Volume 255, number 2, 427-430 FEB 07648 September 1989

Work overload induced changes in fast and slow skeletal muscle myosin heavy chain gene expression

Matthew J. Morgan and Paul T. Loughna

Department of Veterinary Basic Sciences, Royal Veterinary College, London University, London, England

Received 23 July 1989

Work induced hypertrophy of the slow postural soleus and the fast phasic plantaris muscles was produced by tenotomy of the synergistic gastrocnemius muscle. Increases in weight of both muscles were associated with proportionately even larger increases in total RNA and mRNA levels. Alterations in levels of specific myosin heavy chain (MHC) isoform mRNAs were measured using the slot blot procedure with radioactively labelled oligonucleotides as probes. Type 1 MHC gene expression was unaffected in both muscles by work overload, whereas type 2a was deinduced in the soleus and type 2b was deinduced in the plantaris. The neonatal MHC gene was transiently reinduced in the plantaris.

Myosin heavy chain; Gene expression; Hypertrophy; Skeletal muscle

1. INTRODUCTION

Myosin is a major structural component of the striated muscle contractile apparatus. The myosin heavy chain (MHC) subunits of the myosin molecule provide the essential ATPase activity necessary for the force generation. A number of sarcomeric isoforms of MHC exist, each of which is a product of an individual member of a highly conserved multi-gene family, which are expressed in a tightly regulated tissue specific and developmental stage specific manner [1]. The relative proportions of different MHC isoenzymes present in skeletal or cardiac muscle tissue appear to correspond closely with its contractile properties [2]. Adult muscles can show a considerable degree of plasticity both in terms of demonstrating rapid changes in mass and more subtle alterations in contractile protein composition. The relative proportions of myosin isoenzymes in a skeletal muscle have been observed to alter in response to a variety of stimuli, including altered patterns of innervation [3], mechanical overload [4] and hormonal environment [5,6]. In this study we have examined the effect of overload, on total and specific RNA levels, in the rat soleus and plantaris muscles. Using the slot blot technique with MHC isoform specific oligonucleotides we have analysed changes in MHC gene expression, in this system, at the mRNA level.

Correspondence address: M.J. Morgan, Dept of Veterinary Basic Sciences, Royal Veterinary College, London University, Royal College Street, London NW1 0TU, England

2. MATERIALS AND METHODS

2.1. *Animals and experimental protocol*

Overload of the slow postural soleus and the fast phasic plantaris muscles was produced, in male Sprague-Dawley rats weighing approx. 300 g (Tucks), by bilateral removal of the tendon of insertion and the distal portion of the synergistic gastrocnemius muscle, whilst under xylazine/ketamine anaesthesia. Control animals matched on body weight underwent sham surgery. Experimental and control animals were killed after 5, 10, and 20 days, the soleus and plantaris muscles rapidly removed and frozen in liquid N_2.

2.2. *Preparation of synthetic probes*

MHC gene specific oligonucleotides of 20 bases in length were obtained from Oswell DNA service. Oligothymidine (oligo-dT) of 12–18 bases in length was obtained from Sigma. The sequences of the MHC oligonucleotide probes used in this study have been reported previously [7]. They are all sequences located in the 3′ untranslated regions of the specific MHC mRNAs and have been shown to be highly isoform specific

Published by Elsevier Science Publishers B.V. (Biomedical Division)
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[7,8]. The probes were labelled at the 5′ end to a specific activity of approx. 10^7 cpm/μg and separated from [γ-^{32}P]ATP (Amersham) using Sephadex G25 columns.

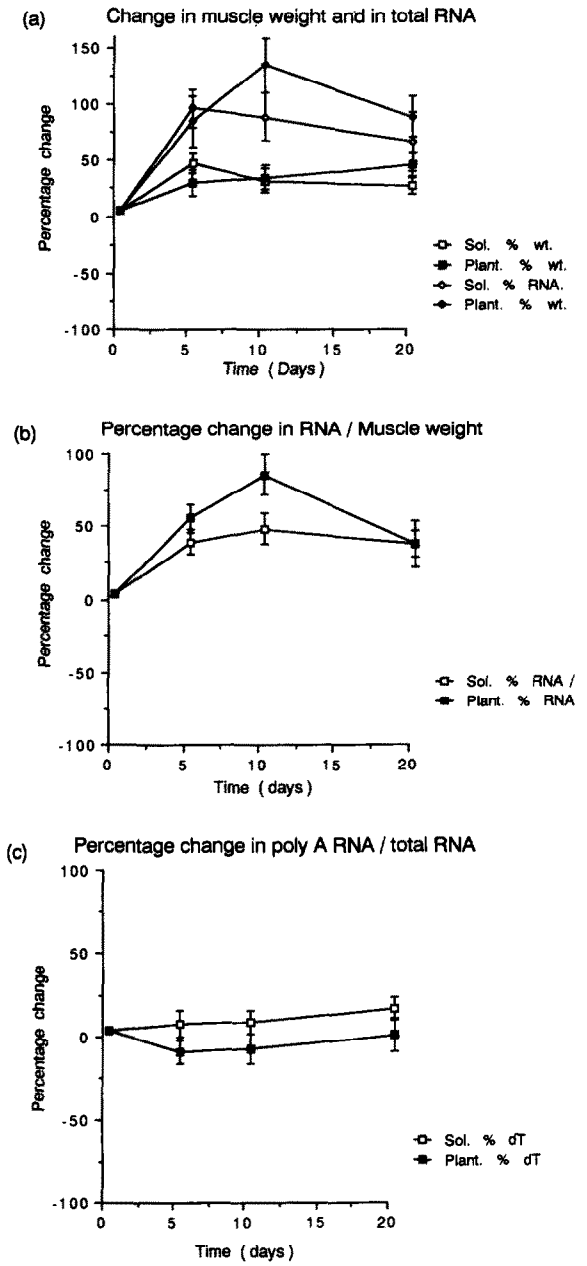

Fig.1. Percentage changes in muscle weights, total RNA and poly(A) RNA from experimental soleus and plantaris muscles are expressed in comparison to control muscles. Each point represents the mean ± SE.

2.3. *RNA analysis and dot blot assay*

Total cellular RNA was extracted by a modification of the hot phenol procedure [9]. 4-μg aliquots of RNA in 10 × SSC were blotted, in triplicate, onto Hybond N (Amersham) using a standard slot blot procedure. The filters were subsequently dried and then fixed with UV irradiation. Filters were prehybridised for 2 h in buffer containing 6 × SSC, 5 × Denhardt's solution, 1 mM EDTA, 1% SDS, 50 mM sodium phosphate, pH 6.5, and salmon sperm (200 μg/ml). Hybridizations were carried out as described previously [7,8]. In the case of hybridizations with oligo-dT probe a 20-fold excess of cold oligo-dT was added to the hybridization fluid. After the filters were washed and dried autoradiography was carried out at −70°C for 2–16 h. Afterwards individual slots were cut from the filters and the radioactivity was determined by liquid scintillation.

2.4. *Statistical analysis*

The data were analysed using analysis of variance. Mean comparisons of significant F-ratios, where appropriate, were evaluated using Newman-Keuls multiple range test. Statistical significance was set at the 95% level of confidence.

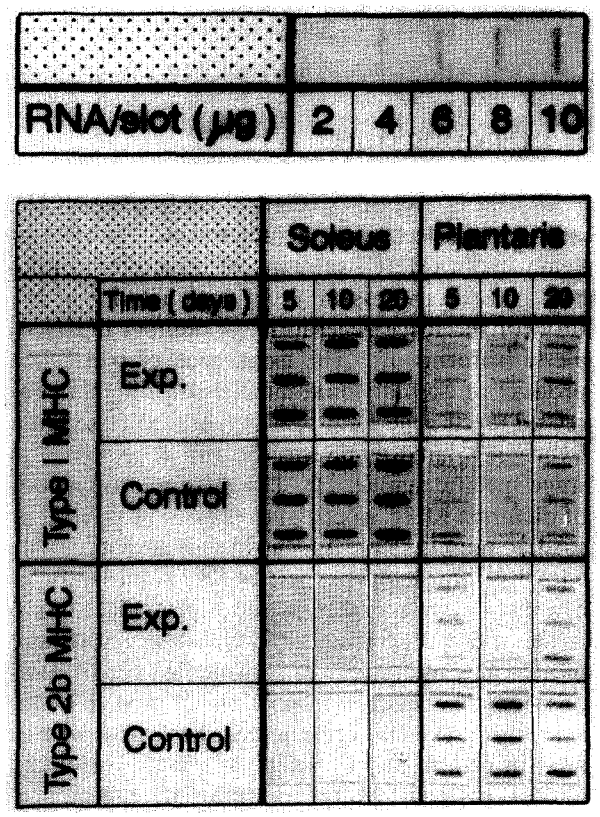

Fig.2. (a) Linear relationship between amount of gastrocnemius RNA in slot blot and hybridization with labelled type 1 MHC oligonucleotide. (b) Single slot blots for RNA from 3 control and experimental muscles hybridised with type 1 and type 2b MHC probes.

3. RESULTS

Both the soleus and plantaris muscles exhibited significant hypertrophy in response to overload (fig.1a). Although the maximum percentage increases in weight when compared to controls were similar in the 2 muscles the peak increase was not reached until after 20 days in the plantaris as opposed to only 5 days for the soleus (fig.1a). The significant increases in total RNA in both muscles were considerably greater than those for muscle weight (fig.1a) leading to rapid increases in total RNA per unit muscle weight which in both muscles reach a maximum after 10 days of overload (fig.1b). However, the significant percentage increase in RNA per unit weight, compared to control levels, in the plantaris at 10 days was twice that observed in the soleus. Despite these dramatic changes in total RNA levels in response to overload the proportion of this RNA present as poly(A) RNA did not significantly differ from control levels in either muscle during the course of this study (fig.1c).

As observed previously [7] the amount of bound synthetic ^{32}P-labelled probes follows a linear relationship with increasing amounts of total cellular RNA of up to 10 μg per slot applied to the filter (fig.2a and data not shown). Thus the amounts of RNA used in this study were well within this range. The levels of specific MHC mRNA species are expressed in relation to levels in muscles from control animals killed at the same time-points. The type 2b MHC mRNA was present at much higher levels in the control fast plantaris muscles than in the slow

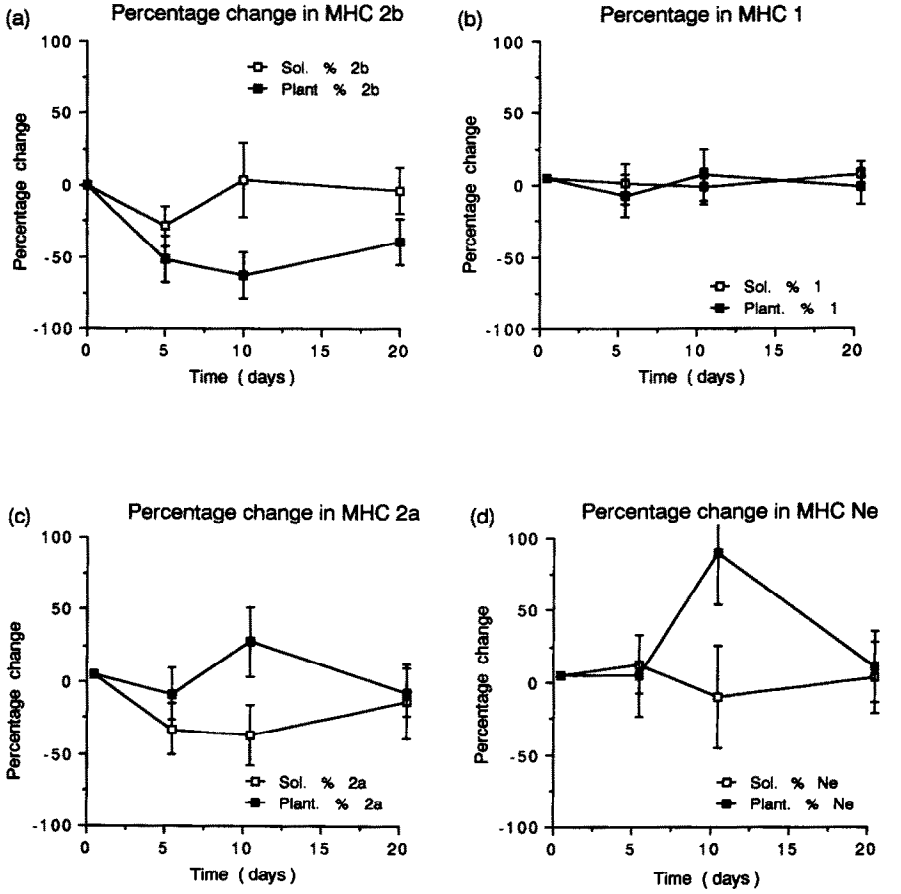

Fig.3. Slot blot analysis of the effect of work overload on hybridization of oligonucleotide MHC probes with RNA from the soleus and plantaris muscles. The values represent the change in filter-bound radioactivity associated with RNA from overloaded muscles as compared to that from muscles of control animals. Each point represents the mean ± SE of triplicate determinations on muscles of 4 animals.

postural soleus muscles (fig.2b). The response of this gene to work overload differed between these 2 muscles. In the plantaris there was a dramatic and significant deinduction of type 2b MHC gene expression that was maintained throughout the course of this study, reaching a maximum at 10 days (fig.3a). In contrast this mRNA species only fell significantly below control levels at 5 days after tenotomy of the gastrocnemius and subsequently exhibited no significant difference. However, for type 1 MHC there was no significant difference from control levels, in either muscle at any time-point in this study (fig.3b). As with type 2b MHC, work overload produced different effects upon type 2a MHC gene expression in the 2 muscles investigated (fig.3c). In the plantaris there was no significant increase in this mRNA when compared to control levels whereas in the soleus there was a significant deinduction of this gene after 5 and 10 days of overload (fig.3c). The only distinct change in levels of neonatal gene expresion was observed in the plantaris muscle where there was a large induction of this gene at the 10 day time-point (fig.3d). Levels of expression of the embryonic gene were very low in both muscles and in neither did overload produce any measurable effects (data not shown).

4. DISCUSSION

Both the soleus and plantaris muscles exhibited similar qualitative responses to work overload, induced by the tenotomy of the synergistic gastrocnemius muscle in terms increased muscle weight, total RNA and mRNA (poly(A)) content. In contrast the responses to overload of the MHC genes differed within each muscle and also, with the exception of the type 1 MHC gene, there was a dissimilar response by the same gene in the 2 muscles. The lack of induction of the type 1 MHC gene in the overloaded plantaris contrasts with the mean increase (78% over 4 weeks) of this isoform at the protein level observed by Gregory et al. [4] using a similar experimental protocol. However, this may be explained by the marked deinduction of the type 2b MHC gene, which is normally expressed at high levels in this muscle, and by the overall changes in muscle RNA levels. Due to the increases in total RNA after 10 days of work overload the amount of total type 1 MHC mRNA per unit wet weight of muscle is increased to about 80% above control levels and the total amount of type 2b MHC is reduced by 34% of control levels. Thus in terms of total mRNA present in this muscle the relative proportions of these 2 isoforms are modulated by work overload in different directions. The effects of work overload upon type 2b MHC gene expression in the plantaris correspond with observations we have made (unpublished) upon the effects of disuse in the soleus muscle. When the soleus is immobilised at less than resting length there is a rapid induction of the type 2b MHC gene suggesting that the normal activity pattern of this slow postural is inhibiting expression of this gene. Correspondingly in this study we observed in the plantaris an increased level of activity deinduced this gene. Interestingly in the soleus increased work overload only induced a transitory deinduction of the type 2b MHC gene. It appears that increased workload causes a deinduction of the fastest isoform present at significant levels in a muscle which for the plantaris is the type 2b isoform and for the soleus is the type 2a isoform.

Due to the normal activity patterns of the two muscles analysed in this study a more dramatic effect on phenotype might be expected in the fast phasic plantaris muscle following tenotomy of the gastrocnemius. This might explain the observed transitory induction of the neonatal isoform in this muscle.

Acknowledgement: This work was funded by a grant from the British Heart Foundation.

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