Volume 326, number 1,2,3, 272–274 FEBS 12706 July 1993

Specific increase of genetic expression of parvalbumin in fast skeletal muscles of mdx mice

Ph. Gailly[a], E. Hermans[b], J.N. Octave[b] and J.M. Gillis[a]

[a]*Department of Physiology and* [b]*Department of Neurochemistry, Catholic University of Louvain, 1200 Brussels, Belgium*

Received 14 May 1993

Parvalbumin mRNA was assayed by Northern blot analysis in muscles from normal and dystropic (mdx) mice. Its content was found to be specifically higher in mdx fast muscles than in control preparations. This suggests an increased expression of the protein in dystrophin-lacking fast fibres. A possible role in calcium homeostasis is discussed.

Duchenne muscular dystrophy; Parvalbumin; Calcium homeostasis

1. INTRODUCTION

Muscles from mdx mice and from Duchenne dystrophy patients are deficient in dystrophin [1], a cytoskeletal protein associated with the plasma membrane [2,3]. The very benign manifestations of the dystrophy in mice sharply contrast with the severe and life-threatening evolution in humans [4,5]. It is of considerable interest to understand the reason(s) of this difference. It has been reported that spontaneous proteolysis was higher in mdx muscle [6], that it could be reduced to normal rate if the external Ca^{2+} concentration was reduced, and exaggerated by increasing Ca^{2+} [7]. This led to the hypothesis that dystrophin-lacking fibres suffer from a chronic Ca overload, resulting in the activation of Ca-dependent proteases [7,8]. Indeed, the total Ca content of mdx muscle is about doubled [9,10], but as far as the cytosolic Ca^{2+} concentration is concerned, reports are contradictory [7,11–13].

Duchenne dystrophy predominantly affects fast glycolytic [14], type IIb, muscle fibres which are progressively replaced by oxidative type I and IIa fibres. This phenomenon, though present, is much less pronounced in muscle from mdx mice. Fast muscles of the mouse, and more precisely type IIb fibres, contain a high concentration of the Ca-binding protein parvalbumin, amounting to about 0.5 mM (as in fast muscles of fish and amphibians) [15,16]. This is unique to very small mammals and the parvalbumin content sharply decreases as the size of the animal increases; it is undetectable in man [16]. Parvalbumin can act as an intracellular Ca-buffer and promote fast relaxation of muscle by binding calcium [17]. We hypothesize that in muscle from mdx mice, parvalbumin prevents a significant rise in cytosolic Ca^{2+} concentration and the subsequent proteases activation so that dystrophy remains benign. In apparent contradiction with the above hypothesis, one group reported that parvalbumin content is reduced in fast muscle from mdx mice, a finding attributed to a large content of immature fibres which poorly express parvalbumin [18]. This interpretation should be re-examined as parvalbumin continuously leaks out muscle cells: its plasma concentration is about ten times higher than for normal old mice [19]. This suggests an increased turnover rate of parvalbumin in mdx muscle. The present report shows that the parvalbumin mRNA content is specifically increased in fast muscle from mdx mice.

Correspondence address: Ph. Gailly, Department of Physiology, UCL 5540, av. Hippocrate, 55, 1200 Brussels Belgium. Fax: (32) (2) 764 53 22.

2. MATERIALS AND METHODS

Tibialis anterior, soleus and ventricle muscles were dissected from 1-year-old C57 and mdx mice, immediately frozen in liquid nitrogen and stored at −80°C. Total cellular RNA was prepared by the guanidium isothiocyanate method [20] and analyzed by Northern blot analysis. 5–10 μg total RNA of each muscle (measured by spectrophotometry) were denaturated with deionized glyoxal [21], fractionated by electrophoresis on 1% agarose gel in 10 mM sodium phosphate pH 7 and transferred to a nylon membrane (Hybond-N, Amersham) by capillarity in 20 × SSC. Parvalbumin DNA probe was excised from the pPVD21 plasmid by *Eco*RI [22]. Glyceraldehyde phosphate dehydrogenase (GAPDH) cDNA [23] and actin cDNA probes were used as controls. The probes were labeled with [α-^{32}P]dCTP by multipriming (Amersham). The blot was hybridized overnight at 68°C in a buffer containing 3.5 × SSC, 1 × Denhardt, 125 mM phosphate buffer, 0.5 mM EDTA and 200 μg/ml salmon sperm DNA, washed and autoradiographed as previously described [23]. Specific radio-labeled bands were quantified by two-dimensional densitometry (Lecphor image analysis program of Biocom, 91942 Les Ulis, France). As a check, radioactive blots were directly counted (Phosphor-Imager, Molecular Dynamics) with the same relative results between the different bands.

Published by Elsevier Science Publishers B.V.

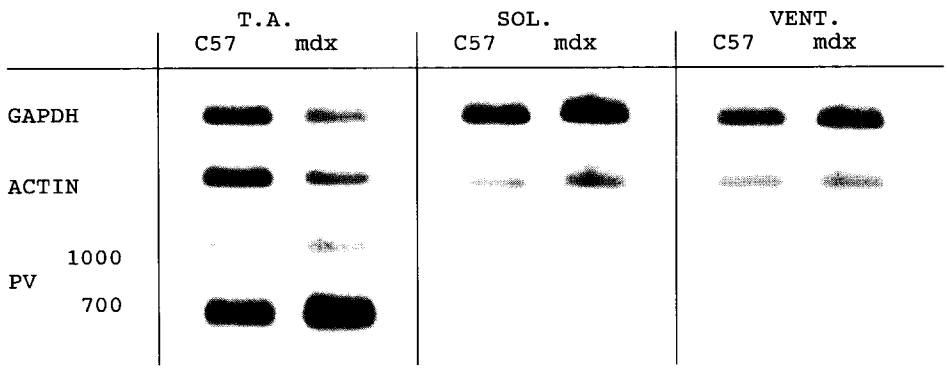

Fig. 1. Montage of 3 Northern blots. According to the molecular weight of the mRNAs, each blot was cut to allow hybridization in separated baths with the different cDNA probes. For each experiment, mdx muscle and its homologous control were simultaneously treated. As said in Section 2, the image was digitalized and the bands were quantified by densitometry.

3. RESULTS

Based on spectrophotometric measurements, total RNA content per muscle weight was found to be 1.6-fold higher in mdx than in C57 skeletal muscles, a value very close to previously reported data [6,24]. This observation reflects a nonspecific increase of the relative rate of protein synthesis [6], even in adult mdx mice, when fibres no longer undergo cycles of degeneration and regeneration [25].

As total RNA contents were higher in mdx than in C57 muscles, the amount of specific mRNAs were expressed per unit actin mRNA taken as a reference. Actin gene was indeed considered as a housekeeping gene, as its mRNA had been shown to be proportional to the total RNA content [24].

In one report, the decrease of parvalbumin content in fast muscles from mdx mice was attributed to an important proportion of immature regenerating fibres which do not express parvalbumins [18,26]. To check this point, GAPDH expression was chosen as a regeneration marker, as it was reported to be specifically activated during skeletal muscle development [27]. We found that GAPDH mRNA content was not specifically increased in adult mdx skeletal muscles in comparison with C57 samples ($104 \pm 8\%$, $n = 4$, see Fig. 1), confirming that the regeneration rate is very low at this age [25].

Specific hybridization with a parvalbumin cDNA probe revealed, in fast muscle preparations, two mRNA bands (1000 and 700 nt) [28]. For both these parvalbumin mRNA types, we noted a specific increase ($150 \pm 10\%$, $n = 5$ and $157 \pm 10\%$, $n = 5$, respectively) in tibialis anterior muscle of mdx mice in comparison with C57 preparations (Fig. 1). As expected, parvalbumin mRNAs were not detected in extracts from slow (soleus) and cardiac muscles, which do not contain parvalbumins [29].

Thus, in mdx muscles, the total RNA content was 1.6-fold increased and the parvalbumin mRNA presented a relative over-expression of 1.5-fold. Altogether, the expression of parvalbumins was thus 2.4-fold higher in fast mdx muscles than in C57. As far as individual fibres are concerned, this probably constitutes an underestimation, as the content of fibres IIb, the only fibres which synthesize parvalbumins [30], is diminished in mdx fast muscles [31].

4. DISCUSSION

In contrast to the evolution of Duchenne dystrophy in humans, muscles in mdx mice do not longer degenerate in the adult state. The increased content of RNA in fast mdx muscle, reported here and in [6,24], suggests that this apparent healthy state results from an increased protein turnover, sufficient to balance an increased protein degradation.

We reported here that parvalbumin mRNA content was higher in mdx fast muscle than in controls. It is not clear which step is critical for this adaptation: increased gene transcription or reduced mRNA degradation. However as translation efficiency seems identical in mdx and C57 mice [6], we can suppose that parvalbumin expression was significantly enhanced in mdx fast fibres. This was not observed in soleus muscle, which, in the mouse, contains both type I and type IIa fibres in about equal proportions [31]. This suggests that the increased expression of parvalbumin is specific of type IIb fibres.

In spite of this increased expression, the parvalbumin content of mdx fast muscle is either decreased (as in tibialis anterior, [18]) or normal (as in extensor digitorum longus, [31]). This reflects a dynamic equilibrium between increased expression and continuous loss of parvalbumin from mdx fibres [19]. Through this parvalbumin efflux, large amounts of calcium may be expelled from the fibres. Indeed, considering intracellular free concentrations of calcium and magnesium around 50 nM [7,12] and 0.5 mM [32], respectively, and K_d's of parvalbumin of 10^{-8} and $10^{-4.1}$ [17] for the two ions respectively, around 40% of the parvalbumin content is

expected to be loaded with calcium (in the resting state). Thus a combination of increased expression and leakage of the protein into the serum could provide mdx fast fibres with a compensatory mechanism against calcium overload.

Acknowledgements: We thank Dr. M. Schleef (University of Bielefeld, Germany) for providing the parvalbumin - pPVD21 plasmid, Drs. B. Lethé and O. Debacker (Institute of Cellular Pathology, Brussels Belgium) for the actin PCR product and Mrs M.Colson for technical assistance.

REFERENCES

[1] Hoffman, E.P., Fishbeck, K.H., Brown, R.H., Johnson, M., Medori, R., Loike, J.D., Harris, J.B., Waterson, R., Brooke, M., Specht, L., Kupsky, W., Chamberlain, J., Caskey, C.T., Shapiro, F. and Kunkel, L.M. (1988) N. Engl. J. Med. 318, 1363–1368.
[2] Koenig, M., Monaco, A.P. and Kunkel, L.M. (1988) Cell 53, 219–228.
[3] Zubrzycka-Gaarn, E.E., Bulman, D.E., Karpati, G., Burghes, A.H., Belfall, B., Klamut, H.J., Talbot, J., Hodges, R.S., Ray, P.N. and Worton, R.G. (1988) Nature 333, 466–469.
[4] Dangain, J. and Vrbova, G. (1984) Muscle Nerve 7, 700–704.
[5] Wessel, H.B. (1990) Ped. Neurol. 6, 3–12.
[6] MacLennan, P.A. and Edwards, R.H.T (1990) Biochem. J., 268, 795–797.
[7] Turner, P.R., Westwood, T., Regen, C.M. and Steinhardt, R.A. (1988) Nature 335, 735–738.
[8] MacLennan P.A., McArdle A. and Edwards R.H.T. (1991) Am. J. Physiol. 260, E594-E598.
[9] Dunn, J.F. and Radda, G.K. (1991) J. Neurol. Sci. 103, 226–231.
[10] Bertorini, T.E., Bhattacharya, S.K., Palmieri, G.M.A., Chesney, C.M., Pifer, D. and Baker, B. (1982) Neurology 32, 1088–1092.
[11] Mongini, T., Ghigo, D., Doriguzzi C., Bussolino, F., Pescarmona, G., Pollo, B., Schiffer, D. and Bosia, A. (1988) Neurology 38, 476–480.
[12] Gailly, Ph., Boland, B., Himpens, B., Casteels, R. and Gillis, J.M. (1993) Cell Calcium in press.
[13] McArdle, A., Edwards, R.H.T. and Jackson, M.J. (1992) Clin. Sci. 82, 455–459.
[14] Webster, C., Silberstein, L., Hays, A.P. and Blau, H.M. (1988) Cell, 52, 503–513.
[15] Gillis, J.M. (1985) Biochim. Biophys. Acta 811, 97–145.
[16] Heinzmann, C.W., Berchtold, M.W. and Rowlerson, A.M. (1982) Proc. Natl. Acad. Sci. USA 79, 7243–7247.
[17] Gillis, J.M., Thomason, D., Lefèvre J. and Kretsinger, R.H. (1982) J. Musc. Res. Cell Mot. 3, 377–398.
[18] Sano,M., Yokota, T., Endo, T. and Tsukagoshi, H. (1990) J. Neurol. Sci. 97, 261–272.
[19] Jockusch, H., Friedrich, G. and Zippel, M. (1990) Muscle Nerve 13, 551–555.
[20] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[21] McMaster, G.K. and Carmichael, G.G. (1977) Proc. Natl. Acad. Sci., 74, 4835.
[22] Schleef, M. (1992) Erbliche Myotonie der Maus: Molekulargenetisdche Analysen deregulierter Gene und ihrer Transkripte. Thesis, University of Bielefeld, Germany.
[23] Hermans, E., Jeanjean, A.P., Laduron, P.M., Octave J.N. and Maloteaux, J.M. (1993) Neurosci. Lett., in press.
[24] MacLennan, P.A. and Edwards, R.H.T. (1991) J. Physiol. 438, 153P.
[25] DiMario, J.X., Uzman, A. and Strohman, R.C. (1991) Dev. Biol. 148, 314–321.
[26] Leberer, E. and Pette, D. (1986) Biochem. J. 235, 67–73.
[27] Edwards, Y.H., Lloyd, J.C., MacMillan, S.L. and Benham, F.J. (1985) Mol. Cell. Biol. 5, 2147–2149.
[28] Berchtold, M.W. and Means, A.R. (1985) Proc. Natl. Acad. Sci. USA 82, 1414–1418.
[29] Baron, G., Demaille, J. and Dutruge, E. (1975) FEBS Lett. 56, 156–160.
[30] Celio, M.R. and Heinzmann, C.W. (1982) Nature 297, 504–506.
[31] Anderson, J.E., Bressler, B.H. and Ovalle, W.K. (1988) J. Musc. Res. Cell Mot. 9, 499–515.
[32] Ng, L.L., Davies, J.E. and Ameen, M. (1992) Metabolism 41, 772–777.