Metabolic fluctuation during a muscle contraction cycle

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Metabolic fluctuation during a muscle contraction cycle. Am. J. Physiol. 274 (Cell Physiol. 43): C846–C852, 1998.—Gated 31P-nuclear magnetic resonance followed the metabolic fluctuation in rat gastrocnemius muscle during a contraction cycle. Within 16 ms after stimulation, the phosphocreatine (PCr) level drops 11.3% from its reference state. The PCr minimum corresponds closely to the time of maximum force contraction. P< sub>1</sub> increases stoichiometrically, while ATP remains constant. During a twitch, PCr hydrolysis produces 3.1 µmol ATP/g tissue, which is substantially higher than the reported 0.3 µmol ATP/muscle twitch. The results reveal that a substantial energy fluctuation accompanies a muscle twitch.

PHYSIOLOGISTS HAVE FOCUSED for many years on the bioenergetics of muscle contraction and the associated reaction steps. They have long attempted to relate chemical reaction kinetics to the time course of energy liberation. In this paradigm, ATP hydrolysis supplies all the energy demand and should equal the energy released into heat and work. Buffering the ATP loss is the creatine kinase reaction driving phosphocreatine (PCr) to ATP (19, 21–23, 32). In fact, the energy account does not always balance. The measured enthalpy production exceeds the available energy from ATP hydrolysis as reflected in the fall of the PCr level, and the initial rate of heat production is much faster than initial rate of PCr breakdown (6, 7, 11, 15). These observations have led researchers to question whether the change in PCr during a twitch (10, 24, 25) is sufficient to account for all the heat and work and to postulate reactions associated with an “unexplained” enthalpy (13, 14, 40).

At issue then is the ΔPCr/twitch. Even though the dynamic PCr change during a twitch underpins many postulated biochemical mechanisms, its quantified value is ambiguous. Many determinations apply a time-averaged technique, which divides the overall PCr change by the total number of twitches. Such analysis assumes that the experimental observation over an averaged vs. transient period does not introduce any significant errors. However, the time to peak force development is ~20 ms, or 2% of the total observation time under 1-Hz stimulation. Sampling the overall PCr change and then dividing by total number of twitches might mask a large but transient fluctuation, which would undermine the validity of the energy balance analysis.

Assessing the ΔPCr/twitch, however, has posed a formidable experimental hurdle. Although optical methods can rapidly track some cellular changes, so far they cannot follow the PCr reaction kinetics (36). Much of the current data on ΔPCr/twitch in muscle arise from the freeze-clamping or immersion method, which overcomes the limitations of the time-averaged measurements but depends critically on sample thickness. It has a time resolution of only 100 ms, much longer than the time to maximum force development (11, 17, 18, 38). Initial experiments on turtle and frog muscles at 0°C revealed a negligible change in ATP even during the isotonic contraction, which presumably should consume more energy than isometric contraction (32).

Later, with the improved time resolution of the freeze-clamping technique, ATP changes, as reflected in the ΔPCr of 0.36 µmol/g muscle, became apparent and exhibited a linear relationship between the amount of work and the amount of breakdown (3, 18).

However, the well-resolved, dynamic profile of ATP utilization during a twitch is still uncertain, raising a residual question about whether the PCr breakdown occurs during contraction or relaxation or whether the energy is produced at the beginning or is available during the contraction-relaxation cycle. Even with advanced freeze-clamping methods, energy production and PCr split typically are compared after a complete contraction-relaxation cycle or after many such cycles. Such measurements cannot reveal accurately the energy changes during the early stages of a twitch, in particular at time points below 100 ms (11, 17, 18, 38), yet these are exactly the critical time points that are essential in mapping the mechanical-chemical energy coupling process during muscle contraction.

With 31P-nuclear magnetic resonance (NMR), many studies have provided insights into the bioenergetics of normothermic muscle in vivo and have raised an opportunity to explore the metabolic fluctuation during a twitch (10, 24, 25). We have undertaken a study to develop a gated 31P-NMR technique with sufficient time resolution to observe the PCr hydrolysis during a contraction cycle in stimulated rat gastrocnemius muscle. Indeed, the PCr level falls rapidly within 16 ms after stimulation and exhibits a kinetics curve that...
mirrors the force development profile. \( P_i \) rises stoichiometrically, while the ATP level remains constant. The data indicate that PCR hydrolysis proceeds much more rapidly, as well as extensively, than previously observed in amphibian muscle at 0°C and may contribute significantly to the initial heat production. The NMR approach presents, then, a unique way to probe the coupling mechanism governing the transient interplay between chemical and mechanical energy in the fundamental unit of muscle contraction (21).

**MATERIALS AND METHODS**

Animal preparation. Sprague-Dawley female rats (260–300 g) were anesthetized with 65 mg/kg body wt pentobarbital sodium and were prepared as described previously (4). A pentobarbital booster was administered in 4.5-mg doses every 45–60 min via an intraperitoneal catheter. The sciatic nerve was exposed, and its proximal ending was crushed so that only the distal limb muscle contracted. A stimulator (Grass S48) then initiated muscle contraction via a bipolar platinum electrode attached to the sciatic nerve. To prevent tissue drying and to insulate the electrode from the surrounding tissues, a thin layer of a mineral oil-petroleum jelly mixture was applied to the tissue incision. Through the Achilles tendon anchoring the gastrocnemius-plantaris-soleus muscle group to the ankle bone, a sewn 4-0 silk suture was connected to a transducer (Grass FT03C), whose signals were monitored by a Gould oscillographic recorder (Gould RS3200) or by a MacLab system, which first digitized the analog signal at 1 kHz. The contraction data were analyzed subsequently with Sigma Plot 5.0 (Jandel Scientific).

The muscle was subjected to an isolated electrical stimulation, and a recorder followed the developed force profile. After each stimulation, the suture was tightened until the developed force no longer increased. At that time, the suture length was tightened by an additional 10%. A series of tetanic stimulated contractions then followed to take up any slack. The muscle length adjustment procedure was then iterated. Typically a 12-V, 50-µs tetanic stimulation was sufficient to produce a supramaximal contraction of the entire gastrocnemius-plantaris-soleus muscle group. With this muscle preparation, the peak twitch force remained constant throughout 2–3 h of 1-Hz stimulation.

NMR Spectra were recorded with a 7T horizontal bore GE Omega spectrometer. The animal was placed supine on a Lucite platform, below which a concentric 1.4/2.2 cm \(^{31} \text{P} \) surface coil system was attached. The \(^1 \text{H} \) coil detected the water signal for magnet shimming, while the \(^{31} \text{P} \) coil followed the high-energy phosphate signals. The gastrocnemius muscle was positioned directly over the surface coil, which was separated from the muscle by a thin Teflon sheet. The foot, knee, and ankle joint were secured by clamps to Lucite plates to reduce motion. The typical stimulation protocol utilized a 12-V, 50-µs pulse at 1 Hz for a duration of 2–4 s, followed by 7–8 min of rest. Twitch characteristics, including the peak twitch force, remained constant throughout the entire 2–3 h of the 1-Hz stimulation.

With each data block, the spectrometer radio frequency pulse was triggered to the electrical stimulus at time point A. A delay, \( t_1 \), after the stimulation elapsed before a signal gated the spectrometer to acquire a signal transient at time point B. The time interval between time points B and C corresponded to \( \text{Acq} \), which was constant throughout the entire experiment. A final time interval, \( t_2 \), was inserted to maintain the relationship \( (t_1 + \text{Acq} + t_2) = 1 \) s. The spectrometer then acquired one data block, comprised of 128 transients and lasting for 2.3 min.

The first experiment started with a \( t_1 \) of 5 ms. In each subsequent experiment, \( t_1 \) was incremented stepwise, while \( t_2 \) was decremented to maintain a constant recycle time \( (t_1 + \text{Acq} + t_2) \). Before the next data acquisition began, 7–8 min elapsed to allow the muscle PCR level to return to its resting state, as observed in the \(^{31} \text{P} \) spectra. All other experimental conditions were kept exactly the same to create an identical time course for the gradual PCR decline during the entire stimulation protocol. About 10–14 data blocks, corresponding to time points after the stimulation, were then collected. Each data block was analyzed, and the results were used to reconstruct the various metabolic profiles. The final \( t_1 \) between stimulation and signal acquisition was incremented up to 400 s. This last point helped map precisely the steady-state level in the recovery phase.

In the experiments to determine the steady-state effect at 1-Hz stimulation, the number of scans per block was reduced to 64, and the total acquisition time was decreased to 30 s. Other signal acquisition and processing parameters, as well as the stimulation protocol, remained identical to the ones used in the transient experiments. A block of \(^{31} \text{P} \) data was then acquired at 30-s intervals. The total protocol yielded 10 blocks of data.

Peak area analysis utilized the Omega 6.2 curve-fitting algorithm to integrate the NMR signals. To account for motional artifacts during stimulation, the normalization procedure assumed that total \(^{31} \text{P} \) metabolite content should remain constant under the experimental conditions and set the total \(^{31} \text{P} \) signal as the normalizing factor in each spectrum, consistent with previous analysis procedures (24). Under no circumstances did the total peak area of the control or fully recovered spectra, before and after the stimulation episodes, differ by more than \( \pm 5\% \).

Intracellular pH was calculated from:

\[
\text{pH} = \text{pK}^+ + \log \left( \frac{\delta_A - \delta_0}{\delta_0 - \delta_B} \right)
\]

where \( \text{pK}^+ = 6.9 \), \( \delta_A \) is the chemical shift (ppm) of [HPO\(_4^2–\)] at 3.290 ppm, \( \delta_B \) is the chemical shift (ppm) of [HPO\(_4^2–\)] at 5.805 ppm, and \( \delta_0 \) is the chemical shift (ppm) of Pi referenced to PCR as 0 ppm. Intracellular Mg concentration was calculated from the chemical shifts of \( \alpha \)-ATP and \( \beta \)-ATP (27). The ADP level was derived from \(^{31} \text{P} \)-NMR parameters and the creatine kinase equilibrium constant of \( 1.66 \times 10^8 \text{ M}^{-1} \) (26).
Figure 1 shows clearly the 31P signal response at 20°C (24). The observed PCr-to-Pi reaction appears to be stoichiometric. There are 3.1 and 3.2 µmol/g wet tissue, respectively, for 1- and 0.2-Hz stimulation, respectively. On the basis of the reported resting metabolism, the Mg concentration and NMR data to determine the kinetics time constants, SD, and SE. Statistical significance was assigned when Student’s t-test indicated P < 0.05. For the steady-state kinetics analysis of PCr, the nonlinear fitting utilized the following equation for the stimulation and recovery phases, respectively:

\[
PCr(t) = PCr(ss) + [PCr(0) - PCr(ss)]e^{-\gamma t}
\]

where \(PCr(t)\) is PCr level at a given time after beginning of muscle stimulation, \(PCr(ss)\) is steady-state PCr level, \(PCr(0)\) is initial PCr level in the resting muscle, \(t\) is time after muscle stimulation, and \(\gamma\) is the exponential time constant for PCr breakdown during muscle stimulation.

The extrapolated energy cost per twitch utilized the first order derivative of Eq. 1, evaluated at \(t = 0\) (10):

\[
dPCr(t)/dt = (-1/\gamma)(PCr(0) - PCr(ss))e^{-\gamma t} \bigg|_{t=0}
\]

**RESULTS**

Figure 1 shows a set of 31P spectra from rat gastrocnemius muscle. The control 31P spectrum from resting muscle is shown in Fig. 1A. Signal averaging with an NMR acquisition trigger set at 20 ms distal to the sciatic nerve stimulation pulse produced the spectrum in Fig. 1B. The PCr signal intensity declines, whereas the Pi signal increases. ATP level remains constant. Figure 1C shows clearly the 31P signal response at 20 ms after stimulation in a difference spectrum (Fig. 1B – Fig. 1A).

During a twitch, the PCr level declines initially and then recovers to 92.1 ± 3.0% of the resting level. With recovered PCr level as the reference state, under 1-Hz stimulation, the PCr falls during a twitch from 92.1 ± 3.0 to 80.8 ± 5.8% of the control level within 16 ms (Fig. 2A). A similar profile is observed at 0.2-Hz stimulation. The PCr profile is a slightly time-shifted mirror image of the force contraction response, which exhibits a maximum at 25 ms. The corresponding ATP levels remain constant, at 98.1 ± 5.9 and 95.2 ± 3.0% of control for 1- and 0.2-Hz stimulation, respectively. On the basis of the ATP chemical shift analysis, the Mg concentration does not show any detectable alteration (27).

As PCr level falls, Pi concentration increases (Fig. 2B). During a twitch, Pi rises ~1.8 times or 447.3 ± 138.3% of control before returning to 248.9 ± 54.5% of control. The dynamic Pi profile is inversely related to the PCr profile. On the basis of the reported resting state values of PCr and Pi, of 27.1 and 2.8 µmol/g wet tissue in rat gastrocnemius muscle, the calculated changes in PCr and Pi, after saturation factor correction, are 3.1 and 3.2 µmol/g wet tissue, respectively (24). The observed PCr-to-Pi reaction appears to be stoichiometric.

Concomitantly, pH appears to shift by 0.06 pH units, from 7.14 ± 0.02 to 7.08 ± 0.11. However, the limited accuracy of the measurement does not indicate that the change is statistically significant and precludes at this time any definitive interpretation. The experimental data are summarized in Table 1.

In contrast, the nongated, continuous acquisition of the 31P signals under 1-Hz stimulation reveals a different picture (Fig. 3, A and B). PCr gradually declines within 2.3 min to 79.8% and reaches a steady-state level of 74.8% of control. ATP still remains constant, but the Pi level rises and then falls inversely with respect to the PCr profile (Fig. 3B). The pH shows a gradual decline from 7.15 to 7.04, consistent with previous reports (10). The analysis of the PCr contraction and recovery phase kinetics indicates that the corresponding kinetics time constants (\(\gamma\)) are 1.36 ± 0.07 and 0.89 ± 0.19 min. The average PCr value during a 2.3-min interval is then 89.9% of control, matching closely the observed 92.1% twitch recovery value observed in the transient, gated experiments (Table 1). The analysis of the data from the nongated, continuous signal acquisition experiments extrapolates to a ΔPCr/twitch of 0.3% or 0.08 µmol·twitch⁻¹·g tissue⁻¹ (Eq. 2), consistent with previous reports (10, 29).
**DISCUSSION**

Detection of metabolic fluctuation. The experimental results indicate that the gated NMR technique can detect with 1-ms time resolution the pronounced PCr fluctuation during a contraction cycle. Figure 1 clearly shows that high-energy phosphate signal intensities change sharply: PCr falls, Pi rises, and ATP remains constant. Such a distinct set of signal responses would argue strongly against a significant contribution from motional artifact as the muscle contracts. An overall sample movement away or toward the homogeneous detection volume should also produce a concerted change in the signal intensities as well as a substantial line broadening. Neither is observed. Moreover, the overall integrated signal areas in the 31P spectra are constant.

If the detection volume shifts between fast- and slow-twitch fibers, a contrasting set of 31P spectral changes can occur. The decrease in PCr during a twitch would imply a transition from detecting fast-twitch fibers to detecting slow-twitch fibers, which would produce changes in the NMR-observable 31P metabolite levels. In the transition from detecting fast- to detecting slow-twitch fibers, PCr will decrease from 35 to 17 mM, Pi will increase from 3 to 10 mM, but ATP will decrease from 9 to 5 mM (21, 25, 28, 31, 33). The magnitude and direction of the spectral changes, however, are not consistent with the observed experimental data: ATP level remains constant; PCr and Pi changes are inconsistent with predicted direction and values; the conversion of PCr to Pi is stoichiometric. Moreover, the constant overall integrated signal areas of the 31P-NMR spectra argue against any significant shift in the detection volume. The interpretation is consistent with the unlocalized NMR detection scheme that cannot discriminate fiber type differences with a 31P surface coil's sampling volume, which encompasses the entire muscle bed.

Because the NMR technique is not a one-shot detection scheme and depends on signal averaging over an acquisition time of 2.3 min, the research design must take into account the gradual PCr decline during the stimulation protocol. That indeed has occurred. The experimental condition for every acquisition data block matches identically the stimulation protocol and recycled time. Only the receiver acquisition trigger is stepped up incrementally. Sufficient time elapses to allow for full PCr recovery to its resting level, before the next block of data acquisition commences. Given such a protocol, the PCr decline during the course of stimulation should then be identical for each data block, even though the receiver acquisition trigger has advanced.

Indeed, both the continuous accumulation and 0.2-Hz gated experimental results confirm the above suppositions. Under nongated, continuous signal acquisition at 1-Hz stimulation, PCr declines within 2.3 min to a

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**Table 1. Metabolic changes and contractile parameters during a muscle twitch**

<table>
<thead>
<tr>
<th>Metabolic parameters</th>
<th>Steady State</th>
<th>Transient (Maximum Change Point)</th>
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<tbody>
<tr>
<td>PCr</td>
<td>92.1 ± 3.0% (17)</td>
<td>80.8 ± 5.8%* (5)</td>
</tr>
<tr>
<td>ATP</td>
<td>98.1 ± 5.9% (16)</td>
<td>95.2 ± 3.2% (5)</td>
</tr>
<tr>
<td>Pi</td>
<td>248.9 ± 54.5% (17)</td>
<td>447.3 ± 138.3%* (5)</td>
</tr>
<tr>
<td>ADP</td>
<td>41.4 ± 3.8% (16)</td>
<td>51.0 ± 12.1% (5)</td>
</tr>
<tr>
<td>pH</td>
<td>7.14 ± 0.02 (17)</td>
<td>7.08 ± 0.11 (5)</td>
</tr>
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<table>
<thead>
<tr>
<th>Contractile parameters</th>
<th>Steady State</th>
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<tbody>
<tr>
<td>Time to maximum peak tension, ms</td>
<td>24.5 ± 3.8 (8)</td>
</tr>
<tr>
<td>Half-relaxation time, ms</td>
<td>10.5 ± 2.8 (8)</td>
</tr>
<tr>
<td>Peak force, g/g body wt</td>
<td>1.10 ± 0.08 (8)</td>
</tr>
</tbody>
</table>

Values are means ± SD for no. of animals given in parentheses. Control values originate from fully relaxed 31P-spectra. Steady-state values (% of control) are from data recorded at 100, 120, 150, 200, 500, and 900 ms. Maximal change is relative to steady-state value. Corresponding relative maximum change occurs as follows: phosphocreatine (PCr), 16 ms; Pi, 16 ms; ADP, 16 ms; pH, 21 ms; ATP, 200 ms. Control pH = 7.11. *Significant change, P < 0.05 by paired t-test. All other reported changes are insignificant.
steady-state level corresponding to 74.8% of the resting control level. If overall PCr decline is divided by 2.3 min, the average PCr value during the course of the experiment is 89.9% of the resting control level. The value matches closely the observed 92.1% recovery level observed in the gated experiments and is in excellent agreement with literature studies of rat gastrocnemius muscle (10). At 0.2-Hz stimulation, the PCr declines less and reaches a higher steady-state level.

After a twitch contraction, PCr recovers much faster than after the end of stimulation, implicating distinct recovery mechanisms. On the basis of the in vitro $V_{\text{max}}$ for the phosphofructokinase, glycolysis does not appear to account for all the energy recovery after twitch (5). Either oxidative phosphorylation activity contributes to the twitch PCr recovery or the in vivo $V_{\text{max}}$ for phosphofructokinase is much higher than the in vitro values. The glycolytic source is most likely not the phosphofructose kinase, since the glyogen content in muscle can directly support only a few contractions. After the end of stimulation, the PCr recovery kinetics is consistent with a predominant role of oxidative phosphorylation. These observations warrant further study.

Some support for an aerobic energy contribution emerges from the relative increase in oxygen consumption during muscle contraction as reported in the literature (6, 25). The basal $V_{\text{O}_2}$ for cat biceps and...
soleus (glycolytic vs. oxidative fibers) is between 0.07 and 0.08 µmol O₂·g tissue⁻¹·min⁻¹. At 0.5-Hz stimulation, the VO₂ increases six to seven times; at 1 Hz, it increases ~10 times. For glycolytic muscle, 0.5-Hz stimulation can raise the ADP level to 4.1–30 µmol/g tissue, a factor of 50, whereas for oxidative fiber the ADP level can rise from 1.3 to 65 µmol/g tissue, a factor of 7.3 (25). These relative changes would imply that ADP levels can shift dramatically. Such a change is within the adenine translocase Michaelis constant range of 6–66 µmol/g tissue (20, 25). The phosphorylation potential has also decreased, but assessing its impact is less clear, since the redox potential can also mediate respiratory control (8, 39).

Many muscle studies have shown a low absolute oxygen consumption rate, consistent with a slow oxidative phosphorylation component (21, 25). The observation supports the current paradigm that aerobic energy mobilization is associated primarily with the recovery phase. However, the VO₂ values depend highly on the experimental model and conditions. In perfused rat hindlimb muscle, basal VO₂ rises to 0.37 µmol O₂·g tissue⁻¹·min⁻¹, a factor of five greater than those observed in superfused cat muscle experiments, and increases to 1.73 µmol·g tissue⁻¹·min⁻¹ during 1-Hz stimulation (16). Dividing the VO₂ by the number of twitches per minute yields 0.03 µmol O₂·g tissue⁻¹·twitch⁻¹ or 0.18 µmol ATP·g tissue⁻¹·twitch⁻¹, given a P-to-O ratio of 3:1 (16, 35). Perfused rat hindlimb muscle experiments, however, have led to an extrapolated oxygen consumption rate of 0.35 µmol O₂·g tissue⁻¹·contraction⁻¹ or 2.1 µmol ATP·g tissue⁻¹·contraction⁻¹ (34). Certainly the muscle aerobic respiratory capacity, mitochondrial content, temperature-dependent respiration rate, experimental model, and oxygen consumption measurement techniques can give rise to a range of VO₂ values.

The gated NMR data have led to a calculated enthalpy from ATP hydrolysis (based on −34 kJ/mole) of −102 kJ·twitch⁻¹·g tissue⁻¹ (7, 40). Although the enthalpy contribution from the high-energy phosphate splitting is much higher than previous observations, the experimental conditions, such as model, temperature, fiber type, and contraction duration, are sufficiently different to preclude a firm comparison and interpretation at this time (21). One perspective on the magnitude of the energy change in muscle contraction emerges from isometric tetanus study of rat soleus and extensor digitorum longus muscle. During isometric tetanus, PCr decreases by 2.13 µmol/g wet weight of muscle, while the heat production reaches 110 µW/g at 17−18°C (12). In rat extensor digitorum longus muscle at 27°C, isometric tetanus produces 154 mJ/g (37). Additional experiments with matching NMR, VO₂, and myometric measurements on the identical nonthermoregulated rat muscle group in situ will be crucial in clarifying the mechanisms underlying the bioenergetics of a contraction.

In conclusion, this report outlines a gated NMR methodology to explore transient metabolic fluctuation during a twitch and shows a substantial PCr hydrolysis within 16 ms of stimulation. P i increases stoichiometrically, while ATP remains constant. The rapid change in PCr raises questions about energy mobilization and balance within a muscle contraction cycle.

REFERENCES


