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Three fast myosin heavy chains in adult rat skeletal muscle

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A new fast myosin heavy chain isoform was electrophoretically detected in adult rat skeletal muscles. It was present at high levels in diaphragm and, therefore, designated as MHCIId. Appreciable amounts of MHCIId were detected in tongue musculature, the extraocular muscles, and in the deep red portions of various fast muscles. Its concentration in fast-twitch muscle was greatly increased by chronic stimulation.

Myosin heavy chain isoform; Skeletal muscle; Diaphragm; Extraocular muscle; (Fetal, Neonatal, Adult)

1. INTRODUCTION

The number of myosin heavy chain (HC) isoforms detected to date in skeletal muscle may still be incomplete. Slow (type I) fibers contain HCI, and fast fiber types IIA and IIB contain HCIIa and HCIIb, respectively [1–4]. In addition, embryonic and neonatal HC isoforms (HC_{emb}, HC_{neo}) are expressed in developing muscles [5]. As shown for the rat, these latter two isoforms also exist, as well as a specific extraocular myosin HC (HC_{eom}), in adult extraocular muscles [6]. With the improved electrophoretic separation used in the present study we were able to show, in rat fast-twitch muscle fibers, the existence of an additional, previously undetected HC isoform.

2. MATERIALS AND METHODS

Muscles were excised from embryonic, newborn and adult Wistar rats. Chronic low-frequency stimulation (10 Hz, 10 h daily) of tibialis anterior (TA) was performed as described [7]. In addition, the soleus muscle of Sprague-Dawley rats and various skeletal muscles of White New Zealand rabbits were examined. Muscles were frozen and pulverized under liquid N_2. Crude extracts were prepared by homogenizing the muscle powder 1:7 (w/v) in the following medium: 0.3 M KCl, 0.1 M KH_2PO_4, 50 mM K_2HPO_4, 10 mM EDTA, pH 6.5. After stirring for 15 min on ice, the homogenate was centrifuged at 10000 *g*. The supernatant fraction was two-fold diluted with glycerol and stored at −20°C. Protein was determined according to [8].

Polyacrylamide gel electrophoresis in the presence of SDS (SDS-PAGE) was performed according to [9,10] using a 0.75 mm thick 5–8% gradient separating gel and a 3.5% stacking gel. Aliquots of the extracts containing 0.1–0.5 μg of protein were loaded for electrophoresis after having been incubated for 10 min at 56°C in a final volume of 5 μl lysis buffer: 10% (w/v) glycerol, 5% (v/v) β-mercaptoethanol, 2.3% (w/v) SDS, 60 mM Tris-HCl, pH 6.8. Electrophoresis lasted 24 h at 120 V. Gels were silver-stained as described in [11]. Protein bands were tentatively identified according to their apparent molecular masses compared with those of marker proteins. In addition, myosin preparations purified from muscles predominantly composed of type I (soleus, Wistar rat) or type IIB (levator ani) fibers, were used to aid in myosin HC identification.

Electrophoreses were densitometrically evaluated to estimate the percentage distribution of the various HC isoforms.

3. RESULTS

Gradient gel electrophoresis enhances small differences existing between the various MHC isoforms. Thus, a maximum of four different MHC isoforms were electrophoretically discerned in various adult skeletal muscles of the rat (fig.1). Of these, the slow MHCI isoform represented the fastest migrating isoform, followed by the fast MHCIIb and MHCIIa isoforms. An additional MHC isoform was detected in a variety of fast- and slow-twitch muscles. This hitherto unknown isoform displayed the lowest electrophoretic mobility. Its relative concentration was highest in

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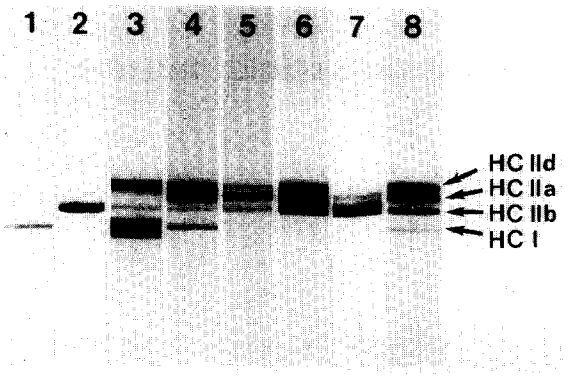

Fig.1. Gradient PAA (5–8%) gel electrophoresis of rat muscle myosin heavy chain (MHC) isoforms in the presence of SDS. Lanes: 1, soleus (Wistar); 2, levator ani; 3, soleus (Sprague-Dawley); 4, diaphragm; 5, extraocular muscles; 6, tongue; 7, untreated tibialis anterior; 8, low-frequency stimulated (28 days) tibialis anterior.

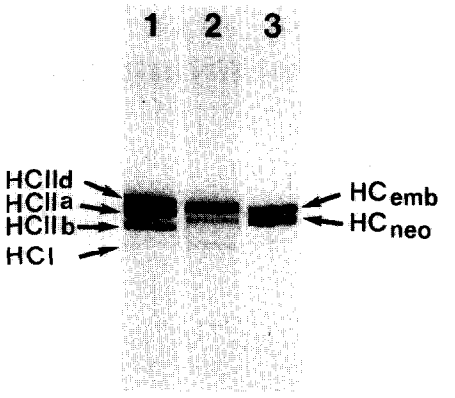

Fig.2. Gradient PAA (5–8%) gel electrophoresis of embryonic, neonatal and adult rat myosin heavy chain (MHC) isoforms. Lanes: 1, low-frequency stimulated (28 days) tibialis anterior; 2, late fetal hindlimb muscles; 3, newborn hindlimb muscles. Densitometric evaluation gave the following percentage distributions: 79% HC_{emb}, 17% HC_{neo} and 4% HCI in late fetal muscle (2), and 54% HC_{emb}, 43% HC_{neo} and 3% HCI in newborn muscle (3).

diaphragm. Therefore, it was tentatively designated as MHCIId. Considerable amounts of the MHCIId were also present in the deep portions of masseter, gastrocnemius and vastus lateralis, as well as in tongue musculature, mylohyoideus and the extraocular muscles (table 1). Relatively small amounts of MHCIId were present in the investigated fast-twitch muscles of the hindlimb. It was undetectable in levator ani and soleus (Wistar rat) muscles which uniquely contained the MHCIIb and MHCI isoforms, respectively (fig.1). However, appreciable amounts of MHCIId were present in soleus muscle of the Sprague-Dawley rat (fig.1).

Table 1

Densitometrically evaluated percentage distributions of myosin heavy chain isoforms in various muscles of adult Wistar rats and soleus muscle of Sprague-Dawley rats

Muscle	% MHCI	% MHCIIb	% MHCIIa	% MHCIId
Levator ani	0	100	0	0
Vastus (superfic.)	0	98	2	0
Gastrocnemius (superfic.)	0	88	12	0
Psoas (superfic.)	0	88	12	0
Plantaris	3	51	46	0
Tibialis ant.	0	77	19	4
Psoas (deep)	0	71	23	6
Tongue musculature	0	20	72	8
Extraocular muscles	0	24	67	9
Mylohyoideus	0	56	34	10
Gastrocnemius (deep)	10	26	50	14
Soleus	100	0	0	0
Soleus (Sprague-Dawley)	83	3	0	14
Masseter (deep)	0	7	74	19
Vastus (deep)	6	23	50	21
Diaphragm	14	1	53	32
Tibial. ant. (28 day stim.)	1	22	39	38

The expression of MHCIId in fast-twitch TA was greatly enhanced by low-frequency stimulation (fig.1). Its relative concentration increased from approx. 6% in untreated to 39% in the 28-day stimulated muscle. Interestingly enough, MHCIId was undetectable in chronically stimulated TA and diaphragm and various skeletal muscles of the rabbit (not shown).

To verify that the newly detected MHCIId was

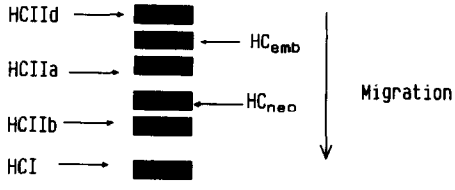

Fig.3. Schematic illustration of the electrophoretic mobilities of embryonic, neonatal, adult fast and slow myosin heavy chain isoforms of the rat.

not identical with either HC_{emb} or HC_{neo} MHC isoforms, analyses were performed on fetal and newborn muscles (fig.2). Both HC_{neo} and HC_{emb} displayed mobilities distinct from MHCIId (fig.3).

4. DISCUSSION

The distribution of the newly detected MHCIId appears to be opposed to that of MHCIIb. It was undetectable in the levator ani muscle (purely MHCIIb) and, concomitant with a reduction in the tissue level of MHCIIb, it increased in chronically stimulated TA muscle. Its relatively high concentration in low-frequency stimulated muscle and also in normal diaphragm suggests that its expression relates to fast-twitch fibers capable of sustained contractile activity. Its presence in extraocular muscles raises the question of a possible identity with the previously described HC_{eom}. This isoform was detected at the mRNA level by a specific cDNA clone in extraocular muscles, but was not found in other skeletal muscles of the rat [6]. Since the investigated skeletal muscles were not specified in that study [6] and appreciable amounts of MHCIId exist only in the red deep portions of fast skeletal muscles (table 1), it may be that mRNA coding for MHCIId remained undetected. Another possibility is that MHCIId is identical with the MHC-2X which was described in a preliminary form by Schiaffino et al. [12,13] in adult rat skeletal muscles. According to these authors, MHC-2X is an additional fast MHC isoform, intermediate between MHCIIa and MHCIIb. It is predominantly expressed during stimulation-induced fast-to-slow and slow-to-fast fiber transformation [13]. Although the present results clearly demonstrate the existence of an additional fast MHC isoform in rat skeletal muscle, its identity remains to be elucidated in further studies.

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