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MAXIMAL SHORTENING VELOCITIES, ISOMYOSINS AND FIBRE TYPES IN SOLEUS MUSCLE OF MICE, RATS AND GUINEA-PIGS

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SUMMARY

1. Guinea-pig soleus contains only type I fibres and slow isomyosin, SM_2. Rat and mouse soleus contain about 70% of type I fibres and a mixture of isomyosins: slow, SM_2 and intermediate, IM. Many rat soleus muscles contain a third isomyosin of a slow type, SM_1.

2. The maximal velocity of unloaded shortening, V_0, is largest in mouse soleus (6·11 L_f s^{-1}), slowest in guinea-pig soleus (1·67 L_f s^{-1}) and intermediate in rat soleus (4·16 L_f s^{-1}) (L_f = fibre length).

3. In guinea-pig soleus, V_0 is equal to the maximal velocity (V_{max}) computed using the Hill force–velocity relationship; V_0 is approximately twice as large as V_{max} in mouse and rat soleus.

4. V_0 measures the unloaded shortening velocity of the fastest fibres whereas V_{max} is a function of the force–velocity characteristics of all the fibres contained in the muscle.

5. V_0 increases according to the isomyosin composition of the fibres in the sequence $SM_2 < SM_1 + IM < IM$.

INTRODUCTION

Maximum shortening velocity is one of the most useful mechanical parameters for characterization of the various types of muscle fibres. It can be estimated by extrapolating the hyperbolic force–velocity relationship (Hill, 1938) to zero external load, V_{max}. It can also be measured from the time it takes a fully activated muscle fibre to take up a slack, V_0 (Edman, 1979). Julian, Rome, Stephenson & Stritz (1986) showed that V_0 equals V_{max} in experiments on frogs where both velocities were carefully determined for the same single skeletal muscle fibre. Claflin & Faulkner (1985) measured both velocities in rat soleus muscle, a whole-muscle preparation with a heterogeneous fibre population, and observed that V_0 was 60% larger than V_{max}. They concluded that V_0 is a measure of the unloaded velocity of the fastest fibres whereas V_{max} is a function of the force–velocity characteristics of all the fibres within a skeletal muscle preparation.

To test this interpretation we studied soleus from animals belonging to three species of the same taxonomic order (rodents). One preparation, from guinea-pig,

had a homogeneous population of fibres; the other two, from rat and mouse, were heterogeneous. V_{max} and V_0 were measured sequentially on the same muscle. It is important to specify the conditions under which V_{max} is measured since the discrepancy between V_{max} and V_0 might be quite small if V_{max} is determined by extrapolation to zero load of the velocities measured at very light loads (Julian *et al.* 1986). For this reason, we have estimated V_{max} by the ratio of the velocity constant, b, to the force constant, a/F_0, of a force–velocity relationship in which the loads were varied between 95–98 and 10–20 % of the maximal isometric force F_0.

In isolated single fibres V_0 depends on the myosin heavy chain types and the content in myosin light-chain LC_3f (Reiser, Moss, Giulian & Greaser, 1985; Sweeney, Kushmerick, Mabuchi, Stréter & Gergely, 1988). Since myosin heavy and light chains can combine in many ways to form an isomyosin (Staron & Pette, 1987), it is desirable to correlate V_0 with the complete myosin molecule. For this reason, the muscle was subjected to fibre typing and to electrophoretic analysis of isomyosins under non-denaturing conditions after the mechanical experiments. Besides confirming and extending the findings of Claflin & Faulkner (1985), our results enabled us to evaluate the relationships between V_0 and the isomyosin composition of the muscles.

METHODS

Muscle preparation and stimulation

Experiments were performed on whole soleus muscles isolated from fourteen rats (Wistar; body weight, 130–360 g), twenty-four mice (NMRI; body weight, 30–40 g) and eleven guinea-pigs (body weight, 110–150 g). The animals were anaesthetized with a subcutaneous injection of 2 ml kg^{-1} body weight Thalamonal. The right soleus muscle was dissected free from the animal. The animals were killed by ether after the dissection. The distal tendon was tied to a fixed frame, which supported two parallel rows of eight platinum electrodes spaced 2 mm apart. The muscle was placed between the rows of electrodes. The proximal tendon was tied to a glass rod (250 mg in weight). The frame was placed in thermostated chamber containing a buffered physiological salt solution (in mM): NaCl, 118; $NaHCO_3$, 25; glucose, 5; KCl, 5; $CaCl_2$, 2; $MgSO_4$, 1; KH_2PO_4, 1; the pH of the solution was adjusted to 7·4 by equilibration with a gas mixture of 95 % O_2 and 5 % CO_2 and its temperature was kept at $20 \pm 0{\cdot}5$ °C. The glass rod was attached to the force transducer of an electromagnetic ergometer. The muscle was slightly stretched at rest, until it resisted with a small resting force of approximately 5 mN. It was stimulated with capacitor discharges of alternating polarity to avoid electrolysis (time constant, 5 ms; output impedance, 100 Ω, 1 H_z). Its length was finely adjusted until the maximum isometric twitch force was obtained; the corresponding length defined the optimal length, L_0.

Measurement of V_{max} and V_0

The force–velocity relationship was obtained with the method of isovelocity releases (Cecchi, Colomo & Lombardi, 1978), using an ergometer described in an earlier work (Maréchal & Plaghki, 1979). The muscle was maximally tetanized at the optimal length. The isometric force attained a plateau, F_0, after an initial duration of stimulation of 0·6 s for mouse, 1·2 s for rat and 3 s for guinea-pig. The muscle was released at very high velocity (70 mm s^{-1}) in order to discharge the series elastic elements. The amplitude (25–100 μm) of the quick release was adjusted to the velocity of the following shortening at controlled speed in order to minimize the transients (see Fig. 1, insets). The force F was measured at the beginning of the release at controlled velocity, just after the rapid fall caused by the quick release, and it was expressed relative to F_0. The tetanus with releases were repeated eighteen to twenty-two times, at different controlled velocities adjusted for each species, to cover the span of the force–velocity relationship (see Fig. 1) and arranged in a quasi-mirror order to minimize the influence of fatigue. The fall of force between the first and last tetanus averaged 5 % and was never higher than 10 %. The ratio F/F_0 was fitted to the velocities of release by a rectangular hyperbola (Hill, 1938; Fig. 1) using a non-linear least-squares regression based on the

Gauss–Newton method (Yamaoka, Tanigawara, Nakagawa & Uno, 1981). V_{max} was computed as b (velocity constant) divided by a/F_0 (force constant).

V_0 was determined by the slack test (Edman, 1979) as applied to whole muscle by Claflin & Faulkner (1985). Nine changes in length were applied during the plateau of an isometric tetanic contraction. The velocity of release was 70 mm s^{-1}. Step sizes, in order of application within each series, were 0·44, 0·88, 1·32, 1·78, 2·20, 1·98, 1·54, 1·10 and 0·66 mm. In all cases, the final length was L_0. The duration of unloaded shortening associated with each release, t_L, was measured from records obtained with a fast UV recorder (ME-100) as the interval between the instant of release and the point at which the force started to redevelop. This point was sometimes difficult to determine, due to the slow rise of the force and to some low-amplitude oscillations (Fig. 2, upper panels) at low frequency, approximately 500 Hz, originating in the electromagnetic device. In these cases, t_L was estimated as the interval between the onset of the release and the intercept between a linear extrapolation of the rising force and the baseline. The unloaded shortening time intervals increased linearly with release amplitudes in the soleus muscles of all three species (Fig. 2, lower panels). The V_0 value was calculated as the inverse of the slope of the resulting line.

After the mechanical experiment, the muscle was blotted and weighed. It was placed on a plastic sheet and stretched to L_0; under a binocular microscope, the length of five to ten superficial fibres were averaged, giving the fibre length, L_f. We observed a mouse L_f of $6{\cdot}9 \pm 0{\cdot}1$ mm, nearly equal to that reported by Luff (1981), $6{\cdot}84 \pm 0{\cdot}20$ mm. The ratio 'muscle fibre length, L_f/muscle length, L_0' was 0·68 for mouse soleus (in agreement with the published values of 0·69 for mouse soleus, Brooks & Faulkner, 1988) and 0·72 for rat and guinea-pig soleus. The mean cross-sectional area was estimated by dividing the muscle weight by its fibre length, assuming a muscle density equal to one (Close, 1972); the maximum tetanic stress, S_0, was calculated as the maximum tetanic force divided by the mean cross-sectional area. The muscle was rapidly frozen in isopentane at −150 °C and stored at −80 °C until further use.

Fibre typing and isomysin analyses

Eighteen of those frozen muscles, six of each species, were simultaneously analysed for their fibre types and native isomyosins. Cryostat sections (10 μm thickness) were obtained from three levels: proximal, central and distal. Three serial sections from each level were used for fibre typing by the classical myosin ATPase reaction at three pH values, 10·0, 4·45 and 4·28 (Brooke & Kaiser, 1970). Twenty sections from each level were homogenized at 4 °C and extracted with 100 μl of Guba–Straub solution (in mM): NaCl, 300; NaH_2PO_4, 100; Na_2HPO_4, 50; $MgCl_2$, 1; $Na_4P_2O_7$, 10; NaN_3 0·1%; 2-mercaptoethanol, 0·1%; pH 6·5; centrifuged (10 min, 10000 r.p.m., 4 °C); then, 100 μl of glycerol were added to the supernatant and 50 μl of the mixture were diluted with 50 μl of buffer ($Na_4P_2O_7$, 200 mM; glycerol, 50%; bromophenol, 0·015%; 2-mercaptoethanol, 0·01%; pH 8·5; all percentages w/v) and 20 μl of an extract of taenia coli (added to provide an interval standard for the electrophoretic mobility). Finally, 10 and 20 μl of this mixture were put on top of a pyrophosphate polyacrylamide gel for electrophoretic analysis under native conditions (Hoh, McGrath & White, 1976). The mobility of the isomyosins and their relative proportions were estimated by computerized densitometry. Since no differences were observed between the sections from the three different levels, the data were pooled and averaged. In the remaining frozen muscles ($n = 29$), native isomyosins were separated and quantified as described in Maréchal, Schwartz, Beckers-Bleukx & Ghins (1984).

Statistical analyses

Results were expressed as mean ± S.E.M. Non-linear regressions were computed using a Truebasic program and statistical calculations were performed with the SPSS statisical package, both on a XT microcomputer.

RESULTS

Examples of experiments to estimate V_{max} by the force–velocity relationship and V_0 by the slack test method are presented in Figs 1 and 2. Isomyosin patterns and corresponding densitograms of the gels are shown in Fig. 3. Histochemical myosin

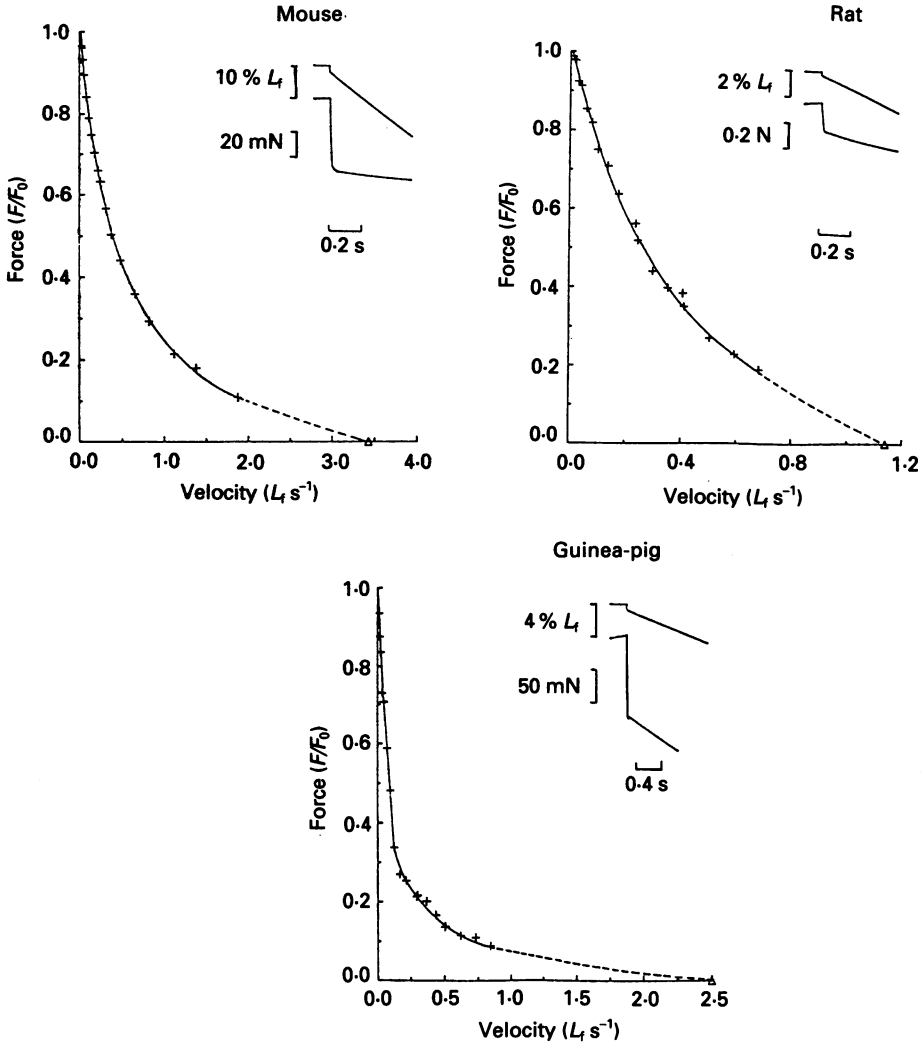

Fig. 1. Force–velocity relationships of soleus muscles of mouse (upper left), rat (upper right) and guinea-pig (lower). Insets: isometric tetanus with release at a constant velocity. The graph begins before the release when the tetanic isometric force, F_0, is maximal. Upper curves, muscle length; the velocity of release equals 0·17 fibre length s^{-1} (L_f s^{-1}) for the mouse soleus, 0·08 Lf s^{-1} for rat soleus and 0·02 L_f s^{-1} for guinea-pig soleus. Lower curves, force maintained during the release. Main graphs: force–velocity relationships. The ratio F/F_0 (+) is used as the estimate of the force maintained by the muscle when released at a constant velocity. The continuous lines show the Hill's hyperbolas computed by a least-squares method; the dashed lines point toward V_{max}(△), computed as the ratio of b to a/F_0. *Mouse*: age, 120 days; body weight, 31 g; muscle weight, 9 mg; fibre length, 6·2 mm; maximum tetanic force, 194 mN; b = 0·52 L_f s^{-1}; a/F_0, 0·15; V_{max}, 3·4 L_f s^{-1} V_0, 7·2 L_f s^{-1}; SM_2, 68 %; SM_1, 0 %; IM, 32 %. *Rat*: age, 97 days; body weight, 325 g; muscle weight, 186 mg; fibre length, 21 mm; maximum tetanic force, 1050 mN; b = 0·46 L_f s^{-1}; a/F_0, 0·43; V_{max}, 1·14 L_f s^{-1}; V_0, 2·5 L_f s^{-1}; SM_2, 73 %; SM_1 18 %; IM, 9 %. *Guinea-pig*: age, 21 days; body weight, 120 g; muscle weight, 70 mg; fibre length, 14 mm; maximum tetanic force, 551 mN; b = 0·10 L_f s^{-1}; a/F_0, 0·04; V_{max}, 2·5 L_f s^{-1}; V_0, 1·9 L_f s^{-1}; SM_2, 100 % SM_1, 0 %; IM, 0 %.

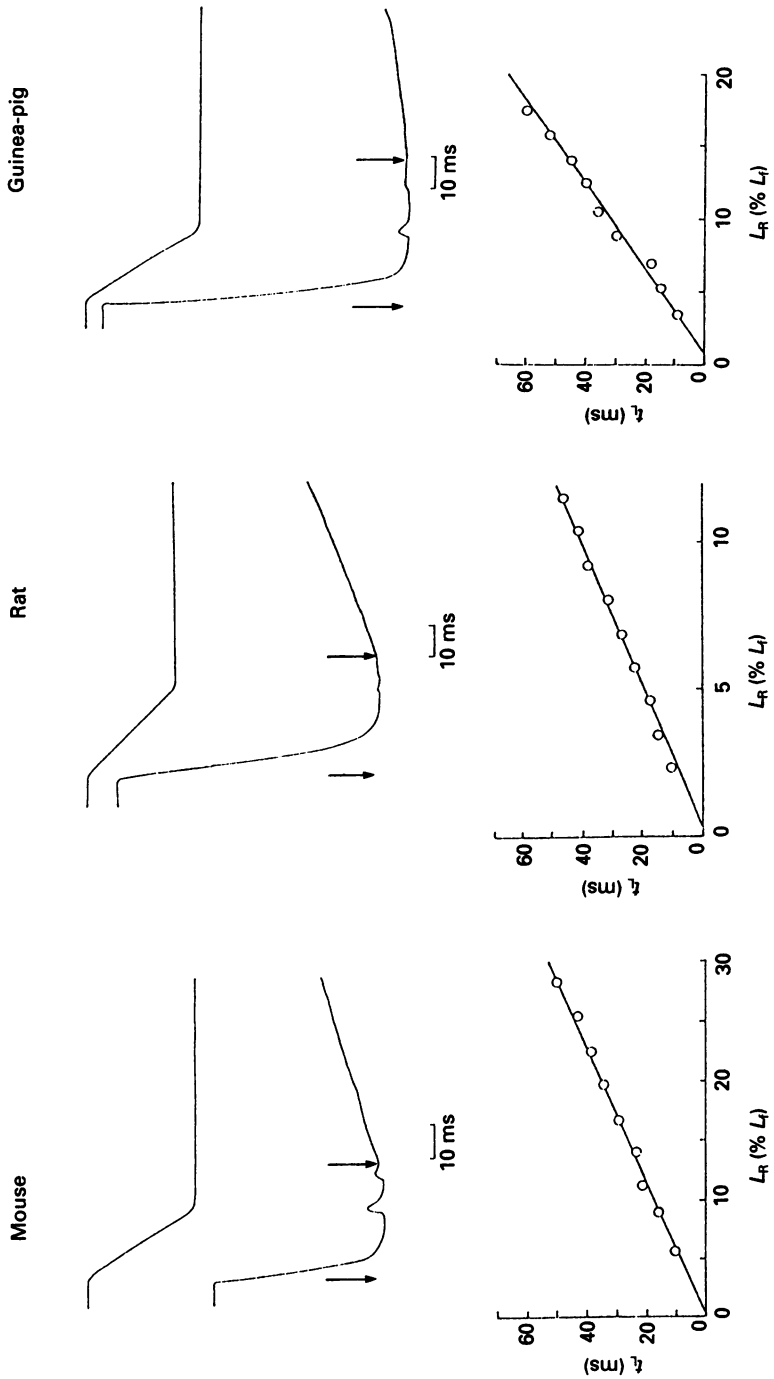

Fig. 2. Determination of unloaded shortening velocity (V_0) by the slack test of the muscles shown in Fig. 1. Upper panels, imposed length changes (upper curves) and resulting force response (lower curves). The release amplitude, L_R, equals 17 % L_f for mouse soleus, 7 % L_f for rat soleus and 10 % L_f for guinea-pig soleus. The time t_L required to take up the imposed slack is indicated by the arrows. Lower panels, t_L for nine releases at various amplitudes. The line represents the least-squares regression of t_L upon L_R. The unloaded shortening velocity (V_0) is computed from the slope of this line; it is equal to 5·4 L_f s^{-1} for the mouse soleus, 2·5 L_f s^{-1} for the rat soleus and 2·3 L_f s^{-1} for the guinea-pig soleus.

ATPase patterns are not shown, since they do not differ from numerous examples previously reported in the literature.

Table 1 summarizes the results obtained in all experiments. Soleus muscles from the three species exhibited the same maximum isometric stress, S_0; it appears to be

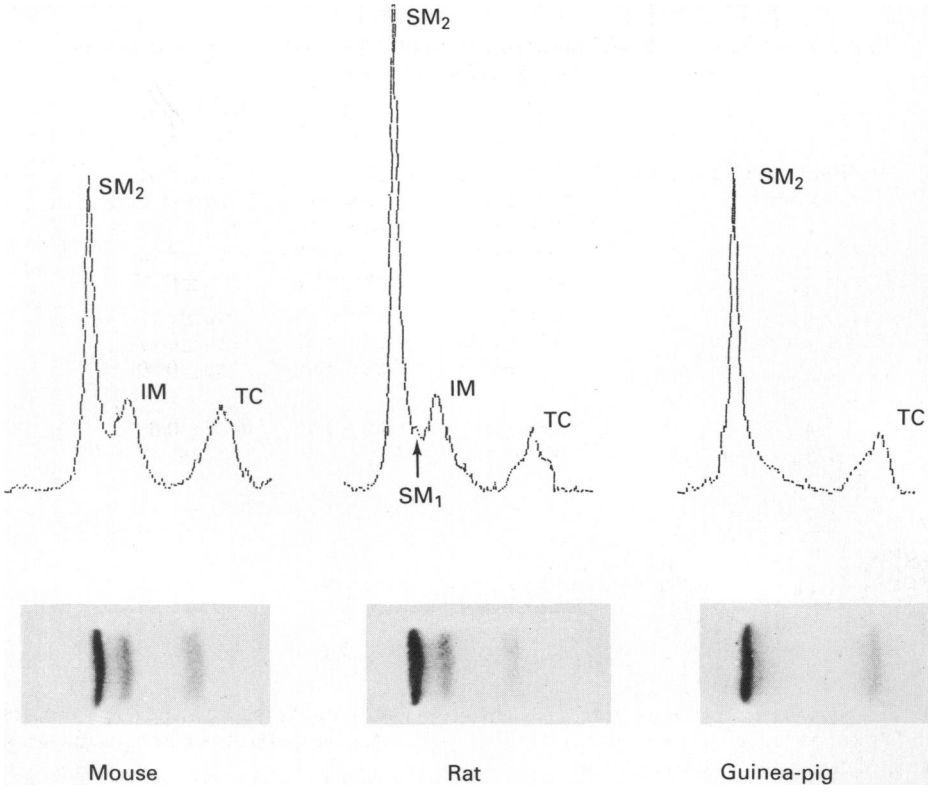

Fig. 3. Isomyosins of soleus muscles of mouse, rat and guinea-pig. Twenty μl of a muscle homogenate (final dilution 1:300 w/v) in a pyrophosphate buffer were put on top of the gel, underwent electrophoresis, were stained with Coomassie Blue and scanned with a densitometer. Photographs of the gels are shown in the lower panels and their absorbance at 535 nm, in arbitrary units, is shown in the upper panels. The mouse shows two zones, the rat three zones (the arrow points toward the small third zone) and the guinea-pig one zone. In every case, the zone to the right is taenia coli (TC) myosin, used as an internal standard to standardize the mobility of the other zones, its electrophoretic mobility being arbitrarily set at 147. The relative proportions of the soleus isomyosins were obtained by computer integration of the densitograms using the drop line method. *Mouse*: SM_2, 70%; IM, 30%; *rat*: SM_2, 79%; SM_1, 7%; IM, 14%; *guinea-pig*: SM_2, 100%.

rather low, but several workers have reported similar low values for soleus muscles (for instance, in mice: Rowe & Goldspink, 1969; Luff, 1981; Anderson, Bressler & Ovalle, 1988; in rats: Close, 1972; Witzmann, Kim & Fitts, 1983; and in guinea-pigs: Powell, Roy, Kanim, Bello & Edgerton, 1984). The Hill velocity constant b of the guinea-pig soleus reaches only one-third that of the rat and the mouse. V_0 and V_{max} are also lower in the guinea-pig. V_0 is approximately twice as large as V_{max} in the rat and in the mouse whereas these two estimates of the maximum velocity are almost equal in the

guinea-pig. In accordance with previous reports, we find only one fibre type in the guinea-pig soleus muscles using the histochemical myosin ATPase assay, whereas the mouse and rat soleus are mixed muscles, containing type I and type IIA fibres (Close, 1967; Ariano, Armstrong & Edgerton, 1973; Lewis, Parry & Rowlerson, 1982).

TABLE 1. Mechanical, biochemical and histochemical characteristics of soleus muscles of various rodents

	Mouse ($n = 24$)	Rat ($n = 14$)	Guinea-pig ($n = 11$)
Muscle weight (mg)	11·0 ± 0·16	145·0 ± 9·0	72·9 ± 2·8
L_f (mm)	6·9 ± 0·1	17·6 ± 0·3	14·6 ± 0·3
S_0 (N cm^{-2})	14·8 ± 0·8	16·8 ± 1·1	14·7 ± 2·2
b (L_f s^{-1})	0·50 ± 0·04	0·54 ± 0·05	0·13 ± 0·02
a/F_0	0·15 ± 0·01	0·33 ± 0·05	0·10 ± 0·02
V_{max} (L_f s^{-1})	3·42 ± 0·24	1·98 ± 0·16	1·53 ± 0·28
V_0 (L_f s^{-1})	6·11 ± 0·30	4·16 ± 0·26	1·67 ± 0·17
V_0/V_{max}	1·75 ± 0·12	2·28 ± 0·24	0·98 ± 0·20
SM_2			
Mobility (%)	99·8 ± 0·5	98·2 ± 0·4	99·8 ± 0·6
Proportion (%)	69·9 ± 3·0	76·2 ± 5·2	100
SM_1			
Mobility (%)	—	107·8 ± 0·8	—
Proportion (%)	0	11·1 ± 3·1	0
IM			
Mobility (%)	110·6 ± 1·1	112·9 ± 0·4	—
Proportion (%)	30·1 ± 3·0	12·7 ± 3·2	0
Type I (%)	67 ± 5·3	73 ± 1	100
Type IIA (%)	33	27	0

Results are reported as mean ± S.E.M. The electrophoretic mobility is expressed as percentage of the mobility of taenia coli myosin, arbitrarily set at 147; the proportions of isomyosins are as a percentage of the sum of all isomyosins.

The proportion of type I fibres in mouse soleus in markedly higher than that reported in other works, probably because the strain used here (NMRI) is different (C57BL/6J in Dribin & Simpson, 1977 and in Anderson *et al.* 1988). One isomyosin, SM_2, is present in the soleus muscles of all three species; this form migrates very slowly (electrophoretic mobility = 100). A second form, SM_1, which migrates faster (electrophoretic mobility = 108), is present only in rat soleus, in which it is not abundant (11% of total myosin) and not constant (observed in ten samples out of fourteen). A third form is observed in rat and mouse soleus; since its electrophoretic mobility (equal to 112) is intermediate, falling between that of the slow isoforms $SM_1$and $SM_2$and that of the fast muscle myosin isoforms(FM_1, FM_2 and FM_3, with electrophoretic mobilities between 116 and 130; see Maréchal, Biral, Beckers-Bleukx & Colson-Van Schoor, 1988), it has been called 'intermediate isomyosin', IM. Thus, guinea-pig soleus contains only one isomyosin, SM_2 (in agreement with d'Albis, Pantaloni & Bechet, 1979), mouse soleus contains two isomyosins, SM_2 and IM (in agreement with Fitzsimons & Hoh, 1983), and rat soleus contains three isomyosins, SM_1, SM_2 and IM. The fast muscle myosin isoforms (FM_1, FM_2 and FM_3) are absent from the soleus muscles of the three rodents.

DISCUSSION

Comparison of V_{max} and V_0

In this study, values of V_{max} ($1{\cdot}98 \pm 0{\cdot}16\ L_f\ s^{-1}$) and V_0 ($4{\cdot}16 \pm 0{\cdot}26\ L_f\ s^{-1}$) obtained for rat soleus are in fair agreement with those reported by Claflin & Faulkner (1985), $3{\cdot}2 \pm 0{\cdot}1\ L_f\ s^{-1}$ and $5{\cdot}0 \pm 0{\cdot}1\ L_f\ s^{-1}$, respectively. The ratio V_0/V_{max} is somewhat hig er in our experiments: $2{\cdot}28 \pm 0{\cdot}24$, to compare with $1{\cdot}6 \pm 0{\cdot}1$ in Claflin and Faulkner's experiments. This agreement is satisfactory since the methodology used in the two laboratories markedly differs. Claflin and Faulkner used controlled isotonic releases to evaluate V_{max}, whereas we used releases at controlled velocities. Additionally, they performed the slack tests at a higher velocity (700 mm s^{-1} instead of 70 mm s^{-1}). Claflin and Faulkner concluded that V_0 measures the unloaded shortening velocity of the fastest fibres only, whereas V_{max} represents a complex function of all the fibres within the muscle. They predicted that V_{max} will only be equivalent to V_0 in muscles that are homogeneous with respect to the shortening velocities of their fibres. We tested this hypothesis by measuring V_{max} and V_0 on guinea-pig soleus, which is homogeneous in its fibre type composition. For the sake of comparison, we also determined V_{max} and V_0 on two heterogeneous muscles (rat and mouse soleus). In strong support of the hypothesis, we observed that V_{max} equals V_0 in the homogeneous guinea-pig soleus, whilst V_{max} is roughly twice as small as V_0 in heterogeneous muscles.

Fibre types, myosin isoenzymes and V_0

All fibres of guinea-pig soleus are of type I and they contain only one isomyosin: SM_2 (Table 1). Therefore, SM_2 is associated with the slowest observed value of V_0 ($1{\cdot}67\ L_f\ s^{-1}$; 20 °C). Mouse soleus contains about 70 % type I fibres and 30 % type IIA fibres. Since the proportion of each fibre type is equal to that of the isomyosins SM_2 and IM respectively, it is reasonable to assume that a given fibre contains only one isomyosin: SM_2 being localized in type I fibres and IM in type IIA fibres. As isomyosin SM_2 of the mouse has the same subunit composition as that of the guinea-pig (Maréchal *et al.* 1988) it is very likely that type I fibres have the same value of V_0 in both animals. The high V_0 ($6{\cdot}11\ L_f\ s^{-1}$) observed in mouse soleus would thus characterize the isomyosin IM. The case of the rat soleus is more difficult to interpret since its *two* types of fibres (I and IIA) must accommodate *three* varieties of isomyosins (SM_2, SM_1 and IM). In the absence of direct determinations of the isomyosin content of individual fibres, we have to examine two hypotheses: (i) the fibres are homogeneous in the sense that they contain myosin heavy chain MHC_I, present in the isoforms either SM_2 or SM_1 (which differ by their light chain composition, Maréchal *et al.* 1988), whilst type IIA fibres contain myosin heavy chain MHC_{IIA}, present in the isoform IM. As there is no reason to think that isomyosin IM is different for rat and mouse, we expect V_0 to be equal in rat and mouse soleus. Since this is not the case, mouse soleus being 47 % faster, we have to postulate that rat IIA fibres cannot contract at the characteristic V_0 observed for mouse IIA fibres either because they are intrinsically slower or being few (no more than 11 % on the basis of IM proportion) they have to pull the slower fibres and thus contract under a sizeable internal load. (ii) Fibres are heterogeneous, containing a mixture of isomyosins with

two different heavy chains: type IIA fibres would contain isomyosins SM_1 and IM and perhaps some isomyosin SM_2. Their characteristic V_0 ($4{\cdot}16\ L_f\ s^{-1}$) would be intermediate between those of the slow SM_2 of guinea-pig ($1{\cdot}67\ L_f\ s^{-1}$) and the fast IM of mouse ($6{\cdot}11\ L_f\ s^{-1}$). The presence of SM_1 in IIA fibres would thus lower the characteristic high V_0 of isomyosin IM, a reasonable assumption since we expect SM_1 to be a slowly contracting isomyosin according to its subunit composition, which is identical to SM_2 except for the substitution of one slow alkaline light chain, LC_1s, by a fast one, LC_1f (Maréchal *et al.* 1988). The second hypothesis seems more likely because several groups have reported that many single fibres contain a mixture of various myosin heavy and light chains (Billeter, Heizman, Reist & Jenny, 1981; Danielli-Betto, Zerbato & Betto, 1986; Staron & Pette, 1987; Biral, Betto, Danielli-Betto & Salviati, 1988).

In conclusion, the characteristic V_0 of isomyosins increases in the sequence: $SM_2 < SM_1 + IM < IM$. The subunit composition of these isomyosins is identical in mice, rats and guinea-pigs (d'Albis *et al.* 1979; Fitzsimons & Hoh, 1983; Maréchal *et al.* 1988). Using this information, we conclude that V_0 increases according to the following sequence: $MHC_I(LC_1s)_2 < MHC_I(LC_1s,LC_1f) + MHC_{IIA}(LC_1s,LC_1f) < MHC_{IIA}(LC_1s,LC_1f)$.

Taken together, all these results indicate that the shortening velocity of a fibre is controlled not only by the nature of its myosin heavy and light chains but also by the relative proportions of the isomyosins mixed in it.

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