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Force-velocity relation and myosin isozymes of rat muscles

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Summary

The proportions of three myosin heavy chains in rat soleus and EDL are used to compute a triple linear regression which predicts correctly the maximal mechanical power and approximately the parameters b and a/Fo of the Hill's equations.

Introduction

Slow-twitch muscle as well as fast-twitch muscle contain several myosin isozymes in various proportions (review in Swynghedauw, 1987). Isomyosins have three kinds of heavy chains: myosin heavy chain 1 (MHC1), myosin heavy chain 2A (MHC2A) and myosin heavy chain 2B (MHC2B), which are combined with several forms of light chains. It is expected that the relative proportions of the three heavy chains control the mechanical parameters of the force-velocity relation. This expectation should be true not only when comparing slow-twitch with fast-twitch muscle, but also when comparing slow-twitch (or fast-twitch muscles) which differ by the relative proportions of myosin heavy chains. It is the purpose of this paper to check this expectation.

Methods

Soleus (SOL) or extensor digitorum longus (EDL) were dissected free from outbred Wistar rats 95 to 105 days of age. They were tetanically stimulated in an oxygenated Krebs solution at 20 ± 0.5°C every 3 minutes. Their length was controlled by an electromagnetic ergometer and recorded together with the force on a fast UV recorder (2). The length was adjusted at the start of the experiment so that the resting force was 5±2% of the maximum isometric force Fo; fiber length (Lf) was evaluated by microscopic measurements of a few superficial fibres. The force-velocity data were fitted to a Hill's hyperbola (F + a/Fo)(V + b) = b(1 + a/Fo), using an iterative least square method based on the method of Gauss-Newton (3). The maximum power, Pmax, was computed as $b.Fo(1 + 2a/Fo - 2\sqrt{[(a/Fo)^2 + a/Fo]})$. The myosin heavy chains of a crude extract in a Guba-Straub solution were separated by electrophoresis on glycerol-SDS-polyacrylamide gels (4); the zones were quantified by computerized densitometry. The statistical calculations were performed using the Statistical Package for Social Sciences (SPSS-PC+).

Results

1. The force-velocity relationship and the myosin heavy chains.

The muscle is isometrically stimulated for 0.5 s (for EDL) or 1.2 s (for SOL) and released (see inset of Fig. 1) at a constant velocity after a brief release (20 to 100 µm) at high velocity (70 mm/s) which discharges the series elastic elements (5). The tetanus are repeated 20 times every 3 min, using each time a different velocity of shortening. The force F maintained at the beginning of the release is referred to the isometric force Fo measured just prior to the release. The ratio F/Fo is plotted as a function of the velocity of release in Fig. 1, and the parameter a/Fo and b (in Lf/s) are given in Table 1. Fatigue, as measured by the decline in Fo between the first and the last tetanus is quite small for SOL (mean -2% Fo) and somewhat higher for EDL (mean -16% Fo).

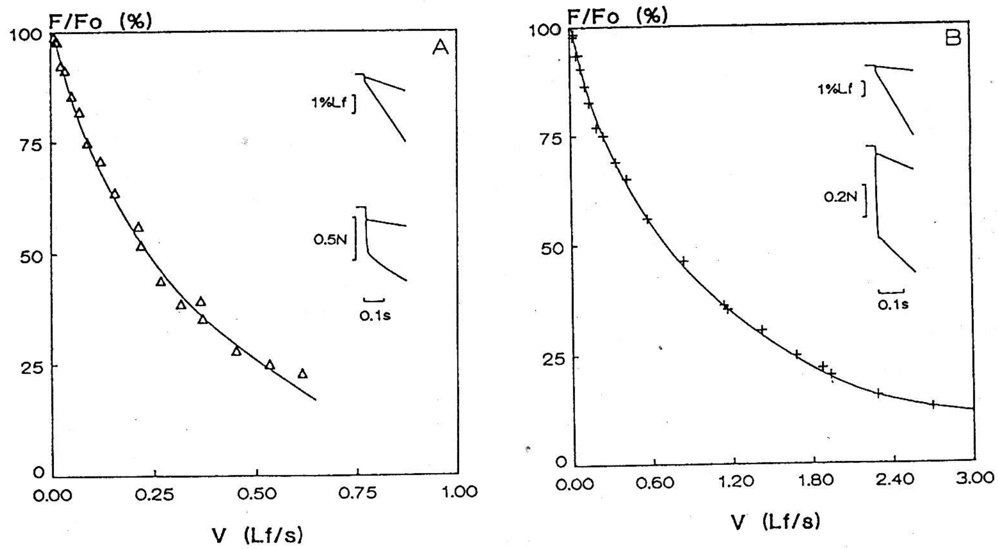

Fig. 1.- Force-velocity relations.
A. right soleus of a male rat 325 g of body weight, 97 days of age; muscle weight: 186 mg; fiber length: 21 mm; resting force: 3 g; maximal tetanic force: 154 g; proportions of myosin heavy chains (in % of total): MHC1: 95, MHC2A: 5; MHC2B: 0. The inset shows releases (upper curves: length; lower curves: force) at two velocities (0.05 and 0.25 Lf/s). The curve is an hyperbola: b = 0.46(±0.05)Lf/s; a/Fo = 0.43 (±0.10); residual variance: 118.
B. left EDL of a male rat 405 g of body weight, 99 days of age; muscle weight: 190 mg; fiber length: 22 mm; resting force: 4 g; maximal tetanic force: 107 g; proportions of myosin heavy chains (in % of total): MHC1: 0, MHC2A: 58; MHC2B: 42. The inset shows releases (upper curves: length; lower curves: force) at two velocities (0.05 and 0.63 Lf/s). The curve is an hyperbola: b = 0.97(±0.02)Lf/s; a/Fo = 0.16 (±0.01); residual variance: 28.

After the experiments the myosin heavy chains are separated and quantified (Table 1). MHC1 varies from 82 to 97% in SOL and is absent in EDL. MHC2A varies from 3 to 18% in SOL and from 26 to 58% in EDL. MHC2B varies from 0 to 3% in SOL and from 42 to 74% in EDL.

Table 1
Mechanical parameters and myosin heavy chains of rat skeletal muscle (means ± standard deviation)

	SOL (n=8)	EDL (n=7)
Weight (mg)	173 ± 35	148 ± 36
Lf (mm)	24 ± 1	30 ± 2
Fo (mN)	1073 ± 280	1176 ± 306
Stress (mN/mm^2)	124 ± 35	164 ± 15
b (Lf/s)	0.50 ± 0.13	0.74 ± 0.13
a/Fo	0.44 ± 0.19	0.12 ± 0.06
Pmax ($\mu W/mm^3$)	14 ± 1	38 ± 6
MHC1 (% total myosin)	92 ± 5	0
MHC2A (% total myosin)	7 ± 5	64 ± 11
MHC2B (% total myosin)	1 ± 1	36 ± 11

2. Correlations between mechanical and biochemical variables.

We observe a strong correlation (Fig. 2A; $r=0.96$; $P < 0.001$) between the Hill's parameters a/Fo and b for SOL, whilst those of EDL are less strongly correlated ($r=0.65$; NS). Examination of the theoretical curves computed for the case of SOL muscle showed that the differences between the curves are quite small, being much less impressive than would suggest the wide variation in their parameters (Fig. 2A). In EDL to the contrary, the rather small changes in the Hill's constants (Fig. 1A) correspond to sizeable changes of the force-velocity curve. This observation demonstrates that large changes in the <u>coupled</u> values of a/Fo and b do not necessarily reflect large changes in the shape of the force-velocity relation.

The linear correlations between mechanical and biochemical parameters are reported in Table 2. Since they are statistically significant and quite large, we may examine the hypothesis that a/Fo, b and Pmax are linear functions of the three myosin heavy chains:

$$Yz = b_1\,[MHC1] + b_{2A}\,[MHC2A] + b_{2B}\,[MHC2B] \qquad \text{(Eq 1)}$$

where Yz is the predicted variable and the b's are the coefficients of a multiple regression. Predicted variables bz and Pmaxz agree satisfactorily with the experimental variables b and Pmax (Fig. 2D, 2C,); the agreement is less good between az/Fo and a/Fo (Fig. 2B).

The correlations within muscles are weak or not significant, and, therefore, it is not possible to check whether the coefficients of the multiple regression (Eq. 1) are also valid for SOL and EDL considered separately.

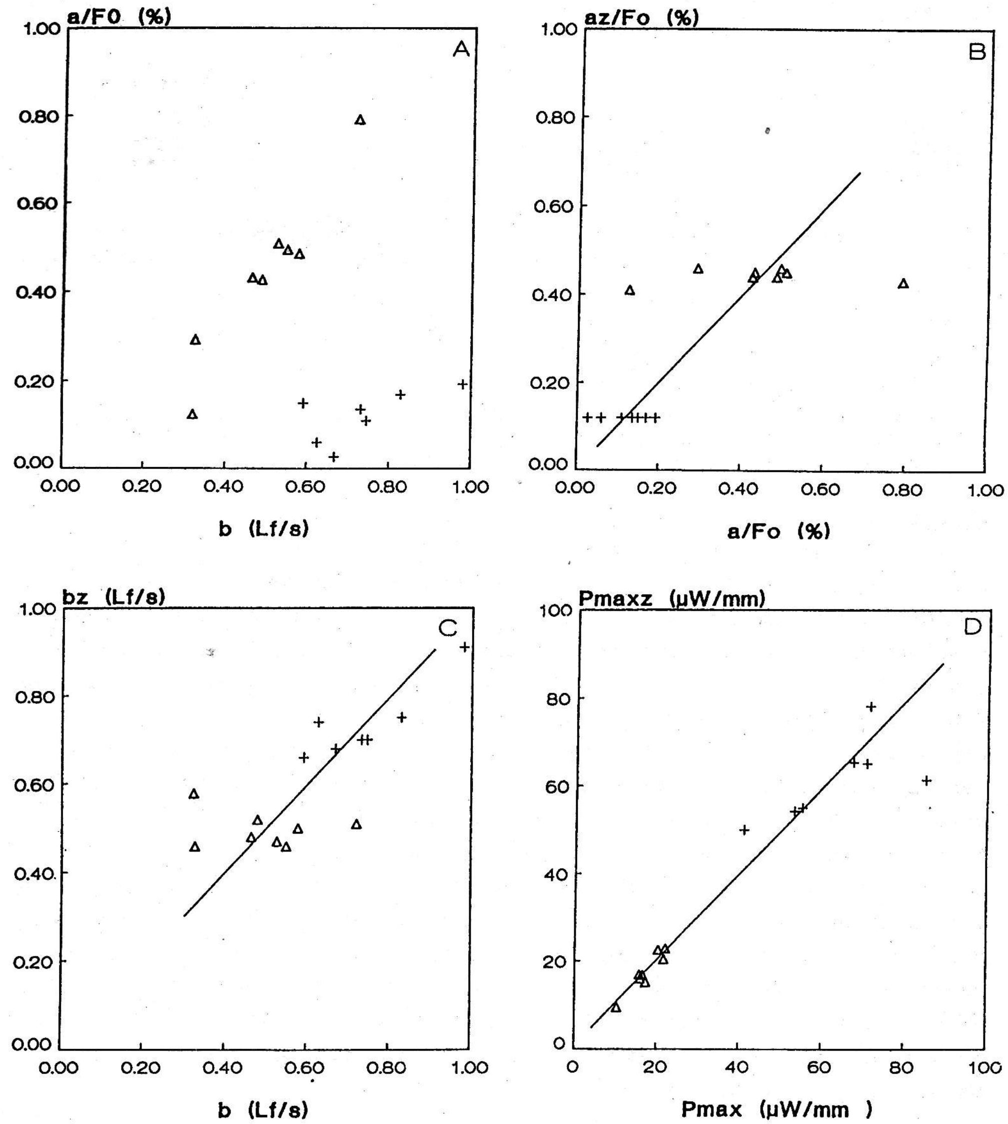

Fig. 2.- Correlations between mechanical and biochemical data.

A. Correlations between Hill's velocity constant b and the force constant a/Fo: they are strongly correlated for SOL (triangles) but weakly correlated for EDL (crosses).

B,C,D. Comparisons between the parameters predicted (az/Fo, bz, Pmaxz) using equation 1 (in ordinates) and the parameters experimentally measured (in abscissae). SOL: triangles; EDL: crosses. The linear coefficients of correlation are 0.774 (case B), 0.770 (case C) and 0.961 (case D). The expected result is shown by the identity lines of slope 1.

Table 2

Linear correlation coefficients between Hill's parameters and the myosin heavy chains.

	a/Fo	b	Pmax
MHC1	0.77**	0.70*	-0.95**
MHC2A	-0.69*	0.77**	0.88**
MHC2B	-0.76**	0.61*	0.91**

Coefficients of the multiple linear regression (Eq 1)

	a/Fo (%)	b (Lf/s)	Pmax ($\mu W/mm^3$)
b1	0.005 ± 0.001	0.005 ± 0.001	0.13 ± 0.05
b2A	0.001 ± 0.003	0.012 ± 0.003	0.75 ± 0.25
b2B	0.001 ± 0.002	0.005 ± 0.002	0.57 ± 0.15
F	25**	129**	85**

Probability: *: < 0.01; **: < 0.001. Data of SOL and EDL are confounded (n=15). F: F-test for multiple linear regression (degrees of freedom: 3 for regression; 12 for residual).

Discussion

We have shown in this paper that it is possible to construct a triple linear regression which allows to predict the mechanical parameters of the Hill's equation (a/Fo, b) and the maximal mechanical power (Pmax) as a function of the relative proportions of the three adult myosin heavy chains (MHC1, MHC2A, MHC2B). The exactness of the fit may be estimated from the square of the coefficient of linear correlation, which is an approximate measure of the proportion of the variable variance "explained" by the multiple regression. Thus, 92% of the variance of the maximal mechanical power (Pmax) is accounted for by the varying proportions of the myosin heavy chains, whilst this latter accounts for only 59% of the variance of the Hill's parameter a/Fo and b: the content in myosin light chains is a likely complementary factor which might account for the variance unexplained by the myosin heavy chains. These facts suggest an interesting hypothesis which could be tested by further experiments, namely that myosin heavy chains control the maximal mechanical power whilst myosin light chains control the shape of the force-velocity relationship.

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