Isometric Tension Differences in Fibers of Red and White Muscles

Abstract. *Measurements of maximum isometric tension and rate of onset of tension in fibers of glycerinated rat muscle indicate that the red soleus fibers develop significantly higher tensions than do white medial gastrocnemius fibers. Gastrocnemius fibers develop tension at a significantly faster rate than soleus fibers, however. These differences seem to be related to formation of actin-myosin cross-links.*

Differences between fibers of predominantly red and predominantly white skeletal muscles have long interested researchers. Biochemically, red muscle has more myoglobin (*1*), greater mitochondrial activity (*2*), and more sodium and less potassium (*3*) than has white muscle; it has greater uptake of glucose and synthesizes glycogen more readily (*4*); and its myosin has lower activity of actin-activated and EDTA-activated (EDTA, ethylenediaminetetraacetic acid) adenosine triphosphatase, and lower activities of Ca^{++}-activated adenosine triphosphatase and inosine triphosphatase (*5*). Physiologically, red muscle has a slower contraction-relaxation cycle, which has been attributed to differences in the motoneurons innervating the muscles (*6*). We now present evidence showing that differences in development of isometric tension and in rate of onset of tension exist even in glycerinated red and white fibers, and may be an expression of differences in actin-myosin relations.

Thirty-one female rats of the Holtzman strain were quickly killed with ether. The musculature of the left hind leg was exposed, and the medial head of the gastrocnemius was freed *in situ*; it was tied at resting length to a toothpick with nylon thread, removed, and placed in a 50-percent solution of glycerol for extraction in the cold. Soleus muscle was treated similarly. Extracting muscles were kept at 4°C for 3 days, with daily change of glycerol, before transferral to glycerol at −18°C. For 19 animals, extraction proceeded for 23 days; for 12 animals, extraction proceeded for 90 days to eliminate any effects of relaxing factor.

Table 1. Mean isometric tension and rate of development thereof in soleus and medial gastrocnemius rat muscles extracted with glycerol; 31 rats were used. Statistical evaluation on the basis of comparison of pairs of muscles. Gastr, gastrocnemius.

Muscle	Isometric tension	
	Mean, S.E. (g/mm^2)	Rate of development, S.E. (mg/min)
Gastr	18.43 ± 1.04	230.0 ± 27.7
Soleus	22.78 ± 1.96	140.6 ± 18.7
	t	
	3.7	4.25

The apparatus and methods used for measuring isometric tension have been reported (*7*). Extracted samples of muscle are transferred to 20-percent glycerol for 30 minutes at room temperature and teased into bundles containing two to six fibers; their ends are cemented to flat aluminum discs with Duco cement. Mounted fibers, floating on a drop of buffer, are examined microscopically for continuity; the fibers are counted and the diameters are measured with a calibrated eyepiece. On the assumption of a circular shape for individual fibers, the total cross-sectional area is calculated. One end of the mounted bundle is connected to a rigid muscle holder; the other, to a thin glass rod attached to a Grass FT.03C transducer. The mounted bundle is submerged in 2.7 ml of phosphate buffer in a glass tube, the buffer consisting of 0.03*M* KH_2PO_4, 0.05*M* KCl, and 0.005*M* $MgCl_2$, with a total ionic strength of 0.11 and a *p*H of 7.45 at 27°C; a water bath at 26.8°C surrounds the tube. About 10 mg of tension is applied to the bundle to remove slack. Isometric tension is developed by addition to the medium of adenosine-5′-triphosphate disodium made up in the buffer so that the final concentration of adenosine triphosphate is 0.0004*M* at *p*H 7.2. Output of the FT.03C transducer is recorded on a Grass model-5 polygraph.

Because it is difficult to establish a tangent to the curve of initial development of tension, a method was devised that utilized tension 10 seconds after onset as a measure of the initial rate of development of tension. Tension is measured at a point 10 seconds after its onset and, this rate is extended linearly to 1 minute. The rate of tension can thus be expressed directly in milligrams per minute. Such a method bears no direct relation to the ultimate tension developed, but is sensitive to changes in the initial rate of development of tension.

Table 1 contains the measurements of both total isometric tension and rate of development of tension. Since no difference was observed in maximum tension after 23-day extraction (gastrocnemius, 18.2 g/mm^2 ± 1.45 S.E.; soleus, 22.6 g/mm^2 ± 2.15) or 90-day extraction (gastrocnemius, 18.9 g/mm^2 ± 1.17; soleus, 23.0 g/mm^2 ± 2.13), the data were combined. Mean soleus tension exceeded mean gastrocnemius tension by 4.35 g/mm^2. When soleus is compared with gastrocnemius from the same animal, this difference is significant at the $< .01$ level. With our method of measurement, gastrocnemius fibers exceeded soleus fibers in rate of development of tension by 89.4 mg/min on the basis of comparison of pairs of muscles; this difference is highly significant ($P < .001$) and seems to be in line with the description of white fibers as "fast" and of red fibers as "slow."

Development of tension in extracted fibers could depend on relative amounts of actin and myosin present, but Barany *et al.* (*5*) have shown the concentrations of these proteins to be the same in soleus and medial gastrocnemius of rabbits. Our observations might be explained by differences in the number or nature of actin-myosin cross-links formed. Since the rate of development of tension in extracted red and white muscle fibers parallels the rate of contraction-relaxation in vivo, we feel that the differences in these types of muscle fiber must be at a more fundamental level than innervation.

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