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Effects of acute creatine kinase inhibition on metabolism and tension development in isolated single myocytes

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Kindig, Casey A., Richard A. Howlett, Creed M. Stary, Brandon Walsh, and Michael C. Hogan. Effects of acute creatine kinase inhibition on metabolism and tension development in isolated single myocytes. *J Appl Physiol* 98: 541–549, 2005. First published August 27, 2004; doi:10.1152/japplphysiol.00354.2004.—This study investigated the effects of acute creatine kinase (CK) inhibition (CK_i) on contractile performance, cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$), and intracellular P_{O_2} ($P_{I_{O_2}}$) in *Xenopus laevis* isolated myocytes during a 2-min bout of isometric tetanic contractions (0.33-Hz frequency). Peak tension was similar between trials during the first contraction but was significantly ($P < 0.05$) attenuated for all subsequent contractions in CK_i vs. control (Con). The fall in $P_{I_{O_2}}$ ($\Delta P_{I_{O_2}}$) from resting values was significantly greater in Con (26.0 ± 2.2 Torr) compared with CK_i (17.8 ± 1.8 Torr). However, the ratios of Con to CK_i end-peak tension (1.53 ± 0.11) and $\Delta P_{I_{O_2}}$ (1.49 ± 0.11) were similar, suggesting an unaltered aerobic cost of contractions. Additionally, the mean response time (MRT) of $\Delta P_{I_{O_2}}$ was significantly faster in CK_i vs. Con during both the onset (31.8 ± 5.5 vs. 49.3 ± 5.7 s; $P < 0.05$) and cessation (21.2 ± 4.1 vs. 68.0 ± 3.2 s; $P < 0.001$) of contractions. These data demonstrate that initial phosphocreatine hydrolysis in single skeletal muscle fibers is crucial for maintenance of sarcoplasmic reticulum Ca^{2+} release and peak tension during a bout of repetitive tetanic contractions. Furthermore, as $P_{I_{O_2}}$ fell more rapidly at contraction onset in CK_i compared with Con, these data suggest that CK activity temporally buffers the initial ATP-to-ADP concentration ratio at the transition to an augmented energetic demand, thereby slowing the initial mitochondrial activation by mitigating the energetic control signal (i.e., ADP concentration, phosphorylation potential, etc.) between sites of ATP supply and demand.

oxygen consumption; muscle energetics; skeletal muscle fiber

CREATINE KINASE (CK), located both in the cytosol and mitochondrion, catalyzes the reversible transfer of a high-energy phosphate moiety between creatine (Cr) and ADP as

$$PCr + ADP + H^+ \leftrightarrow Cr + ATP$$

where PCr is phosphocreatine. In striated muscle, the most abundant form of CK (MM-CK) is located throughout the cytosol and is functionally coupled to the major sites of energy usage [e.g., myofibril ATPases, sarcoplasmic reticulum (SR) Ca^{2+} ATPases, sarcolemmal Na^+-K^+-ATPases]. The maximal activity of MM-CK is severalfold higher than that of the ATPases, and the close proximity of MM-CK to the sites of ATP hydrolysis is believed to maintain a desirable [ATP] (brackets denote concentration) and therefore a desirable Gibbs free energy (ΔG) for ATP hydrolysis in the vicinity of the ATPases by rapidly rephosphorylating ADP. Mitochondrial CK is an additional isoform of CK found in striated muscle (predominantly in highly oxidative muscle). The functional coupling of mitochondrial CK to adenine nucleotide translocase on the outside of the inner mitochondrial membrane creates an environment favorable to PCr formation. It is believed that a shuttle exists between these two isoforms to provide a rapid transport of PCr from the mitochondrion to the ATPases and Cr in the reverse direction without necessitating large changes in free [ADP] (for an explanation of the PCr shuttle, see Refs. 41, 44). Therefore, CK is believed to play an important role in maintaining sufficient energy for contraction at the onset of work (62).

Several investigations have studied the effect of CK deletion on skeletal muscle function in knockout (KO) mice (e.g., Refs. 14, 22, 51, 59). Roman et al. (51) demonstrated, in CK-KO mice, that skeletal muscle contractile force was similar for the first contraction to that in wild-type mice. However, force fell precipitously thereafter for the ensuing few contractions. Subsequently, force recovered partially over the duration of the 2-min contraction bout (51). On the basis of mathematical modeling, the authors predicted that oxidative phosphorylation would need to be activated at contraction onset more rapidly in KO compared with the wild type, to meet initial ATP demands (51). Indeed, a more rapid O_2 uptake ($\dot{V}O_2$) response to an elevation in metabolic demand has been demonstrated previously in cardiac muscle of CK-KO mice compared with wild-type controls (24). Although these experiments have provided seminal information regarding the role of CK in cellular function, experiments in KO animals should be interpreted with caution because of compensatory adaptations incurred as a result of the KO. Indeed, skeletal muscle in CK-KO mice demonstrates both increased mitochondrial volume density (and oxidative capacity) and structural reorganization of mitochondria within the cell (59, 61). It is believed that these adaptations occur to allow a more direct channeling of ADP and ATP between the sites of energy production and usage without the PCr shuttle (33), all of which may be responsible, in part, for more rapid $\dot{V}O_2$ onset kinetics. Therefore, CK-KO mice may not be ideal for elucidating the direct role of CK in muscle energetics.

Another, arguably preferential, manner in which to study the direct effects of CK deficiency minus the compensatory adaptations in KO muscle is via acute CK inhibition (CK_i; e.g., Refs. 6, 28, 66). The purpose of the present investigation was to study the effects of acute CK_i on contractile performance, cytosolic $[Ca^{2+}]$ ($[Ca^{2+}]_c$), and intracellular P_{O_2} ($P_{I_{O_2}}$) in *Xenopus* isolated single myocytes. Because frog muscle lacks myoglobin, in accordance with Fick's law of diffusion the fall in $P_{I_{O_2}}$ in these single fibers is proportional to the net increase in $\dot{V}O_2$ (30). To investigate the effects of CK_i in muscle, we

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subjected isolated single myocytes to two ~2-min contraction bouts, one control (Con) and one subsequent CK_i trial. We tested the hypotheses that CK_i would result in *1*) an immediate reduction in peak tension and peak $[Ca^{2+}]_c$ after the first contraction, *2*) a more rapid fall in $P_{I_{O_2}}$ indicative of accelerated oxidative phosphorylation at contraction onset, and *3*) a more rapid $P_{I_{O_2}}$ recovery at contraction cessation.

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 ($n = 32$) were isolated and prepared as described previously (29). 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 physiological Ringer solution consisting of (in mM) 112 NaCl, 1.87 KCl, 0.82 $CaCl_2$, 2.38 $NaHCO_3$, 0.07 NaH_2PO_4, 0.1 EGTA, pH = 7.0. Cells were injected via micropipette pressure injection (PV830 pneumatic picopump, World Precision Instruments, Sarasota, FL) with either a solution consisting of 0.5 mM Pd-*meso*-tetra-(4-carboxyphenyl) porphine bound to bovine serum albumin (for phosphorescence quenching) and the Ca^{2+} indicator dye fura 2 (10 mM; Molecular Probes, Eugene, OR) or fura 2 alone (for fluorescence microscopy). Following microinjection, cells were given a minimum of 30 min recovery.

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, and the contralateral was attached to an adjustable force transducer (model 400A, Aurora Scientific, Aurora, ON, Canada), allowing the muscle to be set at optimum muscle length (i.e., length at which maximal tetanic tension was produced). 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% CO_2 and ~4% O_2 in N_2 balance. Constant perfusion was maintained throughout the protocol to maintain the extracellular P_{O_2} at ~30 Torr and to reduce the occurrence of an appreciable unstirred layer surrounding the cell. Tetanic contractions were elicited by 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. Myocytes were subjected to trials of ~100–120 s at a ~0.33-Hz stimulation frequency with a 15-min recovery period between trials.

Because of the irreversible nature of both drugs used to inhibit CK, order randomization was not possible. Thus one set of experiments was performed in which the Con trial was performed first, followed by the CK_i trial for both $[Ca^{2+}]_c$ ($n = 9$) and $P_{I_{O_2}}$ ($n = 7$). Additionally, Con-Con trials were performed to determine whether an order effect existed in regard to either the $[Ca^{2+}]_c$ ($n = 9$) or $P_{I_{O_2}}$ ($n = 7$) response to contractions. In the first protocol, myocytes were treated with iodoacetamide (IA; 2 mM) to inhibit CK for the phosphorescence quenching studies. In the second protocol, muscle fibers were subjected to 2,4-dinitrofluorobenzene (DNFB; 10 μM dissolved in DMSO; Ref. 66) to inhibit CK during the $[Ca^{2+}]_c$ fluorescence studies. These drugs were chosen because IA affected adversely the fluorescent signal and the DNFB disrupted the phosphorescence quenching signal.

Cytosolic [Ca^{2+}] Measurement

$[Ca^{2+}]_c$ was measured by use of an epifluorescent microscope system that consisted of a Nikon inverted microscope with a ×40 fluor objective and a DeltaScan illumination and detection system (Photon Technology International, South Brunswick, NJ) as described previously (58). Fibers injected with fura 2 were illuminated sequentially (20 Hz) with two excitation wavelengths of 340 and 380 nm, and the resulting fluorescence emission was measured at 510 nm. The ratio of 340- to 380-nm fluorescence was used to obtain the Ca^{2+}-dependent signal (23).

Assessment of $P_{I_{O_2}}$

Each myocyte was observed with a Nikon ×40 fluor objective (0.70 numerical aperture). 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 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). The O_2 dependence of phosphorescence quenching is described by the Stern-Volmer equation where

$$\tau_o/\tau = 1 + k_q{*}\tau_o{*}P_{O_2}$$

$$\text{thus } P_{O_2} = (\tau_o/\tau - 1)/(k_q{*}\tau_o)$$

where τ_o and τ are the phosphorescence lifetimes at anoxia and a given P_{O_2}, respectively, and k_q, the quenching constant (in Torr/s), is a second-order rate constant that is related to the frequency of collisions between O_2 and the excited triplet state of the porphyrin and the probability of energy transfer when collisions occur. The constants k_q and τ_o were respectively set at 690 Torr/s and 100 μs for Pd-*meso*-tetra (4-carboxyphenyl) porphine bound to albumin in solution for this preparation as established previously (29). Phosphorescent decay curves were recorded every 4 s from each cell throughout the experimental period.

Data Analysis

After experimental procedures, the mean response time (MRT) was calculated as the time to 63% of both the fall in $P_{I_{O_2}}$ with contractions (on) and $P_{I_{O_2}}$ recovery after contractions (off). Both peak tension and $[Ca^{2+}]_c$ data were normalized to the initial Con point of the first trial.

Statistical Analysis

Data are presented as means ± SE. Differences between trials in regard to the $P_{I_{O_2}}$ fall and on- and off-kinetics were tested via a paired *t*-test. Changes in peak tension and $[Ca^{2+}]_c$ were tested via a repeated-measures one-way ANOVA. When significant *F* values were present, the Bonferroni post hoc test was employed for determination of between-group differences. Statistical significance was accepted at $P < 0.05$.

RESULTS

Peak Tension

Peak tension tracings for one muscle fiber performing two identical Con tetanic contraction bouts with a 15-min recovery (*top*) as well as peak tension for another myocyte that initially performed a Con contraction bout followed 15 min afterward by an IA-induced CK_i trial (*bottom*) are shown in Fig. 1. Peak tension was not significantly different ($P > 0.05$) at any time point between the initial Con bout and a second Con bout (Fig.

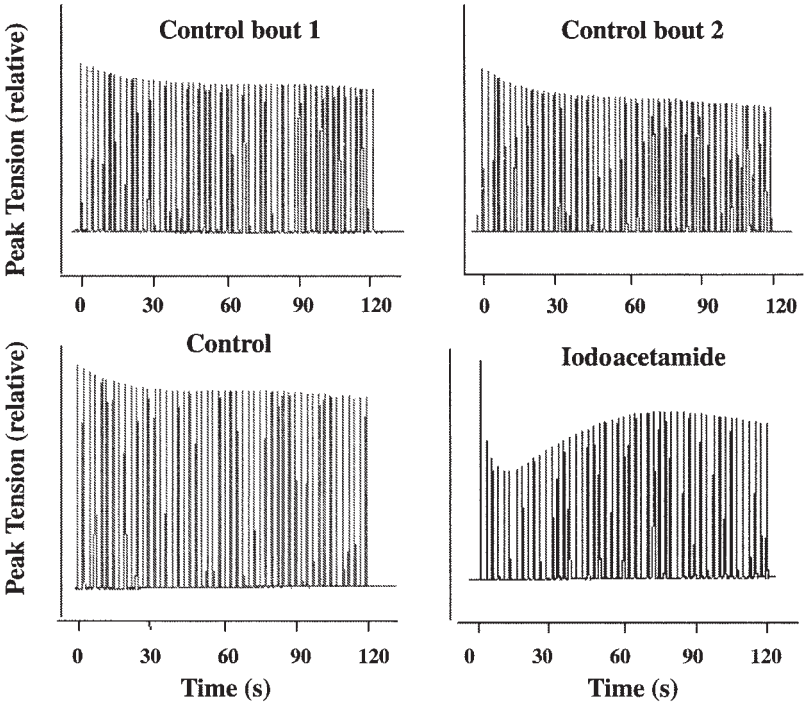

Fig. 1. Peak tension from 2 representative single muscle cells. *Top*: peak tension tracing for an isolated single muscle fiber performing two tetanic control (Con) contraction bouts (first on *left* and second on *right*) with 15 min between bouts. *Bottom*: peak tension for a separate myocyte that was first subjected to a Con contractile bout and then performed the second trial 15 min afterward in which creatine kinase (CK) was inhibited with iodoacetamide (IA).

2, *top*). Peak tension in the first (initial) contraction did not differ significantly ($P > 0.05$) with CK_i via either IA or DNFB administration compared with Con (Fig. 2, *middle* and *bottom*). However, both IA and DNFB significantly reduced ($P < 0.05$) peak tension in the second contraction. This attenuation in peak tension was maintained throughout the duration of the contraction bout (all time points, $P < 0.05$; Fig. 2, *middle* and *bottom*).

Cytosolic [Ca^{2+}]

Peak $[Ca^{2+}]_c$ was slightly yet significantly (all time points, $P < 0.05$) reduced across the duration of the contraction bout in the second Con bout compared with Con 1 (Fig. 3, *top*). Similarly, peak $[Ca^{2+}]_c$ was significantly reduced ($P < 0.05$) across the bout of contractions with DNFB administration compared with Con (Fig. 3, *middle*). Because of the irreversible nature of CK_i, all DNFB trials followed the initial Con trial. Given the apparent order effect for $[Ca^{2+}]_c$, the DNFB (CK_i) trial was compared statistically to the second Con trial from the subset of Con-Con myocytes (Fig. 3, *top*) and is shown in Fig. 3, *bottom*. Initial peak $[Ca^{2+}]_c$ after DNFB administration was not different ($P > 0.05$) from the second Con value; however, every point thereafter was significantly reduced ($P < 0.05$) in the DNFB compared with the second Con trial (Fig. 3, *bottom*). Resting $[Ca^{2+}]_c$ was not different ($P > 0.05$) between the 2 Con trials (Fig. 3, *top*). However, during the DNFB trial, baseline $[Ca^{2+}]_c$ became significantly elevated ($P < 0.05$) at the 90-s time point and end point of contractions compared with Con (Fig. 3, *middle*).

Peak Tension-to-Peak Cytosolic [Ca^{2+}] Ratio

The ratio of peak tension-to-peak $[Ca^{2+}]_c$ was not different ($P > 0.05$) between the Con and DNFB trial during the initial contraction (Fig. 4). However, thereafter, a trend existed for the ratio to be lower in the CK_i bout compared with Con. Indeed, the peak tension-to-peak $[Ca^{2+}]_c$ ratio was significantly lower ($P < 0.05$) with DNFB compared with Con during the fourth and fifth contractions but did not reach statistical significance at any time point thereafter (Fig. 4).

Intracellular P_{O_2}

Mean data for the $P_{I_{O_2}}$ response at rest, during contractions, and after contractions are shown in Fig. 5. Additionally, the $P_{I_{O_2}}$ response to the initial 30 s of contractions is shown in Fig. 6. Because a "priming effect" has been reported previously in the *Xenopus* myocyte $P_{I_{O_2}}$ response to contractions with 5 min recovery between bouts (29a), a subset of myocytes ($n = 7$) was subjected to two identical contraction bouts with 15 min between trials. There were no significant differences (both $P > 0.05$) for either the fall in $P_{I_{O_2}}$ (Con 1, 19.3 ± 2.2; Con 2, 18.5 ± 1.3 Torr) or the MRT of the fall (Con 1, 54.6 ± 4.2; Con 2, 58.1 ± 9.3 s) in response to contractions between the two Con bouts. There was a significantly greater ($P < 0.01$) fall in $P_{I_{O_2}}$ in the Con (26.0 ± 2.2 Torr) compared with the IA (17.8 ± 1.8 Torr) trial (Fig. 7). However, because end-peak tension was significantly reduced in the IA compared with the Con trial (Fig. 2, *middle*), the ratio of Con-to-IA end-peak tension (1.53 ± 0.11) was not different ($P > 0.05$) from the ratio of Con-to-IA $P_{I_{O_2}}$ fall with contractions (1.49 ± 0.11), suggestive of an unaltered aerobic metabolic cost of contractions between groups. In addition, the speed of the fall in $P_{I_{O_2}}$ at contraction onset (Con MRT = 49.3 ± 5.7; IA MRT = 31.8 ± 5.5 s) as well as the speed of the $P_{I_{O_2}}$ recovery after contraction cessation (Con MRT = 68.0 ± 3.2; IA MRT = 21.2 ± 4.1 s) was significantly faster (both $P < 0.05$) in the IA compared with the Con trial (Fig. 7).

DISCUSSION

This is the first investigation to study, simultaneously, the effects of CK_i on the aerobic metabolic cost and the contractile

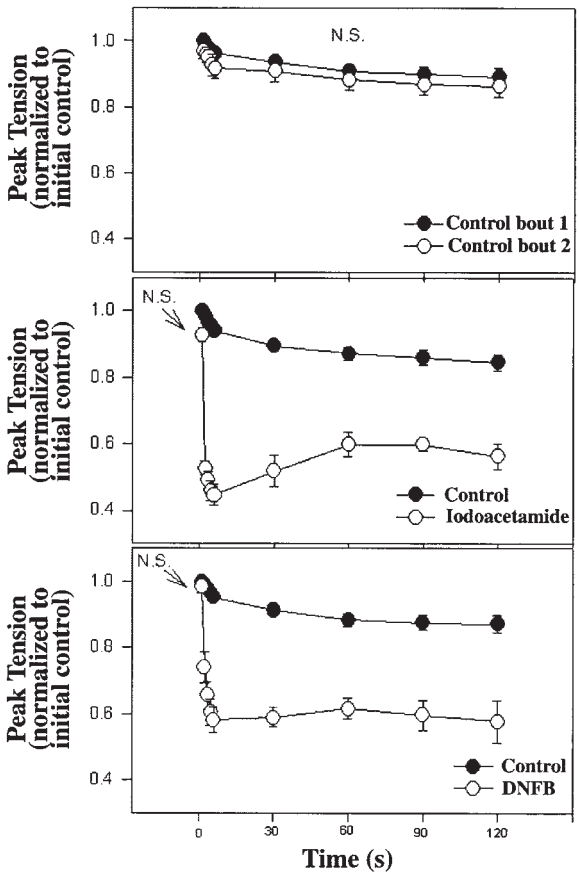

Fig. 2. Peak tension profiles under control (Con) and creatine kinase inhibition (CK_i) conditions. *Top*: peak tetanic tension (means ± SE; $n = 9$) in *Xenopus* isolated single muscle cells was not significantly different (NS) at any time point between 2 matched Con contraction bouts. *Middle* and *Bottom*: regardless of the method of CK_i, be it via IA (means ± SE, $n = 7$) or 2,4-dinitrofluorobenzene (DNFB; means ± SE, $n = 9$), peak tension in the initial contraction was not different (NS, $P > 0.05$) from the matched Con trial. However, peak tension fell precipitously in the second contraction (both $P < 0.05$) and remained significantly depressed (all time points, $P < 0.05$) for the remainder of the bout compared with Con. Note the similarity of peak tension profiles regardless of either IA or DNFB use for CK_i.

fatigue profile in isolated single muscle cells. Key novel findings in this study include *1*) a more rapid fall in $P_{I_{O_2}}$ with CK_i compared with Con, indicative of accelerated $\dot{V}O_2$ onset kinetics (see *$P_{I_{O_2}}$ as an analog of $\dot{V}O_2$*) as postulated for CK-KO skeletal muscle (51), *2*) significantly faster $P_{I_{O_2}}$ offset kinetics, and *3*) similar aerobic metabolic cost as demonstrated by a similar delta $P_{I_{O_2}}$-to-peak tension ratio between CK_i and matched Con. As these data were obtained from wild-type muscle, and, thus, absent of the augmented muscle mitochondrial oxidative capacity in CK-KO mice, these data reveal that PCr hydrolysis at exercise onset acts to provide a temporal buffer for initial ATP demand, thereby effectively slowing the onset of oxidative phosphorylation by delaying the key energetic controlling signal(s) between sites of ATP hydrolysis and the mitochondrion.

Methodological Considerations

Percent of CK inhibition. In the present investigation, molar concentrations of DNFB and IA were 10 μM and 2 mM, respectively. These concentrations are considered supramaximal in regard to CK inhibition but are not expected to induce side effects. Indeed, 0.4 mM IA treatment inhibited 97% of total CK activity in isolated heart (28) and 2 mM IA treatment "essentially eliminated" CK activity in skeletal muscle (6). Additionally, because both DNFB and IA evoked contractile responses (Fig. 2) that were remarkably similar to those reported in CK deficient skeletal muscle (e.g., Refs. 51, 59), it

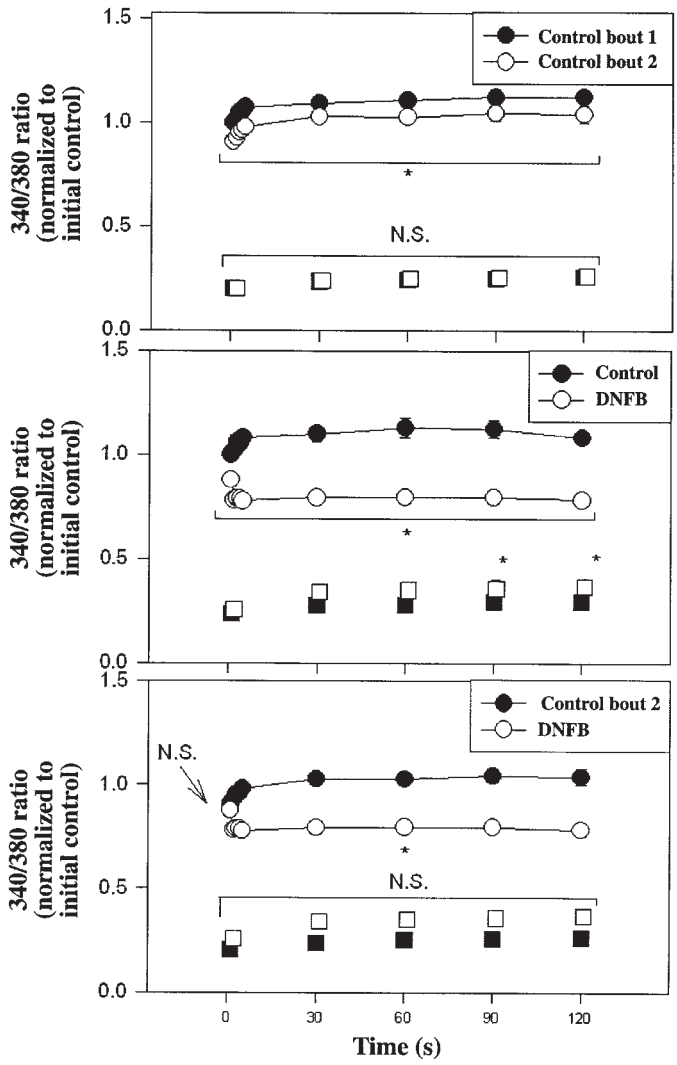

Fig. 3. Effect of CK_i on cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) in contracting single myocytes. Circles represent peak cytosolic $[Ca^{2+}]_c$ and squares represent baseline $[Ca^{2+}]_c$. *Top*: *Xenopus* single myocyte (means ± SE; $n = 9$) peak $[Ca^{2+}]_c$, as assessed via the ratio of 340- to 380-nm fluorescence (340/380 ratio), was significantly (*all $P < 0.05$) reduced at each time point during the second control contraction bout compared with the first bout; however, all baseline values were unchanged (NS, $P > 0.05$). *Middle*: peak $[Ca^{2+}]_c$ (means ± SE; $n = 9$) was significantly reduced (*$P < 0.05$) after DNFB (a CK inhibitor) administration compared with Con ($n = 9$) at each time point. Baseline $[Ca^{2+}]_c$ was elevated significantly (*$P < 0.05$) at the 90- and 120-s time points of the contraction bout compared with the initial Con trial. *Bottom*: as an order effect for $[Ca^{2+}]_c$ was evidenced (*top*), the second Con bout from the upper figure was compared with the CK_i trial from the *middle*. Although the initial peak $[Ca^{2+}]_c$ was not different (NS, $P > 0.05$) between trials, peak $[Ca^{2+}]_c$ was reduced significantly (*$P < 0.05$) with DNFB at every time point thereafter. There were no significant differences in resting $[Ca^{2+}]_c$ between trials.

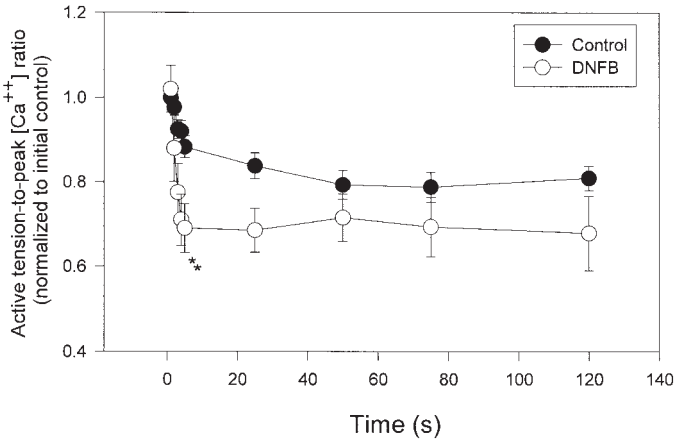

Fig. 4. Effect of CK_i on the peak tension-to-$[Ca^{2+}]_c$ ratio. The peak tension-to-$[Ca^{2+}]_c$ ratio (means ± SE) was significantly reduced ($*P < 0.05$) during the fourth and fifth contractions after DNFB (a CK inhibitor) administration compared with Con in *Xenopus* isolated single myocytes ($n = 9$). At all other time points, no significant differences existed ($P > 0.05$) between the DNFB and control trial.

is likely that the vast majority, if not all, of CK activity was inhibited in the present study.

Nonspecific effects of IA and DNFB. One concern in the present investigation is that the drugs used to inhibit CK may have induced an altered metabolic and/or contractile response because of mechanisms not associated with CK_i. Recent work has demonstrated that IA does not inhibit oxidative phosphorylation maximal capacity (isolated mitochondria preparation; Ref. 28) or alter either glycolytic flux (60) or myofibrillar ATPase activity (26). Furthermore, the immediate fall in tension as demonstrated herein is similar to that reported in skeletal muscle of CK-KO mice (e.g., Refs. 13, 14, 51, 59), suggestive of a direct CK_i effect rather than a potential nonspecific effect.

$P_{I_{O_2}}$ as an analog of $\dot{V}_{O_2}$. In the present study, $\dot{V}_{O_2}$ was not measured directly. However, the relationship between $\dot{V}_{O_2}$ and

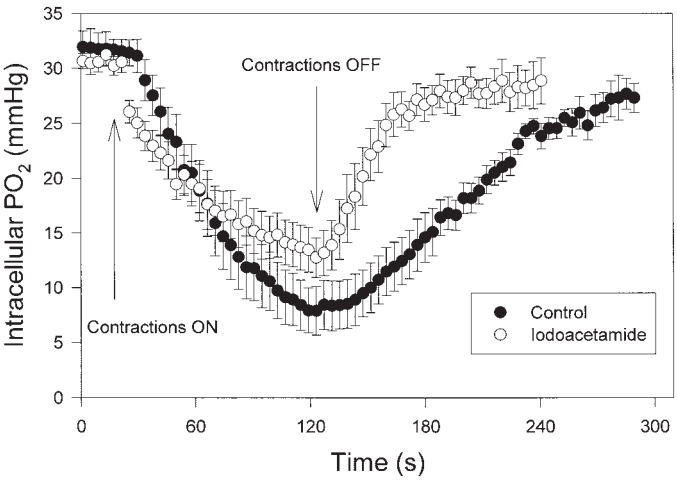

Fig. 5. Effect of CK_i on intracellular P_{O_2} ($P_{I_{O_2}}$) profiles. *Xenopus* isolated single myocyte $P_{I_{O_2}}$ (means ± SE; $n = 7$) before, during, and after matched bouts of repetitive tetanic contractions under control and CK_i (IA) conditions. Both the speed of the $P_{I_{O_2}}$ fall with contractions and speed of the $P_{I_{O_2}}$ recovery after contractions were significantly faster with IA administration compared with control.

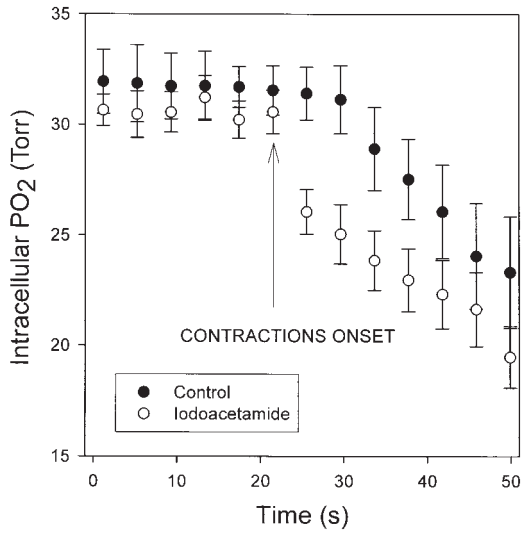

Fig. 6. Fall in $P_{I_{O_2}}$ from baseline over the initial 30 s of contractions in *Xenopus* isolated single myocyte $P_{I_{O_2}}$ (means ± SE; $n = 7$) under control and creatine kinase inhibition (CK_i; IA) conditions. Note immediate fall in $P_{I_{O_2}}$ with CK_i (at least within 4 s) compared with no discernible fall for the initial 8 s under control conditions.

$P_{I_{O_2}}$ for single myocytes lacking myoglobin, such as in *Xenopus* muscle, is described by Fick's law of diffusion as

$$\dot{V}_{O_2} = D_{O_2}*(P_eO_2 - P_{O_2}mito)$$

where D_{O_2} is the muscle O_2 diffusion constant (assumed to remain constant between Con and CK_i trials) and P_{O_2}mito and $P_{E_{O_2}}$ represent mitochondrial and extracellular P_{O_2}, respectively. Assuming little or no gradient between cytosolic and mitochondrial P_{O_2}, the difference between $P_{E_{O_2}}$ and $P_{I_{O_2}}$ is proportional to the net increase in $\dot{V}_{O_2}$ as demonstrated previously in our laboratory (30). Thus in our single muscle fiber preparation, the fall in $P_{I_{O_2}}$ is considered to reflect linearly the net rise in $\dot{V}_{O_2}$.

Peak Tension and Cytosolic $[Ca^{2+}]$

In the present investigation, acute CK_i resulted in a precipitous fall in tetanic peak tension after a normal first contraction. The attenuation of peak tension subsisted for the remainder of

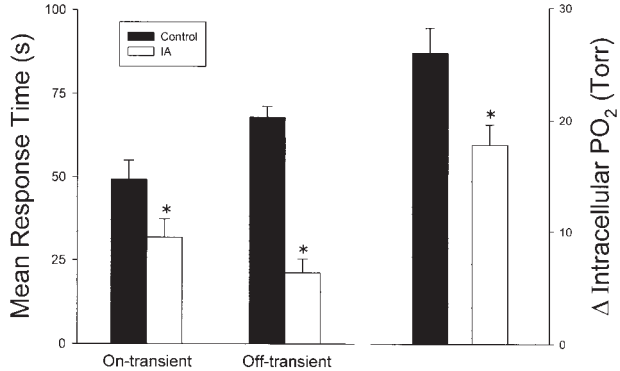

Fig. 7. $P_{I_{O_2}}$ dynamics with contractions. *Left*: mean response time for the fall in single myocyte $P_{I_{O_2}}$ (means ± SE; $n = 7$) with contractions and recovery after contractions was significantly ($*P < 0.05$) faster during creatine kinase inhibition CK_i compared with a matched control run. *Right*: fall in $P_{I_{O_2}}$ (Δ; means ± SE; $n = 7$) was significantly ($*P < 0.05$) greater during the control compared with the CK_i trial.

the contraction bout (Fig. 2). This reduction in peak tension was associated with a fall in peak $[Ca^{2+}]_c$ (Fig. 3). Additionally, there was a transient reduction in $[Ca^{2+}]_c$ sensitivity over the initial few contractions (i.e., the fourth and fifth contractions; Fig. 4). These findings are in general agreement with the work of Steeghs et al. (59), which demonstrated that both the release and uptake of Ca^{2+} are affected by the absence of CK. The absence of functioning CK is expected to alter the relationship between rate of work and any of the metabolites in the CK equilibrium (i.e., [ADP], [ATP], [P_i], and pH) during transitions from rest to work. These metabolites have also been suggested to be putative mechanisms in the diminished contractility during fatigue and may explain the decreased contractile performance after CK_i.

In the presence of dysfunctional CK, glycolysis and oxidative phosphorylation are the major pathways through which ADP is rephosphorylated. These pathways, unlike PCr hydrolysis, require P_i to phosphorylate ADP. Therefore, the CK_i should attenuate increases in net cytosolic free [Pi] resulting from PCr hydrolysis. P_i is a metabolite that is often implicated in decreased contractile function and has been shown to reduce maximum cross-bridge force production and mitigate myofibrillar Ca^{2+} sensitivity (21, 45). Furthermore, P_i can affect SR function in numerous ways, including activation of a Ca^{2+} efflux pathway resulting in SR Ca^{2+} pump inhibition, precipitation of calcium phosphate within the SR, thereby reducing the Ca^{2+} available for release, as well as modulation of SR Ca^{2+} release channel activation (16, 19, 64). Indeed, although elevated resting [P_i] has been demonstrated in CK-deficient skeletal muscle (14, 59), previous work in CK-KO (15) and CK-inhibited (60) muscle has shown that P_i was actually reduced compared with Con during contractions. Therefore, it is expected that CK_i did not induce a P_i-associated decrement in force.

A fall in pH could potentially affect SR function and contractility (18). Indeed, it is well accepted that PCr is an important H^+ buffer at the onset of exercise. However, research has shown little difference in pH between CK-deficient muscle and Con (51, 59, 60). Furthermore, findings remain equivocal with regard to the effect of pH on contractile performance and appear, in single fibers, to rely heavily on muscle temperature (65).

In the absence of energetic buffering provided by CK, it is expected that [ADP] will increase, and [ATP] decrease, more rapidly to meet a given energetic demand. Indeed, it has been shown that skeletal muscle [ADP] is significantly elevated (e.g., Refs. 51, 60, 66), and [ATP] may be decreased (at least transiently, e.g., Refs. 1, 15) during a series of contractions with impaired CK compared with Con. Additionally, it has been demonstrated that metabolites in the vicinity of the myofibril and SR ATPases do not rapidly equilibrate throughout the cytosol (34, 55). Rather, it is believed that compartmentalization of metabolites occurs within the cell and that changes in [ADP] and [ATP] during exercise may be of greater magnitude in the immediate vicinity of the myofibril and SR (2, 5, 12, 19, 32, 35, 47, 52, 63).

In agreement with the idea of metabolic compartmentalization, it has been shown that local energy production (via locally bound CK or glycolytic enzymes) is necessary to maintain a desirable phosphorylation potential (i.e., [ATP]/[ADP], [P_i]) at both the SR and myofibrils (5, 12, 17, 32, 35, 36, 38, 47, 52). Therefore, it appears plausible that compartmentalization of adenine nucleotides may be radically changed with CK_i such that during contraction [ATP]/[ADP] becomes much lower in the area of the SR Ca^{2+} ATPases and myofibrillar ATPases. The possible mechanisms of inhibition of SR and myofibril function by an altered phosphorylation potential are numerous. A decreased phosphorylation potential will reduce the free energy of ATP hydrolysis, affecting both SR and myofibril function. In particular, it has been shown that the SR ATPase pumps function in both the forward and reverse directions and are highly dependent on the free energy of ATP (56). It is also possible that an accumulation of ADP could result in myofibril or SR inhibition. For example, ADP has been shown to inhibit myofibril maximum shortening velocity (11, 66), and recent data in skinned muscle fibers also suggest that ADP impairs the ability of the SR to resequester Ca^{2+} (40, 46). Furthermore, MM-CK is coupled to a number of ATP-sensitive sites in the SR that may be involved in the failure of Ca^{2+} release. Therefore, the decline of tetanic $[Ca^{2+}]_c$ may have been due to a direct inhibition of SR Ca^{2+} release due to reduced [ATP] (or increased [Mg^{2+}]) (4). Along a similar line of thought, impaired function of Na^+-K^+-ATPase (57) and/or ATP-sensitive K^+ channels (48) may reduce SR Ca^{2+} release via failing action potentials. Although data in this investigation cannot discern whether these mechanisms are responsible for the initial or residual tension reduction with CK_i, from the data presented herein and results from previous studies it is evident that functional CK is necessary for adequate maintenance of tension development, which is likely coupled to maintaining desirable ATP/ADP at the level of the SR and myofibrils.

PI_{O_2} On-Transient Response

Using an electrical analog model transformed to a chemical model to describe muscle respiratory control in which the CK reaction serves as the capacitance and oxidative phosphorylation is the current, Meyer (42) predicted that a decrease or removal of the capacitance (i.e., PCr available for hydrolysis) should reduce the time constant for changes in metabolism. Indeed, Roman and colleagues (51) predicted, from mathematical modeling, that $\dot{V}O_2$ kinetics at contraction onset would be dramatically faster in CK-KO mice compared with wild-type Con. To avoid compensatory adaptations (i.e., augmented oxidative capacity) in KO muscle (59, 61) that would be expected to speed $\dot{V}O_2$ on-kinetics regardless of functional CK (25) (which is analogous to increasing the resistor of the simple analog system, Ref. 42), we studied the effects of acute CK_i. Our data demonstrate that CK_i results in a marked speeding of PI_{O_2} on-kinetics in single myocytes, indicative of a more rapid onset of oxidative phosphorylation at contraction onset. Similar findings have been demonstrated previously in CK-KO mouse heart (24) and also in heart treated with IA (28). Although the present findings are in apparent agreement with that of cardiac muscle, it should be noted that metabolic control differs significantly between heart and skeletal muscle.

Mechanisms likely responsible for attenuation in tension development discussed in the above section may also be involved in the more rapid $\dot{V}O_2$ response (described by the change in PI_{O_2}) at contraction onset as demonstrated in CK_i muscle. These putative respiratory controlling mechanisms include kinetic limitation by [ADP] (and/or [P_i]) in accordance with Michaelis-Menten kinetics (9), nonequilibrium thermody-

namic control via the phosphorylation potential (3), alterations in Gibbs free energy of cytosolic ATP hydrolysis (43), and increases in cytosolic and/or intramitochondrial [Ca^{2+}] (27). It has been demonstrated repeatedly that, in the absence of PCr breakdown, [ADP] will rise more rapidly (51, 60, 66). As the current literature suggests that [P_i] and pH appear to remain relatively unchanged (as discussed above), and cytosolic [Ca^{2+}] was not elevated (actually diminished), then the rate-controlling stimulus for mitochondrial activation at the transition to an elevation in metabolic demand in these experiments was likely [ADP] or phosphorylation potential. The classic view of metabolic control suggests that a decrease in ATP/ADP near the myofibrils and SR will propagate to the mitochondrion and stimulate respiration. In normal muscle, the rephosphorylation of ADP by PCr will slow the rise in [ADP]. However, previous work has suggested an important role for the PCr shuttle in expediting the immediate onset of oxidative phosphorylation at the transition to an elevation in metabolic demand (e.g., Ref. 62). The finding in the present study of more rapid $P_{I_{O_2}}$ kinetics at contraction onset would argue against the concept of a more rapid signal transduction by PCr/Cr ratio rather than ATP/ADP. Rather, these data support the concept of cytosolic CK being a high-capacity temporal buffer that normally delays the mitochondrial response to rapid energy demand increases, thereby maintaining normal [ADP] within the cell and buffering a more rapid activation of the mitochondria every time an increase in metabolic demand is incurred.

Aerobic Metabolic Cost of Contractions

Previous work comparing the $\dot{V}O_2$-to-work ratio (specifically, the rate-pressure product) between CK_i and Con muscle, which has, to date, been limited to cardiac muscle, has been conflicting. Specifically, Saupe et al. (54) demonstrated unchanged $\dot{V}O_2$ per unit work whereas Gustafson and Van Beek (24) demonstrated a reduced $\dot{V}O_2$ cost per unit in the CK-KO cardiac muscle compared with wild-type Con. In the present investigation, the reduced peak tension in the CK_i trial was matched by a proportionate reduction in the overall fall in $P_{I_{O_2}}$, resulting in an unchanged peak tension-to-$P_{I_{O_2}}$ ratio, in agreement with the previous work of Saupe and colleagues (54). As the ATP produced per O consumed (P:O ratio) is not altered by CK_i (26, 28), one would not expect any change in the aerobic ATP contribution to an elevation in metabolic demand, and thus the difference reported by Gustafson and Van Beek (24) may be due, in part, to some compensatory mechanism within the KO muscle.

$P_{I_{O_2}}$ Off-Transient Response

It has often been demonstrated that the activation of oxidative metabolism at the onset of moderate-intensity exercise is matched by a similar time course of recovery at the end of exercise, suggesting that oxidative metabolism is controlled by a single rate-limiting step (i.e., first order system) (10, 37, 39, 49, 50). However, asymmetry between the on- and off- transients of O_2 consumption is not uncommon (7, 53). Rossiter et al. (53) demonstrated slower off-transients than on-transients in humans, similar to results found in the Con trial of the present study, suggesting a system with multiple substrate controllers.

In the present investigation, both the on- and off-transients for $P_{I_{O_2}}$ were significantly faster with CK_i (Fig. 7). Although the more rapid $\dot{V}O_2$ onset can be explained by [ADP] kinetics alone (see *$P_{I_{O_2}}$ On-Transient Response*), the finding of asymmetry suggests more complex control (e.g., ATP/ADP or phosphorylation potential). Postexercise $\dot{V}O_2$ may be considered to be required to meet the energetic cost of Ca^{2+} resequestration, lactate metabolism, and rephosphorylation of Cr (8, 20). Thus, with no requisite Cr rephosphorylation during CK_i, it can be postulated that the [ADP] recovery rate will be more rapid compared with Con, thus speeding $P_{I_{O_2}}$ off-kinetics. However, CK_i increased the speed of the off-transient $P_{I_{O_2}}$ kinetics to a much greater extent than the on-transient (69% vs. 35% faster vs. Con for off- and on-transients, respectively). Therefore, because higher-order control models often imply faster off- than on-kinetics (such as [ADP] $\times$ [Pi], phosphorylation potential, or [ADP^2]; Ref. 31), it would seem that the present findings of asymmetry would suggest more complex control (than simple [ADP] feedback) in the absence of CK activity. However, control of oxidative phosphorylation can be multifactorial, and under different conditions, any of the substrates of respiration may play a primary regulatory role.

Conclusions

In the present investigation, the effects of acute CK_i were studied in *Xenopus* isolated single muscle cells. These data demonstrate that functional CK is necessary for the maintenance of tetanic peak tension over a bout of repetitive contractions. These data also demonstrate that the loss of peak contractile tension is due primarily to a concomitant reduction in Ca^{2+} release from the SR. Furthermore, $P_{I_{O_2}}$ on- and off-kinetics, analogous to $\dot{V}O_2$ transients, were markedly faster with CK_i compared with matched Con. These latter data suggest that CK-catalyzed breakdown of PCr at exercise onset moderates the rise in [ADP] in a manner that attenuates the initial rate of oxidative phosphorylation increase. This suggests that PCr hydrolysis is responsible, in part, for the $\dot{V}O_2$ "inertia" seen at the onset of an elevation in metabolic demand.

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