Volume 335, number 2, 243–245 FEBS 13330 December 1993

Electrophoretic analysis of myosin heavy chain isoform patterns in extraocular muscles of the rat

Gerhard Asmussen**, Irmtrud Traub, Dirk Pette*

Fakultät für Biologie, Universität Konstanz, Postfach 5560-M641, D-78434 Konstanz, Germany

Received 28 September 1993

Six oculorotatory muscles and the levator palpebrae muscle of the rat were analysed by SDS-PAGE for their myosin heavy chain (MHC) isoform patterns. Oculorotatory muscles display a marked predominance of fast MHC isoforms. They contain, in addition to the slow (MHCI) and fast (MHCIIb, MHCIId, MHCIIa) skeletal MHCs, the neonatal MHC_{neo} and the extraocular MHC_{eom}. The levator palpebrae, generally assumed to be a member of the extraocular muscle group because of its innervation by the ocolumotor nerve, does not contain MHC_{neo} and MHC_{eom}. It resembles a fast-twitch skeletal muscle with a predominance of MHCIId.

Electrophoresis; Extraocular muscle; Myosin heavy chain isoform; Rat

1. INTRODUCTION

Mammalian extraocular muscles are highly specialized. They are capable of performing very slow vergence and fusion movements, but their contractions are also among the fastest when compared to other cross-striated muscles. They are characterized by low force output and high fatigue-resistance [1]. Furthermore, they display properties normally observed only in neonatal or denervated muscles, e.g. acetylcholine contractures [2]. As revealed by enzyme histochemical, immunocytochemical and electron microscopic studies, these properties relate to specific fiber types and a characteristic fiber architecture [3,4].

To date, only a few studies exist on the myosin composition of extraocular muscles. Wieczorek et al. [5] studied pooled extraocular muscles of the rat and were able to delineate at the mRNA level six myosin heavy chain (MHC) isoforms, an embryonic, a neonatal, a slow, one specific extraocular, and two fast skeletal MHC isoforms. The MHC_{eom} isoform was also identified by immunoblotting and immunocytochemistry at the protein level [6]. To our knowledge, MHC_{eom} has as yet not been identified electrophoretically. We were interested to characterize its electrophoretic mobility and also to investigate its distribution in various oculorotatory muscles of the rat. The levator palpebrae, assumed to be a member of the extraocular muscle group because of its innervation by the oculomotor nerve, was included in our study.

*Corresponding author. Fax: (49) (7531) 88 39 40.

***Permanent address:* Carl-Ludwig-Institut, für Physiologie, Universität Leipzig, Liebigstr. 27, D-04103 Leipzig, Germany.

2. MATERIALS AND METHODS

2.1. *Animals and muscles*

Six young adult Wistar rats were anesthetized and killed by exsanguination, and the six oculorotatory muscles, as well as the levator palpebrae muscle were dissected under a stereomicroscope. The muscles were collected separately, frozen in melting isopentane (−159°C).

2.2. *Myosin extracts and myosin heavy chain electrophoresis*

Frozen muscles were pulverized under liquid N_2 in a micromortar [7], homogenized in 0.3 M KCl, 0.1 M KH_2PO_4, 50 mM K_2HPO_4, 1 mM EDTA, pH 6.5, and centrifuged at 12,000 × *g*. The supernatant fractions were two-fold diluted with glycerol and stored at −25°C. Protein concentrations were determined according to Lowry et al. [8]. MHC isoforms were electrophoretically separated on 7–10% gradient polyacrylamide gels in the presence of sodium dodecyl sulfate as previously described [9]. Several (3–6) electrophoreses were performed on the extracts prepared from each muscle. Extracts of the deep portion of the gastrocnemius muscle served as marker for the electrophoretic mobilities of the skeletal MHC isoforms. Gels were silver-stained [10] and the percentage distributions of the various MHC isoforms were evaluated densitometrically. The results were expressed as means ± S.D.

3. RESULTS

The electrophoretically separated MHC patterns of all six oculorotatory muscles differed markedly from those of normal fast-twitch skeletal muscle (Fig. 1). The oculorotatory muscles contained the fast-twitch (MHCIIa, MHCIId, MHCIIb) and slow-twitch (MHCI) skeletal isoforms. However, they contained two additional MHC isoforms, not present in skeletal muscles. One of these migrated between MHCIId and MHCIIb. Due to its proximity to MHCIId, its separa-

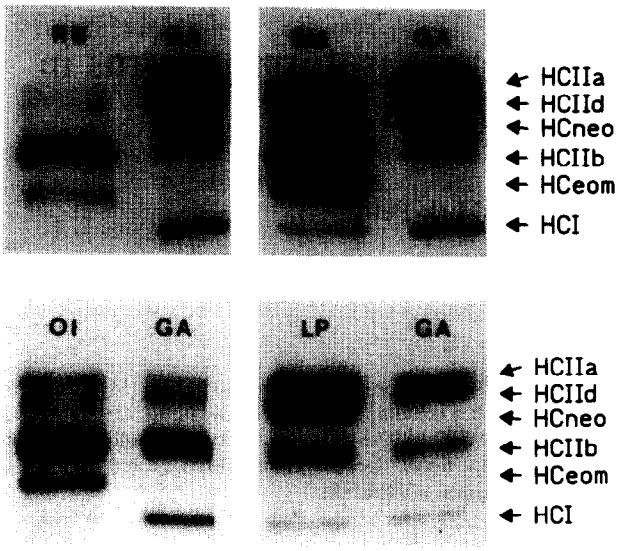

Fig. 1. Silver-stained SDS-PAGE of MHC isoforms in three oculorotatory muscles, levator palpebrae, and gastrocnemius muscles of the adult rat. Abbreviations: GA, gastrocnemius; LP, levator palpebrae; OI, obliquus inferior; RM, rectus medialis; RS, rectus superior; HCI, slow MHC; HCIIa, HCIIb, HCIId, fast skeletal MHCs; HC_{eom}, extraocular MHC; HC_{neo}, neonatal MHC.

tion was not always complete. Based on previous studies [11], this band was identified as the neonatal MHC, MHC_{neo}. The second additional MHC isoform displayed an electrophoretic mobility intermediate between the fast MHCIIb and the slow MHCI. Thus, the electrophoretic mobilities increased in the order of MHCIIa < MHCIId < MHC_{neo} < MHCIIb < MHC_{eom} < MHCI.

The MHC_{eom} was never detected in skeletal muscles and was also not found in the levator palpebrae muscle (Fig. 1). We suggest that this band corresponds to the MHC isoform, MHC_{eom}, specific to extraocular muscles [5,6].

Results from densitometric evaluations are summarized in Table I. A characteristic of the oculorotatory muscles was their extremely low content of the slow MHCI. All six muscles displayed similar amounts (15%–20%) of MHC_{eom}. MHCIIb was the predominant isoform. When the relative concentration of MHCIIb was related to that of MHCIIa, small variations became evident. However, these differences were less pronounced when antagonistic muscles were compared (Table I). Thus, superior and inferior rectus muscles exhibited the highest relative amounts of MHCIIb and the lowest amounts of MHCIIa, MHCIId, and MHC_{neo}. The oblique muscles contained higher amounts of MHCIIa, MHCIId, and MHC_{neo} than superior and inferior rectus muscles, the lateral and medial rectus muscles being intermediate. The levator palpebrae differed from the oculorotatory muscles as it did not did not contain MHC_{neo} or MHC_{eom}. It resembled that of a skeletal muscle with a predominance of MHCIId (Fig. 1, Table I).

4. DISCUSSION

To our knowledge, this investigation is the first comparative electrophoretic study of MHC patterns in different extraocular muscles. In addition, we characterize the electrophoretic mobility of MHC_{eom}, an isoform specific to extraocular muscles [5,6]. This isoform is present in similar amounts in all oculorotatory muscles, but undetectable in the levator palpebrae muscle. In addition to MHC_{eom}, oculorotatory muscles are characterized by the predominance of fast MHC isoforms and the presence of MHC_{neo}.

On the whole, these results are compatible with the immunocytochemically assessed fiber type distributions [3,5,6,12]. Small deviations may result from the fact that fiber types differ in their cross-sectional areas. Therefore, percentages of fiber types and MHC isoforms may not be fully compatible. As to the existence of slow-tonic fibers [3], we were unable to identify this specific

Table I

Distribution of myosin heavy chain isoforms in six extraocular muscles and the levator palpebrae muscle of adult rat as evaluated by densitometry of silver-stained SDS-PAGE

MHC isoform	Percent of total MHC isoforms					
	HCIIa	HCIId	HC_{neo}	HCIIb	HC_{eom}	HCI
Obliquus superior	10 ± 2	18 ± 2		57 ± 5	14 ± 3	≈ 1
Obliquus inferior	13 ± 1	8 ± 2	8 ± 2	51 ± 1	20 ± 1	≈ 1
Rectus superior	2 ± 2	5 ± 2		75 ± 6	18 ± 4	≈ 1
Rectus inferior	2 ± 1	4 ± 3	2 ± 1	69 ± 2	21 ± 2	2 ± 1
Rectus medialis	7 ± 1	16 ± 1		54 ± 1	20 ± 1	3 ± 1
Rectus lateralis	5 ± 3	16 ± 8		59 ± 2	19 ± 1	≈ 1
Levator palpebrae	16 ± 3	64 ± 8	0	17 ± 4	0	3 ± 1

Values are means ± S.D. (n = 3–6).

MHC isoform. Because slow-tonic fibers represent an extremely small population in the oculorotatory muscles, their MHC isoform may have been below electrophoretic detectability. It is also possible that $MHCI_{ton}$ displays an electrophoretic mobility similar to that of MHCI and, thus, escaped detection.

The very high shortening velocity of rat oculorotatory muscles [13] suggests that this property relates to MHC_{eom}. This isoform should be the fastest and display the highest ATPase activity. It has been demonstrated that extraocular muscles exhibit higher ATPase activities than skeletal muscles [14]. The sum of MHC_{eom} and MHCIIb, the fastest among the skeletal MHC isoforms [15], amounts to 70–90% of the total MHC isoforms in the oculorotatory muscles. This predominance of fast isoforms is the basis for fast cross-bridge cycling. Speculating on this line, the superior and inferior rectus muscles with 90% relative concentration of MHC_{eom} and MHCIIb, might be the fastest oculorotatory muscles.

The finding that the levator palpebrae displays a MHC pattern different from that of those oculorotatory muscles innervated by the same (oculomotor) nerve, is relevant in view of the role innervation is thought to exert on muscle fiber phenotypes [16]. Obviously, the differences in myosin expression between these muscles is intrinsically programmed and relates to their developmental origin from different cell lineages

Acknowledgements: This study was supported by the Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 156.

REFERENCES

[1] Asmussen, G. and Gaunitz, U. (1981) Pflügers Arch. Eur. J. Physiol. 392, 183–190.
[2] Asmussen, G. and Gaunitz, U. (1981) Pflügers Arch. Eur. J. Physiol. 392, 191–197.
[3] Rowlerson, A.M. (1987) in: Transact. 16th Europ. Strabismolog. Assoc. (Kaufmann, H. Ed.) pp. 19–26, Giessen.
[4] Spencer, R.F. and Porter, J.D. (1988) in: Neuroanatomy of the üsoculomotor system (Büttner-Ennever, Ed.) pp. 33–79, Elsevier Science Publishers BV, Amsterdam.
[5] Wieczorek, D.F., Periasamy, M., Butler-Browne, G.S., Whalen, R.G. and Nadal-Ginard, B. (1985) J. Cell Biol. 101, 618–629.
[6] Sartore, S., Mascarello, F., Rowlerson, A., Gorza, L., Ausoni, S., Vianello, M. and Schiaffino, S. (1987) J. Muscle Res. Cell Motil. 8, 161–172.
[7] Pette, D. and Reichmann, H. (1982) J. Histochem. Cytochem. 30, 401–402.
[8] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[9] Bär, A. and Pette, D. (1988) FEBS Lett. 235, 153–155.
[10] Oakley, B.R., Kirsch, D.R. and Morris, N.R. (1980) Anal. Biochem. 105, 361–363.
[11] Termin, A., Staron, R.S. and Pette, D. (1989) Histochemistry 92, 453–457.
[12] Pierobon-Bormioli, S., Sartore, S., Vitadello, M. and Schiaffino, S. (1980) J. Cell Biol. 85, 672–681.
[13] Close, R.I. and Luff, A.R. (1974) J. Physiol. 236, 259–270.
[14] Bogatzki, M., Dieckhues, B. and Hollwich, F. (1968) Z. Klin. Chem. 6, 53–55.
[15] Bottinelli, R., Schiaffino, S. and Reggiani, C. (1991) J. Physiol. 437, 655–672.
[16] Pette, D. and Vrbová, G. (1985) Muscle Nerve 8, 676–689.