Gene expression of myogenic factors and phenotype-specific markers in electrically stimulated muscle of paraplegics

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Submitted 18 October 2004; accepted in final form 1 March 2005

Vissing, Kristian, Jesper L. Andersen, Stephen D. R. Harridge, Claudia Sandri, Andreas Hartkopp, Michael Kjaer, and Peter Schjerling. Gene expression of myogenic factors and phenotype-specific markers in electrically stimulated muscle of paraplegics. J Appl Physiol 99: 164–172, 2005. First published March 3, 2005; doi:10.1152/japplphysiol.01172.2004.—The transcription factors myogenin and MyoD have been suggested to be involved in maintaining slow and fast muscle-fiber phenotypes, respectively, in rodents. Whether this is also the case in human muscle is unknown. To test this, 4 wk of chronic, low-frequency electrical stimulation training of the tibialis anterior muscle of paraplegic subjects were used to evoke a fast-to-slow transformation in muscle phenotype. It was hypothesized that this would result from an upregulation of myogenin and a downregulation of MyoD. The training evoked the expected mRNA increase for slow fiber-specific markers myosin heavy chain I and 3-hydroxyacyl-CoA dehydrogenase A, whereas an mRNA decrease was seen for fast fiber-specific markers myosin heavy chain IIX and glycerol phosphate dehydrogenase. Although the slow fiber-specific markers citrate synthase and muscle fatty acid binding protein did not display a significant increase in mRNA, they did tend to increase. As hypothesized, myogenin mRNA was upregulated. However, contrary to the hypothesis, MyoD mRNA also increased, although later than myogenin. The mRNA levels of the other myogenic regulatory factor family members, myogenic factor 5 and myogenic regulatory factor 4, and the myocyte enhancer factor (MEF) family members, MEF-2A and MEF-2C, did not change. The results indicate that myogenin is indeed involved in the regulation of the slow oxidative phenotype in human skeletal muscle fibers, whereas MyoD appears to have a more complex regulatory function.

spinal cord; low-frequency stimulation; fast-to-slow transition; metabolic genes; myosin heavy chain

SKELETAL MUSCLE IS COMPOSED of different muscle fiber phenotypes. These different phenotypes differ in their speed of contraction [commonly distinguished by the myosin heavy chain (MHC) isoforms] and by different abilities to participate in either oxidative or glycolytic metabolism. Although it is generally accepted that adult skeletal muscle cells have the potential to transform from one phenotype to another in response to various stimuli (6), the underlying mechanisms are still largely unclear. In recent years, myogenic transcription factors, in general, and members of the myogenic regulatory factor (MRF) family of basic helix-loop-helix transcription factor proteins, in particular, have been suggested to play an important role in the differentiation processes of the adult skeletal muscle cells through transcriptional control of phenotype-specific proteins (7, 11, 31, 37). In rats, MyoD mRNA has been shown to be most prevalent in fast glycolytic muscles, whereas myogenin mRNA has been shown to be most prevalent in slow oxidative muscles, and this relationship followed phenotype transition caused by cross-innervation (19). These results lead to the theory that MyoD and myogenin control fast and slow fiber-type-specific expression, respectively. Overexpression of myogenin in transgenic mice causes an increase in the activity levels of oxidative enzymes like 3-hydroxyacyl-CoA dehydrogenase and succinate dehydrogenase, whereas glycolytic enzyme activity levels of glycolytic enzymes, like glyceraldehydedehyde dehydrogenase (GDPDH) and lactate dehydrogenase (LDH), decrease (13, 18). Also, Siu and coworkers (41) found a relationship between the upregulation of myogenin and the oxidative enzyme citrate synthase (CS) in endurance-trained rats. These results indicate a role of myogenin to participate specifically in at least a part of a fast-to-slow fiber-type transition.

Other roles of MRF transcription factors are important to consider. Accordingly, MyoD and myogenin have been observed to undergo substantial upregulation in response to denervation of rat muscle (20, 21, 48). Because acetylcholine receptor subunits are also observed to be upregulated in response to denervation and because MRF transcription factors are believed to exert transcriptional control over their expression, it is believed that this constitutes a mechanism of disuse of acetylcholine receptor supersensitivity to minimize skeletal muscle atrophy (1, 12, 15, 47). Muscle damage is also known to induce expression of MRF transcription factors, which are then thought to drive satellite cells through a transcriptional program resembling myogenesis during an ongoing repair process (9). However, although both denervation and muscle damage result in a change toward faster and/or less oxidative muscle phenotypes (2, 23), a link to the changes in MRF under such circumstances has not been thoroughly investigated.

Analysis of promotor regions of phenotype-specific genes indicates the coupling between transcription factors of the MRF family and transcription factors of other transcription
factor families (30), and it has been suggested that different collaborative units of transcription factors might exert transcriptional control of functionally related (and perhaps phenotype-specific) subsets of genes (43). Of special interest is the myocyte enhancer factor (MEF)-2 family of transcription factors, which has been shown to interact with the MRF transcription factors (29). In studies on tissue culture as well as on transgenic mice, MEF-2 isoforms have been shown to participate in slow phenotype-specific gene regulation, possibly through a calcineurin-dependent pathway (49).

However, most of the knowledge on the exact functions of MRF and MEF-2 transcription factors relates to developing muscle and/or are based on in vitro and animal studies. There is some evidence that adult human skeletal muscle fibers are also able to transform from one phenotype to another in response to altered usage patterns when judged by the expression of both contractile and metabolic molecular markers (6). However, in vivo studies on the transcriptional role of MRF and MEF-2 transcription factors in adult human muscle are lacking.

Human patients suffering from a spinal cord injury are unable to activate large parts of their skeletal muscle, and, as a direct result, the affected muscle fibers gradually transform into a muscle phenotype composed primarily of fast fibers with reduced oxidative capacity (2). Electric stimulation of muscles of such patients initiates a transformation of the muscle fibers toward a phenotype with a slower contractility and a higher degree of oxidative capacity (2, 36). Thus, because the state of spinal cord injury resembles an extreme degree of muscle inactivity, spinal cord patients subjected to electrical stimulation provide an interesting model in the investigation of transcriptional regulation of muscle processes. In the present study, we have chosen this model to investigate the expression pattern of phenotype-specific markers and to relate this with the transcriptional control of functionally related (and perhaps phenotype-specific) genes.

MATERIALS AND METHODS

Subjects and study design. Six male subjects, age 39 ± 9 yr (mean ± SD), participated in the study. The individuals had sustained a spinal cord injury (at levels ranging from C7 to T10, 2–22 yr previously). Approval of the experimental procedure was obtained from the Municipal Ethics Committee of Copenhagen. All subjects gave written, informed consent before participation in the study, which conformed to the standards set by the Declaration of Helsinki (1996).

The preferred leg of each subject was used for training under isometric conditions in a purpose-built dynamometer. The tibialis anterior muscle was chronically stimulated each weekday for 4 wk, using percutaneous electrodes at a frequency of 10 Hz, with a duty cycle of 5 s/5 s. Stimulation time rose from 2 h/day at the beginning of week 1 to 6 h/day in week 4. For details on the training protocol, please refer to Harridge et al. (16).

Needle muscle biopsies were obtained from the tibialis anterior muscle before and after 2 and 4 wk of electrical training using the needle biopsy technique (4). The posttraining biopsies were obtained ~24 h after the last training session. Following sampling, the tissue was embedded in Tissue-Tek and frozen in isopentane cooled by liquid nitrogen and stored at −80°C until sectioning for in situ hybridization and RNA purification.

Northern blotting: probe preparation. The Pfu polymerase (Stratagene, La Jolla, CA) was used to amplify PCR products from human muscle cDNA (see Table 1). The PCR products were cloned into the Smal site of pBlueScript II SK(+) (see plasmid specifications in Table 1). From these plasmids, single-stranded probes were generated as previously described (24). Briefly, 5′ biotinylated and nonbiotinylated flanking M13 primers were used to amplify the insert by PCR. The biotinylated strands were then retained by use of streptavidin-coated Dynabeads. The original antisense primer for the PCR product was added, and complementary strand resynthesis was then achieved by mixing with [α-32P]dATP (3,000 mCi/mmol) and exonuclease-free Klenow polymerase (24). For the MHCs, single-stranded oligo probes were made from 5′ biotinylated oligonucleotides corresponding to the 3′ untranslated region, which is specific for the different isoforms, as described by Higginson et al. (17), but otherwise by a protocol similar to the one for the PCR probes (probe information is stated in Table 1).

Northern blotting: blotting and hybridization. Total RNA was extracted from the muscle biopsies (9). By principles previously described (24), RNA was mixed with formaldehyde loading buffer and then loaded as 200 ng/well on a denaturing formaldehyde agarose gel. The gel was stained in SYBRgreen II and captured on a fluorescence scanner for verification of RNA integrity. The gel was then blotted onto a nylon membrane by alkaline capillary transfer. Probe diluted to a final concentration of 2 × 106 cpm/ml was hybridized to the membrane during overnight rotation at 50°C (and 42°C) for MHC probes. Blots were then washed at high stringency and exposed on phosphor screens. The signal was captured on a phosphor imager for analysis.

mRNA expression of each specific target was quantified and normalized to GAPDH mRNA. The GAPDH was chosen for normalization, as it was considered the least likely “housekeeping gene” to change (22). Compared with the ribosomal RNA loaded on the gel, the GAPDH mRNA was constant or perhaps with a slight tendency to an increase over time. To express data as fold changes to pretraining, all data were divided by the average pretraining value. Due to the large heterogeneity of the individual muscles from the spinal cord subjects, we did not normalize to the individual pretraining value for each subject.

In situ hybridization. In situ hybridization was performed on cryosections (10 μm) from the biopsies. Probes specific for human...
Table 1. Northern probe information for synthesis of PCR probes and oligo probes for selected mRNA targets

<table>
<thead>
<tr>
<th>Target</th>
<th>PCR Primer Sequences</th>
<th>Plasmid ID</th>
<th>Insert Orientation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MyoD</td>
<td>Sense: GCT CCG AGG GCA TGA TGG</td>
<td>CM171</td>
<td>Z+</td>
</tr>
<tr>
<td></td>
<td>Antisense: TAA AGC GCT GTT GGG AGG</td>
<td>pCM175</td>
<td>Z+</td>
</tr>
<tr>
<td>Myogenin</td>
<td>Sense: GCA GCA TCA AGA AGG TGA AT</td>
<td></td>
<td>Z+</td>
</tr>
<tr>
<td></td>
<td>Antisense: ATG GAT GAG GAA GGG GAT AG</td>
<td>pCM179</td>
<td>Z+</td>
</tr>
<tr>
<td>myf-5</td>
<td>Sense: AAG AGG AAG TGC ACC ACC AT</td>
<td></td>
<td>Z+</td>
</tr>
<tr>
<td></td>
<td>Antisense: AGC CTT CTT CTT GCT Gtg TA</td>
<td>pCM183</td>
<td>Z+</td>
</tr>
<tr>
<td>MRF4</td>
<td>Sense: CGA AAG GAG GAG GCT AAA GA</td>
<td></td>
<td>Z+</td>
</tr>
<tr>
<td></td>
<td>Antisense: GCA CAA AAG GAT CAC GTT AAT</td>
<td>pCM230</td>
<td>Z+</td>
</tr>
<tr>
<td>MEF-2A</td>
<td>Sense: TCA GCA TCA AGT CCG AAC CG</td>
<td></td>
<td>Z+</td>
</tr>
<tr>
<td></td>
<td>Antisense: AAG CCT TAG GTC ACC CAC GC</td>
<td>pCM109</td>
<td>Z+</td>
</tr>
<tr>
<td>MEF-2C</td>
<td>Sense: TGG CAA CAG CAA CAC CTA CA</td>
<td>pCM236</td>
<td>Z+</td>
</tr>
<tr>
<td></td>
<td>Antisense: GCC TGG ACT GAG GGA CTT TG</td>
<td></td>
<td>Z+</td>
</tr>
<tr>
<td>HADHA</td>
<td>Sense: AGG TGC CTC CTA AGT CTT GAG AAG</td>
<td></td>
<td>Z+</td>
</tr>
<tr>
<td></td>
<td>Antisense: ACT TCA CGA GGA AGG AGA GAT</td>
<td>pCM126</td>
<td>Z+</td>
</tr>
<tr>
<td>CS</td>
<td>Sense: CCT GTT GT TGT TCG TGC ACG C</td>
<td></td>
<td>Z+</td>
</tr>
<tr>
<td></td>
<td>Antisense: CTC TTT GCC CAG TCT TTT GA</td>
<td>pCM143</td>
<td>Z+</td>
</tr>
<tr>
<td>mFABP</td>
<td>Sense: CAG CCT AGC CCA GCA TCA CT</td>
<td></td>
<td>Z+</td>
</tr>
<tr>
<td></td>
<td>Antisense: TCA ATT AGC TGC GGG AGA CCA A</td>
<td>pCM186</td>
<td>Z+</td>
</tr>
<tr>
<td>GOPDH</td>
<td>Sense: ACC TCA CTA CTA CTT GCT AT</td>
<td></td>
<td>Z+</td>
</tr>
<tr>
<td></td>
<td>Antisense: CCA GTG AAA CGA TGA GGA GA</td>
<td>pCM132</td>
<td>Z+</td>
</tr>
<tr>
<td>LDH-A</td>
<td>Sense: CAA CAG GAT TCT AGG TGG AGG</td>
<td></td>
<td>Z+</td>
</tr>
<tr>
<td></td>
<td>Antisense: CTT GGA TAG TTG GTC GAA TTG</td>
<td>pCM5</td>
<td>Z+</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Sense: GAA CAT CAT CCG TGC CTC TAC T</td>
<td></td>
<td>Z+</td>
</tr>
<tr>
<td></td>
<td>Antisense: GTC TAC ATG CCA ACT GTG AGG A</td>
<td></td>
<td>Z+</td>
</tr>
</tbody>
</table>

For PCR probes, the following are stated: PCR primer sequences, plasmid identification (ID), and insert orientation [Z+ and Z− refer to insert orientation in the same or the opposite direction of LacZ gene of the pBlueScript II SK(+)/vector, respectively]. For oligo probes, the following are stated: oligo probe primer sequence and oligo template sequence (complementary to the 3′ untranslated region). Myf-5, myogenic factor 5; MRF4, myogenic regulatory factor 4; MEF, myocyte enhancer factor; HADHA, 3-hydroxyacyl-CoA dehydrogenase A; CS, citrate synthase; mFABP, muscle fatty acid binding protein; GOPDH, glycerol phosphate dehydrogenase; LDH, lactate dehydrogenase; MHC, myosin heavy chain.

MHC I, MHC IIa, and MHC IIx were synthesized from previously made plasmids (42), whereas probes specific for the myogenic factors, MyoD, myogenin, Myf5, and MRF4, were synthesized from linearization of the plasmids stated in Table 1, by using restriction enzymes and polymerases stated in Table 1, by using restriction enzymes and synthesized by RNA polymerases to produce probe sizes of 170–693 bp.

Table 2. In situ probe information for myogenic factors

<table>
<thead>
<tr>
<th>Name</th>
<th>Linearization</th>
<th>Polymerase</th>
<th>Probe Size, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>MyoD</td>
<td>BamHI</td>
<td>T1 RNA</td>
<td>693</td>
</tr>
<tr>
<td>Myogenin</td>
<td>PstI</td>
<td>T1 RNA</td>
<td>368</td>
</tr>
<tr>
<td>myf-5</td>
<td>Hinfl</td>
<td>T1 RNA</td>
<td>170</td>
</tr>
<tr>
<td>MRF4</td>
<td>XhoI</td>
<td>T1 RNA</td>
<td>459</td>
</tr>
</tbody>
</table>

The plasmids for MRF family members stated in Table 1 were linearized by selected restriction enzymes and synthesized by RNA polymerases to produce probe sizes of 170–693 bp.
RESULTS

Metabolic phenotype-specific gene markers. Human muscle fibers exhibit a relatively higher capacity for oxidative metabolism in the fiber-type order type I > type IIA > type IIX (38). As such, the oxidative enzymes, HADHA and CS, and the lipid transporter, mFABP, represent markers toward the type I fiber order of the spectra. The results for oxidative metabolic markers following electrical stimulation training are shown in Fig. 1A. HADHA mRNA did not change after 2 wk, but was upregulated 1.7-fold after 4 wk (P < 0.05). CS mRNA showed a tendency to upregulation (P < 0.1) by the same expression pattern as HADHA.

mFABP mRNA showed a tendency to upregulation (P < 0.1) after 2 wk with no further change after 4 wk.

In contrast, fast muscle fibers exhibit a higher capacity for glycolytic metabolism in the order type IIX > type IIA > type I (38). The glycolytic enzymes GOPDH and LDH-A, therefore, represent markers toward the type II fiber order of the spectra. Results for glycolytic metabolic markers are shown in Fig. 1B. GOPDH exhibits two splice variants, the smaller one of which (~1.7 kb) was downregulated after 2 wk (P < 0.05) to one-half the expression level before stimulation, after which it returned back to the baseline level of expression after 4 wk. The other larger splice variant (~3.0 kb) showed a tendency to upregulation after 4 wk (P < 0.1). LDH-A showed a tendency to upregulation after 2 wk (P < 0.1), with no further change after 4 wk.

Contractile phenotype-specific gene markers. MHC I, MHC IIA, and MHC IIX represent the three isoforms of the myosin motor protein in human skeletal muscle that determine speed of fiber contractility. Northern blot analysis revealed an upregulation of the slow MHC I gene after 4 wk of training (P < 0.05) (Fig. 1C). When judged by in situ hybridization (see Fig. 3), this upregulation seemed more evident, which could be explained by the fact that increased expression was specifically located to a limited amount of cells. In contrast, judged by in situ hybridization, MHC IIX seemed to be downregulated. This downregulation was, however, not significant when quantified by Northern blotting, most likely due to large variation in expression levels (Fig. 1C). MHC IIA expression was not observed to change by either method.

MRF and MEF-2 transcription factors. The mRNA data for the MRF family members of transcription factors are shown in Fig. 2A. Myogenin levels were significantly higher after both 2 wk (2.5-fold) and 4 wk (further 2-fold) of electrical stimulation training. MyoD did not change after 2 wk, but was upregulated twofold after 4 wk (P < 0.05). MRF4 tended to increase (P < 0.06) after 2 wk, with no further change after 4 wk. Myf-5 did not change. As determined from in situ hybridization, MyoD was located both around myonuclei in the surrounding cell membrane and inside the transversely sectioned muscle cells and with less obvious upregulation after 4 wk than showed by Northern blotting (see Fig. 3). Myogenin, on the other hand, was specifically located around the myonuclei and upregulated in most subjects throughout the entire stimulation period (Fig. 3). MRF4 was the most expressed MRF transcription factor and otherwise distributed in a diffuse manner inside the muscle cells, but with no major visible changes in expression throughout the stimulation period (results not shown). The myf-5 expression level was very low and, in almost all samples, indistinguishable from the general background level (results not shown). No correlation was seen between time since injury and the initial expression level of the specific MRF transcription factors (data not shown).

Northern results for MEF-2 family member MEF-2A and both the larger (~7.3 kb) and the smaller (~3.9 kb) MEF-2C splice variant (Fig. 2B) showed no regulation.

DISCUSSION

This study demonstrates that the training-induced fast glycolytic-to-slow oxidative transition in human muscles is asso-
...and mFABP and CS both showed tendencies (25, 34, 40). In accordance, in our study, mRNA for oxidative response to electrical stimulation or endurance-type exercise types has previously been shown to be upregulated in vivo in our selected metabolic markers of slow oxidative fiber skeletal muscle phenotype. mRNA level and/or enzyme activation levels along with markers of contractile and metabolic transcription factor families were quantified for mRNA expression over time.

Myogenin overexpression in mice has been shown to induce an overall increase in activity levels of oxidative enzymes (13, 18), with a reversed overall effect on glycolytic enzymes (18). This overexpression of myogenin alone did not, however, induce alterations in MHC protein expression in the mice. As expected, we observed a similar overall pattern of effects on oxidative and glycolytic metabolic markers. However, added to that, we also observed an upregulation of MHC I and a tendency to downregulation of MHC IIx mRNA. The need to process lactate during such conditions might overrule otherwise expected downregulation of the LDH enzyme.

The selected mRNA markers of contractile phenotype were constituted by MHC I, MHC Ila, and MHC IIX. Whereas MHC IIX expression is known to decrease in response to electrical stimulation and exercise (2, 32), much more profound stimulation seems required to be able to induce MHC I alterations (33). In this regard, it is interesting that we observed an upregulation of the slow type myosin, MHC I. Fast fiber-type MHC IIX seemed to decrease, as judged by in situ hybridization, but the variation between the relatively few subjects (as seen by Northern blotting) was too large to validate this phenomenon.

If myogenin and MyoD regulate slow and fast fiber-specific expression, respectively (19), we should expect to see the changes in the transcription factors and the metabolic and contractile markers simultaneously.

Interestingly, our results show that the mRNA expression of the myogenic transcription factors exhibited a somewhat different pattern from that expected: this being that both MyoD and myogenin exhibited significant upregulation, with myogenin being the most responsive. As for other MRF and MEF-2 transcription factors, MRF4 showed a minor upregulation, which was not significant (P < 0.1), and neither myf-5, MEF-2A, or MEF-2C responded to the stimulation. Results by Hughes et al. (19) demonstrated that MyoD expression was higher in fast fibers, whereas myogenin was highly expressed in slow fibers. In a very recent study, Siu et al. demonstrated a correlation between the change in expression of myogenin and markers of slow fiber phenotype, but no change in the expression of MyoD after endurance training in rats (41). Our results are somewhat contradictory in the sense that not only myogenin, but also MyoD is upregulated in response to a slow fiber-type-specific stimulus.

Myogenin overexpression in mice has been shown to induce an overall increase in activity levels of oxidative enzymes (13, 18), with a reversed overall effect on glycolytic enzymes (18). This overexpression of myogenin alone did not, however, induce alterations in MHC protein expression in the mice. As expected, we observed a similar overall pattern of effects on oxidative and glycolytic metabolic markers. However, added to that, we also observed an upregulation of MHC I and a tendency to downregulation of MHC IIX mRNA.

Although the mRNA for some metabolic enzymes demonstrated a shift toward oxidative metabolism after 2 wk together with myogenin (mFABP and GOPDH), other enzyme mRNAs...
were not upregulated before 4 wk, e.g., HADHA and CS. This suggests that myogenin does not regulate HADHA and CS directly, but requires a delayed additional or alternative signal for activation, as illustrated in Fig. 4. MyoD mRNA increases at 4 wk, and, therefore, this might be involved in the activation of the HADHA, CS, and MHC I genes. Furthermore, the GOPDH expression returns to baseline at 4 wk, suggesting that myogenin and MyoD might have opposite regulatory effects on the GOPDH gene. MyoD upregulation, however, might also be the result of accumulated electrical stimulation activating some unknown factor, which then activates both MyoD and the other targets upregulated at 4 wk. Alternatively, it cannot be ruled out that the higher level of myogenin at 4 wk is required for the activation of HADHA, CS, and MHC I mRNA.

Other aspects must be taken into consideration in the interpretation of our results. First of all, variability in age and time since spinal cord injury might influence expression patterns. In the studies by Hyatt et al. (20) and by Ishido et al. (21), MyoD and myogenin exhibited immense upregulation during the immediate time course postdenervation in rat muscle. Because
MRF transcription factors are believed to control the expression of acetylcholine receptor subunit mRNAs, this is supposed to constitute a compensatory mechanism against muscle atrophy (15), but studies on rats by Adams et al. (1) show that such denervation-induced upregulation of MRF transcription factors and acetylcholine receptor subunits is only transient and diminishes a few months after the injury. Because none of the subjects had suffered spinal cord injury less than 2 yr before our experiments, the MRF expression levels would, therefore, be expected to have leveled off at the time of onset of our experiments, as also indicated by the lack of correlation between time since injury and the pretraining level of MRF mRNA. Although our results indicate that myogenin and/or MyoD might participate in driving the slow oxidative phenotype-specific gene expression, our data do not link either of these transcription factors to a specific muscle fiber type. Rather, when judging from the results of the in situ hybridization analysis, MyoD and myogenin seem evenly distributed across all muscle cells. Because the MRFs are also known to induce the acetylcholine receptors (47), perhaps part of the upregulation of myogenin and MyoD after electrical stimulation is the fiber’s attempt to establish new connections to neurons. It could be similar to the upregulation of MRFs and acetylcholine receptors shortly after denervation (1). Another explanation for a non-phenotype-specific upregulation of MyoD and myogenin might rely on a persistency of a high relative proportion of hybrid fibers a long time (years) after spinal cord injury, as indicated in a study by Talmadge et al. (44). Thus an immediate MRF-to-phenotype relationship might not be clear from histochemical techniques.

Another aspect to consider relates to MRF expression in myonuclei and satellite cells. Studies on the effect of low-frequency stimulation of fast muscle of hypothyroid rodents show that increased satellite cell activation levels are accompanied by increased expression of myogenin as well as MyoD (35), which imply that MRF transcription factors participate in driving satellite cells through stages of proliferation and differentiation in a manner corresponding to myogenesis to uphold a constant nuclei-to-cytoplasmic ratio (14, 28). Also, quiescent satellite cells are activated in response to muscle damage, leading to upregulation of MRF transcription factors (9). Thus the MRF expression patterns that we observe in our study could be argued to relate primarily to satellite cell activation in response to low-frequency innervation or damage from previous biopsy sampling. However, the number of activated satellite cells are previously reported to constitute only a small number compared with the number of myonuclei (26, 45). Because, from our in situ hybridization data, we observe expression at multiple locations within each fiber, MRF expression appears to be located to myonuclei as well and, therefore, presumably participate in phenotype expression.

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In conclusion, electrical stimulation of human skeletal muscle induced a fast-to-slow fiber-type transformation. This change in phenotypic expression pattern was accompanied by increased expression of myogenin mRNA, indicating that myogenin is involved in regulating the metabolic genes in human skeletal muscle. In contrast, the upregulation of MyoD indicates that MyoD plays another role besides just determining the fast phenotype.

ACKNOWLEDGMENTS

Ann-Christina Henriksen and Flemming Jessen are thanked for excellent technical assistance.

Fig. 4. Hypothetical model for timing-specific MRF regulation of phenotype-specific genes. Two weeks of electrical stimulation induce myogenin, which upholds positive control of mFABP and LDH-A, while exerting negative control of one GOPDH splice variant. Additional stimulation is then thought to induce an unknown factor (?) or increase myogenin above a required threshold level, which upholds positive control of MHC I, HADHA, and CS.
GENE EXPRESSION OF STIMULATED HUMAN PARAPLEGIC MUSCLE

GRANTS

The financial support came from the Danish National Research Foundation (J. nr. 504–14), Rigshospitalet H:S (the Copenhagen Hospital Corp.), University of Copenhagen, and the Novo Nordisk Foundation.

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