TECHNIQUE FOR STABILIZING THE STRIATION PATTERN IN MAXIMALLY CALCIUM-ACTIVATED SKINNED RABBIT PSOAS FIBERS

BERNHARD BRENNER
Institute of Physiology, University of Tübingen, D-7400 Tübingen, Federal Republic of Germany and the Laboratory of Physical Biology, National Institute of Arthritis, Diabetes, and Digestion and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205

ABSTRACT A procedure was developed for stabilizing the striation pattern of fully activated skinned rabbit psoas fibers. Inhomogeneities of the striation pattern that develop during the rise in tension and also during isometric steady state are completely reversible by this procedure. The striation pattern can be stabilized for ~2 h. During this period isometric tension and speed of isotonic shortening decrease <10–20% of their initial values.

INTRODUCTION

An important prerequisite for doing physiological experiments on skinned muscle fibers is to keep the sarcomere pattern aligned and stable. This is critical for all parameters that are affected by filament overlap. In skinned muscle fibers, there is a tendency for the striation pattern to deteriorate or even to disappear within seconds of full activation. This is probably due to the development of inhomogeneity of sarcomere length. In the past, two methods were used to deal with this problem. In one, the preparations were activated for as short a time as possible, allowing only a single measurement before relaxation (Hellam and Podolsky, 1968; Julian, 1971). Another technique was to use partial activation whenever possible since this delays (but does not prevent) deterioration of the striation pattern (Julian and Moss, 1981; Gulati and Podolsky, 1980). To stabilize the striation pattern for up to 2 h, a new experimental technique was developed in which the fibers are cycled between isometric steady state and lightly loaded isotonic shortening. This technique has briefly been mentioned as an alternative way of activating skinned skeletal muscle fibers (Brenner, 1980) and was tested in cardiac preparations as well (Brenner and Jacob, 1980).

METHODS

Fiber Preparation

In earlier experiments single rabbit psoas fibers were prepared according to Brenner (1980), but recently a modified procedure was tested as well: When fibers were to be used immediately, membranes were made permeable by incubation of small fiber bundles of ~0.5-mm diam in "skinning solution" (Table I), similar to that described by Eastwood et al. (1979) for 1–4 h at 4°C. Single fibers were then dissected from these bundles and soaked for 20–30 min in a 3:1 (vol/vol) mixture of the skinning solution with glycerol to ensure membrane permeability for larger molecules such as creatine phosphokinase. Bundles to be stored for periods up to 1 wk were transferred directly from skinning solution to a 1:1 (vol/vol) glycerol:skinning solution after 24 h, and were stored at −20°C. For dissection of single fibers the bundles were transferred to relaxing solution via a 1:3 glycerol:skinning intermediate solution. This modified procedure did not yield results significantly different from those obtained by the method described by Brenner (1980).

Skinned fiber segments 4–6 mm in length were mounted to the force transducer and lever system using cyano-acrylate glues. Slight disorder of the striation pattern within ~0.5 mm of the attachment points was unavoidable. This could be of concern in very short segments where only overall fiber length is monitored but is of little consequence when sarcomere length is monitored by laser diffraction or an equivalent technique.

The cross section of the fiber segments was determined by light microscopy (magnification 100X) during the mounting procedure at which time it was possible to measure the smallest and largest diameter by twisting the fiber. All experiments were performed at 5°C and at pH 7.00 ± 0.05.

Apparatus

The modified moving coil galvanometer, used to apply variable loads on single muscle fibers, was identical with the one used in an earlier study (Brenner, 1980), except for the addition of an electronic damping system that reduced oscillations shortly after the changes in load. The force transducer was a semiconductor strain gage element (model 801 Aksjejelskapet, micro-electronic, Horkn, Norway). Sarcomere length was measured by laser diffraction (4 mW HeNe laser, Spectra-Physics Inc., Santa Clara, CA). The position of the first order of the diffraction pattern was monitored using a Schottky barrier photodiode (United Detector Technology Inc., Santa Monica, CA, PIN SC/10D) as the position-sensitive element. The calculation of the actual sarcomere length by an analog circuit was based on the thin grating equation. Variation of the

TABLE I
COMPOSITION OF SOLUTIONS (IN mM)

Solution	KCl	Imidazole	$MgCl_2$	ATP	EGTA	DTT	$CaCl_2$	CrP	CPK
Relaxing solution	100	20	5	5	5	0–1	—	20	200–350*
Activating solution	100	20	5	5	5	0–1	5	10	200–350*
	K propionate	KH_2PO_4	Mg acetate						
Skinning solution	150	5	5	3	5	1			

*In U/ml, where one unit transfers 1.0 μmol of phosphate from CrP to ADP per minute at pH 7.4 and 30°C.

incidence angle of the laser beam was used to test for Bragg artifacts in the records (Rüdel and Zite-Ferenczy, 1979).

Fibers were examined and photographed on Kodak technical pan film 2415 (Eastman Kodak Co., Rochester, NY) with an inverted microscope (Unitron Instruments Inc., Plainview, NY, model BR - BMIC) using an objective with a long working distance (NMIL 32/0.60, E. Leitz KG, Wetzlar, FRG). The condenser was moved away from the fiber to increase contrast.

Cycling Technique

To cycle activated fibers between isometric steady state and isotonic shortening, the galvanometer coil current was periodically lowered to <1/10 of the value necessary to balance the isometric force (P_o). Slight temporary slack in the fibers for coil currents close to zero had no substantial negative effect on the results described in this paper. The fibers were restretched to their original length by reapplying the coil current. This caused the lever to move back to a stop which set the isometric length of the fiber segments (see Brenner, 1980). The fiber segments were restretched directly from isotonic shortening. The restretch generally took 5–10 ms to complete. A typical cycle is shown in the inset of Fig. 3 *a*. The cycling period varied from 5–30 s.

The pronounced curvature of the length trace during long range isotonic shortening and the effect of activation on the amount of this curvature (Brenner, 1980) made it difficult to predict the minimum sarcomere length reached during shortening. Therefore, sarcomere length was monitored by laser diffraction and the duration of isotonic shortening (usually between 250 and 1,000 ms) was adjusted so that the minimum sarcomere length was longer than 1.8 μm.

RESULTS

Characterization of Fibers during the Rise in Force and the Early Isometric Steady State

Compared with the relaxed state (Fig. 1 *a*), fibers held isometric during the rise in force after changing from relaxing solution to activating solution (pCa = 4.5) show a large dispersion in the sarcomere length along and across the fiber (Fig. 1 *b*). As seen in Figs. 1 *b–d* this deterioration, if not allowed to progress too far, can be restored by a

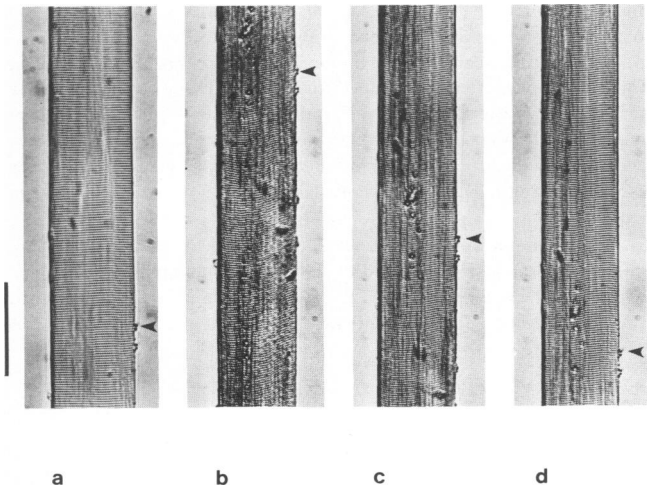

FIGURE 1 Effects of cycling on sarcomere disorder. (*a*) Relaxed fiber, (*b*) disorder of striation pattern during rise in tension (no cycling), active force ~⅓–½ of force at full activation, (*c*) reordering of striation pattern by cycling, micrograph taken during isometric steady state after second cycle of unloaded shortening, (*d*) striation pattern with fiber fully activated and cycled for 10 min. Length of vertical bar = 100 μm. Arrowheads point to the same marker on the fiber in all micrographs. Single rabbit psoas fiber, segment length 5.2 mm, cross-section 75 × 80 μm. Isometric tension 0.80 kg/cm². pCa = 4.5 (*b–d*) and >8.00 (*a*). T = 5°C.

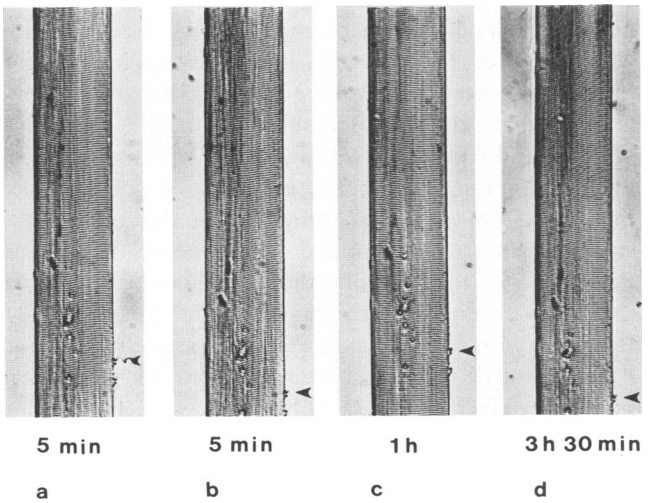

FIGURE 2 Striation pattern during steady-state activation. Same fiber as in Fig. 1, all micrographs taken during full activation. *a*, *c*, and *d* represent the fiber immediately after the restretch; *b*, 30 s after the restretch. Note the slight decrease in sarcomere length and movement of the marker (arrow) between *a* and *b* due to a give of sarcomeres close to the attachments. Also note the slight changes in the orientation (tilt) of the striation. The small inhomogeneity in *b* is reversed back to *a* by cycling. The time given for each micrograph shows the duration of full activation when micrograph was taken.

few cycles of unloaded shortening. The displacement of the marker in Fig. 1 *b* is due to force gradients along the fiber during exchange of solutions, causing parts of the fiber segment to shorten while others elongate. As shown by Fig. 1 *c* and *d* this imbalance during the rise in force is largely reversible within a few cycles. Fibers that continue to be cycled show a well-aligned striation pattern for several hours (Fig. 2 *d*), hardly distinguishable from the relaxed patterns for the best fibers.

When fibers are held isometric until force reaches a steady state and then cycled, the steady-state force and the speed of isotonic shortening actually increase further, compared with the values measured from the first isotonic quick release. This increase is sometimes as large as 25%. This effect is probably related to the initial imbalance of activation and dispersion of sarcomere length along the fiber that is restored by the cycling procedure.

To avoid unnecessary damage to the fibers, cycling normally was started even before changing to activating solution so that any developing inhomogeneities could be restored even during the rise of activation. The cycling period during the rise in activation was usually set to 5 s.

Morphological and Physiological Parameters during Steady Activation

After isometric tension and shortening velocity have reached steady-state values there is still an instability of the striation pattern when the muscle is held isometric. This instability, however, is much less pronounced than during the rise in force (compare Fig. 2 *b* with Fig. 1 *b*). The resulting inhomogeneities arising during such an isometric period can be reversed by lightly loaded shortening and restretching to the initial length (Fig. 2 *a*).

Fibers kept fully activated continually show a decrease in isometric tension and isotonic shortening of <10% within the first hour and 10–20% during the first two hours (Fig. 3 *a* and *b*). During the same period the striation pattern shows no signs of deterioration (Fig. 2 *a*, *c*, *d*). Fibers kept fully activated for more than 2–3 h, however, begin to show a steeper decrease in isometric tension and active shortening. This is accompanied by deterioration of the striation pattern, seen especially in myofibrils close to the fiber surface. These slowly increasing inhomogeneities are not reversible by either the cycling technique described in the present paper or by relaxing the fibers.

When the fibers were relaxed after full activation with the cycling method, no residual tension was detected as long as the striation pattern remained ordered. Residual tension seemed to appear when a substantial and irreversible disorder in the striation pattern was present as, for example, after prolonged full activation.

Laser diffraction patterns obtained in fully activated fibers (Fig. 4 *b*) show no significant broadening of the first-order intensity maximum, and only a slight increase in the background intensity compared with the relaxed pattern (Fig. 4 *a*). This suggests that for cycled fibers neither

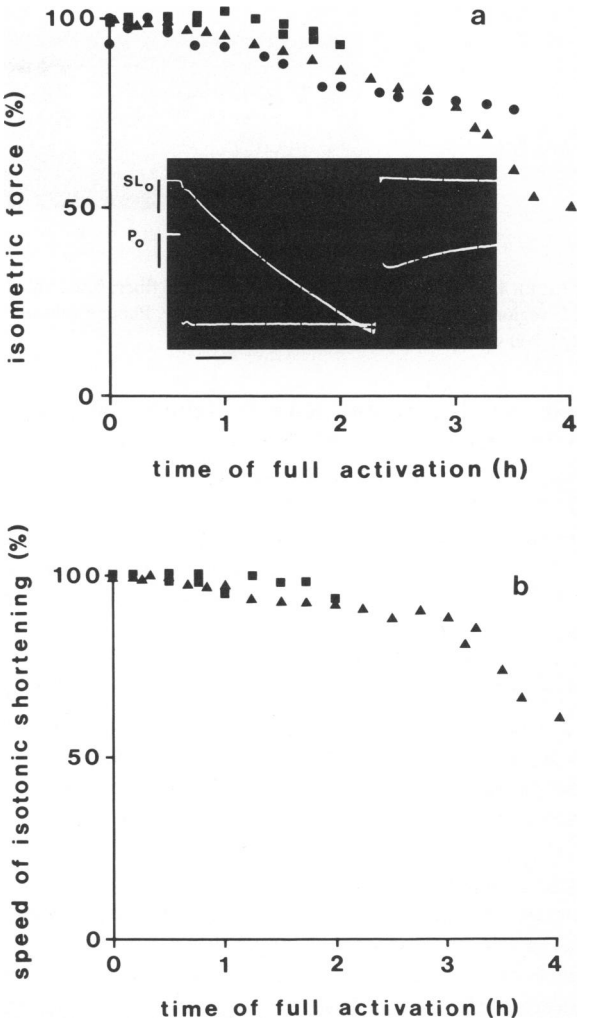

FIGURE 3 Isometric force (*a*) and speed of unloaded shortening (*b*). Different symbols represent different experiments. Active force (pCa 4.5) ●, 0.80 kg/cm2; ■, 0.85 kg/cm2; ▲, 0.95 kg/cm2. Shortening velocity (measured as relative filament sliding) ●, not measured; ■, 1.4 μm/s (load ~1.5% P_o); ▲, 1.0 μm/s (load ~5% P_o). *Inset*, original record of an unloaded isotonic quick release with following restretch to original length. Upper trace, sarcomere length measured by laser diffraction (SL_o, sarcomere length during isometric steady state, vertical bar 0.1 μm/sarcomere). Lower trace, force (P_o, isometric force, vertical bar 50 mg). Load during isotonic shortening ~2.5% P_o. Horizontal bar 50 ms. Segment length 4.6 mm. Cross-section 160 × 95 μm. Isometric force 1.09 kg/cm^2 (pCa 4.5). Initial relative filament sliding 1.1 μm/s.

the variation in length of different sarcomere populations nor the sarcomere disorder increases substantially.

In a few experiments this technique was also used at higher temperatures. At 15°C steady activation (pCa 4.5) for ~30 min did not introduce significant deterioration in the striation pattern. At higher temperatures, 20°–25°C, the persisting disorder seemed to appear earlier than at 5°C.

DISCUSSION

The tendency to develop sarcomere inhomogeneities, up to complete disappearance of the striation pattern, within

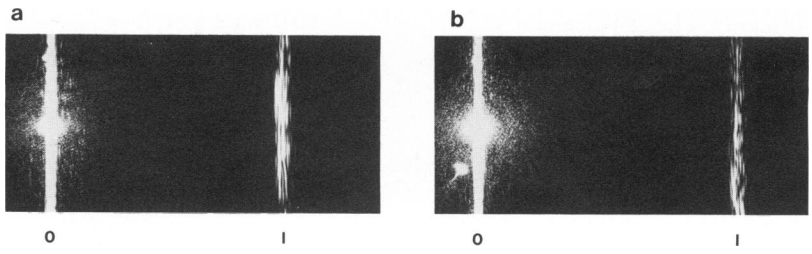

FIGURE 4 Laser diffraction pattern. *a*, relaxed fiber; *b*, activated fiber (pCa 4.5). Note in *b* the slightly increased 0–I distance due to give in the regions close to the attachments of the fiber. Photographs obtained by projecting pattern onto 35 mm film (no objective). Exposure time 1/1,000 s.

seconds of full activation, makes it useful to find a way to reset the striation pattern during activation itself. The reason for trying the present technique was that low force levels during isotonic shortening might allow the sarcomeres to redistribute to their ordered resting pattern. This view is supported by the observation that the striation pattern appears reordered following the period of free isotonic shortening.

The inhomogeneities that develop during the rise in tension (Fig. 1 *b*) are probably caused by Ca^{++} gradients along and across the fiber during this phase. Unless these inhomogeneities are reversed, there is progressive deterioration of the striation pattern, probably because of the negative slope of the force-sarcomere length relation, for sarcomere lengths >2.5 μm in rabbit fibers. Disorder in the striation pattern, which develops slowly during steady isometric tension (Fig. 2 *a*, *b*), is probably due to slight variations in the sarcomere length always present in the preparations, which again become more and more pronounced due to the negative slope of the force-sarcomere length relationship. Fig. 1 shows that the cycling procedure reorganizes the striation pattern to give a stable, uniform preparation, well suited for mechanical measurements.

The more rapid deterioration of the striation pattern following 2–3 h of full activation is probably due to structural damages accumulated during the period of activation. This could explain the progressive decrease in isometric force and speed of isotonic motion. An exhaustion of the ATP regenerating system during prolonged activation seems unlikely as repeated stretching of a fiber in the presence of inadequate ATP supply causes serious damage to the core of fibers, which is easily visualized under the light microscope (own unpublished experiments without regenerating system). This characteristic appearance was not seen in fibers cycled for 3 h in the presence of an ATP regenerating system.

In summary, by using this technique it is possible to stabilize the striation pattern for several hours with only a slight deterioration of the mechanical parameters and increase in sarcomere disorder. This allows sampling of x-ray diffraction patterns from fully activated fibers, which has been extremely difficult, if not impossible, in the past. The persistence of an excellent laser diffraction pattern also makes direct sarcomere length control possible.

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