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MECHANICAL POWER AND ISOMYOSINS IN TRANSPLANTED SOLEUS MUSCLE OF MICE

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Following transplantation, the grafted muscle first degenerates to some extent and then undergoes regeneration (review in 5). Until now, graft recovery is mainly evaluated in terms of maximal isometric force and maximal velocity of contraction. However, in normal life, the animal does not usually contract its muscles at maximal isometric force or maximal velocity. Maximal mechanical power is a more physiological parameter. Therefore, we have used it to evaluate the effect of transplantation on muscle tissue. To this end, we have studied the correlation between isomyosin composition and each of the 3 factors that determine maximal mechanical power (see below).

Methods.

Donor mice of the C57BL/10 strain were killed by cervical-dislocation. Soleus muscles were dissected with a maximum length of tendons and grafted orthotopically in anesthetized host mice of the same age (± 60 days) by tying the tendons from the grafted muscles to host ones. 60 days post-surgery, transplanted and contralateral muscles (which served as control) were dissected and mounted on an electromagnetic puller. Muscle length was adjusted for a resting tension of 5 ± 1 mN. The force-velocity relationship was obtained from 19 to 22 tetani with iso-velocity releases (2). Velocity and force constants (b and a) were computed according to Hill's hyperbolic equation. Maximal mechanical power was computed with the equation described by Maréchal and

Beckers-Bleukx (3):

$$Pmax = So * b * f(a/So) \qquad \text{(Eq. 1)}$$

where So is maximum isometric stress, b is velocity constant and f(a/So) is a function of the force constant. After mechanical measurements, the muscles were frozen in liquid nitrogen. The myosins were extracted at 0 °C in a pyrophosphate containing Guba-Straub solution. Isomyosins were separated by non-denaturating PPi-PAA gel electrophoresis. The myosin heavy chains were separated by the method of Carraro and Catani (1).

Results and discussion.

Table 1 gives the mechanical parameters of control and transplanted muscles.

Table 1. Mechanical properties of control and transplanted soleus muscle of mice.

	Control	(n=7)		**Transplant**	(n=9)
Variables	**Mean**	**SEM**		**Mean**	**SEM**
So (mN/mm^2)	119.6	8.3	***	70.5	8
b (Lf/s)	0.51	0.03	***	1.06	0.11
a (% So)	0.09	0.02		0.088	0.024
Pmax ($\mu W/mm^3$)	34.1	3.3		45.7	7.7

So = maximal isometric stress; **b** and **a** are the velocity and force constants, computed from Hill's hyperbolic equation; **Pmax** is the maximal mechanical power. *** $P < 0.001$.

Table 1 shows that transplantation induces a significant decrease of the stress, a significant increase of the velocity constant b and no change of the force constant a. However, the maximal mechanical power as defined by Eq. 1 is not affected by transplantation.

The results of chemical analyses given in table 2 indicate a decrease in the amount of myosin heavy chain 1 (MHC1) and a slight increase of myosin heavy chain 2A (MHC2A) as a result of grafting.

Table 2. Myosin heavy chains and isomyosins in control and transplanted soleus muscles of mice.

	Control			Transplant	
MHC (% of total)	**Mean (n=7)**	**SEM**		**Mean (n=9)**	**SEM**
MHC1	40.4	3.2	***	16.7	4.2
MHC2A	59.6	3.2		64.5	5.8
MHC2B	0	0		18.8	6.6
Isomyosins (% of total)	**Mean (n=6)**	**SEM**		**Mean (n=5)**	**SEM**
SM2	40.3	4.2	***	9.7	4.8
SM1	0	0		6.9	5.4
IM	59.7	4.2	*	26.4	14.5
FM3	0	0		21.5	6.5
FM2	0	0		28.3	10.7
FM1	0	0		7.2	3.1

* $P < 0.05$ *** $P < 0.001$.

Furthermore, transplanted muscles contain a significant amount of myosin heavy chain 2B (MHC2B) which is absent in control muscles. The lower part of table 2 shows that grafted muscles contain significantly reduced amounts of isomyosins SM2 and IM together with isoforms that are not present in control muscles: the slow isoform SM1 and the fast isoforms FM3, FM2, FM1.

The MHC contents of control and transplanted muscles have been plotted against So (Fig. 1). It appears that So is strongly correlated with the percentage of MHC1 ($r = 0.711$; $P<0.002$). Thus the decrease in maximal isometric stress in transplanted soleus might be explained by a lower percentage of MHC1.

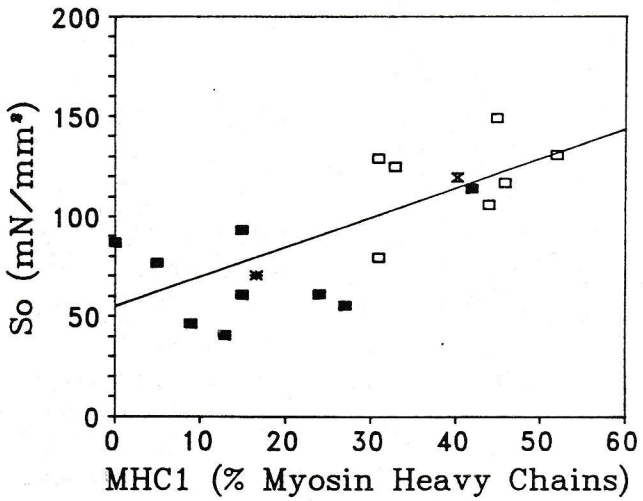

Fig. 1. Maximum isometric stress (So) as a function of the proportion of MHC1. Filled squares (■): transplanted muscles; Open squares (□): control muscles. ★ Mean of transplanted muscles; ⧖ Mean of control muscles.

Previously, Maréchal and Beckers-Bleukx (3) have shown that the velocity constant is also correlated with the percentage of MHC1. Here we find (Fig. 2.) that, while the control muscles fit the regression line calculated by Maréchal and Beckers-Bleukx (communication at this meeting) the transplanted soleus display a higher velocity constant than expected based on their MHC1 contents.

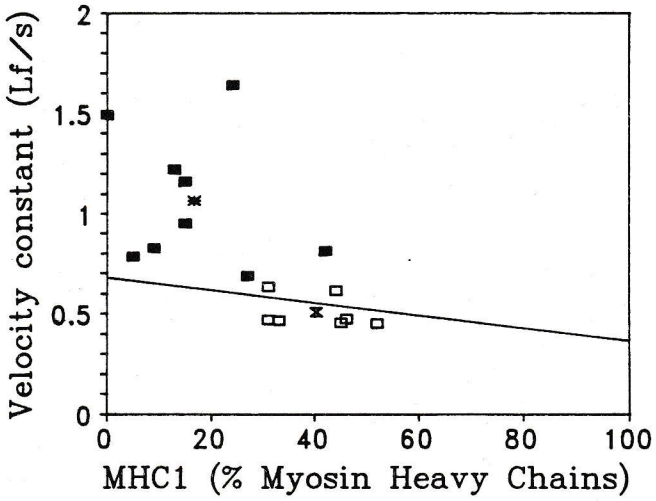

Fig. 2. Velocity constant (calculated according to Hill's equation) as a function of the percentage of MHC1. Filled squares (■): transplanted muscles; Open squares (□): control muscles. ★ Mean of transplanted muscles; ⧖ Mean of control muscles.

This increase of the velocity constant (b) could be caused by the presence of MHC2B (see table 2). However, we found no correlation between b and the percentage of MHC2B (not shown). When we compared the proportions of MHC with

isomyosins, we found that in control muscle, the percentages of MHC1 and MHC2A were close to those of SM2 and IM respectively, as was demonstrated earlier (4). In transplanted muscles, although the percentage of MHC1 is close to that of isomyosins containing MHC1 (SM2 + SM1), the percentage of MHC2A is higher than that of IM. This additional component, absent in control soleus muscles, could be evaluated by subtracting the amount of MHC2A present in IM (hereafter called MHC2As) from the total amount of MHC2A. This additional component (MHC2A-MHC2As) migrating as MHC2A could be either MHC2A or MHC2d.

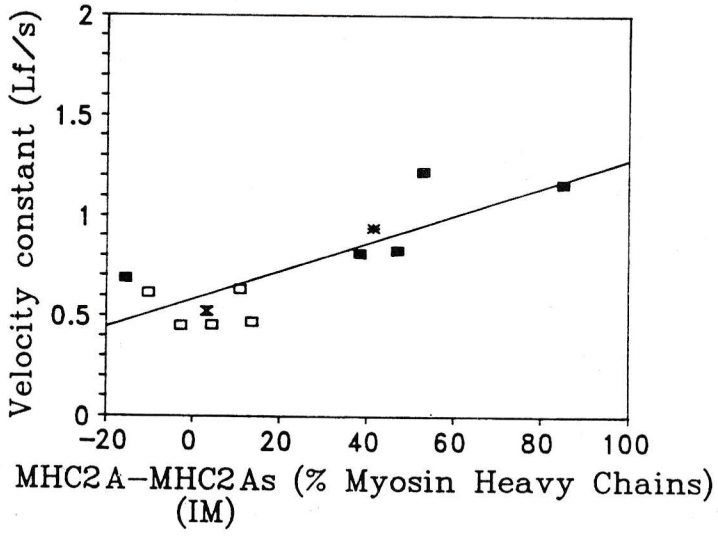

Fig. 3. Velocity constant (calculated according to Hill's equation) as a function of the percentage of MHC2A subtracted from the percentage of MHC2A present in isomyosin IM. Filled squares (■): transplanted muscles; Open squares (□): control muscles. ★ Mean of transplanted muscles; ⧖ Mean of control muscles.

Figure 3 shows that this MHC (MHC2A-MHC2As) is strongly correlated with the velocity constant ($r=0.843$; $P<0.001$). Extrapolation to 100% myosin heavy chain gives the value of $b=1.3$ Lf/s. This value of b could account for almost the entire shift, with respect to their controls, of transplanted muscles towards muscles with faster isomyosins.

Conclusions.

60 days after grafting, transplanted soleus muscles develop maximal mechanical power equal to that of normal muscles. A decrease in isometric stress is balanced by an increase of the contraction velocity of the muscle. However, the velocity at which this power is produced is probably different in transplant and normal muscles. The maximal isometric stress in transplanted muscles is lower than in control muscles: to maintain its maximal power, the strategy developed by the muscle seems to be a modification in the myosin expression as a means to modulate the velocity of contraction and compensate for the decrease in stress.

References.

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