Relationship between intracellular Po2 recovery kinetics and fatigability in isolated single frog myocytes

Casey A. Kindig, Brandon Walsh, Richard A. Howlett, Creed M. Stary, and Michael C. Hogan

University of California-San Diego, Department of Medicine, Physiology Division, La Jolla, California

Submitted 1 April 2004; accepted in final form 25 January 2005

Kindig, Casey A., Brandon Walsh, Richard A. Howlett, Creed M. Stary, and Michael C. Hogan. Relationship between intracellular Po2 recovery kinetics and fatigability in isolated single frog myocytes. J Appl Physiol 98: 2316–2319, 2005. First published February 3, 2005; doi:10.1152/japplphysiol.00355.2004.—In single frog skeletal myocytes, a linear relationship exists between “fatigability” and oxidative capacity. The purpose of this investigation was to study the relationship between the intracellular Po2 (PiO2) offset kinetics and fatigability in single Xenopus laevis myocytes to test the hypothesis that PiO2 offset kinetics would be related linearly with myocyte fatigability and, by inference, oxidative capacity. Individual myocytes (n = 30) isolated from lumbrical muscle were subjected to a 2-min bout of isometric peak tetanic contractions at either 0.25- or 0.33-Hz frequency while PiO2 was measured continuously via phosphorescence quenching techniques. The mean response time (MRT; time to 63% of the overall response) for PiO2 recovery from contracting values to resting baseline was calculated. After the initial square-wave constant-frequency contraction trial, each cell performed an incremental contraction protocol [i.e., frequency increase every 2 min from 0.167, 0.25, 0.33, 0.5, 1.0, and 2.0 Hz until peak tension fell below 50% of initial values (TTF)]. TTF values ranged from 3.39 to 10.04 min for the myocytes. The PiO2 recovery MRT ranged from 26 to 146 s. A significant (P < 0.05), negative relationship (MRT = −12.68TTF + 168.3, r2 = 0.605) between TTF and PiO2 recovery MRT existed. These data demonstrate a significant correlation between fatigability and oxidative phosphorylation recovery kinetics consistent with the notion that oxidative capacity determines, in part, the speed with which skeletal muscle can recover energetically to alterations in metabolic demand.

O2 uptake (Vo2) onset kinetics are considered dependent, at least in part, on maximal aerobic capacity. Indeed, aerobic exercise training that induces significant increases in muscle oxidative capacity also speeds Vo2 kinetics (4, 8, 10). However, this can occur before any discernible increase in maximal Vo2 and muscle oxidative capacity (24). To date, no rigorous investigation studying the relationship between maximal aerobic capacity and Vo2 off-kinetcs exists. However, given that PCr and Vo2 kinetics appear to be reasonably well matched (1, 19, 26), it is possible that a strong relationship exists between maximal aerobic capacity and Vo2 off-kinetcs.

The purpose of the present investigation was to study the relationship between single myocyte fatigability, which we reported previously to be linearly related to mitochondrial volume density (29) and oxidative phosphorylation recovery [assessed via intracellular Po2 (PiO2) kinetics; Ref. 11] after a bout of repeated tetanic contractions. We tested the hypothesis that the time course for PiO2 recovery after a 2-min bout of moderate-intensity contractions would be inversely proportional to the time to fatigue (TTF) during an incremental contraction protocol.

METHODS

Female adult Xenopus laevis were used in this investigation. All procedures were approved by the University of California-San Diego animal use and care committee and conform to National Institutes of Health standards.

Myocyte preparation. Single muscle cells (n = 30) were isolated and prepared as described previously (11). Briefly, frogs were doubly pithed and the lumbrical muscles (II-IV) were removed from the hind feet. Single myocytes were dissected with tendons intact in a chamber of physiologial Ringer solution at a pH = 7.0. Cells were injected via micropipette pressure injection (PV830 pneumatic picopump, World Precision Instruments, Sarasota, FL) with a solution consisting of 0.5 mM Pd-meso-tetra (4-carboxyphenyl) porphine bound to bovine serum albumin and the Ca2+ indicator dye fura 2.

Experimental protocol. Platinum clips were attached to the tendons of each myocyte to facilitate fiber positioning within the Ringer solution-filled chamber. One tendon was fixed, whereas the contralateral was attached to an adjustable force transducer (model 400A, Aurora Scientific, Aurora, Ontario, Canada), allowing the muscle to be set at optimum muscle length. The analog signal from the force transducer was recorded via a data acquisition system (AcqKnowledge, Biopac Systems, Santa Barbara, CA) for subsequent analysis. Fibers were perfused throughout the experiment with Ringer solution equilibrated with 5% CO2 and 4–5% O2 in N2 balance. Constant perfusion was maintained throughout the protocol to maintain the extracellular Po2 and to reduce the occurrence of an appreciable unstirred layer surrounding the cell. Tetanic contractions were elicited using direct (8–10 V) stimulation of the muscle (model S48, Grass Instruments, Warwick, RI). The stimulation protocol consisted of ~250 ms trains of 70-Hz impulses of 1-ms duration.

Initially, myocytes were subjected to a trial of ~2 min at either 0.25- or 0.33-Hz stimulation frequency, during which time PiO2 was measured continuously and throughout the PiO2 recovery to baseline levels. After the initial “square-wave” contraction trial, each cell performed an incremental contraction protocol [i.e., frequency increase every 2 min from 0.167, 0.25, 0.33, 0.5, 1.0, and 2.0 Hz until peak tension fell below 50% of initial values (TTF)] under ambient

Address for reprint requests and other correspondence: R. A. Howlett, Univ. of California-San Diego, Dept. of Medicine, Physiology Division, 9500 Gilman Dr., MC0623a, La Jolla, CA 92093-0623 (E-mail: rhowlett@ucsd.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
conditions. After the incremental trial, TTF was then determined offline as the time to the first contraction with a peak isometric tension that was below 50% of the initial (nonfatigued) isometric tension.

Assessment of \( \text{PIO}_2 \). \( \text{PIO}_2 \) was measured via phosphorescence quenching techniques as described previously (11). Briefly, each myocyte was observed with a Nikon \( \times 40 \) fluorescence microscope. The phosphorescence quenching of the porphyrin compound within the myocyte was measured via a system consisting of a flash lamp (Oxygen Enterprises, Philadelphia, PA), a 425-nm band-pass excitation filter, a 630-nm cut-on emission filter, and a photomultiplier tube for collection of the phosphorescence signal. To calculate phosphorescence lifetimes from the intracellular \( \text{O}_2 \) probe, the phosphorescent decay curves from a series of 10 flashes (15 Hz) were averaged, and a monoexponential function was fit to the subsequent best-fit decay curve (analysis software from Medical Systems, Greenvale, NY). Phosphorescent decay curves were recorded every 4 s from each cell throughout the experimental period.

Data and statistical analysis. After experimental procedures, the mean response time (MRT) was calculated as the time to 63% of the recovery in \( \text{PIO}_2 \) from contracting values to resting baseline. Time to fatigue was denoted as the time at which peak tension fell to 50% of initial peak values. Data are presented as means \pm SE. MRT and TTF data were regressed linearly by standard least-squares procedures. Statistical significance was accepted at \( P < 0.05 \).

RESULTS

Peak tension development over the duration of the incremental frequency contraction protocol is shown in Fig. 1 for two representative myocytes with widely different TTF values. In the incremental frequency protocol, TTF values for all cells (\( n = 30 \)) ranged from 3.39 to 10.04 min (mean = 7.17 \pm 0.35 min). \( \text{PIO}_2 \) recovery responses from the nadir \( \text{PIO}_2 \) after the brief (2 min) constant-frequency (0.25 or 0.33 Hz) contraction bout are shown in Fig. 2 for the same two representative myocytes.

![Fig. 1. Representative peak isometric tension data for 2 single isolated frog myocytes during the incremental frequency protocol (increasing contraction frequency every 2 min) demonstrating the wide differences in fatigability. Developed tension for a fatigue-resistant fiber (A) and for a fast-fatiguing fiber (B). In the incremental frequency protocol, time-to-fatigue (TTF) values for all cells (\( n = 30 \)) ranged from 3.39 to 10.04 min (mean = 7.17 \pm 0.35 min).](image)

The \( \text{PIO}_2 \) recovery MRT averaged 77.4 \pm 5.7 s (range = 26–146 s) whereas the difference between end-contracting \( \text{PIO}_2 \) and \( \text{PIO}_2 \) recovery to extracellular values (i.e., \( \Delta \text{PIO}_2 \)) was 26.6 \pm 2.2 Torr.

![Fig. 2. Intracellular \( \text{PO}_2 \) (\( \text{PIO}_2 \)) recovery data for the same representative fast-fatiguing (\( \circ \)) and fatigue resistant (\( \bullet \)) single myocytes as presented in Fig. 1. The recovery kinetics represent the change in \( \text{PIO}_2 \) profile from the end of a bout of tetanic contractions (time = 0) until recovery to resting values. The \( \text{PIO}_2 \) recovery mean response time (MRT) averaged 77.4 \pm 5.7 s (range = 26–146 s) whereas the difference between end-contracting \( \text{PIO}_2 \) and \( \text{PIO}_2 \) recovery to extracellular values (i.e., \( \Delta \text{PIO}_2 \)) was 26.6 \pm 2.2 Torr.](image)

![Fig. 3. A significant \( P < 0.05 \), negative relationship existed (\( n = 30 \)) for the \( \text{PIO}_2 \) recovery time (MRT) after a 2-min constant-frequency contraction bout and TTF (assessed as time for peak tension to fall to 50% of initial levels) in a subsequent incremental-frequency contraction bout.](image)

**DISCUSSION**

It is generally accepted that PCr recovery kinetics provide an index of muscle oxidative capacity (e.g., Refs. 14, 18, 20–23). However, less is known about the relationship between V\( \text{O}_2 \) off-kinetics and muscle oxidative capacity. To our knowledge, this is the first investigation to study the relationship between individual myocyte fatigability (considered a proxy for muscle fiber oxidative capacity as discussed below) and \( \text{PIO}_2 \) recovery kinetics. Our findings demonstrate that TTF and \( \text{PIO}_2 \) recovery MRT in isolated single myocytes were significantly correlated in that myocytes that fatigue rapidly and have low oxidative
capacity require a longer time period for $P_{O_2}$ recovery. These data indicate that, much like PCR resynthesis kinetics, $V\dot{O}_2$ off-kineti cs are at least partly dependent on muscle oxidative capacity.

The relationship between $V\dot{O}_2$ and $P_{O_2}$ for single myocytes lacking myoglobin, such as in *Xenopus* muscle, is described by Fick’s law of diffusion as:

$$V\dot{O}_2 = D_{O_2} \times (P_{O_2} - P_{O_2\text{mito}})$$

where $D_{O_2}$ is the muscle O$_2$ diffusion constant and $P_{O_2\text{mito}}$ and $P_{O_2}$ represent mitochondrial and extracellular O$_2$, respectively. Assuming little or no gradient between cytosolic and mitochondrial $P_{O_2}$, the difference between $P_{O_2}$ and $P_{O_2}$ is proportional to the net increase in $V\dot{O}_2$ (12).

Recently, our laboratory (29) demonstrated a very strong linear relationship ($r = 0.93; P < 0.0001$) between TTF (utilizing the same variable frequency protocol as the present study) and mitochondrial volume density in *Xenopus* lumbrical single myocytes (the same type of myocytes as utilized in the present investigation). This association, which confirms similar results reported previously for single muscle cells (30), demonstrates that fatigue profiles from a single cell can be used to predict oxidative capacity (29). Thus, for the purposes of the present investigation, fatigability profiles in response to the incremental frequency protocol can be considered analogous to the intrinsic oxidative capacity of the cell.

In the present investigation, $P_{O_2}$ recovery kinetics were shown to be correlated ($r^2 = 0.605$) with TTF and, thus, by inference from earlier work (29), oxidative capacity, although the strength of this relationship is not known. There are a number of putative mechanisms for the association between recovery kinetics and oxidative capacity. First, it would be expected that the myocytes with the highest oxidative capacity would evoke less PCR depletion for a given amount of work (5); thus there would be less oxidative cost associated with PCR repletion. Second, whereas the O$_2$ cost per unit work should be similar between myocytes (although some slight variations in this may exist because of fiber type differences; Ref. 6), the work of Mahler (18) and Meyer (22) would suggest that the muscle cells with the greatest mitochondrial density, and thereby the greatest oxidative capacity, can recover most rapidly after an elevated metabolic demand, in concert with first-order respiratory control.

Factors associated with O$_2$ availability may confound peak aerobic capacity and $V\dot{O}_2$ kinetics data obtained in whole muscle and whole body preparations. First, previous investigations have reported that exercise training significantly speeds $V\dot{O}_2$ kinetics (4, 8, 10). However, a tight relationship exists between mitochondrial volume density and fiber capillarization (25) such that it is difficult to dissociate the effects of increased oxidative capacity from the augmented capacity for capillary-to-myocyte O$_2$ flux (i.e., muscle $D_{O_2}$) and possibly other factors (24, 28). Second, peak $V\dot{O}_2$ (often considered analogous to muscle oxidative capacity) may be differentially contingent on O$_2$ availability based on fitness level. Specifically, it has been demonstrated that breathing hyperoxic gas increases peak leg $V\dot{O}_2$ from normoxic levels in trained subjects (16) but may not in untrained individuals (3). Third, not only may peak muscle $V\dot{O}_2$ values be dependent on O$_2$ availability, both PCR (e.g., Refs. 9, 13) and $V\dot{O}_2$ (e.g., Ref. 7) off-kineti cs can be modulated by O$_2$ concentration. One of the particularly pow-

ereful aspects of the present investigation utilizing single myocytes was that fatigue protocols were run under supraphysiologic O$_2$ levels and the constant-frequency protocols under highly controlled $P_{O_2}$ conditions demonstrated previously not to affect $P_{O_2}$ kinetics (15) such that the potentially confounding effects of $O_2$ availability were avoided.

Previous seminal work has demonstrated intrinsic differences in mitochondrial function and respiratory control between muscles of differing fiber type (e.g., Refs. 2, 6, 17, 27). However, ambiguities in fiber typing (i.e., metabolic, myosin heavy chain, etc.) along with differences in recruitment, fatigability, and efficiency confound metabolic control inferences derived from investigations studying whole body and whole muscle. Thus it was interesting to note that the linear relationship between TTF and $P_{O_2}$ off-kineti cs (Fig. 3) is not based on any fiber-type classifications per se.

The present investigation was undertaken to study the relationship between TTF and $P_{O_2}$ off-kineti cs, independent of fiber type, in single myocytes isolated from frog muscle under a highly controlled and homogeneous O$_2$ environment. Our findings demonstrate a significant negative relationship between TTF in response to an incremental frequency protocol (a known proxy for mitochondrial volume density) and $P_{O_2}$ recovery kinetics after a 2-min bout of moderate-intensity contractions. The findings suggest that $V\dot{O}_2$ recovery kinetics are dependent in part on the intrinsic oxidative capacity of the skeletal muscle performing that work.

**GRANTS**

This study was supported, in part, by National Institute of Arthritis and Musculoskeletal and Skin Diseases Grants AR-40155 and AR-48461. C. A. Kindig and R. A. Howlett are Parker B. Francis pulmonary fellows.

**REFERENCES**

9. Haseler LJ, Hogan MC, and Richardson RS. Skeletal muscle phospho-


