Effects of Excitation-Contraction Uncoupling by Stretch and Hypertonicity on Metabolism and Tension in Single Frog Muscle Fibers

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ABSTRACT Metabolism and tension were examined in single fibers of the semitendinosus muscle of Rana pipiens at 15°C after excitation-contraction uncoupling by stretch and hypertonicity. Interrupted tetanic stimulation at 20 Hz for 150 s of control fibers in isotonic Ringer at a rest sarcomere length (SL) of 2.3 μm, resulted in a steadily declining tension, stimulated glycolysis, and significantly reduced fiber phosphocreatine (PCr) and ATP concentrations. Stretching resting muscle fibers to an SL of 4.7 μm did not alter metabolite concentrations, but glucose-6-phosphate rose and PCr fell markedly when the stretched fibers were stimulated tetanically, although tension was absent. Immersion of untetanized fibers in 2.5X isotonic Ringer produced a transient rise in resting tension, an increase in glucose-6-phosphate, and a significant reduction in PCr. During the transient rise in resting tension, PCr consumption per unit of tension-time integral was the same as that in fibers stimulated tetanically in isotonic Ringer. Tetanization of fibers in hypertonic solution did not further alter metabolite concentrations or produce tension. The results indicate that exposure to hypertonicity induces an increase in both tension and consumption of high-energy phosphate bonds (~P) in resting fibers, but stretch does not. During tetanic stimulation, stretch interferes with contraction but does not prevent activation, whereas hypertonicity inhibits activation as well as contraction.

INTRODUCTION

Excitation-contraction coupling in fast-twitch frog muscle involves depolarization of the plasma membrane and membranes of the T-tubule system,
release and reuptake of calcium at the sarcoplasmic reticulum, activation of myofibrillar ATPase, and splitting of ATP (Huxley and Taylor, 1958; Kushmerick et al., 1969; Endo, 1975; Tada et al., 1978; Homsher and Kean, 1978). Excitation-contraction coupling can be altered in vitro either by increasing the sarcomere length to reduce thick and thin filament overlap to zero (stretch) or by exposure to hypertonicity (Gordon et al., 1966; Gordon and Godt, 1970; Vergara et al., 1977), but in neither case is the action potential markedly affected (Hodgkin and Horowicz, 1957; Lännergren and Noth, 1973).

Stretch and hypertonicity have been used in whole muscles to examine the different energy-requiring steps in the excitation-contraction process. For example, measurements of heat production and $\Delta$P consumption in whole muscles at different extensions during stimulation suggest that energy consumption can be divided into length-dependent and length-independent terms, and that the length-independent term, which equals $\sim 25\%$ of net energy consumption at rest length, is caused primarily by activation processes (Aubert, 1956; Sandberg and Carlson, 1966; Kushmerick et al., 1969; Homsher et al., 1972; Homsher and Kean, 1978).

The interpretation of these observations from whole-muscle preparations is limited by several considerations. Whole muscles are composed of a heterogeneous fiber population to which bathing solutions may have differing access and which may have a spectrum of metabolic and contraction properties (Gordon and Godt, 1970). In addition, most whole muscles cannot be stretched sufficiently to completely uncouple excitation from contraction. Finally, both stretch and hypertonicity, by themselves and in the absence of electrical stimulation, have been reported to increase whole-muscle metabolism (Feng, 1932; Daemers-Lambert et al., 1966; Clinch, 1968; Yamada, 1970), and the direct effects of these treatments on metabolism, as compared with effects secondary to excitation-contraction uncoupling, may not be clearly distinguished in a heterogeneous fiber population.

These objections can be avoided by the use of single muscle fibers, which can be stretched to produce complete excitation-contraction uncoupling and can be immersed in hypertonic solutions to produce rapid changes in resting tension (Gordon et al., 1966; Lännergren and Noth, 1973; Vergara et al., 1977; Nassar-Gentina et al., 1981). We therefore decided to examine the effects of hypertonicity and of stretch on the force production and metabolism of single fibers, with and without tetanic stimulation. A preliminary report of this work has been published (Rapoport et al., 1977).

M E T H O D S

Single fibers were dissected at room temperature from the dorsal heads of semitendinosus muscles of female *Rana pipiens* and were mounted in a Plexiglas chamber in flowing oxygenated isotonic Ringer or in 2.5X isotonic Ringer at 15°C. Isotonic Ringer (pH 7.0–7.2, 230 mosmol) contained 115 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl$_2$, 2.15 mM Na$_2$HPO$_4$, 0.85 mM Na$_2$HPO$_4$, and 0.1 mM tubocurarine HCl. Hypertonic Ringer (2.5X isotonic) contained the above concentrations of solutes as well as 162 mM Na$_2$SO$_4$ and had an omsolality of 570 mosmol as measured by freezing-point depression.
One tendon of the fiber was tied to the lever arm of an RCA 5734 transducer (RCA Electro-Optics & Devices, Lancaster, PA) to record tension, the other tendon to a pair of forceps attached to a micromanipulator. Sarcomere length (SL), which was measured at the center of the fiber with a laser beam (Cleworth and Edman, 1972), was set by adjusting overall fiber length with the micromanipulator. Methods of measuring fiber diameter, stimulating the fiber, and recording tension have been described elsewhere (Vergara et al., 1977; Nassar-Gentina et al., 1978; Nassar-Gentina et al., 1981).

Differentiated square pulses (0.3 ms, 0.5 A) were delivered to the fiber through transverse extracellular Pt/Pt black electrodes. The fiber was stimulated at 20 Hz by 15 10-s trains separated by 1-s rest periods, for a net stimulus duration of 150 s. Before and after this interrupted tetanic stimulation, mechanical responsivity was examined periodically by evoking a twitch, followed 1 s later by a 200-ms contraction (4 or 10 pulses in 200 ms). The 200-ms contraction was used to examine the capacity of the fiber to exert maximal tetanic tension before and after a given treatment, whereas the twitch was used to examine changes characteristic of post-tetanic potentiation (Vergara et al., 1977; Nassar-Gentina et al., 1978, 1981).

Within 1 s after a complete physiological study, the fiber was immersed in liquid N2. It then was cut from its mounting and transferred to a test tube dipped in liquid N2. Fiber phosphocreatine (PCr), ATP, and glucose-6-phosphate were measured as described previously (Lowry and Passonneau, 1972; Nassar-Gentina et al., 1978, 1981).

RESULTS

Stretch and Tension

Fig. 1 illustrates mechanical responses to 150 s of interrupted stimulation at 20 Hz, of a fiber at a rest SL of 2.3 μm (top), and when stretched to an SL of 4.7 μm (bottom). A twitch and, 1 s later, a 200-ms contraction (10 pulses), were evoked periodically before and after each treatment. At an SL of 2.3 μm, tension declined progressively with successive 10-s tetani, although recovery was partial after 1-s interruptions of the train. After a 150-s interrupted tetanus, the height of the 200-ms contraction fell as compared with the pre-stimulation height and remained low for 20 min. Fiber responses during and after 150 s of interrupted stimulation have been described previously (Vergara and Rapoport, 1974; Vergara et al., 1977).

Stretch to a central SL of 4.7 μm prevented mechanical responses to a single pulse or 200-ms tetani (Fig. 1, center), and to a 150-s interrupted tetanus at 20 Hz (Fig. 1, bottom). The heights of the twitch and of the 200-ms contraction did not return to pre-stretch values when the fiber was released to a determined SL of 2.3 μm, which indicates that stretch caused irreversible changes (recovery did not occur within up to 15 min after return to rest length). It was necessary, however, to stretch the fiber to an SL of 4.7 μm to produce complete excitation-contraction uncoupling, as extension to a central SL of 3.8 μm reduced the response to tetanic stimulation only by 90%. These findings agree with observations by Gordon et al. (1966) that a fiber must be stretched beyond a central SL of 3.6 μm to eliminate overlap between actin and myosin filaments at the ends as well as at the center of the fiber.
As illustrated in Fig. 2 (top), immersion of a fiber in 2.5X isotonic Ringer produced a transient resting tension that exceeded 1 kg·cm⁻². The time course of this tension is summarized for five fibers in Fig. 3 and corresponds, in the first 4 min of immersion, to a mean tension-time integral equal to 47.6 ± 10.7 (SEM) kg·cm⁻²·s.

**Figure 1.** Fiber tension in relation to stretch and tetanization. The fiber was stimulated at 20 Hz for 150 s, when at a rest SL of 2.3 μm (top). A twitch (a) and a 200-ms contraction (10 pulses) (b) were evoked every 1 min during the procedure. The fiber was stretched to an SL of 4.7 μm, then was returned to an SL of 2.3 μm (center). A twitch and 200-ms contraction were evoked during the procedure. The fiber was stretched to an SL of 4.7 μm, was tetanized during stretch, then returned to an SL of 2.3 μm (bottom).

**Figure 2.** Fiber tension in 2.5X isotonic Ringer, with and without tetanic stimulation. The fiber was placed in hypertonic Ringer for 2 min, then returned to isotonic Ringer (top). A single pulse (a) and, 1 s later, a 200-ms train (4 pulses) (b), were administered throughout the procedure. 10 min later, the fiber was replaced in 2.5X isotonic Ringer and stimulated there for 150 s at 20 Hz, then returned to isotonic Ringer (bottom).

The hypertonic treatment completely uncoupled excitation from contraction. As illustrated in Fig. 2 (bottom), tension was absent when the fiber was stimulated tetanically for 150 s in 2.5X isotonic Ringer. On return to isotonic
Ringer, the heights of the 200-ms contraction and of the twitch became normal within minutes, as described in detail by Vergara and Rapoport (1974).

**Fiber Metabolism**

Table I presents metabolite concentrations in control fibers at a rest SL of 2.3 \( \mu m \) and in fibers subjected to different experimental conditions, illustrated in part by Figs. 1 and 2. Control metabolite concentrations are in the range reported previously in whole muscles and in single fibers (reviewed by Nassar-Gentina et al., 1981). Interrupted tetanic stimulation of fibers at an SL of 2.3 \( \mu m \) reduced PCr concentration from 166 nmol \cdot mg protein\(^{-1}\) to <1 nmol \cdot mg protein\(^{-1}\).

### Table I

**METABOLIC CONCENTRATIONS IN SINGLE FIBERS SUBJECTED TO DIFFERENT TREATMENTS**

| Experimental conditions | Number of Sarcomere | Ringer | Glucose-6- | PCr | ATP |
|-------------------------|---------------------|--------|-----------|-----|-----|
|                         | fibers | length | isotonic | nmol | mg protein\(^{-1}\) | \( \mu m \) | Isotonic | ATP | mg protein\(^{-1}\) |
| (A) Control 5 | 2.3 | 1x | | 165.9±13.3* | 32.7±4.8 |
| (B) 150-s tetanus at 20 Hz 5 | 2.3 | 1x | <1.0 | 105.9±13.3* | 21.6±1.4‡ |
| (C) 4-min stretch 5 | 3.8 | 1x | 3.1±0.4 | 140.4±1.7 | 30.1±1.9 |
| 4-min stretch + 150-s tetanus at 20 Hz 5 | 3.8 | 1x | 3.1±0.4 | 140.4±1.7 | 30.1±1.9 |
| 4-min stretch 4 | 4.7 | 1x | <1.0 | 165.8±17.5 | 39.1±2.3 |
| 4-min stretch + 150-s tetanus at 20 Hz 6 | 4.7 | 1x | 22.6±0.5‡ | 51.5±9.4‡ | 35.0±3.2 |
| (D) 4-min soak 6 | 2.3 | 2.5x | 14.6±2.3 | 106.9±7.8‡ | 30.4±4.2 |
| 4-min soak + 150-s tetanus at 20 Hz 8 | 2.3 | 2.5x | 17.9±3.4‡ | 106.7±8.1‡ | 34.7±3.7 |
| 15-min soak 3 | 2.3 | 2.5x | 18.5±1.4‡ | 81.4±4.7‡ | 22.1±1.0‡ |

* Mean ± SEM.
‡ Differs from control (\( P < 0.05 \)).

mg protein\(^{-1}\), markedly elevated glucose-6-phosphate concentration, and reduced ATP concentration by one-third. A 4-min stretch to an SL of 3.8 or 4.7 \( \mu m \) did not significantly alter fiber metabolite concentrations (\( P > 0.05 \)), but when such stretched fibers were stimulated tetanically for 150 s, PCr fell markedly and glucose-6-phosphate concentration rose, whereas fiber ATP remained unchanged.

Table I also shows that immersion of fibers in 2.5X isotonic Ringer significantly elevated glucose-6-phosphate and reduced PCr, but did not affect ATP concentration. On the other hand, a more prolonged 15-min soak reduced ATP concentration as well. Interrupted tetanization for 150 s, of fibers soaked for 4 min in 2.5X isotonic Ringer, did not change fiber metabolites more than did the Ringer soak alone.
DISCUSSION

Stretch has been reported to stimulate metabolism of whole muscle of *R. pipiens*, but the effect is not always evident (Feng, 1932; Baskin and Gaffin, 1965; Sandberg and Carlson, 1966; Clinch, 1968; Venosa and Horowicz, 1969). Certainly the present studies do not indicate a significant metabolic stimulation in single fibers by 4 min of stretching. On the other hand, 4 min of soaking in 2.5× isotonic Ringer markedly stimulates metabolism, in agreement with findings in whole frog muscles that hypertonicity consistently elevates consumption of O₂ and ~P and the production of heat and lactate (Daemers-Lambert et al., 1966; Yamada, 1970; Lännergren and Noth, 1973; Homsher et al., 1974).

![Figure 3](image)

**Figure 3.** Mean resting tension in five fibers after immersion in 2.5× isotonic Ringer. Points are means ± SEM.

During a 4-min soak in 2.5× isotonic Ringer, the mean tension-time integral of single fibers is 46 kg·cm⁻²·s, and 60 nmol·mg protein⁻¹ of PCr are consumed (Fig. 3 and Table I). The ratio of PCr consumption to the tension-time integral is 1.3 nmol·mg protein⁻¹·kg⁻¹·cm²·s⁻¹, and approximates a value of 1.2 nmol·mg protein⁻¹·kg⁻¹·cm²·s⁻¹ that is reported during the first 50 kg·cm⁻²·s of tension-time integral in tetanized single fibers (Nassar-Gentina et al., 1981). This equivalency indicates that tension is generated
during a hypertonic soak by the same energy-related mechanisms as during electrical excitation.

Energy-dependent tension generation in a hypertonic soak may be induced by calcium release from intracellular stores independent of a change in membrane potential (Hodgkin and Horowicz, 1957; Homsher et al., 1972; Lännergren and Noth, 1973). Under such conditions, twitch relaxation and the active state are prolonged, and electron probe analysis demonstrates that calcium is translocated from the terminal cisternae to the longitudinal reticulum (Vergara and Rapoport, 1974; Endo, 1975; Vergara et al., 1977; Somlyo et al., 1978).

The insignificant changes in fiber PCr and ATP after 50 s of tetanization in 2.5× isotonic Ringer indicate that hypertonicity interferes with energy-requiring activation processes as well as with contraction. The effect may be caused by decreased release and re-uptake of calcium during electrical stimulation, or by swelling of the transverse tubular system and inhibition of signal transmission to the sarcoplasmic reticulum (Freygang et al., 1967; Gordon and Godt, 1970; Andersson, 1973; Gordon et al., 1973; Vergara and Rapoport, 1974; Vergara et al., 1977; Franzini-Armstrong et al., 1978).

Although 2.5× isotonic Ringer can completely block ATP consumption caused by contraction of single fibers, a similar interruption in whole muscle requires 3.3-4× isotonicity (Smith, 1972; Homsher et al., 1974). The higher concentration may be caused by limited diffusion of the hypertonic solute from the bath to the muscle center (Gordon and Godt, 1970).

These studies indicate that hypertonic treatment but not stretch induces active, energy-dependent tension in association with ATP consumption in frog single muscle fibers. Both treatments uncouple excitation from contraction. Stretch uncouples by interfering with contraction itself (Gordon et al., 1966), but does not appear to prevent energy consumption during activation. Hypertonicity uncouples by interfering with activation as well as with contraction, as it completely blocks significant ATP consumption in response to tetanic stimulation.

Received for publication 11 March 1981 and in revised form 6 November 1981.

REFERENCES

ANDERSSON, K.-E. 1973. The effect of hypertonicity on the time course of the active state in single skeletal muscle fibres of the frog. Acta Physiol. Scand. 88:149-159.

AUBERT, X. 1956. Le Couplage Énergétique de la Contraction Musculaire. Éditions Arscia, Bruxelles. 320 pp.

BASKIN, R. J., and S. GAFFIN. 1965. Oxygen consumption in frog sartorius muscle. I. The isometric twitch. J. Cell. Comp. Physiol. 65:19-25.

CLEWORTH, D. R., and K. A. P. EDMAN. 1972. Changes in sarcomere length during isometric tension development in frog skeletal muscle. J. Physiol. (Lond.). 227:1-17.

CLINCH, N. F. 1968. On the increase in rate of heat production caused by stretch in frog's skeletal muscle. J. Physiol. (Lond.). 196:397-414.
Daemers-Lambert, C., F.-M. Debrun, G. Dethier, and J. Manil. 1966. Métabolisme des esters phosphorés dans le sartorius de Rana Temporaria traité par une solution de Ringer hypertonique. Arch. Int. Physiol. 74:374–396.

Endo, M. 1975. Mechanism of action of caffeine on the sarcoplasmic reticulum of skeletal muscle. Proc. Jpn. Acad. 51:479–484.

Feng, T. P. 1932. The effect of length on the resting metabolism of muscle. J. Physiol. (Lond.). 74:441–454.

Franzini-Armstrong, C., J. E. Heuser, T. S. Reese, A. P. Somlyo, and A. V. Somlyo. 1978. T-tubule swelling in hypertonic solutions: a freeze substitution study. J. Physiol. (Lond.). 283:133–140.

Freycang, W. H., S. I. Rapoport, and L. D. Peachey. 1967. Some relations between changes in the linear electrical properties of striated muscle fibers and changes in ultrastructure. J. Gen. Physiol. 50:2437–2458.

Gordon, A. M., and R. E. Godt. 1970. Some effects of hypertonic solutions on contraction and excitation-contraction coupling in frog skeletal muscles. J. Gen. Physiol. 55:254–275.

Gordon, A. M., R. E. Godt, S. K. B. Donaldson, and C. E. Harris. 1973. Tension in skinned frog muscle fibers in solutions of varying ionic strength and neutral salt composition. J. Gen. Physiol. 62:550–574.

Gordon, A. M., A. F. Huxley, and F. J. Julian. 1966. The variation in isometric tension with sarcomere length in vertebrate muscle fibres. J. Physiol. (Lond.). 184:170–192.

Hodgkin, A. L., and P. Horowicz. 1957. The differential action of hypertonic solutions on the twitch and action potential of a muscle fibre. J. Physiol. (Lond.). 136:17P–18P.

Homscher, E., F. N. Briggs, and R. M. Wise. 1974. Effects of hypertonicity on resting and contracting frog skeletal muscles. Am. J. Physiol. 226:855–863.

Homscher, E., and C. J. Kean. 1978. Skeletal muscle energetics and metabolism. Annu. Rev. Physiol. 40:93–131.

Homscher, E., W. F. H. M. Mommaerts, N. V. Ricchiuti, and A. Wallner. 1972. Activation heat, activation metabolism and tension-related heat in frog semitendinosus muscles. J. Physiol. (Lond.). 220:601–625.

Huxley, A. F., and R. E. Taylor. 1958. Local activation of striated muscle fibres. J. Physiol. (Lond.). 144:426–441.

Kushmerick, M. J., R. E. Larson, and R. E. Davies. 1969. The chemical energetics of muscle contraction. I. Activation heat, heat of shortening and ATP utilization for activation-relaxation processes. Proc. R. Soc. B Biol. Sci. 174:293–313.

Lässner, J., and J. Noth. 1973. Tension in isolated frog muscle fibers induced by hypertonic solutions. J. Gen. Physiol. 61:158–175.

Lowry, O. H., and J. V. Passonneau. 1972. A Flexible System of Enzymatic Analysis. Academic Press, Inc., New York. 146–218.

Nassar-Gentina, V., J. V. Passonneau, and S. I. Rapoport. 1981. Fatigue and metabolism of frog muscle fibers during stimulation and in response to caffeine. Am. J. Physiol. 241:C160–C166.

Nassar-Gentina, V., J. V. Passonneau, J. L. Vergara, and S. I. Rapoport. 1978. Metabolic correlates of fatigue and recovery from fatigue in single frog muscle fibers. J. Gen. Physiol. 72:593–606.

Rapoport, S. I., V. Nassar-Gentina, and J. V. Passonneau. 1977. Metabolic changes in response to stimulation of single muscle fibers in which contraction is uncoupled from excitation by stretch and hypertonic solution. Abstr. 7th Annu. Meeting Neurosci. Soc. 3:277.
SANDBERG, J. A., and F. D. CARLSON. 1966. The length dependence of phosphorlcreatine hydrolysis during an isometric tetanus. Biochem. Z. 345:212-231.

SMITH, I. C. H. 1972. Energetics of activation in frog and toad muscle. J. Physiol. (Lond.). 220:583-599.

SOMLYO, A. P., A. V. SOMLYO, H. SHUMAN, B. SLOANE, and A. SCARPA. 1978. Electron probe analysis of calcium compartments in cryosections of smooth and striated muscles. Ann. N. Y. Acad. Sci. 307:523-544.

TADA, M., T. YAMAMOTO, and Y. TONOMURA. 1978. Molecular mechanism of active calcium transport by sarcoplasmic reticulum. Physiol. Rev. 58:1-79.

VENOSA, R. A., and P. HOROWICZ. 1969. Influence of external potassium and stretch on the oxygen consumption of frog's sartorius muscle. Physiologist. 12:382.

VERGARA, J. L., and S. I. RAPOPORT. 1974. Fatigue in frog single muscle fibres. Nature (Lond.). 252:727-728.

VERGARA, J. L., S. I. RAPOPORT, and V. NASSAR-GENTINA. 1977. Fatigue and post-tetanic potentiation in single muscle fibers of the frog. Am. J. Physiol. 232:C185-C190.

YAMADA, K. 1970. The increase in the rate of heat production of frog's skeletal muscle caused by hypertonic solutions. J. Physiol. (Lond.). 208:49-64.