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Maximum shortening velocity and coexistence of myosin heavy chain isoforms in single skinned fast fibres of rat skeletal muscle

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Summary

Myosin heavy chain composition of a large number (288) of single fibres from slow (soleus), and fast (superficial part of tibialis anterior, and plantaris) muscles of adult (3–5-month-old) Wistar rats was determined. A combination of SDS–PAGE and monoclonal antibodies against myosin heavy chains allowed to identify four myosin heavy chain isoforms (1, 2A, 2X, and 2B) and to detect myosin heavy chain coexistence. Four groups of fibres containing only one myosin heavy chain (1 myosin heavy chain, 2A myosin heavy chain, 2X myosin heavy chain, and 2B myosin heavy chain), and five groups containing more than one myosin heavy chain (1 and 2A myosin heavy chains, 2A and 2X myosin heavy chains, 2X and minor amounts of 2B (2X-2B fibres), 2B and minor amounts of 2X (2B-2X fibres), and 2A, 2X, and 2B myosin heavy chain were identified and their relative percentages were assessed. Coexistence of fast myosin heavy chain isoforms was found to be very frequent (50% of the fibres in plantaris, and 30% in tibialis anterior), whereas coexistence of slow and fast (2A) myosin heavy chain was very rare. Maximum shortening velocity (V_0) was determined using the slack-test procedure in a subset of 109 fast fibres from the above population. The values of V_0 formed a continuum extending from 2A to 2X to 2X–2B to 2B–2X to 2B fibres. 2A fibres had the lowest value of V_0 and 2B fibres the highest. Only the differences between 2A and 2B and 2A and 2B–2X fibres were statistically significant. Importantly, the variability of V_0 in fibres containing only one myosin heavy chain and in fibres containing a variable proportion of two myosin heavy chain isoforms was similar and, in some case (e.g. 2B fibres), such to encompass the whole range of variation of fast fibres' shortening velocities. The results of this study demonstrate that the large variability in maximum shortening velocity of fast fibres is not due to myosin heavy chain coexistence, and therefore suggest that it cannot be explained on the basis of myosin heavy chain composition.

Introduction

Several observations have suggested that differences in myosin heavy chain (MHC) isoform composition might be the reason not only of the lower maximum shortening velocity of slow fibres in comparison to fast fibres, but also for the large differences in maximum shortening velocity among fast fibres (Sweeney *et al.*, 1986, 1988; Eddinger & Moss, 1987; Rome *et al.*, 1990; Bottinelli *et al.*, 1991). It has been reported that fibres containing 2A MHC show lower mean values of maximum shortening velocity determined by the slack-test (V_0) than fibres containing 2B MHC (Eddinger & Moss, 1987; Sweeney *et al.*, 1988; Rome *et al.*, 1990; Larsson & Moss, 1993). In a recent work (Bottinelli *et al.*, 1991), maximum shortening velocity has been evaluated by extrapolation to zero load of force-velocity curves (V_{max}). In this study, also the novel 2X fast MHC isoform (Schiaffino *et al.*, 1986, 1989; Bar & Pette, 1988) has been identified, and three groups of fast fibres have been analysed: 2A, 2X and 2B. 2B fibres had mean V_{max} values significantly higher than 2A and 2X fibres. It has been, however, observed that (1) the variability of V_0 (Sweeney *et al.*, 1988) and of V_{max} (Bottinelli *et al.*, 1991) among fast fibres containing the same MHC isoform is very large when compared to the whole range of variability of fast fibres, and that (2) the ranges of variability of V_{max} of 2A, 2X and 2B fibres largely overlap (Bottinelli *et al.*, 1991). These observations are, in our opinion, relevant and still lack a satisfactory explanation.

Uncertain determination of the MHC isoform composition of fast fibres, and undetected coexistence of MHC isoforms in the same fibre could provide a possible basis

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for the large and overlapping ranges of variability of V_0 and V_{max} of groups of fast fibres which are believed to be homogeneous as regard MHC content. The identification of the MHC composition of fast fibres has proved to be difficult. In most of the studies 2X MHC containing fibres have been pooled together either with 2A or with 2B fibres (Eddinger & Moss, 1987; Greaser *et al.*, 1988; Sweeney *et al.*, 1988; Rome *et al.*, 1990). An additional, and possibly more important, problem for all previous works on this matter might have been the presence of more than one MHC in the same fibre. It is well established that different MHC isoforms can coexist in the same single skeletal muscle fibre (Lutz *et al.*, 1979; Danieli-Betto *et al.*, 1986; Staron & Pette, 1987a, b; Termin *et al.*, 1989; De Nardi *et al.*, 1993). Some observations have suggested that this phenomenon could be frequent not only during experimentally induced fibre transformation (Termin *et al.*, 1989), but also under normal conditions (Danieli-Betto *et al.*, 1986; De Nardi *et al.*, 1993). Only two studies on the relation between V_0 and MHC content have reported the presence of fast fibres containing both 2A and 2B MHC (Sweeney *et al.*, 1988; Larsson & Moss, 1993). In these works, however, the 2X isoform of MHC has not been identified, and therefore coexistence of 2X MHC with 2A and 2B could not be detected. The possibility that undetected MHC coexistence has obscured the relation between MHC composition and maximum shortening velocity in fast fibres is still open. Several studies, in fact, indicate that MHC isoform coexistence is potentially important in determining contractile properties. In single skinned fibres containing slow and fast MHC isoforms (Reiser *et al.*, 1985), or two slow MHC isoforms (Reiser *et al.*, 1988) V_0 has been found to be related to the proportion of the fastest MHC isoform. In cardiac muscle a number of studies (Schwartz *et al.*, 1981; Ebrecht *et al.*, 1982; Pagani & Julian, 1984; Cappelli *et al.*, 1989) have demonstrated a correlation between maximum speed of shortening and the ratio between α and β MHC.

An alternative explanation of the large variability in maximum shortening velocity of fast fibres could be found in variations in their alkali myosin light chain (MLC) isoform content. The ratio between the two isoforms of alkali MLC, MLC1f and MLC3f, has been found to affect V_0 (Sweeney *et al.*, 1988; Greaser *et al.*, 1988; Bottinelli *et al.*, 1993b). Large variations in MLC3f/MLC1f ratio in fast fibres containing the same MHC isoform have been shown (Wada & Pette, 1993).

The present work focused on the possibility that MHC coexistence was at the basis of the large variability in maximum shortening velocity among fast skeletal muscle fibres. As mentioned above, this variability was still present even when fast fibres were divided on the basis of MHC isoform composition (Sweeney *et al.*, 1988; Bottinelli *et al.*, 1991). A more sensitive method was, therefore, necessary to analyse MHC isoform composition and discover isoform coexistence. Both polyacrylamide gel electrophoresis (PAGE) and monoclonal antibodies against MHC were used and the results were combined. In this way, all three fast MHC isoforms could be identified and the presence of more than one MHC isoform in the same fibre could be detected. A large population (~ 300) of single fibres isolated from hindlimb muscles (plantaris, soleus, tibialis anterior) of 3–5-month old rats were examined. Unloaded shortening velocity (V_0) of > 100 single fast fibres were determined using the slack-test method (Edman, 1979). Mean V_0 values and variability of V_0 of fibres containing only one fast MHC (2A, 2X, or 2B), or a variable proportion of fast MHC isoforms were compared. No attempt was done to examine the alternative explanation that the large variability in maximum shortening velocity among fast fibres is based on variations in alkali MLC isoform content.

Materials and methods

EXPERIMENTAL PLAN

A large number (288) of single fibres was dissected from slow (soleus), and fast (superficial part of tibialis anterior and plantaris) muscles of adult rats. One hundred and nine of these fibres, all from fast muscles, were subjected to slack-test manoeuvres to determine maximum shortening velocity (V_0). Fibres were characterized as regards MHC composition using two independent methods (SDS–PAGE and monoclonal antibodies against MHC) and combining the results obtained.

SOLUTIONS AND FIBRES DISSECTION

Skinning, relaxing, pre-activating, and activating solutions were prepared according to Bottinelli and colleagues (1993a). Soleus, plantaris and tibialis anterior muscles were dissected from 3–5-month-old male Wistar rats. Under a stereomicroscope (Wild 10-40X), single fibres were isolated from whole muscles while they were immersed in skinning solution at 12–15°C. Only the superficial part of tibialis anterior muscle was used for experiments. Fibres were chemically skinned by exposure to the skinning solution for 2–3 h and finally by exposure to the same skinning solution with the addition of Triton X-100 (10 $\mu l\ ml^{-1}$) for 1 h. Each fibre was cut in two segments about 2 mm long. One segment was used for the determination of V_0 and thereafter characterized as regard MHC composition by monoclonal antibodies. The other segment was used for MHC determination by SDS–PAGE. The latter segment was stored at −20°C in 20 µl of a solution containing: 62.5 mM TRIS-HCl, 2.3% SDS (w/v), 10% glycerol (w/v), 5% mercaptoethanol (Laemmli, 1970).

EXPERIMENTAL SET-UP

The experimental set-up used has been previously described in detail (Bottinelli *et al.*, 1991, 1993b). Three chambers were milled in an aluminium plate which could be lowered, and translated and raised to quickly immerse the fibre in any of the chambers. The three chambers contained relaxing, preactivating and activating solution respectively. Using aluminium clips, one end of the fibre was connected to a force transducer (AE 801, Aksjeselskapet Mikroelektronikk, Horten, Norway, resonance frequency in water 2 kHz), the other end to an electromagnetic puller (model 101 vibrator, Ling Dynamic System, Royston,

UK). The electromagnetic puller equipped with a position transducer and with a feed-back control system could apply quick releases of different amplitude completed in 1 ms. The entire set-up was built on the stage of an inverted microscope (Axiovert 10, Zeiss, Germany). Sarcomere length and fibre diameter could be measured at 320× magnification.

DETERMINATION OF MAXIMUM SHORTENING VELOCITY

Activation and slack test

Fibres were maximally activated (pCa 4.45) at 12°C, 2.7 μm sarcomere length. Activation was obtained by quickly changing the solution around the fibre by lowering and translating and rising the experimental chambers. Fibres were mounted between the hooks of the force transducer and of the electromagnetic puller while in the chamber containing relaxing solution. Care was taken to secure the aluminium clips in the hooks in a way to prevent any movement of the clips on the hooks during activation and releases. Position of the clips was checked before each activation. Fibres were first transferred to the chamber containing pre-activating solution, and then to the chamber containing activating solution. When tension was at its maximum, a release of amplitude varying from 5–12% of initial fibre length was applied to the fibre in 1 ms. Such releases were always able to drop the tension to zero. When the tension had redeveloped at the final length, the fibre segment was relaxed and then elongated to its initial length. Each fibre was activated 5–6 times for 1 min, and each time a release of different amplitude was given. At the end of the mechanical experiment, the fibre was embedded in gelatin, frozen in liquid nitrogen, and stored at −20°C to be later analysed by monoclonal antibodies.

Data recording and analysis

Length and force signals were displayed on a storage oscilloscope (Tektronix 5113, Beaverton, Oregon, USA), on a digital oscilloscope (Nicolet 4094C, Madison, Wisconsin, USA), and, on a slower time base, on a chart recorder (Graphtec WR 3701, Tokyo, Japan). Signals were stored on floppy disks on which analysis was performed. The force developed by the fibre dropped to zero following the imposed release. The fibre went slack and for all the time it was slack it shortened at zero load at maximum speed of shortening. The time during which the fibre remained slack varied with the amplitude of the release given. V_0 was obtained from the slope of the linear regression between the time necessary to take up the slack (X axis) and the amount of shortening imposed (Y axis) according to a procedure previously described (Edman, 1979).

QUALITY CONTROLS OF THE FIBRES

To prevent disorder of the contractile material that can easily develop during maximal activation, and to avoid gross non-uniformities in the behaviour of different areas of the specimen, fibres were quickly and uniformly activated by immersion in the activating solutions. Activations were kept short, and fibres were elongated to their initial length after being relaxed. Fibres were observed at 320× magnification before and after each activation to check the striation pattern and the possible presence of damages. Fibres almost invariably maintained their striation pattern intact and did not show any damage after 5–6 activations. The few fibres that showed altered structure or underwent a decrease in active tension larger than 10% were discarded.

STATISTICAL ANALYSIS

The statistical package *Primer in Biostatistics* (by S. A. Glantz, released by McGraw & Hill, 1989) was used to assess statistical significance of the differences. Variance analysis followed by Student-Newman-Keuls' test were used. A probability of less than 5% was considered to be statistically significant. Data were expressed as means and SDs.

MYOSIN HEAVY CHAIN IDENTIFICATION

SDS–PAGE and monoclonal antibodies allowed to identify nine groups of fibres: (1) slow or type 1 fibres containing type 1 MHC; (2) 2A fibres containing 2A MHC; (3) 2X fibres containing 2X MHC; (4) 2B fibres containing 2B MHC; (5) 1-2A fibres containing 1 and 2A MHC; (6) 2A-2X fibres containing 2A and 2X MHC; (7) 2X-2B fibres containing a higher amount of 2X and a minor amount of 2B MHC; (8) 2B-2X fibres containing a higher amount of 2B and a minor amount of 2X MHC; (9) 2A-2X-2B fibres containing all three fast MHC isoforms.

SDS–PAGE

All fibres were studied as regard MHC content by electrophoresis (SDS–PAGE) on 6% polyacrylamide slab gels as previously described (Danieli-Betto *et al.*, 1986, 1990). Gels were silver-stained. SDS–PAGE could resolve three bands corresponding to 1 MHC, 2B MHC and 2A or 2X MHC. SDS–PAGE could not consistently separate 2A and 2X MHC. The corresponding band was indicated as 2A/X (Danieli-Betto *et al.*, 1986; Schiaffino *et al.*, 1989) (Fig. 1). Several fibres showed more than one band on SDS–PAGE gels (Fig. 1 c,d,f,g). Whereas slow fibres and 2B fibres were unequivocally identified by SDS–PAGE, 2A/X fibres needed further analysis by monoclonal antibodies to be separated in three groups containing only 2A MHC (2A fibres), only 2X MHC (2X fibres), or both 2A and 2X MHCs (2A-2X fibres). Also fibres that showed more than one band were further characterized. Immunocytochemistry was used on fibres that showed the 1 and 2A/X band to determine whether they contained 1 and 2A or 1 and 2X MHCs, and on fibres that showed the 2A/X and the 2B band to assess whether they contained only two MHC isoforms, 2X and 2B, or also 2A in addition to 2X and 2B (see below).

Immunocytochemistry

The preparation and the specificity of the antibodies utilized has been previously described in detail (Schiaffino *et al.*, 1989; Bottinelli *et al.*, 1991) along with the procedure for single fibre immunostaining. Three monoclonal antibodies against MHC (SC-71, BF-35, and RT-D9), whose specificity is reported in Table 1, were used to further characterize 2A/X fibres and fibres showing more than one electrophoretic band.

Combination of immunocytochemistry and SDS–PAGE

2A/X fibres were interpreted as fibres containing exclusively 2A MHC (2A fibres) when they strongly reacted with SC-71 and BF-35, and not with RT-D9, whereas were interpreted as fibres containing exclusively 2X MHC (2X fibres) when they strongly reacted with RT-D9, and not with both SC-71 and BF-35. 2A/X fibres were considered fibres containing both 2A and 2X MHC when they were stained by all three antibodies.

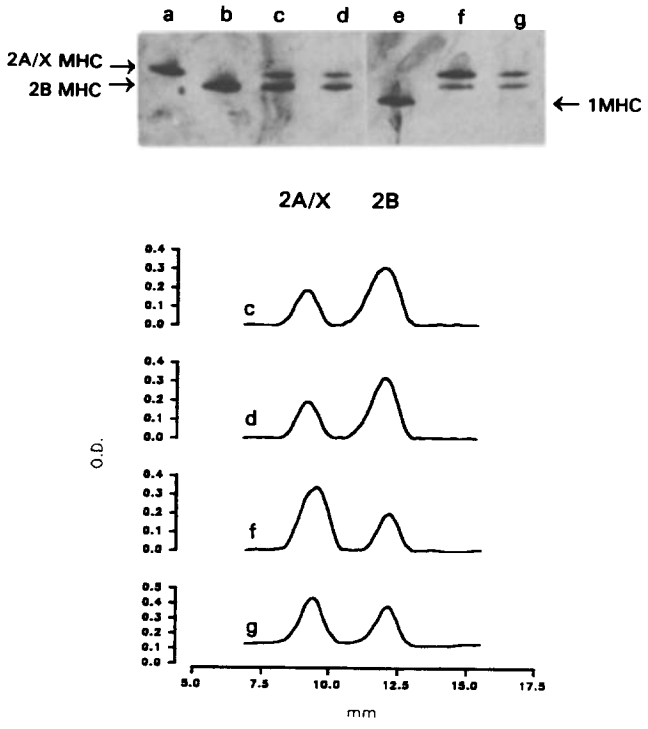

Fig. 1. Top: SDS–PAGE runs of single fibres in the area of MHCs. Fibres in a, b, and e show only one electrophoretic band corresponding to 2A or 2X MHC, 2B MHC, and 1 MHC respectively. Fibres in c, d, f, g show both the 2A/X and the 2B band. Bottom: densitometric traces of runs c, d, f, g showing the two peaks corresponding to the 2A/X and 2B band. Fibres in c, and d contain higher amounts of 2B MHC, whereas fibres in f, and g contain higher amounts of 2A/X MHC.

Few fibres of this kind were found. The use of this procedure is substantiated by a recent study on the distribution of 2A and 2X MHC mRNAs in fibres characterized with respect to antibody staining (De Nardi *et al.*, 1993). Fibres that showed the 2A/X and the 2B band in SDS–PAGE gels were considered as fibres containing 2X and 2B MHCs when they reacted with BF-35 and RT-D9, and not with SC-71, whereas were considered as fibres containing 2A, 2X, 2B fibres when they reacted with all three antibodies. Few fibres were found to belong to the latter group. Among the fibres that were found to contain 2X and 2B MHCs, two subgroups were obtained on the basis of the relative amount of 2X and 2B MHC present. Fibres containing higher amounts of 2B MHC (> 50%) were identified as 2B-2X fibres, whereas fibres containing higher amounts of 2X MHC (> 50%) were identified as 2X-2B fibres. The presence of higher amounts of 2X and 2B MHCs was assessed from densitometric traces of the gels (Fig. 1). No attempt to more precisely quantify the relative amount of the two isoforms was done. Fibres showing the 1 and 2A/X band were considered as containing 1 and 2A MHC since they strongly stained with SC-71 and BF-35 and not with RT-D9. Few fibres containing 1 and 2A/X band were found.

Table 1. Specificy of the three monoclonal antibodies against MHC used in this study

MHC Isoform	Monoclonal antibodies		
	SC-71	*BF-35*	*RT-D9*
2A	+	+	−
2X	−	−	+
2B	−	+	+

Table 2. Percentage distribution of fibre types in 288 single fibres dissected from plantaris, tibialis anterior (superficial part), and soleus muscles of 3–5-month old male Wistar rats. Fibres are identified on the basis of MHC composition determined by combining Mabs and SDS–PAGE. The fibres are also divided according to the muscle of origin

MHC isoform	*Plantaris*		*Tibialis anterior*		*Soleus*	
	n	%	*n*	%	*n*	%
1	3	1.44	0	0	24	88.88
2A	20	9.66	2	3.80	0	0
2X	43	20.77	2	3.80	0	0
2B	21	10.14	34	62.96	0	0
1-2A	3	1.44	0	0	3	11.11
2A-2X	14	6.76	0	0	0	0
2X-2B	58	28.02	1	1.85	0	0
2B-2X	32	15.45	15	27.77	0	0
2A-2X-2B	13	6.28	0	0	0	0
	207		54		27	

Results

MYOSIN HEAVY CHAIN COEXISTENCE IN SINGLE FIBRES

Myosin heavy chain coexistence was studied in 288 single fibres isolated from plantaris, tibialis anterior (superficial part) and soleus muscles of adult rats. All fibres used for mechanical experiments are included. The remaining fibres were characterized as regards MHC composition, but not mechanically characterized. Nine groups of fibres were identified as described in Materials and Methods. Table 2 reports the percentage distribution of the different groups of fibres divided according to the muscle of origin. Slow or 1 MHC coexisted only with 2A MHC; the frequency of this coexistence was very low. About 50% of the fibres in plantaris and 30% in tibialis anterior muscle showed coexistence of fast MHCs. Few fibres were found to contain 2A and 2X MHC (7% in plantaris, none in tibialis) or all three fast MHC isoforms (6% in plantaris, none in tibialis anterior). In tibialis anterior all mixed fibres were found to contain more 2B MHC than 2X MHC (2B-2X fibres). In plantaris muscle 2X-2B fibres, i.e. fibres containing higher amounts of 2X MHC, were more frequent than 2B-2X fibres. As regards fibres containing only one MHC, the percentage distribution of 2B fibres was much higher in tibialis anterior (superficial part) than in plantaris muscle. 2X fibres were the predominant population in plantaris muscle, whereas

they were virtually absent in tibialis anterior. 2A fibres were the least represented fibre type.

V_0 AND MHC COMPOSITION

V_0 of more than one hundred fast fibres from plantaris and tibialis anterior muscles were determined. Table 3 reports mean V_0 values of three groups of fibres containing only one fast MHC (2A, 2X or 2B), and of two groups containing more than one fast MHC in different relative amounts: 2X-B and 2B-2X. Fibres containing 2A and 2X MHCs or all three fast MHC isoforms were not included in the analysis because too few of such fibres were found. 2A fibres had mean V_0 values significantly lower than 2B fibres. 2X were intermediate. The differences between 2A and 2X fibres and between 2X and 2B fibres did not reach statistical significance. 2B-2X fibres had mean V_0 values higher than 2X-2B fibres, but the difference was not statistically significant.

Figure 2 shows the distribution of V_0 values in the fibres of Table 3. It can be seen that the variability in V_0 among fibres containing the same MHC isoform was very large, and that groups of fibres containing different MHC isoforms had overlapping ranges of variability of V_0. Fibres containing only one MHC, particularly 2X and 2B fibres, did not show lower variability in V_0 values than fibres containing more than one MHC isoform (2X-2B and 2B-2X fibres).

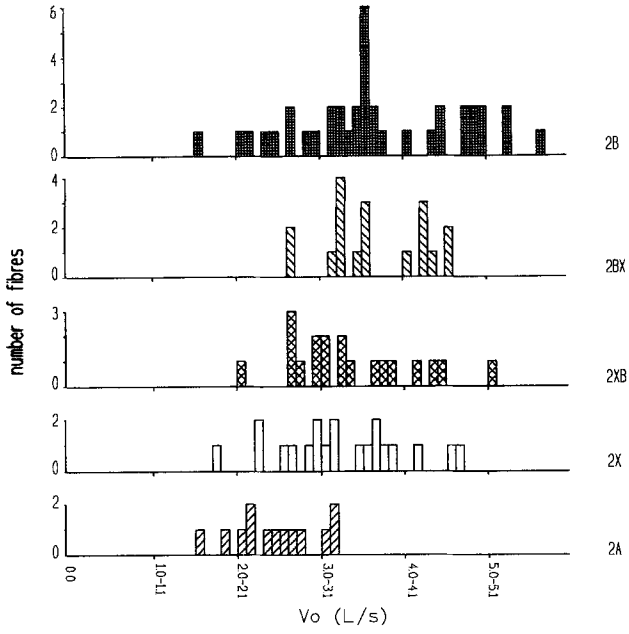

Fig. 2. Histograms showing the distribution of V_0 values in the population of fibres studied. Fibres are classified on the basis of MHC composition determined combining SDS–PAGE and monoclonal antibodies against MHCs. The X axis is the same for each group of fibres. The width of each vertical bar is 0.1 L s^{-1}. The number of fibres is reported on the Y axis. MHC composition of the fibres is indicated on the right of each histogram.

Discussion

In this study, the frequency of MHC coexistence was assessed in hind limb muscles of normal adult rat. Mean values and variability of V_0 of groups of fast fibres containing only one or two fast MHC isoforms in different relative amounts were determined. The results point to the conclusion that the large variability in maximum shortening velocity of fast fibres cannot be accounted for by coexistence of different proportions of two or more MHC isoforms.

Table 3. Unloaded shortening velocity (V_0) and MHC composition in 109 fast fibres from tibialis anterior and plantaris muscles

MHC composition	V_0 ($L\,s^{-1}$)	*n*
2A	2.431 ± 0.594	13
2X	3.234 ± 0.773	20
2X-2B	3.337 ± 0.808	19
2B-2X	3.651 ± 0.603	18
2B	3.700 ± 0.979	39

Myosin heavy chain isoform composition was determined by combining SDS–PAGE and immunocytochemistry. Values are means ± SDs; *n* denotes number of fibres in each group. One way variance analysis indicates that mean V_0 values of 2A fibres was statistically different ($P < 0.05$) from mean V_0 values of 2B and 2B-2X fibres. The other differences were not statistically significant.

The analysis of a large number (288) of single fibres from slow and fast muscles of adult rats shows that fast MHC isoforms frequently coexist in the superficial part of tibialis anterior and especially in plantaris muscle, whereas slow and fast (2A) MHCs rarely coexist. These results confirm and extend previous observations (Danieli-Betto *et al.*, 1986; Staron & Pette, 1993). The finding that 'mixed' fibres in tibialis anterior are all 2B-2X fibres, i.e. they contain higher amounts of 2B than 2X MHC, and in plantaris are more frequently 2X-2B, i.e. they contain higher amounts of 2X MHC, is in agreement with the fact (Table 2) that 2B MHC is the predominant isoform in tibialis anterior, and 2X MHC in plantaris. The small number of fibres containing 2A, 2X and 2B MHC confirms that coexistence of more than two fast MHC isoforms is exceptional and might suggest that even in normal adult rat some fibres undergo type transition (Termin *et al.*, 1989).

In all previous studies on the relation between MHC composition and maximum shortening velocity in fast fibres, the techniques used to identify MHCs were not especially designed to detect MHC coexistence. In most studies, in fact, none of the fibres examined were reported

to contain more than one MHC isoform. The high frequency of fast MHC coexistence reported in this work is consistent with the high frequency of fibres containing mRNAs of different MHC isoforms reported by De Nardi and colleagues (1993). These findings suggest that fibres, that in previous physiological studies were supposed to contain only one MHC, might have contained more than one MHC isoform. It seems well established that maximum shortening velocity of fibres that contain slow and fast MHC isoforms is proportional to the relative content of the fast isoform (Reiser *et al.*, 1985, 1988). The same phenomenon could occur when more than one fast MHC isoform is present. MHC coexistence, therefore, might explain the unexpected large variability in maximum shortening velocity of fast fibres (Eddinger & Moss, 1987; Sweeney *et al.*, 1988; Bottinelli *et al.*, 1991).

The latter hypothesis was tested in this study comparing groups of fibres containing only one fast MHC with one another and with groups of fibres containing more than one fast MHC as regards mean values and ranges of variability of V_0. 2A fibres had mean V_0 values significantly lower than 2B fibres. 2X fibres were intermediate. Fibres containing both 2X and 2B MHCs were intermediate between 2X and 2B fibres. A continuum of increasing V_0 values in the sequence 2A → 2X → 2X-2B → 2B-2X → 2B fibres is suggested by the present data, even though only the differences between 2A and 2B, and 2A and 2B-2X were statistically significant. These data confirm previous observations on 2A and 2B fibres, and extend the results to 2X MHC containing fibres. The higher V_0 values of 2B-2X fibres when compared to 2X-2B fibres seem to confirm that 2B MHC is associated with higher maximum shortening velocities. The most important observation, however, is that the variability in V_0 of 2A, 2X and especially 2B fibres was still very large notwithstanding that these fibres contained only one MHC isoform. The V_0 variability of groups of fibres containing only one MHC was apparently not lower than the variability in V_0 of 2B-2X and 2X-2B fibres that surely contained two different MHC isoforms.

Only two previous studies on the relation between V_0 and MHC composition of fast fibres have detected the presence of more than one MHC in the same fibre. Sweeney and colleagues (1988) has found few rabbit fast fibres containing both 2A and 2B MHCs, and has discarded them from analysis. Larsson and Moss (1993) have very recently found a high frequency of 2A and 2B MHC coexistence in fast fibres from human muscles, and have studied the relation between V_0 and the relative content of 2B MHC. In both studies, however, only two (2A and 2B) of the three fast MHC isoforms have been identified. Therefore, at variance with this study, the large variability in V_0 of fibres supposed to contain the same MHC isoform could still be due to MHC isoform coexistence.

The finding that fibres containing the same fast MHC isoform can be very different as regards their maximum speed of shortening points to some other determinant of the large variability in V_0 among fast fibres. Alkali MLC isoforms are likely candidates. Some studies have suggested that, in fast fibres, the higher the content in the low molecular weight isoform of alkali MLC (MLC3f) the higher the shortening velocity (Greaser *et al.*, 1988; Sweeney *et al.*, 1988; Bottinelli *et al.*, 1993b). The ratio between the two isoforms of alkali MLC has been shown to vary very widely in fibres containing the same fast MHC (Wada & Pette, 1993). Variations in the relative content of the two alkali MLC isoforms (MLC1f and MLC3f) could well explain variations in V_0 of fibres containing the same MHC. It must be also observed that MLC3f has been found to preferentially associate with 2B MHC and MLC1f with 2A MHC (Salviati *et al.*, 1982; Sweeney *et al.*, 1988; Wada & Pette, 1993; Bottinelli, Betto, Schiaffino and Reggiani unpublished observations). This suggests that also the differences in V_0 between 2A, 2X and 2B fibres (and also 2B-2X and 2X-2B fibres) could be due to different alkali MLC content and not to different MHC composition.

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