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A Model Preparation for Studying Fast Mammalian Skeletal Muscles (39871)

ROGER McCARTER,[1] DEBORAH RADICKE, AND BYUNG P. YU

Audie Murphy Veterans Administration Hospital, and Department of Physiology, The University of Texas Health Science Center, San Antonio, Texas 78284

Introduction. Characterizing the contractile properties of the different types of mammalian skeletal muscle fibers is greatly complicated by the absence of suitable preparations. Particular problems are that most mammalian skeletal muscles are composed of fibers of more than one type (1) and that the size of the muscle becomes limiting in other than young animals. The properties of slow-twitch fibers and of red, fast-twitch fibers have been measured using muscles composed mainly of these fibers (2, 3) but, to date, no suitable preparation has been described for characterizing the properties of white, fast-twitch fibers.

The purpose of the present report is to describe dynamic and metabolic properties of the lateral branch of the omohyoideus muscle of the rat, a preparation composed entirely of white, fast-twich fibers. In addition, the small size and ideal geometry render it a unique preparation for studying the contractile properties of fast-twitch fibers in rats of all ages.

Materials and Methods. Male SPF rats of the Fisher 344 strain, obtained from Charles River Breeding Laboratories, Inc., at 28 days of age, were fed ad libitum and were kept behind a barrier until sacrificed at 6 months of age.

The rats were killed by guillotining across the base of the skull. The omohyoideus muscle runs bilaterally from the hyoid bone to the anterior border of the scapula. The entire omohyoideus, hyoid bone, and a section of the anterior border of the scapula were freed of connective tissue and the resting length (RL) of the lateral branch was measured with the rat lying in a horizontal position on its back. The lateral branch was separated from the medial belly by blunt dissection, starting in the clear region at the hyoid bone. Steel yokes were attached to the remaining fragments of the hyoid bone and scapula and the preparation was then placed in a modified mammalian Ringer solution [millimolar concentrations: NaCl, 115.0; KCl, 5.0; $MgSO_4$, 0.65; NaH_2PO_4, 1.20; NaH_2CO_3, 25.0; $CaCl_2$, 2.50; glucose, 11.0; sodium acetate, 10.0; osmolarity = 300 mOsm; pH = 7.4] bubbled with 95% O_2, 5% CO_2, and allowed to equilibrate for 30 min. One of the muscles from each rat was used for the measurement of oxygen consumption and the other for dynamic studies. All experiments were conducted at room temperature (21°).

Mechanical properties were measured following the methods of Jewell and Wilkie (4), modified so as to allow simultaneous measurement of sarcomere spacings, using a 5 mW helium–neon gas laser (Spectra-Physics Model 120). Muscles were mounted on a rigid stainless-steel frame in a Lucite chamber with optically flat sides. Sarcomere spacings could be obtained in a given region of the muscle at any time during the course of an experiment by measurement of the spacing of the first-order lines of the optical diffraction pattern on a screen placed behind the muscle (5). The distal yoke on the muscle was connected to a tension transducer (Grass FT 03C) or to a light aluminum lever system (equivalent mass 500 mg, lever ratio 97:1) by means of prestressed silver chain.

Muscles were stimulated using a multielectrode platinum plate assembly, mounted along the length of the muscle and on either side of it. Supramaximal voltage pulses of 4-msec duration were delivered at 90 Hz for 300 msec in recording maximum tetanic tension. Muscles were mounted at just below the resting length (RL) and successive stretches were applied in determining the

[1] Send reprint requests to Dr. Roger M. McCarter, Department of Physiology, The University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, Tex. 78284.

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isometric length-developed tension diagram. Resting and active sarcomere spacings were measured by photographing the optical diffraction pattern before a contraction and during the plateau of the isometric tetanus. Following the isometric measurements and the establishment of the muscle length (L') corresponding to maximum developed tension, the muscle was connected to the lever system (at L') for a series of load-velocity measurements and quick-release measurements.

Measurements of the rate of resting oxygen consumption were made using standard polarographic techniques (6). The muscles were mounted in a glass chamber (volume 6 ml) at the resting length. Rapid stirring was achieved by a barrier resin (Monsanto L-600)-coated Teflon spin fin (Nalge 6600) and consumption was measured using a Clark-type electrode (Yellow Springs Instrument Co., Model 53).

At the conclusion of the experiments the muscles were blotted several times and weighed. Some preparations were immersed in isopentane (at liquid nitrogen temperature) for rapid freezing and histochemical examination following the methods of Brooke and Kaiser (7). Others were fixed at constant length in a cacodylate-buffered 2.5% glutaraldehyde solution for subsequent counting of fiber numbers (from photographs of thick sections stained with methylene blue) or for electron microscopy of thin sections following standard methods (8).

Results. Results are reported as mean ± standard error, with the number of observations given in parentheses.

Morphology. The average body weight of the rats was 339 ± 27 g (n = 8). The average blotted weight of the lateral omohyoideus (LOMO) muscle was 26 ± 5 mg (n = 8) and the average resting length of the muscle in the body was 2.5 ± 0.2 cm (n = 8). The average number of fibers per muscle was 328 ± 10 and the average fiber diameter was 47 ± 2 μm (n = 200).

The histochemical analysis showed remarkable uniformity of fiber composition (Fig. 1). The fibers exhibited high myofibrillar ATPase staining at pH 9.4 and low staining at pH 4.6. Staining for phosphorylase and NADH tetrazolium reductase showed the fibers to have high glycolytic enzyme activity and low oxidative enzyme activity.

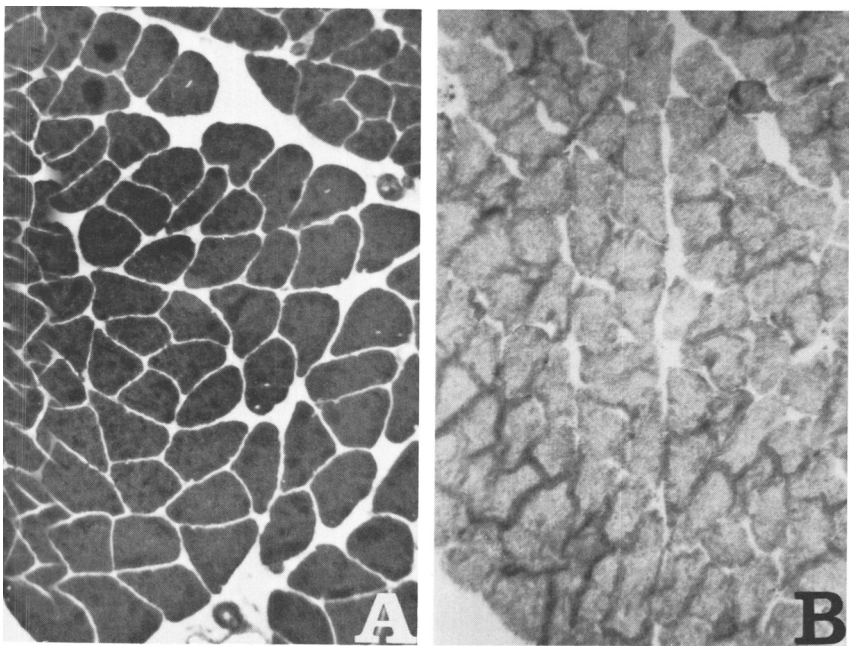

FIG. 1. Transverse section of LOMO muscle, showing uniform staining of fibers. (A) ATPase, pH = 9.4. (B) NADH diaphorase. ×100.

Electron microscopy revealed the usual lattice of thick and thin filaments. Allowing a 15% correction factor for shrinkage during preparation of longitudinal sections, the average lengths of thick and thin filaments were 1.5 and 1.1 μm, respectively. However the absence of well-defined H zones in longitudinal sections indicates either irregular arrangement of the thin-filament lattice or variable thin-filament lengths.

Mechanical properties. Maximum tension (P_0') was developed at lengths (L') slightly greater than the measured resting length *in situ*. This tension was 2.3 ± 0.5 kg/cm^2 (n = 5) and the twitch/tetanus ratio was 0.17 ± 0.02 (n = 5) at L'. Figure 2 illustrates the movement of sarcomeres in the middle of the muscle in an isometric tetanus at different initial lengths. The sarcomere spacing (SS) during the plateau of an isometric tetanus is plotted as a function of the initial (resting) SS. The solid line represents no movement of sarcomeres during contraction. As can be seen, there was a fairly uniform shortening of sarcomeres of about 0.15 μm for most muscle lengths. Since the SS at the optimal length (L') was in the range of 2.8–3.2 μm, the shortening at L' corresponded to a stretching of some series elasticity by about 5%. Figure 3 illustrates the typical behavior encountered in stretching a muscle from below the resting length, measuring initial sarcomere spacing and developed tension.

As can be seen from Fig. 4, the compliance of the series elastic component is nonlinear, except for the lower loads (0–0.2 P_0') where the compliance has a value of 0.13 L_0/P_0', or about 0.02 cm/g. Extrapolation of the results of Fig. 4 gives an expected extension of the series elasticity at maximum isometric tension of approximately 6% L'. The correction applied by Bahler (9) to allow for the effects of inertia of the lever system would have the effect of increasing the maximum extension of the series elasticity to approximately 7% L'.

Figure 5 illustrates the results obtained in force–velocity measurements, with velocity of contraction normalized to the length of the contractile component of the muscles (obtained by subtracting the estimated length of the series elastic component from total muscle length, for a given load). Hill's classic equation (10) does not fit the data. For loads greater than 0.5 P_0', the observed velocity was always greater than values to be expected from the above equation. The extrapolated maximum velocity of unloaded shortening was 7.5 contractile element length/sec. This corresponds to a relative rate of movement of the thick and thin filaments past each other of about 11 μm/sec.

Resting oxygen consumption. Rates of oxygen consumption were determined with the glass chamber filled with the modified Ringer solution at a given oxygen tension, usually about 60% saturated with O_2. The

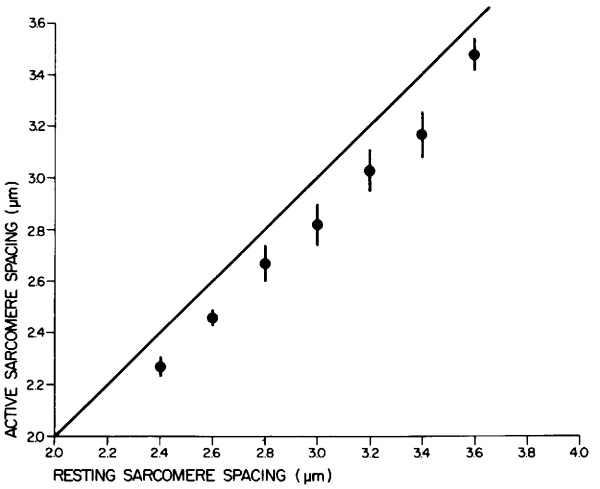

FIG. 2. Sarcomere spacing during plateau of isometric tetanus vs initial (resting) sarcomere spacing (n = 10).

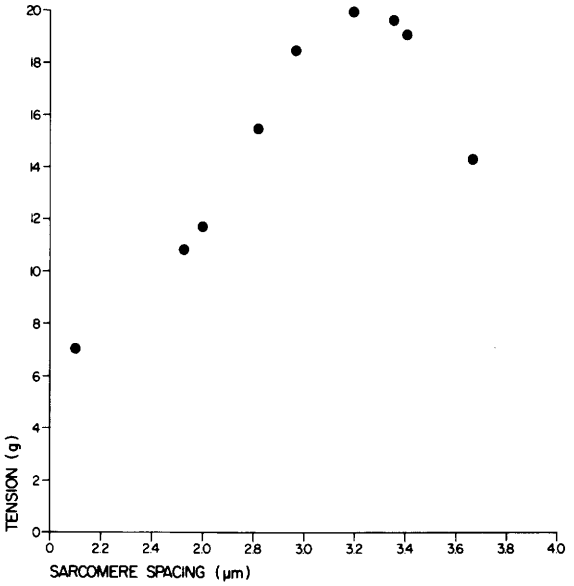

FIG. 3. Variation of active (tetanic) tension with initial sarcomere spacing. Experiment of 6-22-76. Blotted muscle mass, 29.0 mg.

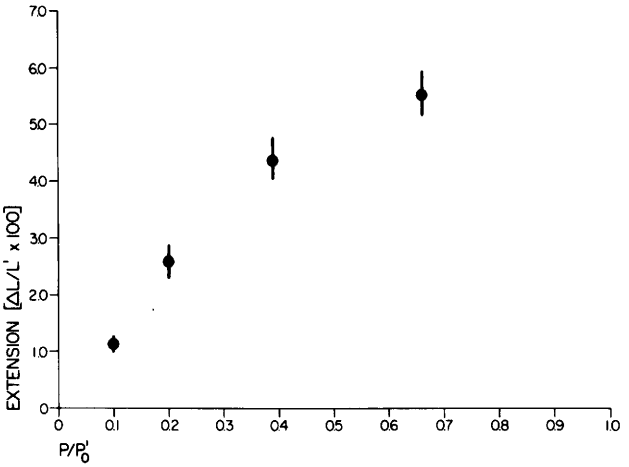

FIG. 4. Extension of series elastic component as a function of load. ΔL = change of length; L' = optimal length; P = load-producing extension; P_0' = maximum isometric tension. n = 5.

rate of resting oxygen consumption was 234 ± 18 μl/g/hr for the muscles and 80 ± 8 μl/g/hr for the bones attached to the muscles (n = 6).

Discussion. On functional and histochemical grounds the LOMO muscle can be classified as consisting of fast-twitch, white muscle fibers or Group IIB fibers (1). The preparation has geometrical simplicity: parallel fibers running from end to end with short tendons connecting these to the bones (the clear separation of lateral and medial branches was seen in rats of the Fisher strain but not in the few Wistar and Sprague-Dawley animals examined). The preparation is sufficiently thin (usually less than 0.5-mm thickness) and free of connective tissue and transverse blood vessels that good optical diffraction patterns can be obtained. The relative absence of connective tissue is manifested in the extremely low resting tensions observed at even high degrees of stretch.

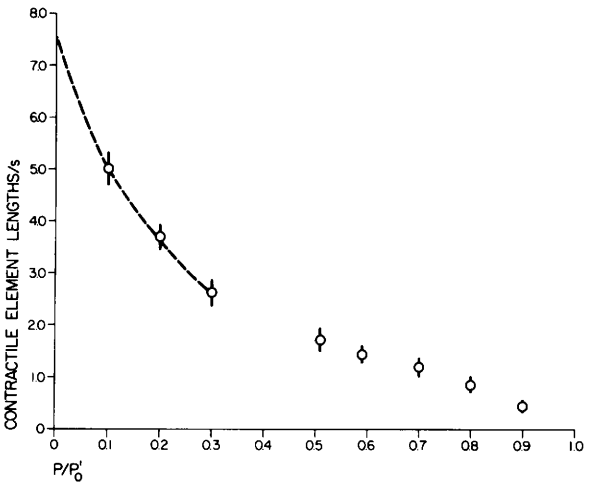

FIG. 5. Force-velocity curve (n = 10). The broken curve shows the extrapolation used to determine V_{max}. Load (P) is expressed realtive to the maximum isometric tension (P_0').

The thin preparation also allows rapid diffusion of oxygen into the interior for the measurement of oxidative metabolism.

The muscles developed 2.3 kg/cm^2 maximum tension at initial sarcomere spacings in the range 2.8–3.2 μm. Since the final contracted length of the sarcomeres at L' was still >2.3 μm, the data indicate that the simple optimal overlap of thick and thin filaments, which determines the length for maximum tension in frog muscle, is not present (11). A possible explanation is that the thin filaments are indeed of variable length, or arranged irregularly, so that the optimal length represents a statistical optimization of overlap of thick and thin filaments in sarcomeres in different parts of the muscle.

Values for the series elasticity of about 7% L' agree with results obtained for other mammalian muscles (1). Since the measured shortening of the sarcomeres in the middle of the muscle amounted to about 5% of their initial values, greater contraction must have taken place in other parts of the muscle to account for the higher value measured for the whole muscle. This presumably resides in sarcomeres in other parts of the muscle which shorten more, indicating a nonuniform behavior of sarcomeres during isometric contraction at the resting length.

It was surprising that the measured values of load and velocity did not conform to the hyperbolic relation (10) found to apply to so many other skeletal muscles. However, similar behavior has been reported for frog sartorius muscles (12) and is certainly found in papillary muscles (13). In view of the simplicity of the present preparation, the phenomenon may be more widely applicable to muscles in general than is currently thought, rather than the usually assumed hyperbolic behavior (1).

The value obtained for the rate of resting oxygen consumption is similar to that reported for the rat extensor digitorum longus (EDL) muscle at high oxygen tension (6, assuming a Q_{10} of 2.0). This implies that the rates of resting metabolism of the red and white fast-twitch fibers are similar, since the EDL is of mixed fiber type (1).

Some of the above results have been obtained in one mammalian preparation or another. The LOMO muscle, however, has the considerable advantage of being sufficiently thin that measurements of oxygen consumption and dynamic sarcomere movements, as well as the classical mechanical studies, can be carried out on the same preparation without using muscles from very young animals only. The parallel arrangement of the fibers and the uniformity of fiber type also greatly simplify the interpretation of the results obtained.

Summary. The use of the lateral branch of the omohyoideus muscle of the rat as a

model fast-twitch preparation for rats of all ages is described. Uniform fiber type, simple muscle geometry, and small thickness permit measurement of mechanical properties, metabolic properties, average sarcomere movements, and unequivocal interpretation of the results obtained.

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