THE EFFECT OF CROSS REINNERVATION ON THE SYNTHESIS OF MYOSIN LIGHT CHAINS*

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Received November 5, 1973

Summary: The reciprocal physiological changes produced in the exterior digitorum longus and soleus muscles upon cross reinnervation are accompanied by changes in the light chain pattern of myosin. These changes are indicative of the synthesis of polypeptide chains characteristic of fast muscle myosin in soleus and slow muscle type chains in exterior digitorum longus.

Cross union of the nerves of slow (soleus) and fast (extensor digitorum longus) muscle of the rat produces a reciprocal change in the intrinsic speed of shortening, force-velocity relation and myosin ATPase activity of these muscles (1-3) and provides further support for the good correlation described earlier between shortening speed and myosin and actomyosin ATPase activity (4). Since slow and fast muscle myosin differ in their light chain complement - 3 light chains in fast, 2 in slow muscle - it was of interest to determine whether the change in myosin ATPase is also accompanied by changes in the subunit structure.

*This work was supported by grants from the National Institutes of Health (HL-5949, HD-06203), the National Science Foundation and the Muscular Dystrophy Associations of America, Inc.
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METHODS

The nerve cross-union operations and the method of determining the physiological characteristics were essentially those described by Close (3). Operative cross-union of nerves to extensor digitorum longus (EDL) and soleus (SOL) muscles was performed on 3-week-old female Wistar rats. Simple cross-union of nerves was carried out as described by Close (3) except that the nerves to anterior tibialis and extensor hallucis longus were reflected and tied back over the thigh; this procedure has been shown to prevent re-innervation of the EDL by "fast" nerve fibers from peroneal nerve (1). The cross-union was always carried out on the right limb; in some animals the EDL and SOL nerves of the left limb were transected and resutured.

The procedure for setting up the preparation and the methods of recording were similar to those described before (1,3). The muscles were carefully checked for the purity of their innervation and,in the case of soleus, for the presence of the "accessory nerve" (1). Muscles with mixed innervation were discarded.

Muscles innervated solely by one nerve were excised and the length of the superficial fibers determined with the muscle held at optimal length. The tendons were cut off near the ends of the fibers, and the muscles blotted, weighed and immediately frozen in liquid nitrogen. The muscles were stored individually in small polyethylene bags between -16°C and -24°C and finally transported by air in dry ice from Canberra to Boston.

Myosin was extracted from pooled samples (normal EDL and SOL, self-innervated EDL and SOL and cross-innervated EDL and SOL) and purified by DEAE cellulose chromatography for SDS-polyacrylamide gel electrophoresis as described earlier (5).

RESULTS

Table I summarizes some properties of the muscles of the normal

Table I. Parameters of Normal, Self-reinnervated and Cross-reinnervated Muscles

Muscle	Days after operation	Body weight (g)	Muscle weight (mg)	F_l (mm)	T_c (msec)	$T_{1/2R}$ (msec)	P_t (g)	P_o (g)	P_t/P_o	P_tF_l/M (kg/cm2)	P_oF_l/M (kg/cm^2)
N-EDL (8)	363 ±10	234.1 ±22.6	115.6 ±9.5	12.6 ±1.0	14.20 ±0.56	9.00 ±0.58	49.55 ±4.56	272.0 ±39.6	0.184 ±0.020	0.543 ±0.078	2.955 ±0.338
S-EDL (8)	378 ±7	228.5 ±16.5	117.2 ±15.7	15.6 ±1.3	13.57 ±0.94	10.57 ±1.59	33.56 ±7.28	184.7 ±53.0	0.187 ±0.030	0.479 ±0.059	2.662 ±0.315
X-EDL (10)	375 ±11	233.0 ±17.6	56.2 ±6.1	15.5 ±0.9	24.84 ±6.01	34.19 ±6.01	19.20 ±4.88	68.1 ±14.9	0.283 ±0.032	0.528 ±0.118	1.880 ±0.391
N-SOL (8)	377 ±41	238.4 ±17.6	108.3 ±10.6	15.6 ±0.5	37.78 ±2.70	58.06 ±5.98	37.65 ±5.77	177.9 ±19.8	0.211 ±0.015	0.544 ±0.066	2.566 ±0.177
S-SOL (5)	377 ±7	224.2 ±13.9	82.1 ±5.5	16.8 ±0.8	45.83 ±3.64	66.63 ±3.64	30.28 ±6.24	131.0 ±12.1	0.230 ±0.030	0.609 ±0.082	2.660 ±0.089
X-SOL (13)	377 ±32	232.9 ±17.2	90.4 ±18.1	20.8 ±1.1	16.19 ±0.81	17.55 ±1.38	18.38 ±3.40	136.4 ±27.6	0.136 ±0.010	0.410 ±0.033	3.027 ±0.198

Mean values ±S.D. of measurements on normal (N-EDL, N-SOL), self innervated (S-EDL, S-SOL) and cross-innervated (X-EDL, X-SOL) extensor digitorum longus (EDL) and soleus (SOL) muscles. Numbers in parentheses indicate the number of muscles. All measurements were made at 35°C and at the optimal length for the isometric twitch. Abbreviations used in the column headings are as follows: F, fiber length; T_c and $T_{1/2R}$, isometric twitch contraction and half-relaxation times, respectively; P_t, maximum isometric twitch; P_o, tetanic tension; M, muscle weight.

and operated animals. It is important to note that the changes in these properties resulting from cross-innervation are essentially similar to those described earlier (3) in muscles in which both force: velocity and isometric characteristics were determined. It will be assumed that the force:velocity properties of the muscles used in the present work were the same as those of the muscles used in earlier work (3). Some of the small differences between these results and those reported earlier (3) can be attributed to the smaller body weight and corresponding smaller muscle weights in the present series. On SDS gel electrophoresis (Fig. 1) the pattern of the self innervated muscles was indistinguishable from the controls (not shown). EDL myosin shows the three light chains (LC) typical of fast muscle while the soleus myosin contains two light chains which move at speeds similar to, but different from, those of LC_1 and LC_2 of fast muscle myosin. Cross innervation of EDL causes the appearance of a band corresponding to LC_1 of soleus, and the LC_2 and LC_3 bands become very weak. Cross innervation of soleus causes the appearance of the fast type muscle LC_1, the disappearance of its LC_2 without replacement by fast type LC_2, and a faint band corresponding to LC_3. Thus both cross-reinnervated muscles show a double LC_1 band and the presence of LC_1 in both the slow and fast types of myosin. Identification of the various light chains is based after cross-reinnervation on their mobility determined according to Weber-Osborne and comparison with control soleus and EDL pattern (6).

These experiments show that the transformation of ATPase activity from the slow to the fast type, and vice versa, following cross-union of the nerve, is accompanied by a change in the subunit structure of the protein. An earlier indication of this type of change was obtained by Samaha, Guth and Albers, although the electrophoresis technique used at that time did not permit a clear cut distinction among light chains of different origins (7).

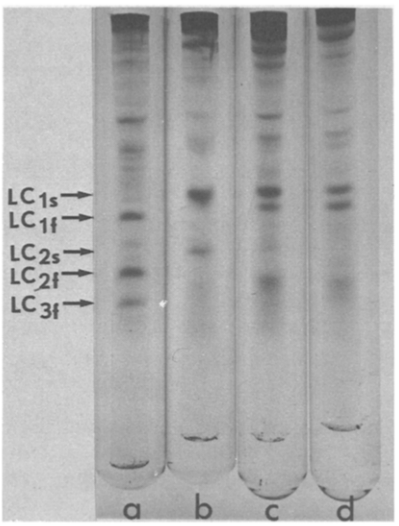

Figure 1. SDS-polyacrylamide gel electrophoresis patterns of self reinnervated EDL (a), soleus (b), cross reinnervated EDL (c), and soleus (d) myosins. Gels containing 10% polyacrylamide, 0.1% SDS sodium dodecyl sulfate (SDS) were prepared and stained with Coomassie blue according to Weber and Osborn (6). Electrophoresis was carried out with a solution containing 0.1M sodium phosphate (pH 7.0) and 0.1% SDS at a constant current of 8 mA per tube for four hours. The amount of protein applied to each gel was 40 μg. The arrows indicate the positions of the fast (f) and slow (s) light chains.

It is of interest that crossing the nerve of a slow muscle to a fast one produces changes that are in part identical with those obtained on chronic stimulation of a fast muscle (8). While, however, stimulation produces slow muscle LC_1 and LC_2 in what appear to be similar amounts, innervation of the fast muscle with the slow nerve leads to the appearance of slow muscle LC_1 only. In the light of previous results on the transformation of the myosin ATPase, this would mean that fully active myosin molecules are formed while the light chain complement contains only one moiety. Since it has been suggested that slow muscle myosin contains 4 light chains per mole, a further implication of this finding is the existence of myosin molecules in which each heavy chain is associated with pairs of the

same light chains. The difference in the light chain pattern following chronic stimulation and cross innervation lends support to the view that, in addition to changes in the activity pattern that can equally be produced by cross innervation or stimulation, there may be specific stimuli attributable to the nerve in determining the expression of genes in muscle.

References

1. Barany, M. and Close, R.I., J. Physiol. 213, 455-474 (1971)
2. Buller, H.J., Mommaerts, W.F.H.M. and Seraydarian, K., J. Physiol. 205, 581 (1969)
3. Close, R., J. Physiol. 204, 331-346 (1969)
4. Barany, M., J. Gen. Physiol. 50 (suppl., part 2) 197-218 (1967)
5. Sarkar, S., Sreter, F.A. and Gergely, J., Proc. Nat. Acad. Sci. (U.S.) 68, 946-950 (1971)
6. Weber, A. and Osborn, M., J. Biol. Chem. 244, 4406 (1969)
7. Samaha, F.J., Guth, L. and Albers, R.W., Experimental Neurology 27, 276-282 (1970)
8. Sreter, F.A., Gergely, J., Salmons, S. and Romanul, F., Nature (New Biol.) 241, 17-19 (1973)