Physiol. Res. 53 (*Suppl. 1*): S57-S61, 2004

Influence of Thyroid Status on the Differentiation of Slow and Fast Muscle Phenotypes

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Received December 10, 2003
Accepted March 1, 2004

Summary

Muscle phenotype is determined by combined effects of intrinsic genetic and extrinsic factors like innervation, hormonal levels and mechanical factors or muscle activity. We have been studying the effect of altered thyroid hormone levels on the expression of myosin heavy chain (MyHC) isoforms in control and regenerating soleus and extensor digitorum longus muscles of euthyroid, hypothyroid or hyperthyroid female inbred Lewis rats. The fiber type composition has been determined according to the mATPase activity and immunocytochemical staining of MyHC isoforms, the content of MyHC isoforms has been determined by SDS-PAGE, the mRNA levels have been measured by RT-PCR and the ultrastructural transformation has been analyzed by electron-microscopy. Our results indicate that although the innervation plays a decisive role in the determination of muscle phenotype, levels of thyroid hormones contribute to the extent of muscle phenotype transformation.

Key words

Muscle fiber types • Muscle innervation • Thyroid hormones • Muscle proteins • Myosin heavy chains• Muscle activity • Mitochondrial GPDH • Calcium transporters

Regulation of muscle phenotype and muscle protein isoforms

Mammalian skeletal muscles are plastic, because the proportions of phenotypically diverse fiber types differs from muscle to muscle reflecting their physiological function and usage. Existence of multiple isoforms of contractile proteins in the muscles contributes to their plasticity. Out of the myofibrillar protein group, the myosin heavy chain (MyHC) multigene family is the best known. It involves at least eight MyHC isoforms, each being the product of a distinct gene, four fast isoforms (2a, 2x/d, 2b and 2extraocular), present in 2A, 2X/D, 2B and extraocular fast muscle fibers, two cardiac (α, β) isoforms, predominantly expressed in cardiac muscle, nevertheless the β-cardiac isoform is also expressed in skeletal slow fibers and designated as β-slow or type 1, whereas the α-cardiac isoform can be found, e.g. in intrafusal fibers of muscle spindles (for review see Soukup *et al.* 1995). The developmental (embryonic and perinatal/neonatal) isoforms can be found in differentiating or regenerating muscle fibers. The expression of a given gene and of its specific protein isoform thus depends on intrinsic programs related to the

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myoblast lineage from which muscle fibers develop and is further regulated by extrinsic influences such as neural, hormonal and mechanical factors including muscle activity (for review see Gutmann 1976, Pette and Vrbová 1985, D'Albis and Butler-Browne 1993, Schiaffino and Reggiani 1996, Buonanno and Rosenthal 1996, Pette and Staron 1997, Soukup and Jirmanová 2000). In our review we focus on the influence of altered thyroid status on the expression of rat slow and fast hind limb muscle phenotypes.

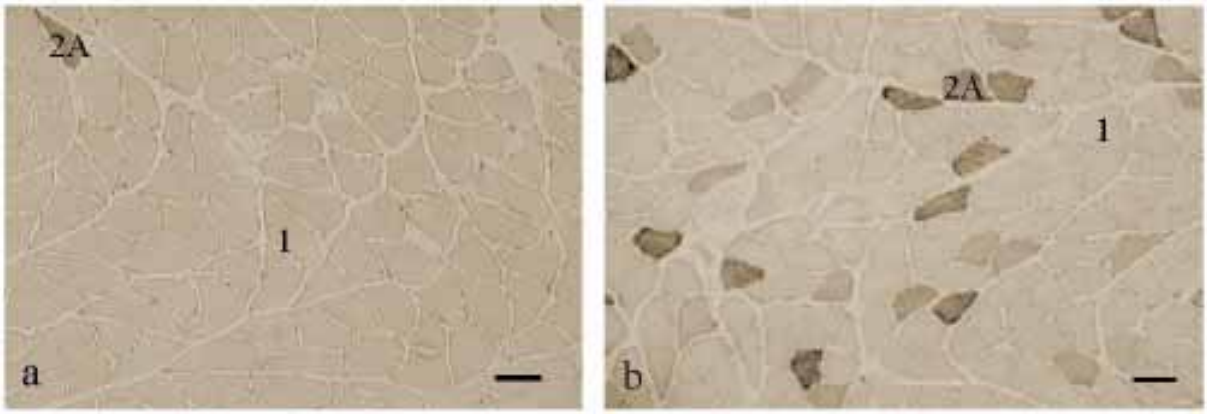

Fig. 1. Serial sections from soleus muscles of the 6-month-old female euthyroid (a) and hyperthyroid (b) rats of the inbred Lewis strain. Sections were stained with monoclonal antibodies specific for type 2a MyHC isoform. Bar, 50 µm.

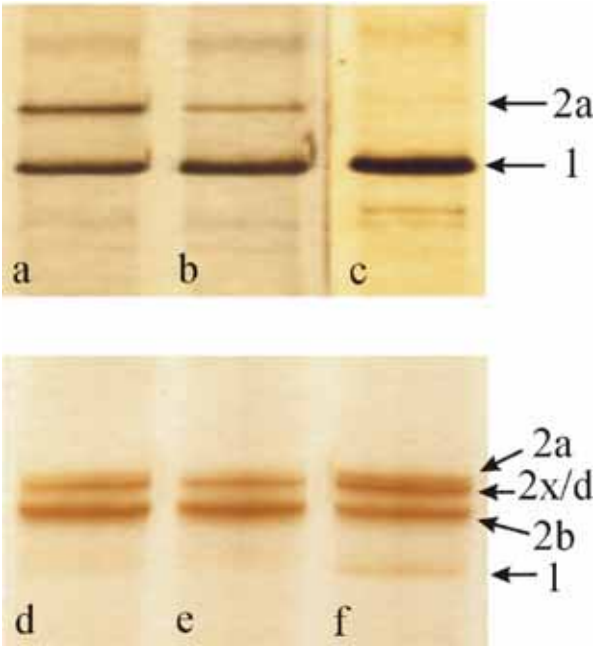

Fig. 2. SDS-PAGE separation of MyHC isoforms from soleus (a-c) and EDL (d-f) muscles of young adult female hyperthyroid (a,d), euthyroid (b,e) and hypothyroid (c, f) inbred rats of the Lewis strain. The 1, 2a, 2x/d and 2b MyHC bands are labelled.

Influence of thyroid hormones on the fiber type and MyHC isoform composition

In our studies (Soukup *et al.* 2002a,b,c, Figs 1 and 2) of a preliminary sample of normal female inbred Lewis rat soleus (SOL) and extensor digitorum longus (EDL) muscles we have shown that in adult euthyroid rats, the SOL muscle contained 97 % of slow type 1 fibers as determined by histo- and immunocytochemistry and 96 % MyHC1 isoform determined by SDS-PAGE. The EDL muscle contained 5.7 % of slow fibers, 24 % of 2A, 37.2 % of 2X/D and 42.9 % of 2B fast fibers and 4.9 % MyHC1, 14.8 % 2a, 33.5 % 2x/d and 47.2 % 2b MyHC isoforms as shown by SDS-PAGE. In hypothyroid rats treated by a 0.05 % solution of methimazole in the drinking water, the SOL muscle contained almost exclusively slow fibers, while the EDL contained 7 % of slow MyHC1 and only 37 % of the fastest 2b MyHC isoform. Conversely, in hyperthyroid rats, injected with 3, 3',5-triiodo-L-thyronine (T_3) (150 µg/kg body weight, 3 times a week), the adult SOL muscle contained 93.5 % of slow fibers (according to mATPase) and 81.3 % of MyHC1 isoform (determined by SDS-PAGE), whereas in the fast EDL the percentage of slow fibers decreased by about a half compared to euthyroid rats. In general, hypothyroid status leads to preferential expression of slower fiber types and MyHC isoforms, whereas hyperthyroid state enhances the content of fast fibers and fast MyHC isoforms. Thyroid status thus significantly affected the muscle phenotype. On the other hand, levels of mRNAs for individual MyHC isoforms were less influenced by altered thyroid status (Vadászová *et al.*

2004). This suggests that thyroid hormones may regulate MyHC isoform expression differently at the translational and transcriptional levels.

Our results of heterochronous isotransplantation (for details see Jirmanová and Soukup 1995) have shown that the regenerated SOL and EDL isografts reinnervated by the "fast" EDL nerve of the host muscle develop into muscles with the fast phenotype. Both grafts contained all three types of fast fibers, but the percentage of slow type 1 and fast 2A, 2X/D and 2B fibers differed according to the thyroid status of the recipient rat (Zachařová *et al.* 1999, Soukup *et al.* 2002c, 2003b). In euthyroid rats, SOL grafts developed into fast muscle containing 95.0 % of fast (2A, 2X/D and 2B) fibers and the transformation was even more pronounced in hyperthyroid rats, as regenerated SOL contained nearly 99 % of fast, predominantly 2B fibers. Conversely, the transformation of the SOL graft into fast muscle was less pronounced in hypothyroid rats, where it contained only about 70 % of fast fibers. We suggest that the reinnervation of the grafts by the host axons triggers fiber type transformation, but the extent of transformation from slow towards fast muscle phenotype is enhanced in hyperthyroid and suppressed in hypothyroid status. Our results also suggest that the satellite cells, giving rise to the new fibers of the graft, which are derived from different fiber types (extrafusal slow type 1 and fast 2A, 2X/D and 2B fibers, intrafusal nuclear bag_1, bag_2 and chain fibers) can express MyHC isoforms in relation to specific extrinsic factors (Soukup and Thornell 1997, Soukup and Jirmanová 2000, Jirmanová and Soukup 2001, for review see Zelená 1994, Soukup *et al.* 1995). These results confirm the fundamental role of the neural influence and a concomitant contribution of thyroid hormones to the differentiation of MyHC isoforms and muscle phenotype and can apply even to studies in humans (Soukup and Thornell 1999, Soukup *et al.* 2003a).

Influence of muscle activity on MyHC isoform composition

Recently we have analyzed the influence of enhanced activity on muscle phenotype of SOL and EDL muscles in Japanese waltzing mice (JWM) of the C57BL/6J-v2J strain in comparison to control (CM) CBA/J mice (Asmussen *et al.* 2003). Electrophoretic analyses revealed a shift towards slower MyHC isoforms in both EDL and SOL muscles of JWM compared to the homologous CM muscles, namely a shift from the fastest MyHC2b to the MyHC2x/d isoform in the EDL muscle and from MyHC2a to MyHC1 in the SOL muscle. These findings show that the enhanced motor activity of the JWM leads to fiber type transitions in the direction of slower phenotypes.

Influence of thyroid hormones on muscle ultrastructure

EM analysis revealed that in the euthyroid rats, the grafted SOL muscle reinnervated by the fast nerve of the host EDL muscle, contained mostly fibers characterized by a low content of mitochondria typical for the fast fibers, in contrast to the control SOL muscle where fibers with a high content of mitochondria corresponding to the slow fibers prevailed (Soukup *et al.* 2002b).

Influence of thyroid hormones on anatomical parameters and liver enzymes

In our study of selected anatomical parameters (Soukup *et al.* 2001, Vadászová *et al.* 2002, Zachařová *et al.* 2002), we have found that compared to euthyroid rats, hypothyroid rats exhibited significantly decreased body weight gain and heart weight, but increased thyroid gland weight, whereas hyperthyroidism led to a significantly increased heart weight and decreased thyroid gland weight. From the metabolic enzymes, we have analyzed the activity of liver mitochondrial flavoprotein-dependent glycerol-3-phosphate dehydrogenase (GPDH). We found a seven- or a three-fold increase of GPDH activity in hyperthyroid female rats after chronic (2-10 months) T_3 or T_4 administration, respectively, compared to euthyroid rats, whereas administration of methimazole reduced the GPDH activity in hypothyroid rats almost to one third of the euthyroid values (Rauchová *et al.* 2004). We thus showed that anatomical parameters and GPDH activity could serve as useful markers for evaluation of hyperthyroid and hypothyroid status in chronic long-lasting experiments on female inbred Lewis rats.

Influence of thyroid hormones on calcium transporting systems

It is well known that modification of thyroid hormone levels has a profound impact on cardiac functions, predominantly through a direct regulation of sarcoplasmic reticulum protein levels. However, less is

known about the effect of thyroid hormones on the calcium transport systems in skeletal muscles. In our study (Hudecová *et al.* 2004) we have found that altered thyroid status can change the gene expression of the Na^+/Ca^{2+} exchanger (NCX) and of type 1 and type 2 ryanodine receptors (RyRs) and inositol 1,4,5-triphosphate receptors (IP_3Rs) in slow and fast rat skeletal muscles. After a period of 2-10 months of T_3 treatment we observed a significant increase in mRNA levels of the NCX and RyRs and IP_3Rs of both types. The increase of NCX and RyRs2 mRNA levels was more pronounced in the slow SOL than in the fast EDL muscle, opposite to RyRs1 and IP_3Rs2 mRNA levels. In hypothyroid rats treated by methimazole for 8-9 months we observed significant decrease in RyR2 gene expression in both muscles and in the NCX mRNA in the SOL muscle. It is tempting to speculate that thyroid hormones may alter the calcium homeostasis in the skeletal muscles and thus influence the excitation-contraction coupling properties.

Future directions

Although the altered protein isoform expression can result in phenotype changes, the basic functional and structural integrity of the sarcomere must be preserved. This implies that different proteins must be synthesized according to the fiber type-specific program of gene expression. It would therefore be useful to analyze simultaneously the expression of MyHC and Ca^{2+} regulatory protein isoforms in developing, regenerating and adult muscles in rats with altered neural, hormonal and mechanical factors. The method of heterochronous (donor and host are of different age) isotransplantation (both donor and host are of the same inbred strain) is especially convenient, because it enables to study the combination of intrinsic and extrinsic factors on a single model. We believe that a deeper insight into regulatory processes controlling muscle gene expression during muscle development, regeneration and differentiation is not only important for the understanding of the process of gene expression, but is also a prerequisite for the prospective clinical muscle transplantations and for the essential improvement in clinical methods such as cardiomyoplasty.

Acknowledgements

We are grateful to Ms V. Semelová for excellent technical assistance, Dr. H. Rauchová for analysis of GPDH activity, Dr. V. Smerdu and Dr. G. Asmussen for collaboration in SDS-PAGE analysis, Dr. O. Križanová and Dr. S. Hudecová for collaboration with RT-PCR, Dr. S. Pavelka for determination of tT_3 and tT_4 levels and Dr. P. Hník for his critical reading of the manuscript. This work was supported by grants of the Grant Agency of the Czech Republic (304/00/1653, 309/03/0752, 305/03/H148), NATO grant (979876), Czech-Slovenian Intergovernmental grant (02-2004-05) and by the Research Project AVOZ 5011922.

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