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The effect of stretch on sarcoplasmic free calcium of frog skeletal muscle at rest

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The concentration of free calcium in the sarcoplasm of a resting frog skeletal muscle is increased by stretch. The magnitude of the rise in free calcium increased with the degree of stretch and the ambient temperature and it was enhanced by caffeine. This phenomenon might play a role in the twitch potentiation and enhanced metabolic rate evoked by stretch.

Stretching a frog skeletal muscle fiber immediately before its stimulation is known to increase the peak tension and duration of the resultant twitch [1,2]. Several hypotheses have been proposed to explain this stretch evoked enhancement of the twitch. Since stretch lowers the membrane potential at which calcium is released from the sarcoplasmic reticulium [3,4], it may increase the amount of calcium released during the action potential. On the other hand, stretch is known to increase the sensitivity of the myofilaments to calcium in skinned fiber preparations and thereby enhance the magnitude of the twitch independent of the amount of calcium released [5,6]. Finally, stretch might increase the myoplasmic concentration of free calcium (Ca_i^{2+}) which would result in a significantly greater number of active cross-bridges formed at the initiation of stimulation thus giving the fiber a head start in the contractile process. Indeed, an increase in Ca_i^{2+} is suggested by the facts that stretch increased the rate of metabolism [7,8] and that there appears to be a tight correlation between Ca_i^{2+} and the rate of metabolism in frog skeletal muscle [9].

In order to test this last hypothesis, the basal Ca_i^{2+} was measured during a stretch in frog skeletal muscle using the calcium-sensitive photoprotein aequorin. Aequorin is the preferred indicator for this type of experiment since it is relatively free of movement artifacts [9,11].

The properties of aequorin and the method of its introduction into the sarcoplasm of single muscle fibers by pressure microinjection have been described [9–11]. Fibers were discarded in which the resting membrane potential or the mechanical responsiveness to electrical stimuli were altered by the microinjection of aequorin.

The aequorin-loaded fiber was suspended by its tendons on two hooks in the aequorin photometer which has been described [9–11]. The distance between the hooks was controlled by a microdrive and the relationship between the distance indicated by the microdrive and the length of the sarcomere was established from the laser diffraction patterns observed at different microdrive settings. Because the length of each fiber differed and because the holes in the tendons for the hooks could enlarge with repeated stretches, this relationship between sarcomere length and stretch was determined for each fiber and at several times

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during the course of an experiment. Furthermore, fibers with a slack length of approx. 0.5 cm could be moved along the same vector as the maximal stretch without changing the magnitude of the light signal.

All fibers were perfused with a media containing 115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, and 3 mM Hepes (*N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid) (pH 7.2). The nitrate medium contained 115 mM $NaNO_3$, 2.5 mM KCl, 1.8 mM $CaCl_2$, and 3 mM Hepes (pH = 7.2). 5 mg% *d*-tubocurarine (Sigma, St. Louis, MO) was present in all media to block the activation caused by acetylcholine which might be released from resealed nerve endings during stretch.

The light signal from the fiber was measured either by an analog device described by Blinks et al. [12] or by a photon counter (Model 1105A; Princeton Applied Electronics, U.S.A.). The analog signal was fed to a tape recorder (Ampex, U.S.A.) and a strip chart recorder. Some of the traces shown in this paper were replotted from the recorded signal using a Nicolet Signal Averager and an Esterline Augus *X-Y* plotter.

An apparent rise in Ca_i^{2+} was observed when a muscle fiber was stretched from a sarcomere length of 2.2 to 2.6 μm (Fig. 1). The increase in Ca_i^{2+} evoked by stretch exhibited a complex time-course. From a base line of 5.2 nA above background the signal increased sharply to 14.8 nA then fell within 30 s to a plateau of 10 nA. After relaxation of the fiber, the signal returned to 5.8 nA. The fact that

Fig. 1. The effect of stretch on sarcoplasmic Ca_i^{2+}. Tibalis anterior muscle fiber (150 μm diameter, 0.45 cm initial length) was stretched from sarcomere length of 2.2 to 2.6 20°C. Verticle bar, 5 nA or 20 mg tension; horizontal bar, 2 min.

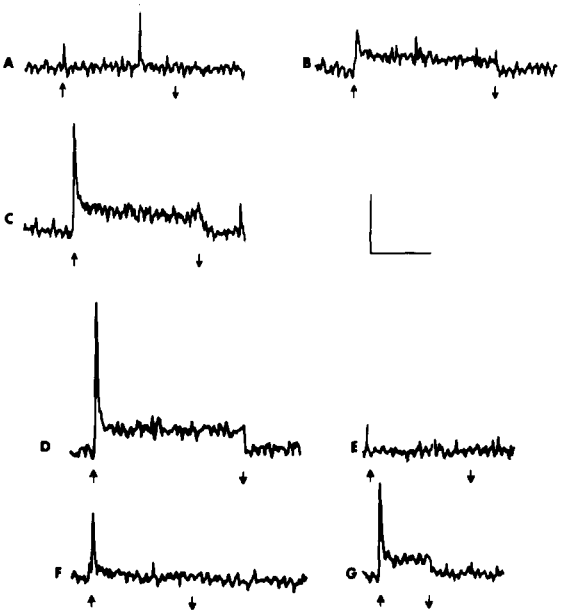

Fig. 2. The effect of final length and temperature on sarcoplasmic Ca_i^{2+}. Tibalis anterior muscle fiber (100 μm diameter, 0.5 cm initial length). Vertical bar, 5 nA; horizontal, 1.5 min. Stretch was applied at the upward arrow and relaxation at the downward arrow. Stretch to 2.1, A; 2.4, B; 2.7, C; and 2.9 μm D were done at 25°C. The temperature was changed from 25°C, D to 15°C in E; to 20°C in F; and to 23°C in G. Stretches from D to E were to 2.9 μm.

the stretch evoked rise in Ca_i^{2+} is fully and consistently reversible (as shown in this and the following figures) indicates that the rise is not due to a leakage of calcium ions through microtears in the sarcolemma created by the injection of aequorin. The lower trace, showing the stretch-evoked changes in tension, first increased from 0 to 11 mg then stabilized at approx. 10 mg.

The amplitude of the spike and of the plateau phase increased with the degree of stretch as shown on Fig. 2 (A through D). Stretch from a sarcomere length of 2.0 to 2.1 μm did not increase Ca_i^{2+} (trace A) while stretches to 2.4, 2.7, and 2.9 μm increased the amplitude of the spike and of the plateau (traces B and D). An increase in temperature enhanced the rise in Ca_i^{2+} evoked by stretch as shown in Fig. 2 (D to G). All of these extensions were from a sarcoma length of 2.0 to 2.9 μm. The temperature was 25°C in trace D, 15°C in trace E, 20°C in trace F and 23°C in trace G.

As shown by Sandow and his colleagues [13]

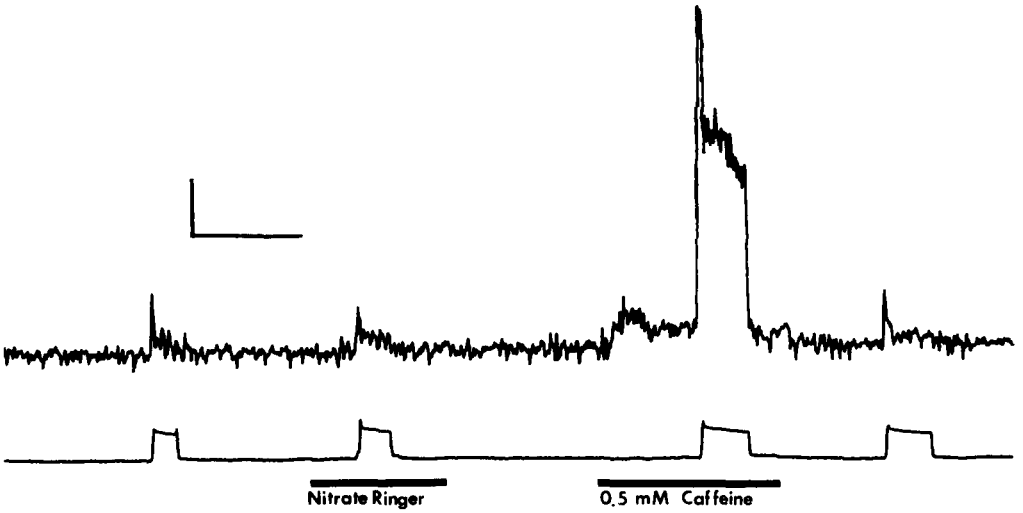

Fig. 3. The effect of nitrate and caffeine on the stretch response. Tibalis anterior muscle fiber (134 diameter, initial length, 0.55 cm) 20°C. Vertical bar, 2 nA and 10 mg; horizontal bar 4 min. The fiber was stretched from 2.0 to 2.8 sarcomere length. Nitrate Ringer refers to a Ringer's solution in which most of the chloride has been replaced by nitrate.

nitrate solution or a subcontracture concentration of caffeine potentiates twitch responses by reducing the extent of depolarization necessary for the release of calcium into the sarcoplasm (i.e., the mechanical threshold). Experiments were done to see if these agents might alter basal Ca_i^{2+} or the amount released by stretch. As shown in Fig. 3, nitrate solution did not alter basal Ca_i^{2+} nor the stretch-evoked increase in Ca_i^{2+} whereas caffeine enhanced both. The influence of caffeine was fully reversible when the drug was removed.

The stretch evoked rise in Ca_i^{2+} could not always be observed in frog muscle fibers kept in cold media but was usually present when the fiber was heated above 25°C. This effect of temperature requires some comment. One could conclude that the decrease of temperature blocks the stretch evoked increase in Ca_i^{2+} but such a conclusion ignores the substantial effect that temperature has on the reaction of aequorin with calcium. Cooling reduces the sensitivity of aequorin to calcium [12] and since basal Ca_i^{2+} of frog skeletal muscle is close to the limit of detection by aequorin, it is possible that the cooling might render aequorin insensitive to a stretch-evoked increase in Ca_i^{2+}. Therefore stretch-evoked increases in Ca_i^{2+} may go undetected by aequorin at low temperatures.

The origin of this calcium released by stretch is hypothesized to be the sarcoplasmic reticulum for the following reasons: First caffeine, an agent that promotes the release of calcium from the sarcoplasmic reticulum [14] elevates basal Ca_i^{2+} and enhances the stretch-evoked release in Ca_i^{2+}; second Brenner [15] observed local releases of calcium upon stretching a chemically skinned muscle preparation with an intact sarcoplasmic reticulum; and third the myofilaments, the other potential source of calcium, do not release calcium ions when stretched [16]. How the calcium is released is the subject of further investigation but the fact that the nitrite solution does not alter it suggest that the stretch-evoked release of calcium does not involve processes normally employed in the release of calcium during contraction.

The mechanical consequences of an increase in Ca_i^{2+} evoked by stretch are clear. The spike like transient rise in Ca_i^{2+} corresponds with the stress relaxation in the mechanical record which suggests that the two phenomna are related. Stretch is also known to increase twitch tension [1,2], to lower the frequency of stimulation necessary to produce a fused tetanus [17], and to enhance the magnitude of potassium contractures [3,4]. Thus it is possible that the slightly higher Ca_i^{2+} reported

here increases the number of cross-bridges formed at rest so that less time is required to stretch out the series elastic component at the initiation of the twitch. A reduction in the time necessary to stretch the series elasticity should increase the duration the fiber spends in shortening against the load and thus enhance its peak twitch tension.

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References

1 Hartree, W. and Hill, A.V. (1921) J. Physiol. (L) 55, 389–411
2 Close, R. (1964) J. Physiol. (L) 173, 74–95
3 Gonzales-Serratos, H., Valle, R. and Cillero, A. (1973) Nature, New Biol. 246, 221–222
4 Lannergren, J. (1975) Acta Physiol. Scand. 95, 347–349
5 Endo, M. (1972) Nature, New Biol. 237, 211–213
6 Moisescu, D.G. and Thieleczek, R. (1979) Biochim. Biophys. Acta 546, 64–76
7 Feng, T.P. (1932) J. Physiol. (L) 74, 441–454
8 Clinch, N.F. (1968) J. Physiol. (L) 196, 397–414
9 Snowdowne, K.W. (1985) Am. J. Physiol. 248, 520–526
10 Blinks, J.R., Rudel, R. and Taylor, S.R. (1978) J. Physiol. (L) 277, 291–323
11 Blinks, J.R. (1982) The use of photoproteins as calcium indicators in cellular physiology, in: Techniques in Cellular Physiology (Baker, P.F., ed.) Vol. P1, Part II, pp. 1–38, Elsevier/North Holland, Amsterdam
12 Blinks, J.R., Mattingly, P.H., Jewell, B.R., Van Leeuwen, M., Harrer, G.C. and Allen, D.G., (1978) Methods Enzymol. 57, 292–328
13 Sandow, A., Taylor, S.R., Isaacson, A. and Sequin, J.J. (1964) Science 143, 577–579
14 Weber, A. (1968) J. Gen. Physiol. 52, 760–772
15 Brenner, B. (1979) Basic Res. Cardiol. 74, 177–202
16 Stephenson, D.G. and Wendt, I.R. (1984) J. Musch. Res. Cell Motility 5, 243–272