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Isoforms of myosin in growing muscles of ky (kyphoscoliotic) mice

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Kyphoscoliotic (ky) mice are spontaneous mutants of the BDL strain whose postural muscles atrophy during post-natal growth, resulting in extensive kyphoscoliosis in adult animals. At 21 days of age, the seven muscles examined were already well differentiated into fast, slow and mixed type on the basis of the proportions of their native myosin isoforms or their subunits. During post-natal growth, from 21 to 120 days of age, the normal pattern of myosin maturation was essentially respected by the ky mutation: fast muscles became faster, slow muscles became slower and mixed muscles specialized in both directions. However, the post-natal increases of myosin heavy chain 2B and fast myosin light chain LC_{3f} were depressed in ky muscles, whilst there was novel expression of slow myosin light chains, LC_{1s} and LC_{2s}, in muscles which normally did not express them. Intermediate native myosin IM was absent in adult ky soleus, but it increased in adult ky tibialis anterior. We conclude that the ky mutation depresses the normal post-natal transition towards faster muscles and results in adult muscles whose myosin isoforms are generally shifted in a fast-to-slow direction.

Keywords: kyphoscoliotic (ky); myopathy; myosin heavy chain; myosin light chain; scoliosis.

Kyphoscoliosis develops in ky mice as an autosomal recessively inherited mutation (Mason and Palfrey, 1977; Venn and Mason, 1986; Venn et al., 1988). Vertebral deformation is preceded by a single postnatal phase of muscle fibre degeneration in postural muscles (e.g. paraspinal and soleus) from postnatal day 1 reaching maximum incidence at 28 days. In slow soleus muscles, fibres are smaller than normal and usually centrally nucleated, characteristic of regenerating mouse muscle. Fast muscles, like extensor digitorum longus, do not degenerate, although they are smaller than normal. There is no ultrastructural evidence of motoneuronal death, abnormal axon myelination, or a deficit in the number of axons (Bridges et al., 1992). Nonetheless, extensive sprouting of motor nerve terminals was seen from about 35–40 days of age within soleus, indicating possible neural involvement in the pathology of ky mice.

The ky locus has been mapped to a small region of distal mouse chromosome 9 (Skynner et al., 1995), a region syntenic with human chromosome 3q21. This position rules out ky as being any previously mapped gene known to be expressed in nerve or muscle, and suggests that ky codes for an as-yet unmapped or unidentified muscle protein.

In an earlier study we showed that adult ky soleus muscles contract more slowly and produce less force/cross-sectional area than do soleus muscles of age-matched normal mice (Maréchal et al., 1995). Moreover, adult ky soleus muscles contain close to 100% slow native myosin (SM_2) whereas normal soleus contain about 82% slow native myosin (SM_2) and 13% intermediate native myosin (IM). This striking difference is associated with the suppression of the fast myosin heavy chain $MyHC_{2A}$ and the sole expression of the slow form $MyHC_1$. On the other hand, adult ky extensor digitorum longus appeared to have quasi-normal contraction velocities and proportions of two fast MyHCs, 2B and 2X, although they produce significantly less total force at particular ages and are lighter than normal. Therefore, the changes observed in the kinetic parameters of adult ky soleus muscles appeared to depend on the suppression of $MyHC_{2A}$ and of native myosin IM.

In this paper we ask the following questions: (a) Are the changes in protein composition observed in ky muscles confined to the soleus or are they characteristic of other postural muscles such as those supporting the spinal column? (b) Are there additional changes in the alkali and essential myosin light chains? (c) Is depression of $MyHC_{2A}$ and IM diagnostic of adult ky muscles? (d) Are the biochemical changes observed during postnatal growth consistent with the view that the primary lesion affecting kyphoscoliotic mice is an inability to withstand loads applied to muscles during postnatal development?

METHODS

Animals. Kyphoscoliotic mice (ky/ky) and congenic normal control mice (BDL +/+) were obtained from a specified pathogen-free colony at Charing Cross and Westminster Medical School (London). They were divided into three groups of mean age (in days ± SEM): 21 ± 1 (range 17–25), 45 ± 2 (range 38–52) and 120 ± 5 (range 105–135). These correspond to the necrotic, regenerating and maturing phases of muscle fibres remodelling in ky soleus muscles. In each age sub-group there were equal numbers of males and females.

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Abbreviations. ky, kyphoscoliotic; SM_1 and SM_2, slow native myosin type 1 and type 2; IM, intermediate native myosin; FM_1, FM_2 and FM_3, fast native myosin type 1, 2 and 3; LC_{1f} and LC_{3f}: essential myosin light chains fast types 1 and 3; LC_{1s}, essential myosin light chain slow type 1; LC_{2f} and LC_{2s}, regulatory myosin light chain type fast and slow; $MyHC_1$, $MyHC_{2A}$, $MyHC_{2X}$ and $MyHC_{2B}$, myosin heavy chain 1, 2A, 2X and 2B.

Mice were killed by cervical dislocation, muscles dissected free, weighed, frozen in liquid nitrogen and stored at −80°C until use. Thirteen muscles were obtained from each mouse. Six muscles were obtained from the left and right sides: gastrocnemius, tibialis anterior, extensor digitorum longus, soleus, paravertebralis anterior and paravertebralis posterior, each pair pooled for analyses. The paravertebralis muscles were dissected from the back of the animal along the erector spinae. The superficial (under the skin) and the deep portions (close to the vertebral body) were combined: the section from C6 to D12 was defined as the paravertebralis anterior; the section from D12 to L5 was defined as the paravertebralis posterior. The tongue was analyzed as one piece.

Analyses of myosin heavy chains and isomyosins. Frozen muscles were pulverized and extracted on ice for 20 min in a Guba-Straub solution (300 mM NaCl; 100 mM NaH_2PO_4, 50 mM Na_2HPO_4, 1 mM $MgCl_2$, 10 mM $Na_4P_2O_7$, 0.1% NaN_3, 0.1% 2-mercaptoethanol, pH 6.5), and centrifuged (10 min at 20000 rpm, 4°C). One volume of glycerol was added to the supernatant. All measurements were made in duplicate using the following methods. Myosin heavy chains were separated by SDS/PAGE, including 33% (mass/vol.) glycerol both in separating (6% polyacrylamide) and stacking (3% polyacrylamide) gels (Talmadge and Roy, 1993). Staining was performed overnight in a Coomassie blue solution (40 mg Serva G250; 3 ml 70% perchloric acid in 100 ml H_2O); gels were destained in 7% acetic acid. This method separated the four myosin heavy chains: $MyHC_{2A}$, $MyHC_{2X}$, $MyHC_{2B}$ and $MyHC_1$ with increasing mobilities. Identification of myosin heavy chains was checked, after immunoblotting, in a few muscles with the antibodies F158 4C10 (anti-neonatal and anti-embryonic, kindly given by Drs F. Pons and J. Léger, Montpellier, France), BA-D5 (anti-1), SC-71 (anti-2A), BF-F3 (anti-2B) and BF-35 (anti-1 and anti-2 except 2X), prepared and characterized by Schiaffino et al. (1989) and purchased from Regeneron Pharmaceuticals. The myosin heavy chains were quantified by densitometry, using an image analyser (Biocom). Myosin heavy chains, myosin regulatory light chains and myosin essential light chains are expressed as a percentage of total sums of myosin heavy chains [$MyHC_{2B}$ + $MyHC_{2X}$ + $MyHC_{2A}$ + $MyHC_1$], of myosin regulatory light chains [LC_{2s} + LC_{2f}] and of essential myosin light chains [LC_{1f} + LC_{3f} + LC_{1s}], respectively. As shown in Fig. 1A, $MyHC_{2A}$ migrated close to $MyHC_{2X}$, also called $MyHC_{2D}$ (Pette and Staron, 1990), but the separation was not successful in many cases; for this reason the two zones were added in all cases, and only their sum $MyHC_{2A/2X}$ is reported in this work.

Alkali (essential) myosin light chains and regulatory myosin light chains were separated by two-dimensional gel electrophoresis (O'Farrell, 1975) of the crude Guba-Straub extract. The density of the spots was directly measured on the gel and quantified as a percentage of the total sum of the essential light chains.

Native isomyosins were separated by polyacrylamide gel electrophoresis in non-denaturing conditions (PP_i/PAGE) (full description by Maréchal and Beckers-Bleukx, 1994). Identification of the bands was based on their electrophoretic mobilities, their reactivity to specific antibodies and, for the native isomyosins, on their myosin light and heavy chain compositions (Maréchal et al., 1989). The relative contents of native myosins were expressed as a percentage of the total sum of all native myosins.

Histochemical typing. Serial 8.0-μm cryostat transverse sections were cut from the mid-belly of muscles and air-dried for 15 min at room temperature. The staining procedure (method of Loughlin, 1993, slightly modified) consisted of incubation at acidic pH (pH 4.6) in sodium acetate/acetic acid followed by incubation at pH 9.4 in a medium containing excess ATP substrate. Inorganic phosphate released was sequentially captured by metal cation (Ca^{2+} then Co^{2+}) solutions and converted to a visible precipitate by incubation in 2% ammonium sulphide solution.

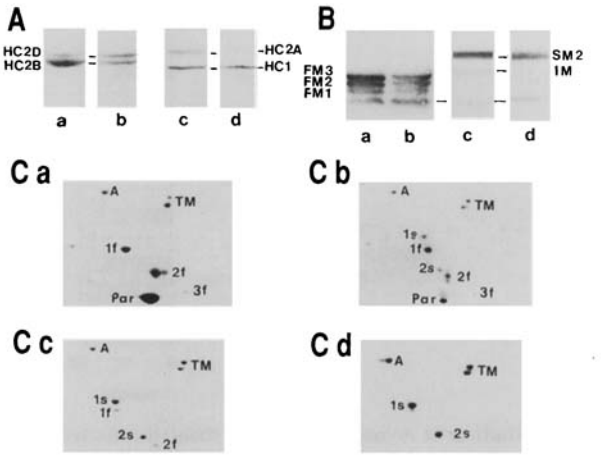

Fig. 1. Myosin subunits and native myosin isoforms of kyphoscoliotic muscles in comparison with normal controls. (A) Myosin heavy chains separated by PAGE/SDS. Extensor digitorum muscles (a, b) and soleus muscles (c, d) from control BDL (a, c) and myopathic ky (b, d) mice aged 120 days. Myosin heavy chains (HC) 1, 2A, 2B; the zone labelled HC2D is a mixture of $MyHC_{2A}$ and $MyHC_{2D}$, (also called $MyHC_{2X}$). Note decrease of $MyHC_{2B}$ and $MyHC_{2A}$ in ky muscles. (B) Native myosin isoforms separated by PAGE/PP_i of the same muscles shown in A. Native myosins of slow types (SM_2 and IM) and of fast types (FM_1, FM_2, FM_3). The unlabelled lower zone is taenia coli myosin, used as an internal standard for R_f estimates. Note absence of IM in ky soleus; note also that fast FM myosins are similar in ky and normal mice. (C) Two-dimensional PAGE of the same muscles showing actin (A), tropomyosins (TM α, the higher spot, and TM β, the lower spot), parvalbumin (Par), essential myosin light chains 1s, 1f and 3f (1s, 1f, 3f) and regulatory light chains 2s and 2f (2s, 2f). Note the presence of 1s and 2s in ky EDL, while these subunits are absent in control BDL muscle. Note also the absence of 1f and 3f in ky soleus muscle, a finding observed in most cases.

Sections were scanned under low magnification (10×) using a Zeiss Axioskop microscope connected to a Hamamatsu CCD camera (model C3077). Image acquisition was achieved using BioView 2.2 software (Improvision) on a Macintosh computer (Quadra 840AV, 32MRAM). Images were recorded using the same gain and offset settings each time. Data files were saved in TIFF format and analyzed using NIH Image 1.53b23 software (NIH shareware). TIFF files were then converted into Photoshop 3.0 files for publication purposes.

Statistics. Means are reported with their standard errors. Student's *t* paired tests were computed to assess the probability of the differences between parameters measured.

RESULTS

Muscles of adult mice. We have analyzed the myosin subunits of seven different muscles of adult kyphoscoliotic mice (mean age 120 days) in order to compare them with age-matched muscles of the normal BDL mice from which the ky mutation arose (Fig. 1A, C). Thus, the data reported in this section are essentially end-stage data obtained at an age when the maturation of non-necrosed and regenerated fibres is complete (Bridges et al., 1992).

We have classified the muscles of control BDL mice in increasing order of their content of $MyHC_{2B}$ (from top to bottom in Fig. 2, white bars). Two groups of muscles emerge: a slow group, composed of only one muscle, the soleus, characterized

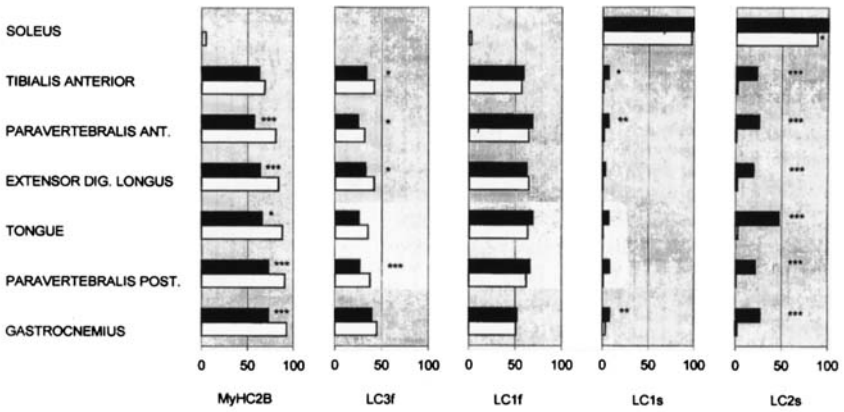

Fig. 2. Distribution of myosin subunits in striated muscles from normal BDL mice (white bars) and kyphoscoliotic mice (black bars) aged 120±5 days. The probability of a difference between BDL and ky muscles is indicated: * $P<0.05$; ** $P<0.01$; *** $P<0.001$. Means of 8–12 experiments. The standard error of the means (not shown) is 2–3, except for LC_{2s} where it is 5 and for the tongue, in which it is twice as high as in the other muscles. Myosin heavy chains, myosin regulatory light chains and myosin essential light chains are expressed as a percentage of total sums of myosin heavy chains [$MyHC_{2B}$ + $MyHC_{2X}$ + $MyHC_{2A}$ + $MyHC_1$], of myosin regulatory light chains [LC_{2s} + LC_{2f}] and of essential myosin light chains [LC_{1f}+ LC_{3f} + LC_{1s}], respectively.

by traces of $MyHC_{2B}$, and a group of fast and mixed muscles, composed of six muscles characterized by high proportions of $MyHC_{2B}$. $MyHC_1$ was present in soleus (78%) and absent in the other muscles. $MyHC_{2A}$ and/or $MyHC_{2X}$ were present in all the muscles, but these isoforms were present at a higher concentration in tibialis anterior (31%) and paravertebralis anterior (19%) than in soleus (17%). On this basis, it is possible to dissociate a group of mixed muscles (tibialis anterior and paravertebralis anterior) and a group of fast muscles (extensor digitorum longus, tongue, paravertebralis posterior and gastrocnemius). All ky muscles showed a decreased content of $MyHC_{2B}$ (Fig. 2, black bars): the mean difference, computed on all seven muscles was $-16.2\pm2.8\%$ ($t_7 = 5.786$; $P<0.001$). The decrease in $MyHC_{2B}$ was compensated for by an increase in the two other fast myosins ($MyHC_{2A/2X}$), except in soleus muscle, where it was compensated for by an increase in $MyHC_1$ such that ky soleus contained only the slow isoform $MyHC_1$.

The fast form of essential myosin light chains LC_{3f} decreased in ky muscles, whilst the slow form LC_{1s} increased and LC_{1f} did not change (Fig. 2). The slow form of regulatory light chain LC_{2s} substantially increased in all muscles (Fig. 2) at the expenses of the fast form LC_{2f}.

Native myosins were analyzed in aliquots of the same muscle extracts in which we had analyzed the myosin subunits (Figs 1B and 3). Myosin SM_2 typical of slow muscle was the major form in soleus, while it was barely expressed in the other muscles. In ky muscles, it became the only myosin expressed in soleus. It is noteworthy that the other ky muscles did not express it above negligible amounts. There were complicated changes of intermediary myosin IM in ky muscles: this myosin disappeared in the groups of fast and slow muscles, but it increased in the group of mixed muscles (tibialis anterior and paravertebralis anterior). Fast native myosins were present as the familiar triplet in the fast and the mixed muscles. In these muscles, FM_3 was systematically higher in ky muscles, and FM_2 was systematically lower. The differences were not statistically significant. However, when all the data were pooled, excepting soleus, the differences between ky and BDL muscles became statistically significant (FM_3, $\Delta = 6.5\pm2.6$, $n = 106$, $P<0.01$; FM_2, $\Delta = 4.4\pm1.2$, $n = 106$, $P<0.01$). FM_1 was lower in ky muscles of the mixed group (tibialis anterior and paravertebralis anterior).

Muscles of growing mice. The development of the mature pattern of myosin specialization seen in adult ky and BDL mice was studied by analysis of muscles from mice at 21 and 45 days of age. These are ages which correspond to the period when postural muscles such as the soleus and paraspinal undergo the most intense muscle necrosis followed by regeneration. We found that young ky and control fast muscles (extensor digitorum longus, tongue, paravertebralis posterior and gastrocnemius) at 21 days of age have similar proportions of myosin subunits and of native isomyosins. Young ky mixed muscles (tibialis anterior and paravertebralis anterior) are also similar to the control muscles, but we observed a remarkable increase of isomyosin IM in the ky mixed muscles: while control muscles contained only traces of IM, ky young tibialis anterior contained a small amount, $3.3\pm1.3\%$ ($n = 12$), and ky young paravertebralis anterior contained a larger amount, $14.5\pm2.0\%$ ($n = 12$). During growth, IM increased in these two ky muscles so that the adult mice (120 days old) contained respectively $19.3\pm2.0\%$ ($n = 12$) and $17.4\pm2.3\%$ ($n = 12$). Young soleus muscles were also somewhat different in the proportions of the myosin heavy chains (respectively for control and ky muscles, $n = 12$, $MyHC_{2B} = 26\pm6\%$ and $16\pm3\%$; $MyHC_{2A/2X} = 50\pm5\%$ and $77\pm4\%$; $MyHC_1 = 24\pm3\%$ and $7\pm2\%$).

At 45 days, control muscles had proportions of myosin subunits and native myosin intermediate between those observed at 21 days and 120 days, while ky muscles were still very similar to the control BDL. Thus most of the differences characteristic of the ky adult pattern arose between 45–120 days of age. We report below the detailed differences observed between the young (21 days) and the adult muscles for control and ky mice, since our data are much more accurate at 21 days than at 45 days, due to the larger number of analyses ($n = 12$ at 21 days; $n = 2-4$ at 45 days).

Fig. 4 reports the changes in myosin subunits which occurred between 21 and 120 days. In growing control BDL mice (white bars), we observed the typical specialization of muscles. There was an increase in $MyHC_{2B}$ and LC_{3f} in the group of fast muscles, a decrease of them in the slow soleus, and a mixed response in the group of mixed muscles. In growing ky mice (black bars), the increase in the fast myosin subunits was less pronounced and often replaced by a decrease, and a general increase in the slow myosin light chains occurred, most noticeable in LC_{2s}.

Fig. 5 reports the changes in native isomyosins observed between 21 and 120 days of age. In the growing control mice, the changes in isomyosins roughly paralleled those observed in the

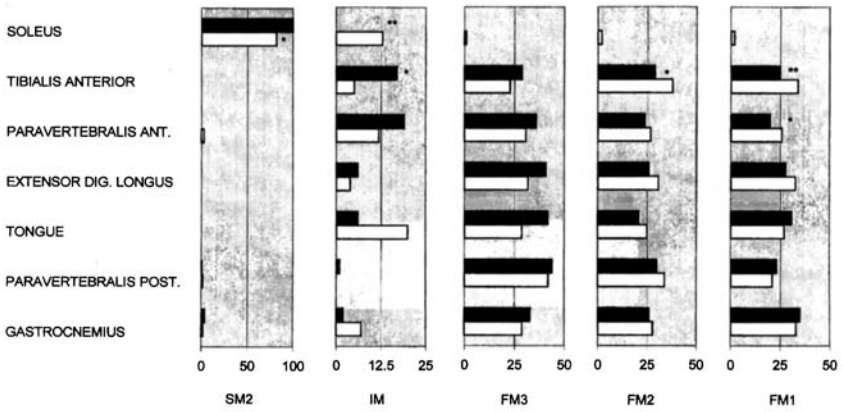

Fig. 3. Distribution of native myosins in striated muscles from normal BDL mice (white bars) and kyphoscoliotic mice (black bars) aged 120±5 days. The probability of a difference between BDL and ky muscles is indicated: * $P<0.05$; ** $P<0.01$; *** $P<0.001$. Means of 8–12 experiments. The standard error of the means (not shown) is 1–2, except for the tongue (3–6). Native myosins are expressed as a percentage of the total sum of native myosins.

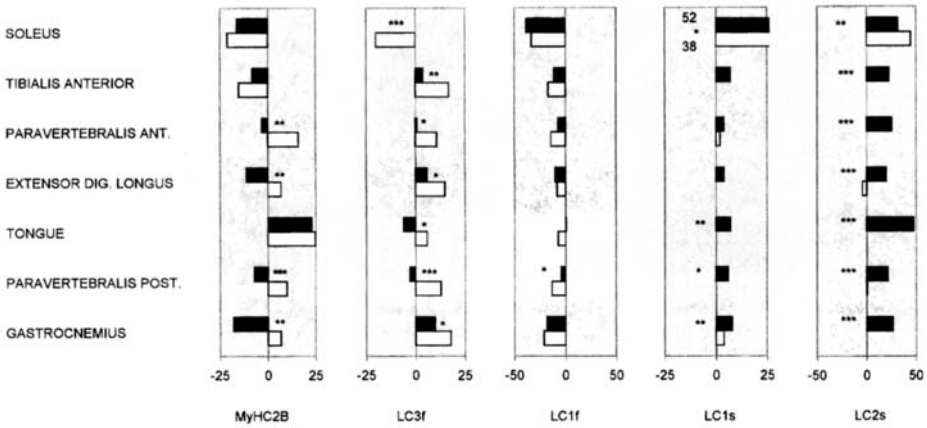

Fig. 4. The increase in myosin heavy chain 2B and myosin light chains between 21 and 120 days of age, in normal BDL mice (white bars) and kyphoscoliotic mice (black bars). The probability of a difference between BDL and ky muscles is indicated: * $P<0.05$; ** $P<0.01$; *** $P<0.001$. Means of 8–12 experiments. The standard error of the means (not shown) is 2–3, except for the tongue (2–5).

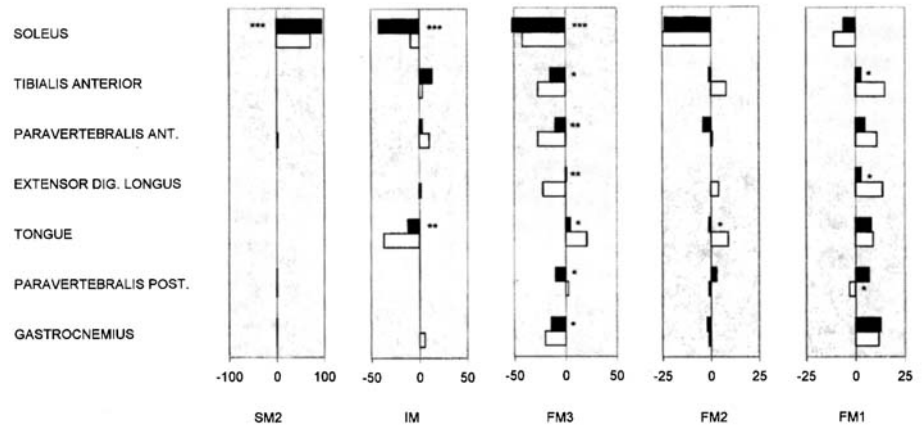

Fig. 5. The increase in native myosins between 21 and 120 days of age, in normal BDL mice (white bars) and kyphoscoliotic mice (black bars). The probability of a difference between BDL and ky muscles is indicated: * $P<0.05$; ** $P<0.01$; *** $P<0.001$. Means of 8–12 experiments. The standard error of the means (not shown) is 2–4.

myosin subunits. In the group of fast muscles and mixed muscles, there was a decrease in FM_3 (except the tongue) and an increase in FM_1; the slow types of isomyosins, SM_2 and IM, were present only in trace amounts at all ages. In the slow soleus, the isomyosins FM disappeared during growth and they were replaced by SM_2 while isomyosin IM decreased slightly. In the growing ky muscles, the changes in FM_3 and FM_1 were smaller in the group of fast and mixed muscles.

Since postural muscles of young ky mice are subjected to a single wave of necrosis and regeneration (Bridges et al., 1992),

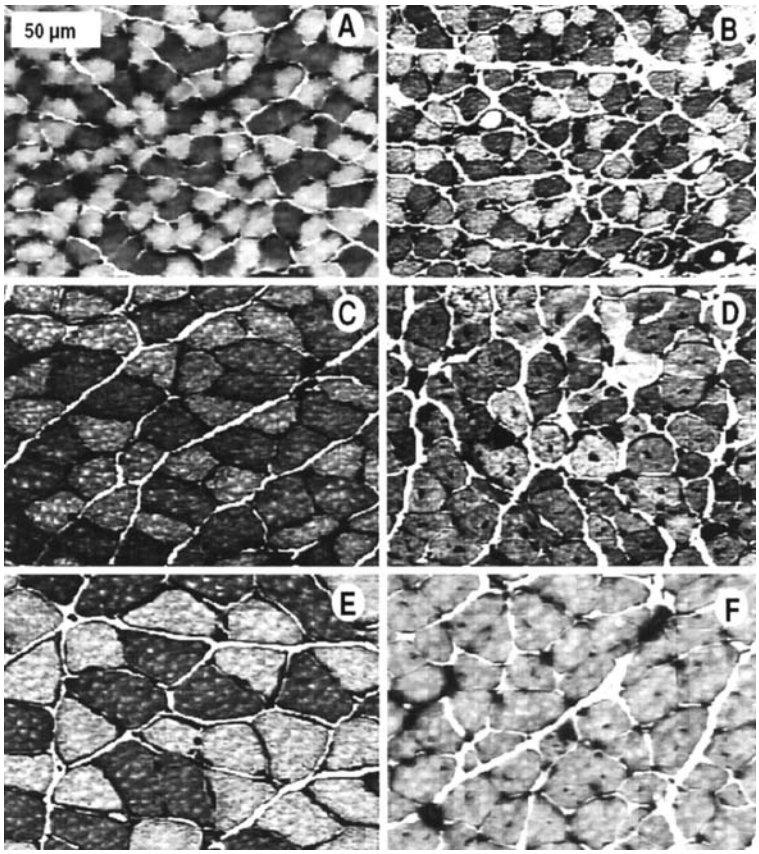

Fig. 6. Digitized images of soleus muscle sections from BDL mice (A, C, E) and ky mice (B, D, F) aged 14 (A, B), 40 (C, D) or 160 days (E, F). The sections were stained by the ATPase histochemical reaction at pH 4.6. Type 1 fibres are pale; type 2A fibres are dark (types 2B and 2X fibres are intermediate, not shown).

it was necessary to enquire whether they contained neonatal myosins. Neonatal native myosin can be easily detected since it migrates slightly faster than the normal fast triplet (Maréchal et al., 1984). We observed none in the young muscles, control and ky alike (results not shown). We also tested for the presence of neonatal or embryonic myosins by immunoblots, using specific antibodies (F158 4C10, given by Drs F. Pons and J. Léger, Montpellier, France). We observed some signal for ky muscles aged 21 and 45 days. It was however impossible to obtain quantitative evaluations.

Histochemical ATPase typing. In order to understand more completely what happened in growing soleus muscle, we histochemically typed muscles at three different ages. Control soleus muscle contained clearly distinct type 2A (dark) and type 1 (pale) fibres (Fig. 6). In good agreement with the data on myosin heavy chains (see above), ky soleus at 14 days was similar to control and old ky soleus (160 days) showed very few or even no type 2A fibres.

The 40-day ky soleus was strikingly different from both control and old or young ky muscles in that most fibres displayed different degrees of intermediate staining intensities. This contrasts with the checkerboard pattern observed in normal muscle and suggests that these fibres contain more than one MyHC (Billeter et al., 1980; Staron and Pette, 1986). Thus at 40–45 days, while the differences in myosin heavy chain content between control and ky soleus were not large at the level of the whole muscle, the distribution of these isozymes inside the fibres was completely modified.

DISCUSSION

Basis of biochemical shifts in ky muscles. We have compared the relative proportions of myosin isoforms of seven different muscles taken from normal and ky mice at three ages during postnatal development. These ages (21, 45 and 120 days) were selected as they correspond to crucial time points in the development of pathology in ky mice. In addition, other studies have shown this to be a period of biochemical specialization in normal mice (e.g. Wigston and English, 1992). Initially, at 21 days of age, both BDL and ky muscles were well differentiated into slow and fast types and they showed little evidence of embryonic or neonatal myosins. During growth of BDL muscles the pattern of distribution of the various isomyosins and their subunits did not change, but their relative amplitude of specialization increased. Thus eventually, all ky muscles contained much less $MyHC_{2B}$ and LC_{3f} than BDL. In contrast, they had higher proportions of LC_{1s} and LC_{2s}. This remarkable accumulation of slow light chains occurred quite late in development as they were not yet expressed at 45 days of age.

Many works (reviews by Pette and Staron, 1990, and Pette and Vrbovà, 1992) have described the control in muscle fibres of the expression of myosin isoforms in response to various external stimuli. The control mechanisms appear to independently modulate the subunits constituting myosin molecules. In the transition fast-to-slow, muscle fibres express myosin heavy chains, myosin light chains and native myosin in the order:

$$MyHC_{2B} \rightarrow MyHC_{2X} \rightarrow MyHC_{2A} \rightarrow MyHC_{1} \quad (1)$$

$$LC_{2f} \rightarrow LC_{2s} \quad (2)$$

$$LC_{3f} \rightarrow LC_{1f} \rightarrow LC_{1s} \quad (3)$$

$$FM_1 \rightarrow FM_2 \rightarrow FM_3 \rightarrow IM \rightarrow SM_2. \quad (4)$$

From our data (Figs 4 and 5), it was possible to distinguish three groups of muscles in control mice. In the first group, comprising gastrocnemius, extensor digitorum longus, tongue and paravertebralis posterior, we observed a slow-to-fast transition during growth. In a second group, typified by soleus, the muscle showed a fast-to-slow specialization. Finally, there existed a third group of mixed muscles consisting of tibialis anterior and paravertebralis anterior where muscle fibres independently specialize in either slow or fast directions.

In the adult ky phenotype, the isoform distribution was abnormally shifted to the right, in favour of slower isoforms. Maturation of ky muscles occurred in the same general direction of phenotypic specialization as for BDL: slow muscles changed in an appropriate fast-to-slow direction, fast muscles became increasingly fast and mixed muscles polarized. Indeed, no slow muscle was wholly transformed into a fast one or vice versa. For example, only the ky soleus muscle contained more than trace amounts of $MyHC_1$. This indicates that the relationship of motor units is essentially normal in ky mice. However ky maturation was characterized by a shift toward a generally slower phenotype in every muscle examined. The amplitude of such change varied with muscle type with the most extreme shift seen in the soleus. In the fast and mixed muscles, either the amplitude of the usual slow-to-fast transition was smaller than in BDL or the direction of maturation was reversed.

In a few cases, we observed that the percentage of some molecules did not change, for instance LC_{1f} or FM_2. These forms being intermediate between faster and slower forms, we interpret this fact to mean that the shift to the right of the faster form is balanced by an equal shift to the right of the slower form.

It is of interest that the increase in slow light chains was not simultaneous with an increase in the slow native myosins (SM_2 or IM) in which they are usually contained (Maréchal et al., 1989). The inference is that the slow light chains must be associated with native myosins that migrate in the FM triplet: i.e., a sizeable fraction of the fast native myosins in ky muscles are hybrid myosins, formed of fast myosin heavy chains 2A, 2X or 2B, combined with slow and fast myosin light chains. The existence of such hybrid forms still remains to be demonstrated in adult ky muscles. Their existence has been proposed in the frame of the continuum hypothesis (Pette and Staron, 1990) and they have been observed in normal adult muscle fibres (Botinelli et al., 1991, 1994; Larsson and Moss, 1993).

Our histological observations also strongly support the conclusion that the change in MyHC composition in ky mice is due to the replacement of MyHC isoforms (transformation) rather than to a replacement of fibres (regeneration). The very high proportion of mixed fibres observed in 40 days ky soleus muscles suggests that the changes in MyHC contents measured in whole muscle extracts are due to modifications of MyHC expression within each muscle fibre and are not due to death of selective fibre types.

The physiological significance of the ky phenotype. Taken together these changes are consistent with a general decrease in the velocity of contraction of ky muscles, since maximum velocity of contraction, V_{max}, is higher in fibres containing $MyHC_{2B}$, (Bottinelli et al., 1991, 1994; Larsson and Moss, 1993) or an increased LC_{3f} (Sweeney et al., 1988; Greaser et al., 1988; Bottinelli et al., 1994), but is lower in fibres expressing more LC_{2s} (Larsson and Moss, 1993). These changes were reflected by concomittant alterations in the level of various native myosins. Soleus is the only muscle in BDL mice which contained more than a negligible level of the slow native myosin SM_2 (two $MyHC_1$, two LC_{2s} and two LC_{1s}) and this increased virtually to 100% in ky soleus. However it did not increase in any other ky muscles examined. All normal adult muscles contained at least traces of intermediate myosin IM, a hybrid containing $MyHC_{2A}$ combined with slow and fast light chains (Maréchal et al., 1989; Larsson and Moss, 1993), which has a higher mobility than SM_2 (Asmussen and Maréchal, 1989; Maréchal and Beckers-Bleukx, 1994). As IM was observed to increase in paravertebralis anterior whilst disappearing in soleus, it invalidates our previous hypothesis that ky mutation may directly or indirectly suppress IM expression (Maréchal et al., 1995). The small increase in FM_3 and the unchanged FM_1 in most ky muscles qualitatively agree with the observed changes in myosin subunits. Thus we would expect ky muscles to tend to shorten with reduced velocity. We observed indeed a 45% decrease in V_{max} for adult ky soleus, but the decrease in V_{max} was much smaller (8%) for ky extensor digitorum longus muscle and it was not statistically significant (Maréchal et al., 1995). Other workers also reported significant alterations in the distribution of fast myosin isoforms not associated with changes in V_{max} (Bottinelli et al., 1991, 1994).

The functional role of individual muscle probably remains normal in ky muscles, with tonic muscles acting to withstand gravity and phasic muscles producing powerful movement. It is interesting that the main histological and biochemical changes we see occur in the postnatal period when they are becoming active; indeed, electromyographic recordings from freely moving rats have shown tenfold increases in the amount of tonic activity of soleus muscles during the first three postnatal weeks (Navarrete and Vrbová, 1983; Slawinska et al., 1995). This period also coincides with the onset of postural function of the hindlimb muscles and quadrupedal locomotion (Navarrete and Vrbová, 1993).

From our previous data we have shown that the gain in mass of ky muscles does not keep pace with that of their body mass and is reflected in a persistently reduced muscle fibre diameter in all muscles (Bridges et al., 1992; Maréchal et al., 1995). Thus, if we presume that neonatal ky mice have a normal behaviour drive to move, they will do so with a proportionally smaller muscle mass. Postural muscles would have a greater proportional mass to support against the gravity and phasic muscles would have to overcome the proportionately greater inertia of limbs in order to move. In normal mice, adaptive muscle hypertrophy would be triggered in such a situation, until the mass of muscle was once again proportional to the loads exerted upon them. The fact that this balance is never achieved in ky muscles suggests that the primary abnormality resulting from the ky mutation affects the ability of muscle fibres to grow under load. The only course of action left to ky muscles would be to stimulate the muscle more intensely in order to recruit more motor units and contract them more frequently. This kind of intense stimulation can produce dramatic shifts to a slower phenotype over several weeks (Green et al., 1983; Pette and Vrbová, 1992). Indeed we have shown that ky muscles are very capable of making adaptative changes in their biochemical makeup. In addition, passive stretch and overload have also been shown to produce shifts to a slower phenotype (Diffee et al., 1993; Loughna et al., 1990; Goldspink et al., 1995). The necrosis observed in ky soleus and other postural muscles may result from the inability of the abnormally growing muscle fibres to supply sufficient load which either produces muscle damage by overstretching or may exceed the ability of the muscle fibres to satisfy the demand for energy for contraction and the maintenance of sarcolemmal integrity. The fact that we see only one phase of necrosis and regeneration is intriguing and implies that regenerating muscle fibres are able to escape the worst effects of the ky mutation,

possibly by being able to make a more dramatic shift to a less fatigable slower phenotype. Such massive shifts in phenotype have also been observed following induction of muscle fibre regeneration by injection of snake toxins (Whalen et al., 1990; Davis et al., 1991). At present we cannot explain the shift to slow in the tongue but, although it is not exposed to gravitational influences in the same way as limb muscles, it is nevertheless a very active muscle during feeding. Thus, our data support the view that the biochemical and structural changes we observe in ky mice are likely to be adaptations to external influences upon the fundamental pattern of this basic biochemical maturation of muscles. As the ky mouse is the only mammal other than man to exhibit inherited idiopathic scoliosis, it will continue to be a valuable system with which to study and understand disease processes leading to spinal curvature.

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