δ-Aminolaevulinate synthase expression in muscle after contractions and recovery

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The synthesis of haem has been postulated to be a key regulatory step in muscle mitochondrial biogenesis. We examined the expression of δ-aminolaevulinate synthase (ALAs), the regulatory enzyme of haem metabolism, in 10 Hz electrically stimulated and non-stimulated control rat tibialis anterior (TA) muscle. ALAs activity and mRNA levels were measured at 0, 18 and 48 h of recovery after 3 h of acute stimulation, or after 7 days of stimulation (3 h/day). ALAs activity in control muscles averaged 7.8 ± 0.8 nmol/h per g ($n = 30$). After 3 h of stimulation and during recovery, no change in ALAs activity occurred. ALAs mRNA during the same time was unchanged except at 48 h of recovery, when it increased 1.3-fold above control ($P < 0.05$). After 7 days of stimulation, ALAs activity was unchanged at 0 h, but increased at 18 and 48 h of recovery to 2.0- and 1.8-fold above control ($P < 0.05$). ALAs mRNA was also increaased, but to a level averaging 1.6-fold above control ($P < 0.05$) at all times, indicating an increased mRNA stability or synthesis. No change in the haem-containing enzyme cytochrome *c* oxidase (CYTOX) activity occurred after 3 h of stimulation in the red section of the TA. After 7 days of stimulation, the increase in CYTOX activity averaged 1.7-fold above control ($P < 0.05$) at all times. Thus the induction of ALAs during recovery after 7 days was regulated by factors which not only change ALAs mRNA content, but which also affect ALAs mRNA at translational or post-translational steps. This induction occurred despite a 1.7-fold increase in CYTOX, implying that a precursor–product relationship does not always exist.

INTRODUCTION

The synthesis of haem begins with the combination of glycine and succinyl-CoA, catalysed within mitochondria by the enzyme δ-aminolaevulinate (ALA) synthase (ALAs) [1]. This is the rate-limiting step in the production of haem [2]. Haem is subsequently incorporated into cytochromes, which are fundamental structural and functional components of the inner membrane. It has been demonstrated that haem synthesis and incorporation into the apocytochromes occur before mitochondrial protein synthesis [3]. Haem is also necessary for the assembly of cytochrome *c* oxidase (CYTOX) subunits into the native enzyme [4], as well as the post-translational import of cytochrome *c* [5,6]. Thus the synthesis of haem may represent a key regulatory step in the overall control of mitochondrial assembly [7].

During conditions of enhanced mitochondrial synthesis, it is reasonable to hypothesize that an elevation in the activity of ALAs could precede, or occur simultaneously with, increases in the levels of the cytochromes which incorporate the haem moiety. This hypothesis has been supported during mitochondrial biogenesis in muscle induced by continuous weight-bearing activity [8], and by treadmill running [7]. The latter study demonstrated increases in ALAs activity in skeletal muscle of exercised animals before any increase in cytochrome content. Other work [9] has demonstrated that the increases in ALAs activity after exercise were only transiently maintained. These studies represented initial steps in the elucidation of the relationship between haem synthesis and haemoprotein assembly. In order to evaluate if haem may be an actual regulator of mitochondrial assembly, we performed a more systematic analysis of changes in ALAs and haemoprotein induction (e.g. the formation of cytochromes) over several time points after contractile activity, mindful of the transient nature of ALAs induction, which appears to peak during the recovery phase after exercise [7,9]. In the present study CYTOX activity was used as a haemoprotein marker enzyme.

We also wished to evaluate the relationship between ALAs activity and its corresponding mRNA expression. Earlier work [8] had suggested a substantial post-transcriptional or post-translational contribution to the increases in ALAs activity observed. However, this was based on analyses at only one time point immediately after continuous muscle overload. Since ALAs mRNA is known to possess an unusually short half-life, we evaluated this relationship over several time points after contractile activity in the present study.

In order to perform this study, we have adopted a model of 10 Hz low-frequency chronic stimulation for 3 h/day, followed by recovery. This model induces large changes in mitochondrial gene expression, including the cytochromes [10–12], in a short period of time. Further, since only one limb is stimulated, while the other is used as the within-animal control, this design permits the elimination of inter-animal differences in hormone levels and developmental stage, both of which affect ALAs actvity [7,13].

MATERIALS AND METHODS

Animals and tissues

Male Sprague–Dawley rats (Charles River, St. Constant, Quebec, Canada) weighing 277 ± 7 g ($n = 37$) were housed individually and were given food and water *ad libitum*.

The surgical procedure described for the unilateral implant-

Abbreviations used: ALA, δ-aminolaevulinate; ALAs, ALA synthase (EC 2.3.1.37); CYTOX, cytochrome *c* oxidase (EC 1.9.3.1); TA, tibialis anterior.

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ation of the electrodes leading to the tibialis anterior (TA) muscle were as previously described [10]. The contralateral limb served as non-stimulated control TA muscle in all animals.

Stimulation protocol and tissue sampling

Two groups of animals were used. The first group was stimulated at 10 Hz for 3 h. Animals were killed immediately ($n = 6$) or after 18 h ($n = 5$) or 48 h ($n = 5$) of recovery. The second group was stimulated for 3 h/day for 7 consecutive days. Animals in this group were killed at the end of the final stimulation period (0 h; $n = 6$) or after 18 h ($n = 5$) or 48 h ($n = 6$) of recovery. Animals were anaesthetized with ketamine hydrochloride (33–44 mg/kg) at the indicated times, and the TA muscle was quickly removed from both the stimulated and the contralateral limbs. TA muscles were separated into red and white portions [14] on the basis of appearance, freeze-clamped and stored at −70 °C. Subsequently, muscles were pulverized to a fine powder in a stainless-steel mortar cooled to the temperature of liquid N_2 [11]. Powders were stored in liquid N_2 until required for analyses.

Enzyme activities

CYTOX activity was measured as the rate of oxidation of fully reduced cytochrome *c*, as done previously [11,15]. One unit of activity is defined as 1 μmol of cytochrome *c* oxidized/min at 30 °C. ALAs activity was measured to estimate the tissue content of ALAs protein by a modification of the method originally described by Briggs et al. [16]. Briefly, freshly excised muscle was homogenized (5%, w/v) immediately in a cold solution of 0.9% NaCl and 10 mM Tris/HCl (pH 7.4). ALAs activity was measured in a 2 ml reaction mixture containing 1 ml of homogenate, 100 mM glycine, 75 mM Tris (pH 7.4), 10 mM EDTA, 0.2 mM pyridoxal phosphate, 1 μCi of [^{14}C]succinate and 0.2 mM succinate. The reaction was initiated by addition of homogenate and was incubated with shaking at 37 °C for 30 min. The reaction was terminated by addition of 0.5 ml of 25% trichloroacetic acid. The mixture was cooled on ice for 10 min, and centrifuged at 1800 *g* (4 °C) for 5 min. The supernate was retained, and the pellet was washed in 5 ml of 1% trichloroacetic acid and centrifuged again. The supernatants were combined and 100 mM succinate, 75 μM ALA and 2.5 mM sodium acetate (pH 4.8) were added. The mixture was then applied to a 5 ml cation-exchange column (Dowex 50W-8H$^+$) and the column was successively washed with 20 ml of 0.1 M sodium acetate (pH 3.9), 20 ml of methanol/0.1 M sodium acetate (pH 3.9; 2:1, v/v), and 10 ml of 0.1 M HCl. [^{14}C]ALA was eluted with 10 ml of 1 M NH_3 and was concentrated to 5 ml by evaporation. [^{14}C]ALA was pyrrolized by addition of 500 μl of acetylacetone and 5 ml of 1 M acetic acid (pH 4.6). The mixture was boiled for 20 min, then allowed to cool to room temperature. To extract the ALA-pyrrole, 12 ml of ethyl acetate (pH 4.6) was added, and the mixture was vortex-mixed for 3 × 30 s. The upper phase was removed and the extraction was repeated. The upper phases of both extractions were then combined. [^{14}C]ALA within the sample extract was determined by counting radioactivity of 5 ml of the total extraction volume in a liquid-scintillation counter. Purified [^{14}C]ALA (Sigma, St. Louis, MO, U.S.A.) was employed to determine the percentage recovery throughout the protocol. Samples were corrected for this recovery, which averaged 88 ± 5% ($n = 16$).

Analysis of ALAs mRNA

Frozen tissue powders were utilized to isolate total RNA [17]. Total RNA samples (5–10 μg) were size-fractionated on denaturing agarose/formaldehyde gels and blotted on nylon filters (Duralon, Stratagene) by capillary diffusion [18]. The RNA was bound to the membranes by exposure to u.v. light. The quantity of ALAs mRNA in total cellular RNA was determined by hybridization analysis [8]. Plasmid pALX containing a 1.1 kb ALAs cDNA fragment encoding the chicken liver ALAs isoform was used to construct a radiolabelled probe. pALX was linearized with *Eco*RI, and an antisense RNA transcript was synthesized by using SP6 RNA polymerase in the presence of [^{32}P]UTP (800 Ci/mmol). The specific radioactivity of the probe was 1×10^9 c.p.m./μg. A second hybridization was performed with plasmid pB [19], with an *Eco*RI fragment of the human gene for the 18 S rRNA. The probe was ^{32}P-labelled by using a random priming kit (Stratagene).

Blots were pre-hybridized for 2–4 h at 56 °C in a solution containing 5 × SSC (1 × SSC = 0.15 M NaCl/0.015 M sodium citrate), 5 × Denhardt's solution, 200 μg/ml denatured sonicated salmon sperm DNA, 0.1% SDS and 50% formamide. Hybridization was initiated by addition of the ^{32}P-labelled SP6 transcript (50000–100000 c.p.m./ml) to the prehybridization solution. The amount of SDS was increased from 0.1 to 0.5%, and the hybridization was carried out for 15 h at 56 °C. The blots were washed at 65 °C for 3 × 15 min each in 1 × SSPE (0.15 M NaCl/10 mM Na_2HPO_4/1 mM EDTA), followed by a single 15 min wash at 65 °C in 0.1 × SSPE. Hybridization conditions and procedures for the 18 S rRNA plasmid probe were the same as noted above, but at 42 °C. Exogenous unlabelled plasmid pB was added to the labelled DNA probe before hybridization to ensure a 3–4-fold molar excess of probe mass relative to the 18 S rRNA on the blot. Blots were washed at room temperature in 2 × SSC for 4 × 15 min, and then at 56 °C in 0.1 × SSC for 4 × 30 min before autoradiography.

The first hybridization was performed with the ALAs mRNA probe. After autoradiography, a second hybridization was performed with the 18 S rRNA probe after the blot had been stripped of the previous probe by a 5 min incubation in 0.1 × SSPE/0.5% SDS at 100 °C.

The radioactivity associated with each band was quantified by using a computerized digitizing scanner (Apple Computer Inc.). Based on pilot experiments, the amounts of RNA used were in the linear range of RNA concentration versus the autoradiographic signal. The total area of the band was measured by cutting out the profiles of the scanned peaks and weighing the paper to the nearest 0.01 mg. Quantification of the 18S rRNA was used to correct for errors arising from the measurement of total RNA sample concentration, loading samples into the gel or transfer of the RNA to the blotting membrane [20]. The amount of 18S rRNA in the total RNA from the stimulated muscle of each animal was expressed as a fraction of the contralateral control, set to a value of 1.00. The peak area of ALAs mRNA was then divided by the corrected masses of 18S rRNA from each total RNA sample.

Statistics

The data were analysed by one-way and two-way analyses of variance, and Student's *t*-tests (alpha level of 0.05).

RESULTS

ALAs activity

ALAs activity in the red portion of the contralateral non-stimulated TA muscles was 7.8 ± 0.8 nmol/h per g ($n = 30$). After 3 h of stimulation, ALAs activities were not significantly

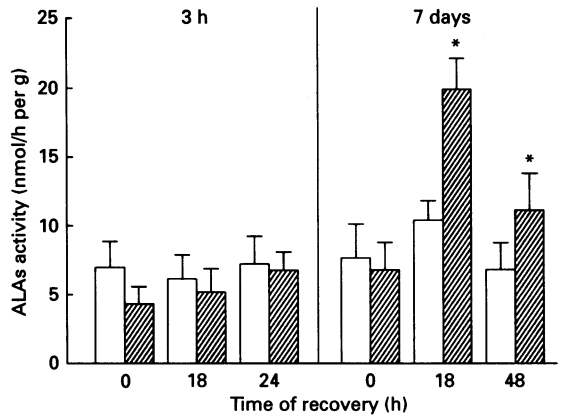

Figure 1 ALAs activity after stimulation and recovery in ipsilateral stimulated and contralateral non-stimulated TA muscles

TA muscles were stimulated at 10 Hz for 3 h, or for 7 days (3 h/day). Muscles were taken at 0, 18 and 48 h of recovery, homogenized immediately, and assayed for ALAs activity as described in the Materials and methods section. □, Non-stimulated control muscle; ▨, stimulated muscle. Values represent means ± S.E.M. ($n = 4$–6 per time point): *$P < 0.05$ versus non-stimulated control.

different from those of contralateral non-stimulated control muscle at 0, 18 or 48 h of recovery (Figure 1). ALAs activity after 7 days of stimulation was not significantly increased above that of control muscle at 0 h of recovery. However, a 2.0-fold increase in ALAs activity above the contralateral control was observed after 18 h of recovery (20.0 ± 2.3 nmol/h per g; $P < 0.05$). This was decreased at 48 h of recovery, but remained 1.6-fold greater than control muscle ($P < 0.05$).

ALAs mRNA

Northern-blot analyses were used to quantify ALAs mRNA levels as a function of recovery time. Quantification of 3–4 blots at each time point revealed that these were not affected by 3 h of 10 Hz stimulation, followed by 0 or 18 h of recovery (Table 1). However, by 48 h of recovery, a 33 % increase in mRNA above the contralateral control was observed in the stimulated muscle ($P < 0.05$). After 7 days of stimulation, analysis of variance indicated that ALAs mRNA increased by 63 % ($P < 0.05$) above non-stimulated muscle across all recovery time points. The largest increase (73 %; $P < 0.05$) was found after 48 h of recovery.

CYTOX activity

CYTOX activity was measured in the red portion of the TA muscle which was used for the assay of ALAs activity. CYTOX activity averaged 11.8 ± 0.8 units/g ($n = 11$) in the red portion of contralateral non-stimulated TA muscles. This was not altered by 3 h of stimulation (0 h) or during the recovery period at 18 or 48 h (Table 2). CYTOX activity in the 7-day-stimulated group was approx. 1.7-fold higher ($P < 0.05$) at 0, 18 and 48 h of recovery than at all three time points after 3 h of stimulation. No effect of recovery time was observed in the red portion of the TA muscle.

We wished to evaluate whether this pattern of change in

Table 1 ALAs mRNA levels in stimulated (stim) and control (con) red TA muscle

Values represent means ± S.E.M. ($n = 3$–4 per time point). ALAs mRNA was quantified from Northern blots and the intensity of the signal was expressed as arbitrary scanner units, corrected for differences in 18S rRNA levels. The ratio Stim/Con indicates the magnitude of the fold increase in ALAs mRNA above control as a function of recovery time. * $P < 0.05$ compared with the corresponding control value at the same time of recovery.

Stimulation...	3 h			7 days		
Recovery time (h)...	0	18	48	0	18	48
Stim	0.166 ± 0.02	0.149 ± 0.01	0.169* ± 0.02	0.150* ± 0.04	0.150* ± 0.01	0.175* ± 0.01
Con	0.169 ± 0.02	0.147 ± 0.02	0.128 ± 0.02	0.094 ± 0.01	0.100 ± 0.02	0.106 ± 0.01
Stim/Con	0.98 ± 0.08	1.07 ± 0.16	1.33 ± 0.06	1.56 ± 0.29	1.55 ± 0.16	1.73 ± 0.09

Table 2 Cytochrome *c* oxidase activities in the red and white portions of TA muscle following 3 h and 7 days (3 h/day) of stimulation

Values are expressed as units/g wet muscle weight and represent means ± S.E.M.; $n = 3$–6/time point. Red and white sections of TA muscle were obtained at 0, 18 and 48 h of recovery. Tissues were pulverized in liquid N_2, homogenized and cytochrome *c* oxidase activity was measured as the rate of oxidation of reduced cytochrome *c*. *$P < 0.05$ compared to corresponding values after 3 h of stimulation, as well as compared to the corresponding control values at the same recovery times (see Results section for control data). ** $P < 0.05$ versus time 0 of recovery at 7 days.

Stimulation...		3 h			7 days		
TA	Recovery time (h)...	0	18	48	0	18	48
Red		10.16 ± 1.56	11.49 ± 1.53	12.70 ± 2.69	18.87* ± 1.28	19.24* ± 2.69	20.64* ± 1.36
White		2.59 ± 0.35	2.21 ± 0.30	3.08 ± 0.70	3.01 ± 0.37	4.00 ± 0.51	5.38** ± 0.76

CYTOX activity was similar in the low oxidative white portion of the TA muscle. CYTOX activity in the white portion of the contralateral non-stimulated control muscle averaged 2.7 ± 0.2 units/g ($n = 11$), only 23 % of the activity found in red muscle (Table 2). Values in the 3 h-stimulated at 0, 18 and 48 h were not significantly different from this value. In contrast with the results found in the red section after the 7-day stimulation protocol, CYTOX activity in the white portion increased as the duration of recovery progressed. At 0 h of recovery, CYTOX was not significantly different from the activity after 3 h stimulation. However, by 48 h of recovery, values were significantly higher (1.8-fold) than at 0 h of recovery ($P < 0.05$).

DISCUSSION

The energy supply for long-term repetitive contractile activity is mainly provided by an increase in flux through enzymic pathways of aerobic metabolism found in the mitochondrion. With daily repeated bouts of contractile activity, a slow rate of mitochondrial biogenesis occurs to enhance the enzymic capacity for flux, and therefore to increase the capacity of the cell for mitochondrial respiration. This requires an increase in protein synthesis, most of which may occur during the recovery phase [21]. One of the first mitochondrial enzymes affected is ALAs [7], which is the rate-limiting step in the synthesis of haem. It has previously been hypothesized that increases in haem synthesis may represent a signal for the initiation of mitochondrial biogenesis in muscle, since haem is required for incorporation into cytochromes [7]. In support of this hypothesis, previous studies in animals treated with the drug allylisopropylacetamide to induce porphyria showed an increased synthesis of haem before any increase in CYTOX activity or cytochrome *b* content [3]. Increases in haem synthesis were also noted to occur both before, and to a greater extent than, the synthesis of mitochondrial proteins in liver [3]. However, few studies have investigated the relationship between haem synthesis and haem-containing enzymes in striated muscle undergoing mitochondrial biogenesis [7,8]. The present study was undertaken to compare the responses of ALAs activity and mRNA expression after a single bout of contractile activity, followed by recovery, with those changes observed after 1 week of daily activity. A haem-containing enzyme (CYTOX) was also measured to determine possible precursor–product relationships between haem synthesis and a protein which has incorporated haem to form the CYTOX holoenzyme complex. The results indicate that enzymic adaptations at the protein and mRNA levels can change as a function of (1) recovery time and (2) the number of previous bouts of contractile activity. The results also provided insight into the mechanism by which ALAs may be induced during the course of mitochondrial biogenesis.

Induction of ALAs activity

ALAs is a unique enzyme in that changes in $V_{max.}$ within a tissue are brought about by rapid alterations in enzyme content, a consequence of the unusually short half-life of the protein and its encoding mRNA [2]. After a single session of 3 h of contractile activity, the only measurable increase in the capacity to synthesize haem was evidenced by a small but measurable increase in ALAs mRNA content after 48 h of recovery. Surprisingly, an increase in ALAs activity was not observed. This contrasts with those observations made by using whole-body exercise [7,9], in which significant increases in ALAs activity in heart and skeletal muscle have been noted between 6 and 17 h after exercise. It is well known that whole-body exercise leads to large changes in circulating hormone levels. In particular, thyroid hormone alone is known to affect ALAs activity [7], and this may be a factor leading to the increase observed during acute whole-body exercise.

The cumulative effect of six additional 3 h contraction periods caused time-dependent increases in ALAs activity. We do not have the time resolution within our experimental design to assess whether or not this represented a peak response. Indeed, Abraham and Terjung [9] found maximal increases of ALAs activity only 6 h after exercise in cardiac muscle. Our choice of experimental conditions was based on previous work [7] which showed increases in skeletal-muscle ALAs activity, using recovery times after exercise similar to those chosen here. Since the half-life of this enzyme in liver is 30–120 min [2], changes might have been evident before 18 h. Comparison of ALAs activity with the corresponding mRNA indicate that these changes cannot be solely due to alterations at the mRNA level, since a relatively constant 63 % increase in ALAs mRNA occurred in the presence of large fluctuations in enzyme activity. Thus the lack of an obvious relationship between protein (as estimated by maximal enzyme activity) and mRNA levels of expression indicates that translational or post-translational mechanisms may play a role in ALAs expression within skeletal muscle. Transient decreases in ALAs degradation, or increases in ALAs protein import into mitochondria between 0 and 18 h, could also account for this.

The pattern evident is likely also to be dictated by differences in the turnover rates of ALAs mRNA and protein. The data illustrated in Table 1 indicate that 7 days of contractile activity can modify the steady-state levels of ALAs mRNA. In contrast with 3 h of stimulation, stable increases in ALAs mRNA at times 0, 18 and 48 h of recovery after 7 days of stimulation were evident. This may be the result of either a progressive rise in the stability of the mRNA or an increase in its synthesis. The cellular content of free haem is one factor known to regulate ALAs mRNA concentration. Changes in haem concentration appear to be inversely related to transcription of the ALAs gene and stability of the ALAs mRNA [22,23]. A decrease in haem content after stimulation could arise because of an increase in the rate of haem degradation, or a greater incorporation of haem into protein. With regard to the latter, a high rate of haem incorporation into cytochromes during recovery after 7 days of stimulation (i.e. CYTOX, Table 2; [10]) could have decreased the free haem concentration, resulting in an increased ALAs mRNA content.

Induction of CYTOX activity

It is evident from a comparison of ALAs and CYTOX activities that CYTOX activity in red TA muscle stimulated for 7 days was augmented at 0 h of recovery in the absence of a detectable change in ALAs activity. Thus an elevated ALAs activity during recovery after 7 days of contractions was not a prerequisite for the increase of CYTOX to occur. Indeed, marked variations in ALAs activity in the 7-day-stimulated and recovery groups were not accompanied, or followed, by detectable changes in CYTOX activity. However, the apparent dissociation between the pattern of changes in these two enzymes may be more related to the markedly different holoenzyme turnover rates. Since CYTOX is composed of 13 individual subunits, which are derived from both the nuclear and the mitochondrial genomes [24], assessment of CYTOX turnover is complex. If all of the subunits are required for catalytic activity, as appears to be the case [25], then changes in the activity measured in the present study should reflect the subunit with the slowest turnover. Although subunit turnover

rates are currently unknown, they are likely to be longer than the very rapid turnover rate of ALAs [2], perhaps resembling more those of other haem-containing proteins such as cytochrome *c* (6–7 days [26]). Our CYTOX data, indicating a 70% increase in activity after 1 week of stimulation, are consistent with this. The elevated levels of CYTOX activity at all time points after 7 days of stimulation may be the result of a slow rise in CYTOX content during the recovery periods of the previous 6 daily stimulation periods. On the other hand, the activity of ALAs apparently follows a different time course of induction during stimulation and recovery. For example, ALAs activity may have been rapidly induced after the sixth bout of contractions, and would presumably remain elevated even by 48 h of recovery. The 3 h contractile-activity intervention at 21 h after the sixth contraction bout apparently attenuated the rise in ALAs activity. This increase is fully evident during recovery after the seventh contraction period. This induction of ALAs occurred despite the presence of elevated CYTOX activities, indicating that the haem synthesized may not be rapidly incorporated as haem *a* into CYTOX, or that haem may be necessary to support an increase in other cytochromes or haemoproteins (i.e. myoglobin) not measured in this study. However, the transient changes in ALAs activity which may be occurring beyond the first day of stimulation may be enough to provide the additional cumulative haem content necessary for the increase in CYTOX to be evident at 7 days. It is also noteworthy that the greatest increase in mitochondrial enzyme activities occurs between 5 and 10 days of stimulation, when stimulation is present for 24 h/day [10]. Comparable increases in CYTOX activity occurred after 7 days in the present study, using only 3 h of 10 Hz stimulation per day.

It is evident from previous work [27] that tension output cannot be maintained without extensive fatigue during 10 Hz stimulation. After 5 min of contractions, tension output decreases to a steady state of approx. 35% of the initial tension [10,27]. This inability to maintain tension, and thus ATP turnover, is likely to be related to the known fibre composition of the TA muscle. It is composed largely of fast-glycolytic (white) muscle fibres (80%), with only a small percentage of fast-oxidative-glycolytic (red) fibres (20% [14]). The tension which is maintained during 3 h of 10 Hz stimulation as performed in the present study must be largely due to the activity of red fibres, whereas the white fibres are probably involved in ATP turnover for only a brief period of time [27,28]. Thus, if these factors are involved in determining the cellular adaptation of muscle to chronic contractile activity, it seems reasonable that the extent, or quality, of the adaptation would differ in these two fibre types. Our data indicate that this was the case, and have implications for typical interpretations made on the adaptive response of muscle to exercise. CYTOX activity in the red section of the TA muscle increased 1.7-fold after 7 days of stimulation, the magnitude of which was unaffected by recovery time. However, after the same number of days of stimulation, no change in CYTOX activity was observed in the white section at 0 h of recovery. Interestingly, an increase was observed in this fibre section only at 48 h of recovery. To our knowledge, this is the first report of an activity-induced increase in a haem-containing enzyme in muscle during a post-contraction recovery period. This suggests that the time after the last bout of muscle contractions is an important factor determining the extent of the mitochondrial adaptation measured within a specific high- or low-oxidative fibre type. It is apparent that more data are needed on the magnitude and duration of changes in enzyme levels as a function of recovery time.

In summary, our data indicate that the experimental conditions of contractile activity followed by recovery can result in a dissociation between changes in ALAs activity and that of a typical haem-containing protein, CYTOX. Further, alterations in ALAs expression at the mRNA and protein levels can also be temporally dissociated. These patterns probably reflect markedly different protein and mRNA turnover rates.

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