Sarcomeric Oscillations in Frog Skeletal Muscle Fibers

Abstract. *Brief asynchronous, small-amplitude, cyclic, longitudinal displacements of the striations of frog skeletal muscle fibers were observed with ordinary light microscopy after application of caffeine and certain quaternary ammonium compounds. With time these oscillations became synchronized and evolved into peristaltic-like movements. The oscillations were influenced by sarcomere length, temperature, external concentration of calcium ions, membrane potential, and disruption of the transverse tubules.*

While investigating the capacity of certain quaternary ammonium compounds to activate and inactivate postjunctional receptors of frog skeletal muscle fibers, we found that external application of the quaternary ammonium ion amyltrimethylammonium (0.2 to 1.0 mmole/liter) initiated oscillatory activity of muscle fiber sarcomeres located at the region of the neuromuscular junction. Similar oscillations have been observed, but not reported, in experiments in which the depolarizing action of acetylcholine (0.027 mmole/liter), applied to the postjunctional membranes of muscle fibers bathed in sodium-free Ringer solution, was tested (*1*).

Because certain quaternary ammonium ions of small diameter can penetrate the activated postjunctional membrane of muscle fibers (*1*), we supposed that amyltrimethylammonium might cause oscillatory activity by exerting some action at an intracellular site, possibly the sarcoplasmic reticulum. Caffeine also penetrates muscle fiber membranes, and it is considered to activate the contractile elements by producing an increase in the intracellular Ca^{2+} concentration (*2*). Therefore we tested the capacity of this drug to produce sarcomeric oscillations.

We found that caffeine (0.25 to 2 mmole/liter) produced sarcomeric oscillations which appeared at both junctional and nonjunctional regions of frog skeletal muscle fibers. Many investigations have shown that caffeine produces muscle fiber contractures when applied in concentrations from 5 to 10 mmole/liter, but, to our knowledge, there are no published reports of the oscillatory phenomena we have observed. We now report our results concerning the oscillatory activity of muscle fibers treated with caffeine and include a survey of some of the factors which influence the production of these oscillations.

Most of the experiments were conducted on frog sartorius muscles mounted at rest length (approximately) in a bath of phosphate- or tris-buffered Ringer solution (18° to 22°C). Caffeine (0.25 to 2 mmole/liter) was added to the bathing solution. The muscle fibers were ordinarily observed with a light microscope (×300 to ×800). A few observations were also made with the phase-contrast microscope (*3*) on small bundles of fibers dissected from the semitendinosus. Conventional means were used for recording membrane potentials from single muscle fibers and for the application of electric current via a second intracellular electrode.

After application of 1 m*M* caffeine, individual sarcomeres of muscle fibers at the lateral edges of the muscle appeared to oscillate. This response could have a latency as short as 10 seconds, but more often it appeared 2 to 6 minutes after the application of caffeine. As a rule only a part of any individual fiber, a region approximately 300 to 500 μm long, exhibited oscillations. The frequency of the oscillatory activity diminished as one moved along the length of the fiber toward the peripheral portions of the active region. Often several adjacent fibers exhibited oscillations. With prolonged exposure to caffeine, fibers in the more central regions of the muscle, both on the surface and in deeper layers, exhibited oscillations. When 1 m*M* caffeine was applied, oscillations were not usually observed in the portions of the fibers nearest the tendons. Furthermore, if a quiescent fiber of a caffeine-treated muscle was impaled for measurement of the resting potential, shortly thereafter (a few seconds to several minutes), oscillatory activity appeared in the fiber at and adjacent to the site of the impalement. Such responses were not seen in control experiments on fibers not treated with caffeine. The oscillations appeared as asynchronous, impulsive longitudinal displacements of individual striation bands. These movements, often less than 1 μm in amplitude, sometimes involved entire striations of a single fiber, but more often independent movements of a portion of a striation occurred. Similar observations were made with phase-contrast microscopy. By means of stroboscopic illumination, the frequency of the oscillatory movements was estimated to lie in the range of 2 to 20 per second.

During the sustained application of 1 m*M* caffeine the pattern of mechanical activity of the fibers changed with time. Oscillations appearing initially had relatively high frequency and seemed asynchronous. Progressively an increased number of oscillating sarcomeres appeared in each active zone, and the frequency of the oscillations diminished. At this stage, the movements of adjacent sarcomeres appeared to be more related to each other. On occasion, striation movements occurred in rhythmic sequence and gave the appearance of a wavelet propagated longitudinally over 100 to 1000 μm.

With prolonged application of caffeine, additional evidence of mechanical coupling between sarcomeres was obtained. Groups of sarcomeres began to generate peristaltic-like movements which traveled longitudinally at low velocity (20 μm/sec) over several millimeters. Muscles displayed such activity for 24 to 48 hours. Zones of muscle fibers which exhibited oscillations often became suddenly quiescent while fresh zones of activity appeared elsewhere. Sometimes, application of 2 m*M* caffeine immediately produced a reversible contracture followed by a relaxation during which oscillations were generated and maintained. The sarcomeric oscillations in a muscle bathed with 2 m*M* caffeine evolved more rapidly into peristaltic activity extending over several millimeters of a muscle fiber. Peristaltic waves traveling toward each other along a muscle fiber were annulled on collision.

We studied the conditions which govern the appearance of oscillatory activity. Our observations, performed on muscles exposed to 1 m*M* caffeine, can be summarized as follows:

1) Cooling to 15°C caused existing asynchronous oscillations to evolve into peristaltic-type movements. Below 4°C and above 25°C, oscillations did not occur. This inhibition was reversed when the temperature of the bath returned to about 20°C.

2) Oscillations in caffeine-bathed fibers whose sarcomere length averaged 2.1 to 2.3 μm were prominent. As the sarcomere length was increased by progressively stretching the muscle fiber, the oscillations became less and less perceptible. At a sarcomere length of 2.6 to 2.7 μm, oscillations were barely discernible; they could not be seen when the sarcomere length was increased to 2.8 μm (average).

3) After a 1-hour exposure to Ringer

solution containing glycerol (400, 800, or 1200 mmole/liter) the muscle was returned to Ringer solution (*4*). This treatment markedly reduced the number of muscle fibers which exhibited oscillatory activity during subsequent application of caffeine, and oscillations disappeared within 20 minutes. Treatment with the highest concentrations of glycerol caused the most marked loss of oscillatory response to caffeine, but the response was never completely inhibited.

4) Previous treatment of the muscle with tetrodotoxin (10^{-7} g/ml) or *d*-tubocurarine (5 mg/liter) sufficient to block neuromuscular transmission did not inhibit the oscillogenic action of subsequently added caffeine. Treatment of a muscle with 3.7 m*M* procaine prevented subsequently applied caffeine from producing oscillations. This action of procaine was rapidly reversed when the anesthetic was washed out of the muscle.

5) Muscles soaked 1 hour in calcium-free Ringer solution containing 4 m*M* ethylenediaminetetraacetate exhibited oscillatory activity when caffeine was added to the bathing solution, but these oscillations disappeared in 2 hours. For a muscle treated for 24 hours in such a Ringer solution, the caffeine-induced oscillations endured only 6 minutes.

6) We have confirmed that 0.25 to 2.0 m*M* caffeine does not produce change in the resting potential of muscle fibers (*5*). Also, the drug does not appreciably alter the rate of rise or the amplitude of the action potential, but produces some slowing in the rate of repolarization (*6*). In a caffeine-bathed muscle, sarcomeric oscillations were observed in fibers in which the resting potential was normal. Muscle fibers whose membrane potentials were reduced to −30 mv or less, by application of increased concentrations of extracellular K^+, showed oscillatory activity when caffeine was added to the bathing solution. In caffeine-treated muscle fibers whose sarcomeres were quiescent, oscillatory activity could sometimes be initiated, with a latency of several seconds or more, when the membrane potential was reduced, by applied electric current, to values near the mechanical threshold (range −60 to −70 mv). Electrical hyperpolarization of a fiber decreased and sometimes stopped sarcomeric oscillations with a latency of a few seconds. In other experiments, oscillatory activity was initiated by application of 0.2 m*M* amyltrimethylammonium. Unlike caffeine, this drug caused rapid reduction of the postjunctional (and therefore extrajunctional) membrane potential to values approaching −50 mv. Thereafter the membrane potential rose to the control value, but despite such repolarization, oscillatory activity persisted throughout.

7) Sarcomeric oscillations were also produced at the region of the neuromuscular junction after application of 0.2 to 0.5 m*M* hexamethonium, 0.27 m*M* carbamylcholine, and several other quaternary ammonium compounds. Oscillatory activity has also been produced by other agents, some of which are structurally related to caffeine (*3*).

We propose that the oscillatory changes in the pattern of striations, as seen with the light microscope, probably are produced by transient asynchronous activation of individual myofibrilar bundles of which each sarcomere is composed. We further assume that groups of myofibrils which make up a sarcomere can be activated synchronously and thereby produce optical and mechanical changes (*7*). Regional activation of contractile elements by local increase in concentration of Ca^{2+} can be most simply accounted for if caffeine and other oscillogenic agents act directly on the sarcoplasmic reticulum and liberate the required Ca^{2+} at individual loci. Alternatively, in caffeine-treated fibers, the overall intracellular Ca^{2+} concentration may increase progressively to the point at which random activation of contractile elements occurs at an increased number of sites. In addition to the above mechanism, agents such as caffeine may act on the transverse tubular membrane at its zones of contact with the lateral sacs of the sarcoplasmic reticulum. At these regions caffeine might cause local oscillations of membrane potential sufficient to initiate Ca^{2+} release.

The generation of sarcomeric oscillations appears to be sensitive to sarcomere length, and the adenosine triphosphatase activity of the activated contractile elements may be stretch-sensitive. Such a mechanism has been suggested for insect flight muscle (*8*), and caffeine-treated muscle fibers may behave in a somewhat similar manner. Stretch-sensitive adenosine triphosphatase activity could help to explain both the oscillations of individual sarcomeres and the more extensive peristaltic-like waves of mechanical activity which we have observed. We propose that stretch-sensitive adenosine triphosphatase activity of contractile elements may be exhibited by normal vertebrate skeletal muscle during the "active state" and that certain important characteristics of the contractile system of skeletal muscle may depend on this property.

LUIS A. MARCO
WILLIAM L. NASTUK
Department of Physiology,
College of Physicians and Surgeons,
Columbia University, New York 10027

References and Notes

1. W. L. Nastuk, *Ann. N.Y. Acad. Sci.* **81**, 317 (1959).
2. C. P. Bianchi, *J. Pharmacol.* **138**, 41 (1962).
3. L. A. Marco and W. L. Nastuk, in preparation.
4. R. S. Eisenberg and P. W. Gage, *Science* **158**, 1700 (1967); J. N. Howell and D. J. Jenden, *Fed. Proc.* **26**, 553 (1967).
5. J. Axelsson and S. Thesleff, *Acta Physiol. Scand.* **44**, 55 (1958); H. C. Lüttgau and H. Oetliker, *J. Physiol.* **194**, 51 (1968).
6. J. Etzensperger, *Compt. Rend. Soc. Biol. Paris* **151**, 587 (1957).
7. A. F. Huxley, *Progress in Biophysics and Biophysical Chemistry*, J. A. V. Butler and B. Katz, Eds. (Pergamon Press, New York, 1957), vol. 7, pp. 257–318.
8. B. R. Jewell and J. C. Ruegg, *Proc. Roy. Soc. London Ser. B.* **164**, 428 (1966); J. C. Ruegg, *Amer. Zool.* **7**, 457 (1967).
9. Supported by NIH grants NB-04988 and special NIH fellowship 2 F11-NB-1847 to Dr. Marco.

27 June 1968

Mosaic Unit Ruler: Does One Exist?

Abstract. *Ancient mosaic patterns tend to be certain absolute sizes (mosaic units). There is evidence that the mosaicists had these lengths marked on rulers. A ruler such as one which an ancient mosaicist might have used has been reconstructed. It is possible that an original may still exist.*

It has been reported (*1*) that geometric patterns in ancient Greek and Roman floor mosaics tend to fall into size groups, and that almost every pattern of a size group has one dimension of (virtually) the same length. Various evidence has been given (*1*) indicating that the ancients probably fixed this length by measurement, thus causing the size-group phenomenon.

Measuring more than 310,000 of these lengths produced the result (*2*) that only 0.6 percent are equivalent to known standard units of length, whereas 89 percent lie (*1*) within ± 3 standard deviations ($\sigma \cong 0.13$ cm) of the following values (mid-interval value of mode, class interval 0.1 cm): 1.2, 2.4, 3.6, 6.0, 9.6, 15.6, 21.6, 25.1, 40.7, 65.8, and 106.5 cm.