Neural control of gene expression in skeletal muscle

Effects of chronic stimulation on lactate dehydrogenase isoenzymes and citrate synthase

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1. The aim of this study was to investigate the effects of neural activity on the expression of fibre-type-specific patterns of metabolic enzymes at the levels of transcription and translation. 2. For this purpose, changes in tissue amounts of citrate synthase (CS) and the H- and M-subunits of lactate dehydrogenase (LDH) were followed in fast-twitch rabbit muscles during low-frequency (10 Hz, 12 h/day) nerve stimulation. These stimulation-induced alterations were correlated with changes in tissue amounts of the total poly(A)⁺ (polyadenylated) RNA, poly(A)⁺ RNAs specifically translatable *in vitro*, yield of total ribosomes and distributions of monosomes and polysomes. The tissue contents of poly(A)⁺ RNAs translatable *in vitro* coding for CS and H- and M-LDH were quantified by immunoprecipitation of their translation products. 3. Increases in total ribosome yields occurred after 4 days' stimulation, reaching a maximum between 14 and 21 days. Stimulation for only 1–2 days greatly increased the amount of monosomes. An increase in polysomes occurred before that in total ribosomes, suggesting that monosomes were integrated into polysomes. 4. Total poly(A)⁺ RNA significantly increased in muscles stimulated for more than 6 days. A maximum increase of 2.5-fold was attained after 14–21 days. 5. Chronic stimulation progressively induced the appearance of LDH isoenzymes containing the H-subunit, with a predominance of LDH-3. This shift corresponded to a slow decay of the M-subunit and a 2-fold steep increase in the H-subunit. These changes correlated with those of the respective poly(A)⁺ RNAs translatable *in vitro*, thus indicating that the re-arrangement of the LDH isoenzyme pattern is mainly due to qualitatively and quantitatively altered transcription. 6. The increase in CS was biphasic and consisted of a moderate rise during the first 4 days of stimulation and a steep rise thereafter. The latter coincided with a steep increase in poly(A)⁺ RNA translatable *in vitro* coding for CS. In view of the early increase in translational capacity, it was concluded that the initial rise in CS resulted from selective post-transcriptional control and enhanced translation *in vivo* of existing mRNA, whereas its steep increase was due to enhanced transcription. 7. These results indicate that the neurally regulated expression of phenotype-specific properties in muscle includes control of both transcription and translation.

INTRODUCTION

Chronic indirect low-frequency stimulation of rabbit fast-twitch muscle induces a fast-to-slow conversion, involving specific cellular systems with distinct time courses (for review see Pette, 1984; Pette & Vrbová, 1985). Transitions in the isoenzyme pattern of lactate dehydrogenase (LDH) and severalfold increases in mitochondrial enzyme activities are among the earliest events (Pette *et al.*, 1973; Reichmann *et al.*, 1985). These changes suggest alterations in gene expression.

This study was undertaken to investigate the effects of altered neural activity on the translational apparatus as well as on the amounts of selected enzyme proteins and their mRNAs. Thus ribosomes were isolated from muscles stimulated for different time periods and were analysed for both yield and monosome/polysome profiles. In addition, transcriptional changes were assessed by measuring the tissue contents of mRNAs (Pollak & Lee, 1980) coding for citrate synthase (CS) and the H- and M-subunits of LDH. CS is a marker for changes in mitochondrial content (Reichmann *et al.*,

1985). The M-LDH subunit was selected because it prevails in fast-twitch fibres, whereas the H-subunit predominates in slow-twitch fibres (Leberer & Pette, 1984). The two LDH subunits therefore serve as cytosolic markers of metabolic conversions during the induced fast-to-slow transition.

Parts of this work have been communicated in a preliminary form (Seedorf *et al.*, 1985).

MATERIALS AND METHODS

Electrostimulation

Experiments were performed on adult male New Zealand White rabbits. The methods for implanting electrodes and for low-frequency (10 Hz) stimulation (12 h/day) have been described (Pette *et al.*, 1973; Schwarz *et al.*, 1983). At the end of each experimental period the rabbits were killed, and left (stimulated) and right (unstimulated control) extensor digitorum longus and tibialis anterior muscles were removed. The muscles were either frozen in liquid N₂ and stored at –80 °C or immediately processed for the isolation of ribosomes.

Abbreviations used: CS, citrate synthase (EC 4.1.3.7); LDH, lactate dehydrogenase (EC 1.1.1.27); poly(A)⁺ RNA, polyadenylated RNA.

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Preparation of antisera

Antisera directed against M-LDH, H-LDH and CS were raised in sheep. LDH isoenzymes 1 (rabbit heart) and 5 (rabbit muscle) were purchased from Boehringer Mannheim. LDH-1 was further purified on a Blue Sepharose (Deutsche Pharmacia G.m.b.H., Freiburg, Germany) column (Nadal-Ginard & Markert, 1975). CS was purified from rabbit heart (Mukherjee & Srere, 1976). The purified enzymes were dissolved in physiological saline (0.9% NaCl; 2 ml, containing 4 mg of protein) and emulsified with 2 ml of complete Freund's adjuvant. The emulsion was intradermally injected into the animal's back. Booster injections without adjuvant were performed 14, 21 and 28 days thereafter. Animals were bled 35 days later. The specificity of the antisera was tested by double immunodiffusion and Western-blot analysis (results not shown).

Purification of poly(A)⁺ RNA

Total muscle RNA was purified by a combination of the methods of Chirgwin *et al.* (1979) and Kirby (1968). Frozen muscle (0.5–2.5 g) was pulverized under liquid N₂. The muscle powder was homogenized with a Polytron homogenizer in 8 ml of the following medium: 4 M-guanidinium thiocyanate, 0.5% (w/v) sodium laurylsarcosine, 25 mM-sodium citrate, 0.1 mM-mercaptoethanol, 0.1% (w/v) Antifoam A (Sigma, München, Germany), pH 7.0. The homogenate was extracted twice with 8 ml of phenol/chloroform/3-methylbutan-1-ol (25:25:1, by vol.). Nucleic acids were precipitated overnight at –20 °C after addition of 0.025 vol. of 4 M-sodium acetate, pH 6.0, and 2.5 vol. of ethanol. The precipitate was collected by 10 min centrifugation at –10 °C in a Sorvall HB-4 rotor at 7000 rev./min (8000 *g*_{av.}). Nucleic acids were then dissolved in 1.5 ml of guanidinium chloride stock solution (7.5 M-guanidinium chloride, 25 mM-sodium citrate, 5 mM-dithiothreitol, pH 7.0). RNA was precipitated overnight at –20 °C after the addition of 0.025 vol. of 1 M-acetic acid and 0.5 vol. of ethanol, and collected by centrifugation in the cold (as above). RNA was redissolved in 1 ml of the guanidinium chloride stock solution and precipitated as above. After two washings in ethanol, RNA was dried under vacuum and dissolved in 300 µl of water. Poly(A)⁺ RNA was prepared by chromatography on oligo(dT)-cellulose (Aviv & Leder, 1972). The integrity of the purified RNA samples was routinely checked by agarose-gel electrophoresis (Maniatis *et al.*, 1982).

Translation *in vitro*

This was performed by using the reticulocyte lysate system (Pelham & Jackson, 1976). For determining mRNA translatable *in vitro* coding for M-LDH, 0.25 µg of poly(A)⁺ RNA dissolved in 2 µl of sterile water was mixed with 13 µl of pre-mix and 10 µl of reticulocyte lysate (NEN). The pre-mix consisted of 100 µl of [³⁵S]methionine (1 mCi; 'translation grade'; ≥ 800 Ci/mmol; NEN), 110 µl of translation cocktail, 40 µl of 1 M-potassium acetate and 10 µl of 25 mM-magnesium acetate. Translation *in vitro* was performed at 37 °C for 45 min. Translation efficiency was determined by trichloroacetic acid precipitation of 1 µl samples before the beginning and after the end of the translation *in vitro*. For controls, one reaction tube contained 2 µl of sterile water instead of RNA and another tube contained 2 µl of a

control RNA (supplied by NEN). For the quantification of mRNAs translatable *in vitro* coding for H-LDH and CS, the reaction volume was increased 3-fold (75 µl).

Immunoprecipitation

After completion of the assay of translation *in vitro* the synthesized M-LDH, H-LDH and CS proteins were immunoprecipitated. For this purpose, 1 vol. of NET buffer [0.15 M-NaCl, 0.05% (v/v) Nonidet P-40, 5 mM-EDTA, 200 kallikrein-inhibitory units of Trasylol/ml, 2 mM-phenylmethanesulphonyl fluoride, 50 mM-Tris/HCl, pH 7.5] and 3 µl of purified LDH-1, LDH-5 or CS (1 mg/ml each) were added. Insoluble material was removed by centrifugation for 15 min at 10000 *g*_{max}; then 10 µl of antiserum (diluted 1:10) was added to the supernatant fraction. To measure CS and H-LDH in the same assay, 10 µl of each antiserum dilution was added (see Fig. 2a). These amounts of antisera were sufficient for complete immunoprecipitation of the antigens. Tubes were incubated for 60 min at room temperature. For precipitation of the antibody-antigen complex, 50 µl of formaldehyde-fixed (Kessler, 1975) *Staphylococcus* a was added. After 3 h incubation at room temperature, immunocomplexes were sedimented by centrifugation at 30 s at maximal speed in an Eppendorf centrifuge. The sediment was washed with 4 × 0.5 ml of NET-sucrose (0.15 M-NaCl, 0.05% Nonidet P-40, 5 mM-EDTA, 0.25 M-sucrose, 50 mM-Tris/HCl, pH 7.5). The antigens were redissolved by suspending the washed immunoprecipitates in 50 µl of lysis buffer [10% (v/v) glycerol, 2.3% (w/v) SDS, 0.2 mg of Bromophenol Blue/ml, 0.0625 M-Tris/HCl, pH 6.8] at 95 °C for 5 min. The *Staphylococcus* a were then removed by centrifugation.

Quantification of immunoprecipitated protein

The supernatant fraction from the preceding centrifugation was applied to a 10% -polyacrylamide slab gel for electrophoresis by the Laemmli (1970) method. The gels were stained with Coomassie Brilliant Blue, incubated in an enhancer solution (EN³HANCE; NEN) for 60 min, and rinsed for 60 min in water. Gels were then dried and exposed on Kodak SB film for 2–10 days.

The amount of [³⁵S]methionine incorporated into the synthesized M-LDH, H-LDH and CS was determined by cutting out the respective bands from the dried gel by using the fluorography and the co-electrophoresed purified proteins as a reference. The excised slices were introduced into counting vessels and incubated overnight in 5 ml of Lipoluma/Lumasolve (Baker Chemical Co.) (10% Lumasolve, 2% water in Lipoluma) at room temperature. Radioactivity was counted on the ¹⁴C channel of a liquid-scintillation counter. An immunoprecipitation performed with pre-immune serum was used as a control.

Preparation of ribosomes

Glassware was silicone-treated and baked, solutions were autoclaved, and other equipment was treated with 0.1 M-NaOH. All solutions contained 100 units of heparin (Serva, Heidelberg, Germany)/ml as a ribonuclease inhibitor. Freshly excised muscles were put into 5 vol. (v/w) of ice-cold homogenizing medium, consisting of 250 mM-KCl, 10 mM-MgCl₂, 5 mM-EGTA, 0.1 mM-EDTA, 10 mM-mercaptoethanol, 50 mM-Tris adjusted to pH 7.6 with HCl (buffer A) plus 250 mM-sucrose. The

tissue was finely minced with scissors, homogenized (2×5 s) with a Polytron homogenizer at medium speed and, after the addition of 10% Triton X-100 (dissolved in homogenizing medium) to a final concentration of 1%, subjected to three strokes in a Potter–Elvehjem homogenizer (glass/Teflon). The homogenate was centrifuged for 10 min in a Sorvall SS 34 rotor at 6500 rev./min ($5100 g_{\max}$). The clear supernatant was filtered through silicone-treated sterile glass wool. The filtrate was diluted to 1% with 10% deoxycholate, stirred gently for 30 min on ice and layered above a discontinuous sucrose gradient (upper layer, 0.5 M-sucrose in buffer A; lower layer, 2.0 M-sucrose in buffer A). Centrifugation at 4°C for 20 h in a Beckman 60 Ti rotor at 35000 rev./min ($87000 g_{\text{av}}$) yielded a clear ribosome pellet, which was drained and gently washed with sterile distilled water after the sucrose medium had been cautiously decanted. The resulting pellet was resuspended in 300 μl of a solution containing 25 mM-KCl, 5 mM-MgCl₂, and 50 mM-Tris adjusted with HCl to pH 7.6 (buffer B). An additional 200–300 μl of buffer B was used to wash the centrifuge tube.

At this point, the preparation still contained some material which could not be resuspended, but was sedimented by 5 min centrifugation in an Eppendorf table centrifuge. After washing the resulting pellet, the combined supernatants contained the highly purified ribosomes. The A_{260} and A_{280} were measured. The sedimented material consisted of chromatin and insoluble muscle proteins, as shown by electrophoretic analyses and protein, RNA and DNA determinations.

Sucrose-density gradient profiles

A sample of the ribosome suspension, usually 100–300 μl , was layered over a linear (0.4–1.5 M) sucrose gradient made up in buffer B. The gradients were centrifuged at 4°C for 2 h in a Beckman SW 40 Ti rotor at 31000 rev./min ($170000 g_{\max}$) and monitored at 260 and 280 nm by upwards displacement with 2 M-sucrose through a Pharmacia flow-cell assembly. The resulting profiles showed distinct monosome and polysome peaks. The peak areas were integrated and used for calculating monosome and polysome portions.

Enzyme activities and determination of H- and M-LDH subunits

Enzyme activities were measured at 30°C by published methods (Leberer & Pette, 1984; Reichmann *et al.*, 1985). Electrophoresis of LDH isoenzymes was performed in 7.7%-polyacrylamide slab gels (Leberer & Pette, 1984). Isoenzyme bands were evaluated by densitometry. The percentage distribution of H- and M-subunits was calculated as described by Thorling & Jensen (1966).

RESULTS

Changes in total poly(A)⁺ RNA

Total poly(A)⁺ RNA significantly increased in muscles stimulated for more than 6 days (Fig. 1). A maximum increase of 2.5-fold was attained after 14–21 days. Poly(A)⁺ RNA was still elevated 1.7-fold after 52 days compared with the unstimulated contralateral extensor digitorum longus.

Fig. 1. Time course of changes in total poly(A)⁺ RNA

Total RNA was extracted from stimulated and contralateral extensor digitorum muscles and its poly(A)⁺ RNA fraction was determined after chromatography on oligo (dT)-cellulose by measuring the A_{260} . Values represent means $\pm$ S.D. for three to six stimulated muscles for each time point, and are expressed as a percentage of the mean of all contralateral unstimulated muscles.

Fig. 2. Time-dependent effects of chronic (10 Hz) nerve stimulation on the expression of CS in rabbit extensor digitorum longus muscle

(a) Poly(A)⁺ RNA translatable *in vitro* coding for CS; (b) tissue content of CS activity. Values represent means $\pm$ S.D. for three animals at each time point.

Optimum conditions for translation *in vitro*

Translation *in vitro* (for the proteins under study) was optimal at 80 mM-K⁺ and 0.5 mM-Mg²⁺. [³⁵S]Methionine incorporation was linear during 45 min of incubation and increased linearly between 0 and 10 μg of added

Fig. 3. Time-dependent effects of chronic (10 Hz) nerve stimulation on the expression of the H-subunit of LDH in rabbit extensor digitorum longus muscle

(a) Poly(A)⁺ RNA translatable *in vitro* coding for the H-subunit of LDH; (b) tissue content of the H-subunit of LDH. The H-subunit content was calculated from the percentage distribution of the LDH isoenzymes. Values represent means $\pm$ S.D. for three animals at each time point.

Fig. 4. Time-dependent effects of chronic (10 Hz) stimulation on the expression of the M-subunit of LDH in rabbit extensor digitorum longus muscle

(a) Poly(A)⁺ RNA translatable *in vitro* coding for the M-subunit of LDH; (b) tissue content of the M-subunit of LDH. The M-subunit content was calculated from the percentage distribution of the LDH isoenzymes. Values represent means $\pm$ S.D. for three animals at each time point.

poly(A)⁺ RNA/ml. The specific [³⁵S]methionine incorporation into trichloroacetic acid-precipitable protein amounted to approx. 50000 c.p.m./ μ l during a 45 min incubation period. The incorporated radioactivity was approx. 6000 c.p.m./ μ l in the absence of added poly(A)⁺ RNA.

H- and M-LDH, as well as CS, were immunoprecipitated from the translation assays *in vitro* by the specific antisera. Electrophoresis of the immunoprecipitates showed a single band for each enzyme (results not shown).

Stimulation-induced changes in specific poly(A)⁺ RNAs translatable *in vitro*

Chronic nerve stimulation led to both increases (CS, H-LDH) and decreases (M-LDH) in translatable *in vitro* poly(A)⁺ RNAs. Poly(A)⁺ RNA translatable *in vitro* coding for CS started to increase after 6 days and attained an 8-fold increase after 14 days (Fig. 2a). Stimulation up to 52 days induced no further increase. Poly(A)⁺ RNA translatable *in vitro* coding for H-LDH was unaltered up to 4 days and then steeply increased almost 2-fold between days 4 and 10 (Fig. 3a). Conversely, poly(A)⁺ RNA translatable *in vitro* coding for M-LDH continuously decreased, beginning 4 days after the onset of stimulation (Fig. 4a), reaching an apparently stable value of 50% of normal after 28 days.

Table 1. Effect of chronic (10 Hz) nerve stimulation on total LDH activity and percentage distribution of LDH isoenzyme subunits in extensor digitorum longus muscles of the rabbit

The percentage of M- and H-subunits was calculated as described by Thorling & Jensen (1966) from the densitometrically evaluated isoenzyme electrophoreses. Data represent mean values $\pm$ S.D. ($n = 3$).

Period of stimulation (days)	Total LDH activity (units/g wet wt.)	LDH subunit (%)	
		M	H
0	852 $\pm$ 121	91.2 $\pm$ 1.8	8.8 $\pm$ 2.0
4	749 $\pm$ 23	86.8 $\pm$ 3.0	13.2 $\pm$ 2.6
8	856 $\pm$ 37	78.8 $\pm$ 2.8	21.2 $\pm$ 3.7
14	635 $\pm$ 48	73.1 $\pm$ 3.7	26.9 $\pm$ 3.2
28	535 $\pm$ 85	63.8 $\pm$ 3.5	36.2 $\pm$ 3.6
50	475 $\pm$ 35	50.4 $\pm$ 3.2	49.6 $\pm$ 5.5

Stimulation-induced changes in CS activity and in H- and M-LDH

Confirming previous observations (Heilig & Pette, 1980), chronic stimulation induced a biphasic progressive increase in CS activity (Fig. 2b). Its initial increase was

Fig. 5. Time course of changes in ribosome yield

The ribosome yield, expressed as A_{260}/g of muscle, was determined in stimulated (●) and contralateral (○) tibialis anterior muscles. Monosome and polysome fractions were separated by centrifugation on sucrose density gradients, and their relative concentrations were evaluated by integrating the respective peak areas of the ribosome profiles. Except for 28 and 60 days, where only two animals were investigated, the values represent means $\pm$ S.D. for three to six muscles for each time point. (a) Total ribosomes; (b) monosomes; (c) polysomes.

moderate, but was significantly enhanced 4 days after the onset of stimulation. After 6 days the increase became steeper, reaching a maximum value (4-fold elevated) after 28 days.

Total activity of LDH progressively decreased with prolonged stimulation. In addition, there was a profound rearrangement of the isoenzyme pattern (Table 1). The unstimulated EDL muscles contained almost exclusively LDH-5. Stimulation induced the appearance of isoenzymes LDH-4, LDH-3, LDH-2 and LDH-1, with a predominance of LDH-3 after prolonged stimulation periods. The decrease in total LDH activity was due to a decrease in the M-subunit, which reached 50% of its normal concentration after 52 days (Fig. 4b). This

decrease was partially offset by an increase in the H-subunit (Fig. 3b). A steep increase in the H-subunit occurred after 4 days, and a 2-fold increase was attained after 10 days. Therefore the initial changes in the isoenzyme pattern were primarily due to an increased synthesis of the H-subunit, whereas the later changes resulted from the slow decay of the M-subunit.

Changes in ribosomes

A steep increase in ribosome yield was seen as early as 2–4 days after the onset of stimulation and then continued at a lower rate until a maximum was reached after 21 days (Fig. 5a). At this time, ribosomes were increased 5-fold. Subsequently ribosome yield decreased, but was still more than 2-fold higher in 60-day-stimulated muscles compared with contralateral muscles, and thus corresponded to that of normal soleus muscle. Evaluation of the ribosome profile by integration of monosome and polysome peak areas showed that the initial increase in ribosomes seen at 2 days was primarily due to a pronounced increase in monosomes (Fig. 5b). Their subsequent increase was less steep and reached a 4-fold elevated plateau between 10 and 21 days. On the other hand, the increase in polysomes occurred with a lag phase of 1–2 days, followed by a significant increase in muscles stimulated for more than 4 days (Fig. 5c). Polysome yield reached a maximum at 21 days, attaining values 6-fold higher than for unstimulated contralateral muscles.

DISCUSSION

The rearrangement of the enzyme activity pattern during the induced 'white-to-red' conversion makes chronically stimulated muscle an excellent model for studying altered gene expression. We used chronic stimulation to assess changes in the mRNA concentrations of selected enzymes. CS is a key mitochondrial enzyme and LDH is a representative of extramitochondrial carbohydrate metabolism. Its H- and M-subunits represent cytosolic markers of the induced metabolic conversion. Both white and red fast-twitch fibres of rabbit muscles predominantly contain the M-subunit, whereas slow-twitch fibres are characterized by the predominance of H-subunit isoenzymes (Leberer & Pette, 1984). However, the rapid increase in the H-subunit observed in the present study cannot be interpreted in terms of a fast-to-slow fibre-type conversion, because the replacement of fast by slow myosin is not detectable at this early phase (Sreter *et al.*, 1973). Thus the early changes in the subunit composition of LDH support the notion that various elements of the muscle fibre may change independently and follow different time courses (Pette, 1984).

Our results demonstrate that alterations in enzyme amounts may occur with and without changes in the amounts of poly(A)⁺ RNAs as assessed by translation *in vitro*. The increase in H-LDH and the decrease in M-LDH follow time courses similar to those of their poly(A)⁺ RNAs translatable *in vitro*. Different time courses exist for CS, where the initial increase precedes the steep increase in the poly(A)⁺ RNA translatable *in vitro* (Fig. 2b). Most probably, the initial increase in CS activity results from enhanced translation *in vivo* of existing mRNA. This suggestion is supported by the finding that chronic stimulation affects the translational

apparatus before increases are detectable in the tissue contents of poly(A)⁺ RNAs translatable *in vitro*.

The ribosome yield increases as early as 2 days after the onset of stimulation (Fig. 5). The initial increase appears to result primarily from an assembly of monosomes, which thereafter are integrated into polysomes. The increase in ribosomes may also be due, in part, to a decrease in degradation. However, the steep increase in ribosomes corresponds to the increase in total RNA (Pette, 1984), and therefore appears mainly to reflect enhanced synthesis. This suggestion is in agreement with the findings of Matri *et al.* (1982). They showed that metabolites and enzymes of the polyamine pathway, including ornithine decarboxylase, are subject to pronounced transient increase as early as 1–4 days after the onset of stimulation. Ornithine decarboxylase, together with high concentrations of polyamines, are thought to enhance the synthesis of rRNA by directly stimulating a RNA polymerase I isoenzyme (Russell, 1983; Tabor & Tabor, 1984). Our results suggest that ribosome assembly and synthesis are among the earliest events during chronic stimulation. Therefore an increase in the capacity and perhaps the efficiency of translation could explain the increase in CS before the increase in its poly(A)⁺ RNA translatable *in vitro*. However, in view of the changes in H- and M-LDH, the early rise in CS cannot be due to a non-selectively enhanced translation of existing mRNA. Therefore, specific regulatory steps must be involved.

The control of eukaryotic gene expression at the stage of translation of mRNA into protein has always been a theoretical possibility. However, like other recent reports (for review, see Hunt, 1985), our results for CS strongly suggest post-transcriptional regulation, possibly including such mechanisms as cytoplasmic RNA precursors (Medford *et al.*, 1980), RNA-discriminatory initiation factors (Ray *et al.*, 1983), translational-control RNA or ribonucleoprotein particles (McCarthy *et al.*, 1983), masked mRNA, complementary RNA, and DNA or proteins which act as repressors or enhancers (Hunt, 1985).

Although the effects of chronic nerve stimulation on protein degradation have so far not been studied, the present results support the view that levels of enzymes involved in energy metabolism in muscle are primarily regulated by synthesis. A similar conclusion was previously derived from studies on enzyme turnover (Dölken & Pette, 1974; Illg & Pette, 1979). Our present results suggest that changes in enzyme synthesis brought about by increased contractile activity are primarily due to alterations at the level of transcription. It cannot be excluded by our results that the observed increases in specific poly(A)⁺ RNAs may also result from stabilization of existing mRNA molecules. However, the time courses of changes in the tissue content of both poly(A)⁺ RNAs translatable *in vitro* and the corresponding proteins point to selective switches in transcription as a major control. In addition, our results suggest post-transcriptional regulation and show that pronounced changes in the translational capacity are involved.

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