Muscle and Motility
Volume 2

Proceedings of the XIXth European Conference in Brussels

Edited by

G. Maréchal and U. Carraro

Intercept
Andover, Hampshire

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Force–Velocity Relationship of Isomyosins SM_2 and IM of Mouse Soleus (Strain NMRI)

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Introduction

Mouse soleus is a slow-twitch muscle although its fibres are mixed, approximately 70% being of type I and 30% of type IIA (1). It also contains two isoforms of myosin: slow-twitch isomyosin SM_2, which is localized in type I fibres, and slow-twitch isomyosin IM which is present in type IIA fibres (2). Isomyosin SM_2 of mouse is similar in its subunit composition to that of guinea-pig soleus, a muscle containing only type I fibres: the myosin heavy chain is of the slow type, MHC_1, combined with slow type myosin alkali light chains, LC_{1s}; isomyosin IM which is absent from guinea-pig soleus has a myosin heavy chain of the fast type, MHC_{2A}, hybrid in its light chains, since it has both slow and fast alkali light chains LC_{1s} and LC_{1f} (3, 4). The unloaded velocity of contraction V_u (5) is higher for type IIA (IM) fibres (6.1 fibre length/s) than for type I fibres (1.7 fibre length/s) according to Asmussen & Maréchal (2). We show in this paper that in spite of the fact that V_u of type IIA fibres is fast, its force–velocity relationship is intermediate between the slow variety of type I fibres and the fast variety of type II fibres of fast-twitch muscle, such as mouse EDL.

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Methods

The experiments were performed *in vitro* at 20°C on whole soleus muscle isolated from NMRI mice. The muscle were pre-stretched at rest to develop 5±2 mN, and stimulated isometrically at 1 Hz for 10 s. The last twitch was recorded and analysed. The force–velocity relationship was obtained from 18 to 22 tetani with iso-velocity releases (6), using an electromagnetic puller. The maximum velocity of unloaded shortening, V_u, was measured by the slack test method of Edman (5). After the experiments, the muscles were frozen in liquid nitrogen. The myosins were extracted at 0°C with a Guba-Straub solution containing 10 mM PPi and separated by PPi-PAA electrophoresis in a nondissociating solution. The myosin heavy chains were separated by the method of Carraro & Catani (7). The relative proportions of the isomyosins and of the myosin heavy chains were measured by densitometry of the gels. The statistical calculations were done with the SPSS package on a AT computer. A more detailed description of our methods is reported in Asmussen & Maréchal (2).

Results

Table 1 shows that the twitch is slow in type; it also shows that the maximum isometric force varies quite a lot between muscles of different animals. These variations are still present when the force is expressed per unit area (maximum

Table 1 Isometric twitch and tetanic forces of soleus muscle of mouse (strain NMRI 20°C).

Variables	Means	Standard Deviation	Minimum	Maximum
Body weight (g)	32.43	5.28	25	42
Age (days)	81.1	17.6	55	120
Muscle weight (mg)	8.45	2.69	4.3	13
Fibre length (mm)	7.41	.64	6.3	8.5
Twitch force (mN)	22.53	7.40	11	37
Twitch time to peak (ms)	65.5	22.6	40	104
Twitch time of half relaxation (ms)	158.4	80.4	40	320
Twitch to tetanus force (%)	12.9	5.0	4.8	23
Maximum isometric force (mN)	186.1	51.8	106	292
Maximum isometric stress (mN/mm^2)	168.4	37.2	100	237
Fatigue index (5)	95.7	14.7	73.7	154.1

The means are computed on 21 experiments. The fatigue index is the ratio of the maximum isometric force of the first tetanus to that of the last tetanus in an experiment (n= 18–22). The maximum isometric force and stress reported in the table are those of the first tetanus in the experiment.

isometric stress). The maximum isometric force of the last tetanus in the experiment is on the average 5% lower than that of the first one (see 'fatigue index').

Table 2 Parameters of the force–velocity relationship of soleus muscle of mouse (strain NMRI, 20°C).

Variables	Means	Standard Deviation	Minimum	Maximum
Force constant a/F_o (%)	0.11	0.05	0.03	0.22
Velocity constant b (fibre length/s)	0.45	0.08	0.32	0.61
SSH	86	62	11	203
Velocity constant ba (with a/F_o=.11) (fibre length/s)	0.47	0.10	.33	0.65
SSHa	105	83	12	308
Maximum power (mW/mm^3)	39.5	9.1	27.4	64.5
V_{max} (fibre length/s)	5.0	3.21	2.0	16
V_u (fibre length/s)	5.7	0.97	3.9	8.2
V_u/V_{max}	1.43	0.58	0.33	2.4

Means of 21 experiments. V_{max} equals b/(a/F_o). ba is the velocity constant computed by setting a/F_o = 0.11 in all experiments. SSH is the residual sum of squares when b and a/F_o are evaluated by a non linear least squares procedure; SSHa is the residual sum of squares when ba is evaluated. The estimates of the parameters of each force-velocity curve are based on 18 to 22 tetani with relases at velocities ranging from 0.1 to 3 fibre length/s. The velocity of release during the slack test (to evaluate V_u) was 267 mm/s, with step sizes ranging from 0.2 mm to 2.0 mm.

Table 2 reports the observations on the force–velocity relationships. The velocity constant, b, and the force constant, a/F_o have been computed by a non-linear least squares procedure based on 18 to 22 tetanus-and-releases. The maximum velocity of shortening, V_{max}, is calculated as the ratio $b/(a/F_o)$. The velocity of unloaded shortening, V_u (5), is 40% higher than V_{max}. We have also computed the velocity constant *ba* by the least squares method when the force constant is set at 0.11 for all experiments. The goodness of fit can be evaluated by the residual sum of squares, i.e. the variance of the data not explained by the fitted curves. SSH and SSHa are the residual sums of squares corresponding to the two estimates b and *ba* of the velocity constant. The two estimates b and *ba* are not different. As expected, SSHa is higher than SSH, but the difference is small: it is thus reasonable to accept that a/F_o is the same for all muscles, whilst b may vary between muscles. This conclusion is supported by the absence of correlation between a/F_o and any of the other variables, whilst b and *ba* show strong correlations, with the isomyosins and the myosin heavy chains (see Table 4).

The maximum power P_{max} (Table 2) is the power per unit volume; it is equal to:

$$P_{max} = S_0 * b * M^2/(a/F_0) \quad (1)$$

in which M^2 is the maximum power normalized for force and velocity (8), F_o is the maximum isometric force and S_o is the maximum isometric stress:

$$M^2 = \{1 + 2\ a/F_0 - 2\ SQR[(a/F_0)^2 + a/F_0]\} * a/F_0 \quad (2)$$

P_{max} varies between muscles quite markedly (Table 2) but these variations are not correlated with the type of isomyosins or myosin heavy chains (see Table 4).

The proportions of isomyosins and myosin heavy chains are reported in Table 3. They are similar for SM_2 and MHC_1 on the one hand, and IM and MHC_{2A} on the other hand. Fig. 1 shows the data for SM_2 and MHC_1 and the line of

Table 3 Isomyosins and myosin heavy chains of soleus muscle of mouse (strain NMRI, 20°C).

Variables	Means	Standard Deviation	Minimum	Maximum
SM_2	66.0	8.9	52	84
IM	34.0	8.9	16	48
MHC_1	64.7	13.9	46	90
MHC_{2A}	33.7	14.0	8	54
MHC_{2B}	1.7	4.3	0	19

Means of 21 experiments, expressed in % of total isomyosins or total myosin heavy chains. The zone labelled MHC_{2B} may contain myosin heavy chain 2X (10) or 2d (11).

Table 4 Coefficients of correlation between the mechanical parameters and weights, isomyosins and myosin heavy chains.

Variables	Muscle weight	SM_2	MHC_1	MHC_{2A}
Twitch force	0.231	-0.231	-0.245	0.213
Time to peak	-0.691**	0.321	0.290	-0.424
Half relaxation	-0.592**	0.368	0.488	0.444
Max. isometric force	0.728**	-0.310	-0.265	0.392
Max. isometric force	0.368	0.390	0.549*	-0.487
a/F_o	0.488	0.403	0.409	-0.428
b	0.454	-0.511*	-0.641**	0.670**
ba	0.638**	-0.621*	-0.728**	0.767**
Max. power	0.327	-0.294	-0.269	0.371

Number of cases: 21. 1-tailed significance: *:0.01; **:0.001. The coefficients of correlation between the mechanical parameters and isomyosin IM are equal to those of SM_2 changed sign.

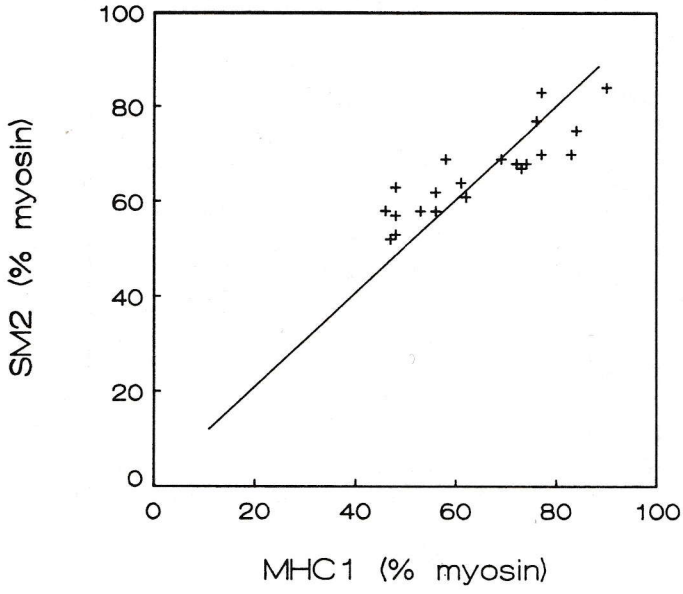

Fig. 1 Content in slow-twitch isomyosin SM_2 (in % of total isomyosins) as a function of the content in slow myosin heavy chain MHC1 (in % of total myosin heavy chain in 21 soleus muscle of mouse (strain NMRI). The slope of the line is 1 (identity line).

identity (slope = 1). This result is expected if all MHC_1 is in SM_2. It also demonstrates that our estimates of the myosin proportions are quantitative.

The correlations between some variables are reported in Table 4. The twitch time to peak and time to half relaxation depend on the muscle weight: apparently heavier animals develop shorter twitches. They do not depend on the proportions in isomyosins: presumably the shape of the twitch is entirely determined by the mechanisms of release and uptake of sarcoplasmic calcium. The force constant is influenced neither by muscle weight nor by the isomyosins. To the contrary, the velocity constants strongly depend on the isomyosins.

We further analysed the correlation between b and the slow myosin heavy chain, MHC_1, (Fig. 2). The regression line was computed on the assumption that both variables have similar coefficients of variation; this means that the slope of the regression line is the mean between the slopes of the regression lines of b on MHC_1 and of MHC_1 on b and the intercept is computed using the means of b and MHC_1. The equation of the best linear regression between b and MHC_1 is:

$$b = 0.85\ (\pm 0.12)\ \ 0.0061\ (\pm 0.0017)\ MHC_1 \qquad (3)$$
$$\text{where } n = 21 \quad r = 0.641 \quad p = 0.002$$

Twelve of the mice were male and 10 were female: except for the body and muscle weights, which were smaller in the female animals, no variable was correlated with sex.

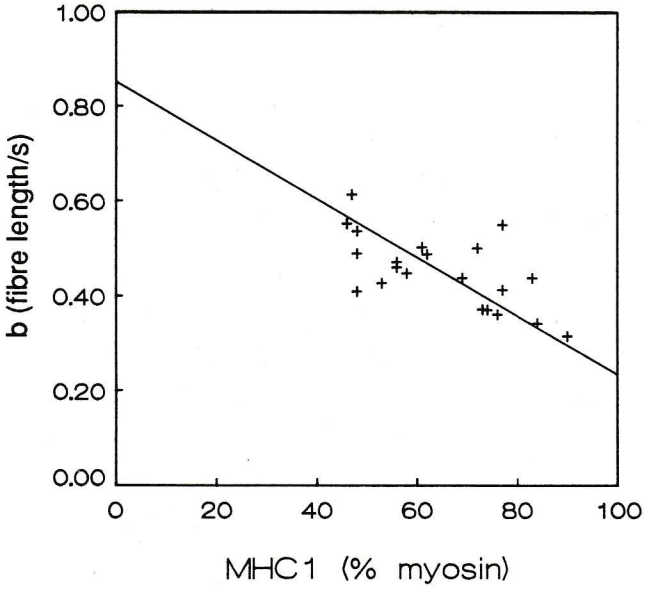

Fig. 2 Velocity constant of the Hill's force–velocity relationship as a function of the proportion in slow myosin heavy chain MHC1. Data of 21 mouse soleus muscles (strain NMRI).

Discussion

Soleus muscle is a relatively simple muscle: its fibres segregate into two varieties of isomyosins (SM_2 and IM), each isomyosin being homogeneous for its myosin heavy chain (MHC_1 and MHC_{2A} respectively). In unpublished experiments, we observed no fibres simultaneously stained by specific antibodies directed against MHC_1 or MHC_{2A} (gift of Dr F. Pons, Montpellier): most of the muscle fibres therefore have only one isomyosin. The muscle fibres being parallel, they add their forces to produce the force–velocity relationship of the whole muscle. It is reasonable to hypothesize that the fibres are subdivided into two homogeneous groups which contract at the same velocity during the tetanus and add their force. It is then justified to evaluate the parameters of the force–velocity of the two populations by an extrapolation of Equation 1. If soleus muscle had only type I fibres, or, equivalently, had only MHC_1, putting $MHC_1 = 100$ in Equation 3 gives an evaluation of the velocity constant of type I fibres. The velocity constant of the type IIA(IM) fibres is obtained if there are no type I fibres in the muscle, i.e., setting $MHC_1 = 0$ in Equation 3. The force constant is the same for all fibres, since it shows no correlation with the isomyosins. V_{max} is computed by the ratio $b/(a/F_o)$. Table 5 summarizes the mechanical properties inferred for the two varieties of fibres.

The parameters of type I fibres are close to those observed in guinea-pig soleus, which is a muscle containing only type I fibres: the velocity constant in

Table 5 Force–velocity parameters of type I and type IIA(IM) fibres of mouse soleus (strain NMRI, 20°C).

Fibre type	b (*Lf*/s)	a/F_o	V_{max} (*Lf*/s)
Type I	0.24	0.11	2.2
Type IIA(IM)	0.85	0.11	7.7

soleus guinea-pig is b = 0.13 ± 0.02 fibre length/s, and its force constant is a/F_o = 0.10 ± 0.02 (2). Type IIA(IM) fibres have the subscript 'IM' to recall that their isomyosin is hybrid in its alkali light chains. The parameters of the force–velocity of type IIA fibres homogeneous in fast light chains 1 is not known, but we know those of a fast-twitch muscle that contains approximately 20% of type IIA fibres, 75% of type IIB fibres and 5% of type I fibres in mouse C57/BL extensor digitorum longus: b = 1.8 ± 0.2 fibre length/s, a/F_o= 0.23 ± 0.03 and V_{max} = 8.2 ± 0.2 fibre length/s (9). We conclude that the substitution of one fast light chain LC_{1f} in type IIA fast fibre by one slow light chain LC_{1s} in type IIA(IM) fibres does not change the maximal velocity of shortening but reduces both b and a/F_o. In molecular terms, this could mean that the alkali light chain does not influence the maximum ATPase activity of the crossbridges, but modulates the energy transfer from the ATPase site to the force-transducing site.

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