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ARTICLE *in* BIOLOGY OF THE CELL · OCTOBER 2003

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Myosin heavy chain isoforms in postnatal muscle development of mice

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Received 5 February 2003; accepted 27 June 2003

Abstract

In this study, using a high-resolution gel electrophoresis technique, we have characterized the myosin heavy chain composition in different skeletal muscle of the mouse during postnatal development. The pattern of myosin heavy chain expression was studied in four hind limb muscles, the diaphragm, the tongue and the masseter. All of these muscles displayed the usual sequential transitions from embryonic to neonatal and to adult myosin heavy chain isoforms but more interestingly these transitions occur with a distinct chronology in the different muscles. In addition, our results demonstrated a transitory pattern of expression for certain adult myosin heavy chain isoforms in the soleus and the tongue. In the soleus muscle IIB and in the tongue IIA myosin heavy chain isoforms were detected only for a short time during postnatal life. Our results demonstrate that muscles of the mouse with different functions are subjected to a distinct programs of myosin isoform transitions during postnatal muscle development. This study describes new data which will help us to understand both postnatal muscle development in transgenic mouse muscles as well as in muscle pathology.

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Keywords: Skeletal muscle; Electrophoresis; Murine development; Muscle plasticity

1. Introduction

Myosin heavy chain (MyHC) is one of the major components of the contractile apparatus of all striated muscles. The functional heterogeneity of muscles can be explained by their MyHC isoform content (Botinelli, 2001). MyHC is encoded by a multigene family, the members of which are expressed in a tissue-specific and developmentally regulated manner (Wydro et al., 1983; Mahdavi et al., 1987). Several MyHC isoforms have been described in skeletal muscles, including two developmental isoforms (embryonic and neonatal/perinatal/fetal), one slow isoform (MyHC I/ß) and three fast isoforms (MyHC IIA, IIX/IID and IIB) (Staron et al., 1993; Pette and Staron, 2000). Other MyHC's have been described in skeletal muscle: four of them, slow tonic, super fast IIM, laryngeal IIL and extraocular myosin (EOM) are expressed in only a few specialised muscles (Wieczorek et al., 1985; Perie et al., 2000) whereas IA MyHC is expressed in slow to fast transforming fibers (Fauteck and Kandarian, 1995).

An important characteristic of skeletal muscle is its plasticity. Because of this property the muscle is able to adapt its fiber type profile to physiological and functional demands. This is reflected in the composition and level of expression of MyHC isoforms within these muscle. Various factors such as development, innervation, increased and decreased neuromuscular activity, physical activity (overloading and unloading), hormones and ageing, have been shown to influence the phenotypic expression of skeletal muscle fibers (Sullivan et al., 1995; Schiaffino and Reggiani, 1996; Pette and Staron, 2000). Skeletal muscle plasticity has been studied extensively by myosin isoform transitions in different experimental animal models, i.e. rat, rabbit, bird models (d'Albis et al., 1989, 1990; Bacou et al., 1996; Blough et al., 1996; Mckoy et al., 1998). However a comprehensive study of the mouse model has not yet been reported.

In this study, we describe the pattern of expression of the different isoforms of MyHC in mouse skeletal muscle during postnatal development by gel electrophoresis. Postnatal muscle development is characterized by important changes in neuromuscular activity, maturation of excitation-contraction coupling and a strong increase in the level of thyroid hormone. After birth, the muscle adapts to these new

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doi:10.1016/S0248-4900(03)00087-X

conditions by progressively changing its MyHC content to meet these new functional demands. In the literature, there is a lack of precise comparative studies concerning MyHC protein expression profile in different mouse muscles. Some studies have analyzed MyHC proteins by immunohistochemistry on leg, head and abdomen at early stages of postnatal development (Lu et al., 1999) and at 1, 5, 10 and 20 days after birth on sections of the leg (Allen et al., 2001). MyHC expression was also studied by electrophoresis at 1, 5, 10 and 20 days after birth on pooled leg muscles (Allen et al., 2001).

In our study, we describe the maturation of different mouse hind limb (soleus, plantaris, extensor digitorum longus [EDL], gastrocnemius), diaphragm and head (masseter, tongue) muscles at 3, 5, 7, 14, 21, 28 and 49 days after birth. We show a sequential expression of embryonic (EMB), neonatal (NN), slow (I/ß) and fast (IIA, IIX/IID, IIB) MyHC proteins the chronology of which is specific for each muscle. This study highlights the variability which exists in the process of maturation in individual muscles. This difference should be taken into consideration when studying both muscle development as well as muscle pathology.

2. Results

2.1. Postnatal muscle development in different mouse skeletal muscles

To determine the pattern of fiber type formation during postnatal muscle development in the mouse, we analysed the expression of the different myosin heavy chain isoforms. The distribution of MyHC's was analyzed in four hind limb muscles (soleus, EDL, plantaris and gastrocnemius), in two head muscles (masseter and tongue) and in the diaphragm from mice at various ages between 3 and 49 days after birth (Figs. 1, 2, Table 1). In mice muscle extracts, 6 MyHCs can be identified following electrophoresis. The resulting bands are, in order of increasing electrophoretic mobility, EMB (embryonic), IIA, IIX/IID, NN (neonatal), IIB and I/ß. The position of the MyHCs band were assessed with western blotting using antibodies directed against different MyHC isoforms (Fig. 3).

In the new-born mice, the developmental myosins (embryonic and neonatal) were the predominant isoforms expressed in all seven muscles, a small amount of slow (I/ß) myosin was expressed in all of the hind limb muscles (Fig. 1),

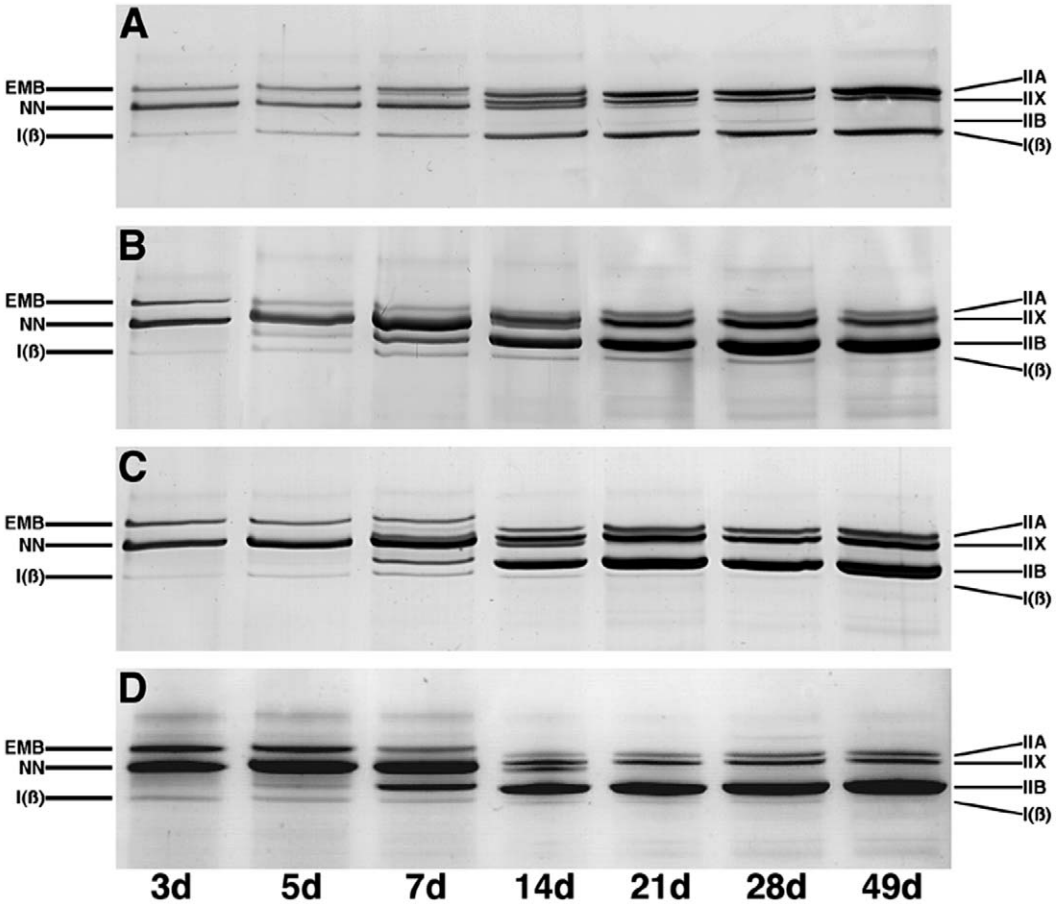

Fig. 1. Electrophoretic separation of MyHCs in mouse hind limb muscles during postnatal development. A) Soleus; B) Extensor digitorum longus (EDL); C) Plantaris and D) Gastrocnemius. By gel electrophoresis in mouse skeletal muscle 6 isoforms of myosin heavy chain can be identified. They are in order of increasing electrophoretic mobility : EMB, IIA, IIX/IID, NN, IIB and I/ß. EMB : embryonic isoform; NN : neonatal isoform; d : days after birth.

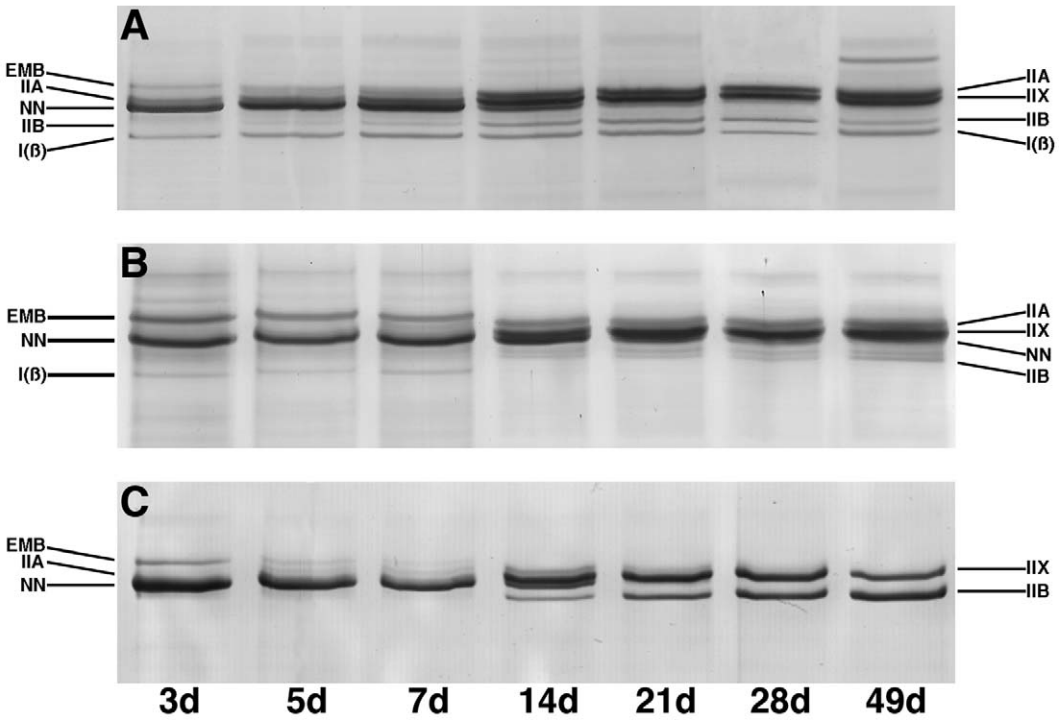

Fig. 2. Electrophoretic separation of MyHCs in mouse skeletal muscles during postnatal development. A) Diaphragm; B) Masseter and C) Tongue. EMB : embryonic isoform; NN : neonatal isoform, d : days after birth.

in the diaphragm (Fig. 2A) and in the masseter (Fig. 2B) but not in the tongue (Fig. 2C). During the next few days, the developmental isoforms, first embryonic and then neonatal MyHC, were progressively replaced by adult isoforms (IIA, IIX/IID, IIB, I/ß). The embryonic isoform represented 30.5±0.8% of the total MyHC content in all of the new-born mouse muscles. This isoform was eliminated from the diaphragm, tongue, masseter, gastrocnemius, plantaris and EDL muscles after 7 days, whereas in the slow contracting soleus muscle it persisted until 14 days (Figs. 1, 2). Neonatal myosin is the major isoform (64.2±1.3%) expressed in new-born mouse skeletal muscle. This isoform was gradually eliminated during post-natal development and maturation and depending upon the muscle was totally absent after 14 and 21 days following birth (Figs. 1, 2). In contrast, in the masseter muscle, the neonatal MyHC isoform persisted and represented 15% of the total MyHC in 3-month-old mice (Table 1).

Four adult MyHC isoforms were detected in mouse skeletal muscles : three fast (IIA, IIX/IID, IIB) and one slow (I/ß) isoform. In all of the new-born mice muscles, except the tongue, a very small amount (3–11%) of slow (I/ß) MyHC was present (Figs. 1, 2). The proportion of this isoform progressively increased in the soleus muscle (Fig. 1A), while in fast contracting hind limb muscles (Fig. 1B–D) and in the masseter (Fig. 2B), it briefly increased and then tended to decrease. In the diaphragm, the proportion of slow myosin remained low but more or less constant (Fig. 2A). Finally, at three months, the slow (I/ß) myosin isoform relative to total MyHC represented 53% in the soleus, 15% in the diaphragm and less then 4% in the fast contracting hind limb muscles (Table 1).

In fast contracting hind limb muscles of adult mice the fast myosin isoforms (IIA, IIX/IID, IIB) represented more than 90% of the total MyHC content (Table 1). Fast IIB MyHC is the major myosin isoform expressed in adult mouse skeletal muscles and was first detected 5 days after birth in the EDL (Fig. 1B) and in the gastrocnemius (Fig. 1D), 7 days after birth in the plantaris (Fig. 1C) and in the diaphragm (Fig. 2A), however in the tongue (Fig. 2C) and in the masseter (Fig. 2B) it was only detected at the beginning of the second week after birth. In the soleus muscle the expression of IIB MyHC was totally different. In the normal adult soleus muscle, IIB MyHC is never observed (Table 1). However in this study, we have demonstrated that there is a transitory expression of the IIB myosin isoform between 14 and 28 days after birth (Fig. 1A, Table 1). The western-blot and the rt-PCR analysis confirmed this observation (Fig. 4). Western-blot analyses were carried out using an antibody specific for IIB MyHC.

The new-born and the adult mouse soleus muscles did not contain IIB MyHC. In contrast, it was present between 14 and 28 days after birth (Fig. 4A). Moreover, rt-PCR analysis showed that IIB MyHC mRNA was first detected 7 days after birth. It was present during 5 weeks but was totally absent in the adult soleus muscle (Fig. 4B).

Two other fast MyHC isoforms (IIA and IIX) are also observed in adult mouse skeletal muscles. These isoforms were first observed during the first week after birth in the soleus (Fig. 1A), in the plantaris (Fig. 1C), in the diaphragm

Table 1
Percentage of the different myosin heavy chains in various muscles of mice. The relative abudance of each MyHC was determined by quantitative densitometry of gels

	Developmental isoforms of MyHC		Fast isoforms of MyHC			Slow isoform of MyHC
	EMB	NN	IIA	IIX/IID	IIB	I/ß
Soleus						
new-born	28.3 (1.5)	65.4 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	6.3 (2.2)
7 d	25.5 (0.5)	38.2 (0.5)	11.8 (1.4)	9.3 (1.4)	0 (0.0)	15.2 (0.4)
21 d	0 (0.0)	6.6 (1.9)	35.8 (3.1)	24.6 (3.3)	1.2 (0.2)	31.8 (1.4)
90 d	0 (0.0)	0 (0.0)	31.2 (6.4)	15.2 (4.4)	0 (0.0)	53.6 (5.4)
EDL						
new-born	31.5 (2.9)	64.8 (4.9)	0 (0.0)	0 (0.0)	0 (0.0)	3.7 (2.0)
7 d	8.4 (3.1)	50.6 (4.2)	0 (0.0)	10.5 (3.4)	24.8 (1.0)	5.7 (2.8)
21 d	0 (0.0)	0 (0.0)	10.5 (1.7)	31.3 (4.3)	54.1 (5.7)	4.1 (0.4)
90 d	0 (0.0)	0 (0.0)	0 (0.0)	9.3 (1.3)	86.8 (1.3)	3.9 (2.5)
Plantaris						
new-born	29.9 (3.3)	66.9 (5.8)	0 (0.0)	0 (0.0)	0 (0.0)	3.2 (0.8)
7 d	13.5 (0.8)	51.1 (5.8)	3.8 (2.6)	10.4 (3.1)	15 (3.6)	6.2 (2.2)
21 d	0 (0.0)	0 (0.0)	19.6 (3.9)	32.4 (3.8)	46.3 (5.4)	1.7 (2.4)
90 d	0 (0.0)	0 (0.0)	21 (4.1)	23.7 (5.7)	53.7 (6.7)	1.6 (1.9)
Gastrocnemius						
new-born	33.4 (2.7)	59.4 (3.5)	0 (0.0)	0 (0.0)	0 (0.0)	7.2 (1.9)
7 d	16.3 (5.6)	48.4 (7.2)	0 (0.0)	0 (0.0)	30 (2.0)	5.3 (1.4)
21 d	0 (0.0)	0 (0.0)	12.9 (3.0)	20.7 (2.3)	62.9 (8.4)	3.5 (5.3)
90 d	0 (0.0)	0 (0.0)	7.6 (4.6)	10.6 (6.7)	78.9 (7.1)	2.9 (3.4)
Diaphragm						
new-born	27.3 (6.3)	61.1 (7.6)	0 (0.0)	0 (0.0)	0 (0.0)	11.6 (4.7)
7 d	5.1 (2.2)	38.6 (3.1)	8.2 (2.6)	26.7 (3.6)	8.4 (0.8)	13 (2.9)
21 d	0 (0.0)	15.6 (1.9)	26 (4.4)	30.6 (6.3)	13 (0.7)	14.8 (2.7)
90 d	0 (0.0)	0 (0.0)	43.6 (7.0)	34.6 (5.0)	6.2 (1.9)	15.6 (2.0)
Masseter						
new-born	32.2 (2.5)	62.4 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)	5.4 (1.1)
7 d	23.3 (3.4)	71.5 (4.9)	0 (0.0)	0 (0.0)	0 (0.0)	5.2 (2.1)
21 d	0 (0.0)	22.9 (6.4)	14 (2.0)	59.5 (7.2)	3.6 (1.4)	0 (0.0)
90 d	0 (0.0)	14.8 (4.1)	29.8 (8.0)	55.4 (14.7)	0 (0.0)	0 (0.0)
Tongue						
new-born	30.7 (5.4)	69.3 (5.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
7 d	7.6 (1.2)	58.7 (3.1)	17.4 (3.2)	11.2 (2.9)	5.1 (3.0)	0 (0.0)
21 d	0 (0.0)	5.3 (5.8)	11.5 (5.4)	49.9 (9.7)	33.3 (10.0)	0 (0.0)
90 d	0 (0.0)	0 (0.0)	0 (0.0)	45.2 (6.5)	54.8 (6.5)	0 (0.0)

(values are mean [SD] ; n = 3 ; d : days after birth)

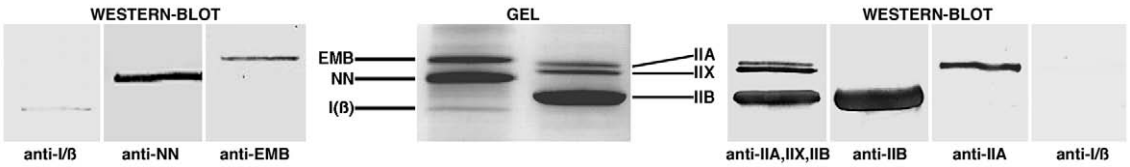

Fig. 3. Determination of the electrophoretic mobility of the different MyHCs in mouse skeletal muscles. The position of the different MyHCs bands were identified with western-blot analysis in adult and new born gastrocnemius muscle.

(Fig. 2A) and in the tongue (Fig. 2C), whereas in the EDL (Fig. 1B), the gastrocnemius (Fig. 1D) and in the masseter (Fig. 2B) then were not detected until the second week after birth. It should be noted that in the tongue there is only a transitory expression of IIA MyHC between 7 and 21 days after birth (Fig. 2C, Table 1).

3. Discussion

MyHC is one of the earliest sarcomeric markers to be expressed in mammalian skeletal muscle. This protein plays a role in the formation of the sarcomere and consequently the production of force. In the present study using a high-

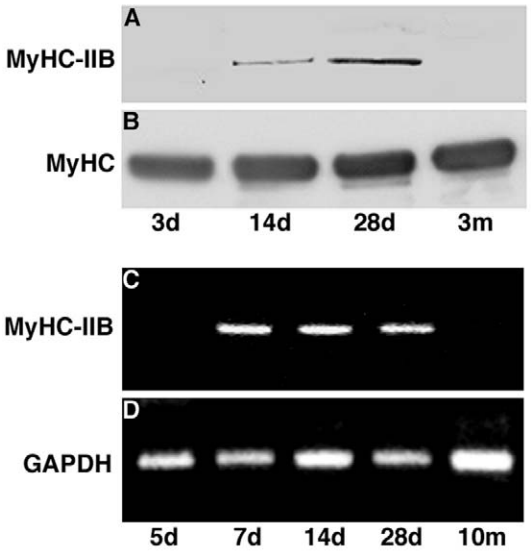

Fig. 4. IIB MyHC protein and mRNA in mouse soleus muscle during postnatal development. A,B) Western-blot C,D) rt-PCR analysis. Western-blot analysis was carried out using antibody specific for IIB MyHC (clone BF-F3) (A) and for MyHC (MF-20) (B). d: days after birth; m: months after birth.

resolution gel electrophoresis technique we have studied the postnatal transitions of the different MyHC isoforms in functionally distinct mouse skeletal muscles to answer the following questions: Are the patterns of MyHC expression identical in functionally distinct muscles? Does the transition from the developmental to the adult MyHC isoforms follow the same chronology in the different skeletal muscles? When does the mouse muscle attain a mature phenotype? Our results demonstrate that in mouse the pattern of MyHC expression at birth is similar in all of the skeletal muscles but the chronology of the maturation and the time at which a mature phenotype is established are different in the functionally distinct muscles. During postnatal muscle development the pattern of MyHC expression featured two successive phases. The first is characterized by a downregulation and elimination of the developmental MyHC isoforms. The second phase is characterized by an up-regulation and more importantly by a stabilization of the adult MyHC phenotype. This sequential transition is controlled by multiple factors such as functional demand, innervation, calcium influx and hormonal signals (Schiaffino and Reggiani, 1996; Pette and Staron, 2000).

In our results, the developmental isoforms of myosin are eliminated later in the muscles which are programmed for slow, repetitive and prolonged activity than the muscles which are programmed for rapid, short-term phasic muscle activity. Therefore in the fast-twitch muscles of the limbs these developmental isoforms were rapidly replaced by the adult fast and slow isoforms during the first few days after birth whereas in the slow-twitch soleus muscle, the diaphragm and the tongue, the embryonic MyHC isoform was eliminated shortly after birth but the neonatal MyHC isoform persisted until 21 days after birth. Finally in the masseter which is used for rapid chewing only at 2–3 weeks after birth, the embryonic isoform was detected until 14 days after birth and the neonatal isoform persisted for at least 3 month in the adult mouse. Several studies (d'Albis et al., 1986; Widmer et al., 2002) have also described the presence of the neonatal MyHC isoform in mouse masseter but the function of this isoform in the adult masseter has not yet been clarified.

Neuromuscular activity plays a key role in the establishment of specific muscle fiber phenotypes during development and in the maintenance of their phenotypic properties (Rubinstein and Kelly, 1978; Pette and Vrbova, 1985). The neuromuscular junction forms at 13–14 days postcoitum (dpc) and its maturation continues during the first few weeks after birth. The elimination of polyneuronal innervation by focal innervation and the increase in size and complexity of the junctional folds are signs that both the muscle fiber and the neuromuscular junctions have attained a mature adult phenotype (Slater, 1982; Jansen and Fladby, 1990). According to Jansen and Fladby (1990), the elimination of polyinnervation in fast-twitch limb muscles occurs much more quickly than in slow-twitch skeletal muscle in the rat. This result could explain the difference in the time of down-regulation and elimination of developmental MyHC isoforms in fast compared to slow skeletal muscles.

MyHC expression during postnatal development is known to be influenced by hormones (Gambke et al., 1983; Mahdavi et al., 1987; Russell et al., 1988). The level of thyroid hormone has been shown to contribute to the regulation of expression of the various myosin isozymes in skeletal muscle (Izumo et al., 1986). During development, hyperthyroidism results in a precocious expression of the adult fast isoforms in skeletal muscle (Gambke et al., 1983; Chizzonite and Zak, 1984; Butler-Browne et al., 1987), whereas hypothyroidism results in an inhibition in the expression of these adult isoforms and a persistence of the developmental isoforms (Butler-Browne et al., 1987; Adams et al., 1999). Thyroid hormones are not detected in the mouse serum before 17 dpc and remain low until birth. The level of thyroid hormone then increases to reached its maximum value at 2–3 weeks after birth and then stabilise at four weeks after birth (d'Albis et al., 1987). This is in agreement with our results which shows that the developmental MyHC isoforms could no longer be detected after the maximum peak of thyroid hormone expression in fast muscles. In these muscles it seems that the increase in thyroid hormone concentration stimulates the replacement of developmental by adult fast MyHC isoforms. Moreover, several studies have recently demonstrated that functionally distinct muscles do not contain the same amount of thyroid hormone receptors (Yu et al., 2000; White et al., 2001) and therefore require different thyroxin concentrations to carry attain a mature phenotype.

At birth, in addition to the embryonic and the neonatal isoforms of MyHC, a very small amount of slow (I/β) myosin was found in all of the muscles except the tongue. The mammalian skeletal muscle is formed as two successive generations of muscle fibers. As has been noted by previous

investigators, the majority of the primary generation muscle fibers initially express the slow isoform (Kelly and Rubinstein, 1980; Lyons et al., 1983; Narusawa et al., 1987; Harris et al., 1989; Condon et al., 1990a, 1990b; Ontell et al., 1993). Synthesis of slow MyHC is an early event in the myogenesis of both future fast and future slow skeletal muscles and is expressed concurrently with embryonic myosin in one population of cells (Condon et al., 1990a, 1990b; Narusawa et al., 1987). The initial activation of the slow MyHC gene is independent of innervation (Condon et al., 1990b). Many primary generation muscle fibers retain their expression of slow myosin whereas others begin to replace slow myosin with neonatal myosin (Condon et al., 1990a).

All secondary generation muscle fibers initially express neonatal as well as embryonic myosin (Condon et al., 1990a, 1990b). The majority of these fibers retain their fast fate and thus augment the number of fast fibers in all muscles as well as contribute to the mosaic distribution of fast and slow fibers. The expression of slow MyHC in secondary generation muscle fibers is dependent on innervation (Condon et al., 1990b).

Our results demonstrate that the proportion of slow myosin progressively increases in the slow-twitch skeletal muscles whereas in fast-twitch skeletal muscles it tended to decrease and a few weeks later the level of this isoform was difficult to detect using this technique. These results demonstrate that the adult phenotype is not necessarily determined before birth but becomes modulated as a mature pattern of motor innervation and physical activity is established. The distinct embryonic origins of axial and craniofacial skeletal muscles may be reflective of different processes of muscle development. According to Dalrymple et al. (1999) the tongue muscle has distinct features of molecular differentiation when compared to limb and axial muscles. The myoblasts that contribute to the formation of the tongue preferentially express Myf-5 during myoblast determination rather than MyoD (Dalrymple et al., 1999). This study also demonstrated the presence of transcription but the absence of translation of slow MyHC gene in the tongue. These results suggest that the tongue muscle represents a distinct myoblast lineage with a different pattern of myogenesis from the limb muscles.

The fast adult MyHC isoforms are first detected at 5 days after birth and in the adult mouse these isoforms predominate. In a previous study (Agbulut et al., 1996) we demonstrated that IIA, IIX and IIB MyHC isoforms are present in all adult mouse skeletal muscles and in general IIB MyHC is the predominant isoform whereas the amount of IIA and IIX MyHC isoforms varies according to the muscles. In addition our present study clearly demonstrated a transitory pattern of IIB and IIA MyHC expression in the soleus and the tongue, respectively during postnatal muscle development. These isoforms are detected for only a few weeks after birth in these muscles. Our results demonstrate that all fast MyHC isoforms are expressed in the skeletal muscles during postnatal muscle development but the presence and the level of expression of each isoforms which will determine the adult phenotype are controlled by extrinsic factors (i.e. hormonal status, functional demand, innervation). These results suggest that initially the skeletal muscles have a rather open pattern of MyHC expression and it is only under the influence of extrinsic factors that a specific pattern of MyHC expression which is correlated with the exact functional needs of the muscle becomes established. It is interesting to note that inactivation of IIB or IIX MyHC genes in the mouse leads to functional and structural defects despite the up-regulation of another MyHC isoform in each null strain to attempt to compensate for the loss of the null isoform (Allen et al., 2000). Together these data support the hypothesis that MyHC isoforms are functionally distinct.

In conclusion, using a modified electrophoretic technique, we have characterized the MyHC isoform composition of new-born, young and adult mice. Once the chronology by which the normal pattern of MyHCs has been defined it can then be compared with that of mice where a gene had been modified either by disease or by genetic manipulation. During development, ageing and disease the analysis of the transitions of the MyHC isoforms is necessary because the muscles become adapted to perform specialized and diverse functions, and this is reflected in the composition and level of expression of MyHC isoforms within the muscle. Consequently we hypothesize that muscle activity, innervation and hormonal status work in concert to produce the highly specialized shifts in MyHC isoform expression during postnatal development characteristic for each muscle.

4. Materials and methods

4.1. Animals and experimental procedure

In this study, C57BL6 (Iffa-Credo, Lyon, France) mice were used. Soleus, EDL, plantaris, gastrocnemius, diaphragm, tongue and masseter were removed from anaesthetised female and male mice at various times between 3–90 days after birth. Following dissection muscles were frozen in liquid nitrogen and stored at –80 °C for further analysis. In this study at least three mice were used for each experimental point.

4.2. Electrophoresis of myosin heavy chain isoforms and densitometric gel analysis

Muscles were extracted on ice for 60 minutes in 4 volumes of extracting buffer (pH 6.5) as previously described (Butler-Browne and Whalen, 1984). Following centrifugation, the supernatants were diluted 1:1 (v/w) with glycerol and stored at –20 °C. MyHC's were separated on 8% polyacrylamide gels which were made in the Bio-Rad mini-Protean II Dual slab cell system as described previously (Agbulut et al., 1996).

The gels were migrated for 31 hours at 72 V (constant voltage) at 4 °C. Following migration, the gels were silver

stained according to Blum et al. (1987). The position of the MyHCs band were assessed with western blotting using antibodies directed against different MyHC isoforms (see below). The gels were scanned using a video acquisition system. The relative level of MyHC protein was determined by densitometric software (Genetools, Syngene, Cambridge, England).

4.3. Western-blot analysis

For western blot analysis the proteins were actively transferred onto nitrocellulose membranes overnight at 30 V (constant voltage) after electrophoresis. Following transfer, the nitro-cellulose membranes were blocked to eliminate non-specific binding in 3% foetal calf serum diluted in phosphate-buffered saline.

The blots were then incubated with the primary antibodies : anti-MyHC-I/ß (BAD5), anti-MyHC-IIA (SC71), anti-MyHC-IIB (BFF3) (Schiaffino et al., 1986), anti-IIA,IIX,IIB (Novocastra, UK), anti-MyHC (MF-20) (Bader et al. 1982), anti-embryonic (Biovalley, France) and anti-neonatal MyHC (Ecob-Prince et al., 1986). The blots were then incubated with a biotin labelled secondary antibody (dilution 1/1000) (Dako, Copenhagen, Denmark), streptABC complex/HRP (Dako, Copenhagen, Denmark) and specific antibody binding was revealed by incubating the membranes in the diaminobenzidine substrate mixture (10 mg DAB, 0.03% H2O2 in PBS pH 7.4) until the bands were sufficiently intense.

4.4. Reverse transcription PCR of MyHC genes

RNA was purified from muscles of wild-type mice using the guanidinium-acid phenol method (Chomczynski and Sacchi, 1987). First-strand cDNA was synthesised from 1 mg of total RNA using reverse transcriptase and 0.2 mg of pd(N)6 primer (First-strand cDNA synthesis kit, Amersham Pharmacia Biotech). Oligonucleotide sequences were those described by Sartorius et al. (1998), taken in the 3' UTR of MyHC-IIB gene. PCR reactions contained 1 µl of cDNA (1/20 RT reaction), 10 pmol of each primer and 1.25 unit of taq polymerase (Taq DNA polymerase kit, Qiagen) in a total volume of 50 µl. After 2 min at 94 °C, amplification conditions were as follows : denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and primer extension at 72 °C for 30 s, for a total of 25 cycles, followed by one cycle of primer extension at 72 °C for 5 min. 10 µl of each reaction were separated on a 3% agarose gel containing ethidium bromide.

Acknowledgements

This work was supported by the "Association Française contre les Myopathies", the CNRS, Université Pierre & Marie Curie Paris VI, the European Community Aging Muscle QLRT-1999-020304. O.A was supported by a fellowship from the "Association Française contre les Myopathies" during this study. We would like to thank Vincent Mouly and Sylvie Bonavaud for the lively discussions we have had during this study.

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