation was lengthened in frog muscle fibers, indicating action potential prolongation (5), which may indicate a potential effect on Na\(^{+}\)-K\(^{+}\)-ATPase activity. Considering that we used only 25 \(\mu\)M BTS and that force did not decrease during the continuous tetani in BTS-treated muscles, it appears unlikely that BTS affected Na\(^{+}\)-K\(^{+}\)-ATPase activity to any significant extent under the conditions studied.

Our current estimate that cross bridges account for only 20% of the total ATP utilization should be considered with respect to three factors: 1) stimulation frequency, 2) temperature, and 3) species. With regard to stimulation frequency, at 50 Hz (30°C), EDL muscle is producing only \(\sim 35\%\) of maximal tetanic force. Studies on skinned muscle fibers have demonstrated that the relative contribution of cross bridges to total ATP consumption decreases markedly as the relative force decreases (37). For example, at maximal force, cross bridges account for 75% of total ATP consumption, whereas at 35% and 10% of maximal force, the cross-bridge component decreases to \(\sim 45\%\) and \(\sim 25\%\), respectively (37). Thus the current findings on intact muscle are in good agreement with results from skinned fibers.

With respect to temperature, most studies that have estimated cross-bridge-dependent and -independent ATP consuming components have been performed at 5–20°C. In this temperature range, the relative ATP consumption of SR Ca\(^{2+}\) pumps increases from 28% at 5°C to 48% at 20°C in skinned fibers (37). With the use of other techniques, Rall (30) estimated that SR Ca\(^{2+}\)-ATPase activity increases in this temperature range, accounting for 56% of total energy liberation at 20°C. Thus temperature could also contribute to the low relative cross-bridge ATP utilization at 30°C in the present study.

With respect to species, most earlier studies that estimated cross-bridge-dependent ATP consumption were performed on frog muscle, although studies on fish, chicken, mice, and rats have also been performed (2, 7, 25, 31, 41, 42). Interestingly, recent studies (38) of fast-twitch (IIA/B and IIB) skinned human muscle fibers (at 20°C) demonstrated that cross bridges accounted for only 35–41% of total ATP consumption, whereas SR Ca\(^{2+}\) pumping accounted for 52–55%. Indeed estimates for SR Ca\(^{2+}\) pump ATP utilization as high as 70% of total (at 20°C) have been reported in skinned mammalian fast-twitch muscle preparations (22). It has also been demonstrated that free myoplasmic [Ca\(^{2+}\)] declines much faster at the end of a tetanic contraction in mouse fast-twitch muscle fibers than in frog fast-twitch fibers, indicating faster SR Ca\(^{2+}\) pumping in the former (44). These data indicate that fast-twitch mammalian fibers exhibit higher rates of SR Ca\(^{2+}\) pumping than amphibian muscles, especially at high temperatures.

The implication from the present findings then is that ion pumping accounts for most (~80%) of the energy consumption during submaximal isometric contraction in mouse fast-twitch muscle at 30°C. Assuming that SR Ca\(^{2+}\)-ATPase is primarily responsible for the non-cross-bridge-dependent energy consumption during contraction (42), we calculate that the SR Ca\(^{2+}\) pumps consumed 10.7 mM ATP/s during the 2-s tetanus in the presence of BTS at 30°C (Table 3). Data from skinned fibers show that SR Ca\(^{2+}\) pumping is near maximal (~90%) already at 35% of maximal force (37). The value of 10.7 mM ATP/s can be compared with estimates derived under comparable conditions for intact mouse fast-twitch fibers, where it was calculated that SR Ca\(^{2+}\) uptake occurred at a rate of 8.7 mM Ca\(^{2+}\)/s at 22°C in a myoplasmic [Ca\(^{2+}\)] of 1 \(\mu\)M (44). Assuming a \(Q_{10}\) of 3.1 for SR Ca\(^{2+}\)-ATPase and 1 ATP hydrolyzed per 2 Ca\(^{2+}\) pumped (37), one calculates a value of 10.8 mM ATP/s at 30°C, which is in good agreement with the results of the present study.

Thus, the results demonstrate that during continuous contractions, cross bridges account for only a small fraction of the total ATP consumption, suggesting that ion pumps are the major consumers of ATP. The relatively low rate of cross-bridge ATP consumption is likely explained by the low stimulation frequency. Possibly, the high temperature and the use of mammalian muscle also contributed to the relatively low rate of cross-bridge ATP consumption.

Considering that at 50 Hz (35% of maximal force), cross bridges accounted for ~20% of total ATP consumption, it might seem surprising that during repeated tetani at 70 Hz (~60% of maximal force) the cross-bridge component was not >25%. One should recall, however, that during repeated tetani, part of the SR Ca\(^{2+}\) pump ATP utilization also occurs during the recovery period between tetani. Therefore, the relative contribution of cross bridges to total ATP utilization during the force-generating phase is >25%.

**ATP turnover during continuous contraction.** It is well established that at the onset of contraction, fast-twitch fibers exhibit a high ATP turnover rate that decreases with time although force is maintained fairly constant (6, 7, 10, 15, 42). Similar results were obtained in the present study at higher temperature and lower relative forces. Traditionally, the decrease in the ATP turnover rate has been attributed to decreases...
in the activity of actomyosin ATPase (6). However, in the present study, the same phenomenon was observed in muscles treated with BTS. Of note, one study (43) estimated that after continuous tetani in mouse fast-twitch muscle fibers (22°C), SR Ca\(^{2+}\) uptake decreases by >50% after a 1-s tetanus compared with a 0.1-s tetanus. Accordingly, our data indicate that both cross-bridge cycling and SR Ca\(^{2+}\) pumping decreased by the same relative extent during continuous tetani. However, because the SR Ca\(^{2+}\) pumps were the major ATP consumers during the contractions, we suggest that the large decrease in the ATP turnover rate was primarily a consequence of a decrease in ATP utilization by the SR Ca\(^{2+}\) pumps in both control and BTS-treated muscles.

In conclusion, these data demonstrate that cross bridges (i.e., actomyosin ATPase) consume only ~20% of the total ATP consumption during contraction in mouse fast-twitch skeletal muscle under near-physiological conditions, suggesting that ion pumping is the major energy-consuming process.

GRANTS
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REFERENCES


