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# Slow-to-fast transitions in myosin expression of rat soleus muscle by phasic high-frequency stimulation

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**Abstract Denervated soleus muscles of euthyroid and hyperthyroid rats were exposed to phasic high-frequency stimulation for periods of up to 40 days and analysed for their myosin heavy chain (MHC) composition. Denervation alone induced appreciable amounts of the fast MHCIId/x and minute amounts of MHCIIb. However, the effects of phasic high-frequency stimulation exceeded by far those of denervation, leading to marked increases of these two isoforms, as well as to pronounced decreases in slow MHCI. In addition, the present study suggested a greater impact of neural activity on myosin expression than thyroid hormone.**

*Key words:* High-frequency stimulation; Impulse pattern; Myosin heavy chain; Rat; Slow-to-fast transformation; Soleus muscle; Thyroid hormone

## 1. Introduction

Skeletal muscle fibers are capable of adjusting their phenotypic properties to altered functional demands [1]. This plasticity relates to the expression of most muscle proteins in various isoform combinations. The expression of these isoform patterns has been shown to be under the control of several factors. A major influence is exerted by the neurally transmitted impulse pattern, but additional factors, e.g., thyroid hormone, seem to be important [1]. The impact of neural activity has been documented by chronic low-frequency stimulation (CLFS) which transforms fast-twitch muscles into slower contracting muscles [2] (for review see [3]). This fast-to-slow transformation encompasses all functional elements of the muscle fiber and involves the exchange of fast-type myofibrillar protein isoforms with their slow counterparts. As for myosin, this process is reflected by sequential transitions in its heavy-chain (MHC) isoforms in the order of MHCIIb → MHCIId/x → MHCIIa → MHCI [4,5]. Conversely, a transformation of slow-twitch muscles into faster contracting muscles is possible by phasic high-frequency stimulation (PHFS) [6,7]. As qualitatively shown for the slow soleus muscle of rat, PHFS leads to an up-regulation of the fast-type MHCIIa and MHCIId/x isoforms, but not of MHCIIb [8]. The authors of that study concluded that slow-twitch soleus muscle is incapable of expressing MHCIIb, the fastest isoform present in rat limb muscles. Since fast rat muscles with a predominance of MHCIIb can be manipulated to express MHCI [9], we asked whether the expression of MHCIIb is truly excluded for rat soleus muscle. We also wanted to analyse quantitatively the effects of PHFS on the two other fast isoforms, MHCIIa and MHCIId/x. To this end, we studied the effects of PHFS on the MHC isoform patterns in soleus muscles and, in addition, investigated to what extent the stimulation-induced changes may be modulated by hyperthyroidism. We have previously shown that elevated thyroid hormone levels counteract the fast-to-slow transformation induced by low-frequency stimulation in fast-twitch muscle of the rat [9]. Therefore, we examined whether an elevation of the thyroid hormone level might amplify the slow-to-fast transition induced by PHFS.

## 2. Materials and methods

### 2.1. Animals, denervation, high-frequency stimulation, hyperthyroidism

Adult male rats (Wistar) weighing between 250 and 300 g were used in three experimental groups (innervated/unstimulated, denervated/unstimulated, denervated/stimulated). Denervation was performed on the left hindlimb by excising 1 cm of the sciatic nerve and sewing back the proximal stump. For stimulation, electrodes were placed around the proximal and distal parts of the soleus muscle; leads were externalized at the neck and connected to a small receiver fixed on the animal's back. Animals were stimulated 24 h daily using a newly developed telestimulation setup for rats. The stimulation protocol consisted of 25 pulses at 150 Hz every 15 min [10]. Stimulation lasted for periods of 18–40 days because in some animals stimulation failed after some time and these animals had to be killed when failure occurred. Stimulation was performed in euthyroid and hyperthyroid animals. Hyperthyroidism was induced 1 week before starting the stimulation by implanting encapsulated triiodo-L-thyronine (1.5 mg) pellets with biodegradable carrier-binder (IRA, Toledo, OH). Implantation of the pellets was repeated after 3 weeks. The unstimulated/innervated soleus muscle of the right hindlimb served as a control. In order to study the effects of denervation alone, a separate group of euthyroid animals underwent denervation of the left hindlimb without stimulation. The duration of denervation matched the time period of stimulation in the euthyroid denervated/stimulated group.

### 2.2. Myosin heavy-chain extraction and electrophoresis

Muscles were powdered under liquid nitrogen and crude myosin extracted in a solution containing 300 mM KCl, 100 mM sodium pyrophosphate, 5 mM EGTA, 5 mM $MgCl_2$ and 1 mM dithiothreitol (pH 6.5). The extracts were diluted (1:1) with glycerol and stored at −25°C. Electrophoresis was performed on gels containing 7% acrylamide, 0.06% bisacrylamide, 29% glycerol, 720 mM Tris-HCl (pH 8.7), and 0.1% SDS. The stacking gel contained 4% acrylamide, 0.18% bisacrylamide, 160 mM Tris-HCl (pH 6.7) and 0.1% SDS. Electrophoresis was performed for 20 h at 120 V in a chamber cooled with tap water. The gels were silver-stained [11] and evaluated by integrating densitometry.

## 3. Results

Phasic high-frequency stimulation was performed on denervated soleus muscle to exclude the influence of the tonic low-frequency impulse pattern normally transmitted to the muscle by its nerve. It was necessary, therefore, to study the effects of denervation alone. The innervated/unstimulated soleus muscles contained MHCI as the predominant isoform and

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Fig. 1. Electrophoretic separation of MHC isoforms in extracts from rat soleus muscles denervated for (a) 27 days, and (b) 34 days. In order to detect isoforms present at low amounts, electrophoresis of the same extracts depicted in (A) was performed at high-protein loading (B). With high loading, which did not lead to a separation of MHCIIa and MHCIId/x, low amounts of MHCIIb and $MHC_{neo}$ were detected. Note the high concentration of MHCIa.

MHCIIa as a minor isoform (Table 1). The major effects of denervation consisted of an increase in fast MHCIIa and an induction of the fast MHCIId/x, concomitant with a decrease in the relative amount of the slow MHCI (Fig. 1A). When large amounts of protein were applied to the gel, also minute amounts of MHCIIb and the neonatal $MHC_{neo}$ were detected (Fig. 1B and Table 1).

Phasic high-frequency stimulation induced marked slow-to-fast transitions with pronounced inductions of MHCIId/x and MHCIIb isoforms (Fig. 2 and Table 1). It should be kept in mind, however, that interanimal variations can be expected when denervated muscles are chronically stimulated via implanted electrodes. Variations may result from slightly different electrode positions and from existing interanimal differences in fiber type composition of soleus muscle at the onset of stimulation. Finally, the muscles under study were exposed to PHFS for various time periods (18–40 days) and this also contributed to the variations observed. However, the stimulation-induced changes displayed the same tendency. Thus, the normally predominant MHCI isoform was reduced on the average to less than 35% and MHCIId/x became the predominant isoform. MHCIIb amounted to approximately 15%. MHCIIa was present at relatively low concentrations, ranging from undetectable levels to 11%.

An additional faint band was often observed, especially when high protein concentrations were loaded on the gel in control, denervated, and in some of the stimulated muscles. This band migrated slightly faster than MHCI, and was assumed to correspond to the previously described MHCIa isoform [12]. Its identity as an α-cardiac-like MHC previously detected in transforming rabbit muscle [13], is highly improbable because preliminary immunohistochemical studies with an antibody raised against the α-cardiac MHC isoform did not detect positive fibers (data not shown).

Hyperthyroidism by itself had only a slight effect on the innervated/unstimulated soleus muscle. The slow MHCI was reduced but remained the major isoform. Its decrease corresponded to the increase in MHCIIa. Detectable levels of MHCIId/x were induced in two animals (Table 1). MHCIIb, the fastest isoform, was never detected in these muscles, even when large amounts of protein were applied to the gel (results not shown). Hyperthyroidism combined with PHFS did not markedly enhance the slow-to-fast transitions produced by high-frequency stimulation alone (Fig. 2 and Table 1).

## 4. Discussion

It is generally accepted that neurally transmitted impulse patterns have a specifying effect on the phenotypes of muscle fibers. A tonic low-frequency stimulus pattern applied to fast-twitch muscles evokes fast-to-slow transitions in myofibrillar protein isoforms [3]. A modulatory influence of the impulse pattern on gene expression is evident also from phasic high-frequency stimulation which, however, produces slow-to-fast transitions. It is noteworthy that the stimulation protocol used in the present study evokes only negligible contractile activity (one 160 ms tetanic contraction every 15 min). Nevertheless, the changes in contractile [6,7] and biochemical [8,10] (present study) properties induced by PHFS are pronounced.

Here we confirm that a denervated slow muscle is converted into a faster muscle under the influence of an impulse pattern characteristic of a fast motoneuron [6,14]. We extend this notion by demonstrating that this conversion covers the whole range of slow and fast MHC isoforms. Thus, soleus muscle exposed to PHFS expresses not only MHCIId/x, as previously shown [8], but also the fastest isoform, MHCIIb. Because PHFS was performed on a denervated muscle, the question arises to what extent the observed alterations may have resulted from denervation. The answer is obvious: compared to the PHFS-induced slow-to-fast transitions in MHC isoform expression, the alterations induced by denervation alone, are minute. The stimulation-induced up-regulation of the fast

Table 1
Densitometrically evaluated relative concentrations of myosin heavy-chain isoforms in innervated/unstimulated (INN/UNST), denervated/unstimulated (DEN/UNST), and denervated/stimulated (DEN/ST) soleus muscles of euthyroid and hyperthyroid rats

| | INN/UNST | | DEN/ST | | DEN/UNST |
|---|---|---|---|---|---|
| | Euthyr. % | Hyperthyr. % | Euthyr. % | Hyperthyr. % | Euthyr. % |
| MHCI | 86.7 ± 6.9 (75.4–98.0) | 75.6 ± 11.0 (55.3–89.7) | 33.4 ± 5.5 (23.5–44.8) | 30.4 ± 6.4 (21.2–42.2) | 41.0 ± 11.7 (28.3–51.2) |
| MHCIa | 2.4 ± 0.8 (1.8–4.1) | 2.9 ± 1.9 (1.25–6.9) | 0.4 ± 0.8 (0–2.0) | 0.7 ± 0.8 (0–2.0) | 5.9 ± 2.3 (3.5–8.0) |
| MHCIIa | 10.8 ± 6.8 (0–21.7) | 19.7 ± 8.9 (8.3–35.2) | 5.3 ± 3.4 (0–10.9) | 4.8 ± 3.2 (0–10.9) | 28.2 ± 4.6 (24.4–33.4) |
| MHCIId/x | 0 | 1.6 ± 4.5 (0–12.6) | 46.0 ± 5.4 (36.6–55.2) | 50.6 ± 4.0 (46.2–57.2) | 17.6 ± 8.4 (11.6–27.2) |
| MHCIIb | 0 | 0 | 14.8 ± 4.6 (7.5–21.2) | 14.7 ± 6.6 (9.1–27.3) | 3.7 ± 0.3 (3.5–4.0) |
| $MHC_{neo}$ | 0 | 0 | 0 | 0 | 3.6 ± 0.6 (3.2–4.3) |

Values are means ± SD from animals stimulated for time periods of 28.3 ± 4.2 days (euthyroid) and 27.3 ± 7.4 days (hyperthyroid). Minimum and maximum values are given in parentheses; $n$=9 for euthyroid and $n$=8 for hyperthyroid animals.

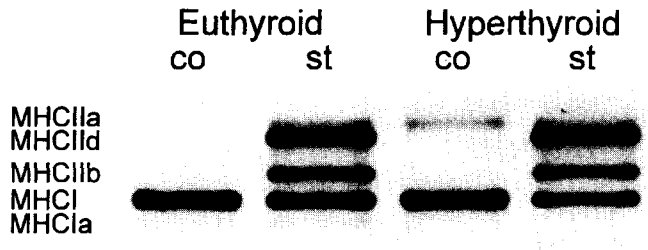


Fig. 2. Electrophoretic separation of MHC isoforms in extracts of denervated soleus muscles of euthyroid and hyperthyroid rats exposed to phasic high-frequency stimulation for 27 days.

MHCIId/x and MHCIIb isoforms at the expense of MHCI and MHCIIa is so strong that the MHC pattern of the denervated/stimulated soleus muscle resembles that of a mixed fast muscle, e.g., the gastrocnemius muscle [15]. In one animal the relative concentration of MHCIIb reached almost 30% and we assume that the slow-to-fast transformation might proceed even further with longer stimulation periods than used in the present study.

A high percentage of hybrid fibers, containing newly synthesized fast MHC isoforms together with MHCI can be expected to exist in the stimulated soleus muscle. Moreover, the relative concentration of MHCIId/x observed in the present study is so high that some fibers must have reached the state of a fast phenotype, i.e., type IID/X. Orienting histochemical analyses (results not shown) are in support of these suggestions.

Hyperthyroidism did not seem to essentially amplify the stimulation-induced slow-to-fast transitions. This might suggest that neural activity has a greater impact on the muscle phenotype than thyroid hormone. The amount of thyroid hormone applied in the present study was lower than in other studies (e.g., [16]) because we aimed to avoid thyreotoxic effects. This may also explain why the slow-to-fast changes in innervated/unstimulated soleus muscle were less pronounced than reported by others. However, an even smaller dosage has been shown in a previous study to suppress the fast-to-slow transitions induced by of chronic low-frequency stimulation in fast muscles of the rat [9].

In summary, we show that, analogously to the fast-to-slow transformation of type II fibers by chronic low-frequency stimulation, a slow-to-fast transformation of type I fibers can be achieved by phasic low-amount, high-frequency stimulation. The changes in relative concentrations of MHC isoforms suggest that the slow-to-fast transformation occurs by sequential transitions in MHC expression in the order of MHCI → MHCIIa → MHCIId/x → MHCIIb.

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## References

[1] Pette, D. and Staron, R.S. (1997) Int. Rev. Cytol. 170, 143–223.
[2] Salmons, S. and Vrbová, G. (1969) J. Physiol. 201, 535–549.
[3] Pette, D. and Vrbová, G. (1992) Rev. Physiol. Biochem. Pharmacol. 120, 116–202.
[4] Termin, A., Staron, R.S. and Pette, D. (1989) Eur. J. Biochem. 186, 749–754.
[5] Leeuw, T. and Pette, D. (1993) Eur. J. Biochem. 213, 1039–1046.
[6] Lömo, T. and Westgaard, R.H. (1974) Proc. R. Soc. Lond. B 187, 99–103.
[7] Eken, T. and Gundersen, K. (1988) J. Physiol. 402, 651–669.
[8] Ausoni, S., Gorza, L., Schiaffino, S., Gundersen, K. and Lömo, T. (1990) J. Neurosci. 10, 153–160.
[9] Kirschbaum, B.J., Kucher, H.-B., Termin, A., Kelly, A.M. and Pette, D. (1990) J. Biol. Chem. 265, 13974–13980.
[10] Gundersen, K., Leberer, E., Lömo, T., Pette, D. and Staron, R.S. (1988) J. Physiol. 398, 177–189.
[11] Oakley, B.R., Kirsch, D.R. and Morris, N.R. (1980) Anal. Biochem. 105, 361–363.
[12] Fauteck, S.P. and Kandarian, S.C. (1995) Am. J. Physiol. 37, C419–C424.
[13] Hämäläinen, N. and Pette, D. (1994) J. Muscle Res. Cell Motil. 15, 189.
[14] Hennig, R. and Lömo, T. (1985) Nature 314, 164–166.
[15] Aigner, S., Gohlsch, B., Hämäläinen, N., Staron, R.S., Uber, A., Wehrle, U. and Pette, D. (1993) Eur. J. Biochem. 211, 367–372.
[16] Larsson, L., Li, X.P., Teresi, A. and Salviati, G. (1994) J. Physiol. 481, 149–161.