

Feedback in the Contractile Mechanism of the Frog Heart

EMIL BOZLER

From the Department of Physiology, Ohio State University, Columbus, Ohio 43210

ABSTRACT Shortening causes a transient decrease, extension an increase, in activity during contractures of the frog ventricle induced by high Ca or by isosmotic K solution. This is shown by the fact that, after the immediate passive shortening, the muscle is extended under isotonic conditions when the load is diminished, and that under isometric conditions quick release causes first a rapid drop, then a further, much slower, fall of tension. Increasing the load or stretching induce the opposite effects. At low temperatures all rapid changes in length produce oscillations of low frequency. These responses are due to a sensitive feedback mechanism similar to that previously demonstrated for insect fibrillar muscle. That this mechanism comes into play in the heart under normal conditions and controls the time-course of the twitch is demonstrated by the observation that relaxation begins earlier the greater the shortening. Thus, during afterloaded isotonic twitches the onset of relaxation is advanced as the load is diminished.

Muscular activity of some muscles is controlled by an intracellular feedback mechanism which causes a transient increase in contraction when the muscle has been lengthened, a decrease after shortening, and which induces oscillations under certain conditions. These properties have been discovered first in insect fibrillar muscle (2, 9) and have more recently also been found in frog skeletal muscle (10). In the experiments described below such a feedback mechanism will be demonstrated in the frog heart. It plays a role in the control of the strength and time-course of the contraction.

METHODS

Rings of the ventricle of the frog (*Rana pipiens* and *Rana catesbeiana*) were used. Apex and base and most of the inner tissue were removed, so that the muscles were about 0.5 mm thick when mounted under moderate tension. The stimuli were rectangular electric currents with a duration of 2 msec.

Mechanical changes were recorded on an inkwriting polygraph. For isotonic recording, a rotary motion transducer (Harvard Apparatus Co., Inc., Millis, Mass.) was used as shown in Fig. 1. The lever consisted of a straw, except for a small piece

of acrylic plastic attached to the axis of the transducer. Results were the same whether the load was a weight or a spring (not shown), both of which were attached close to the fulcrum. The calculated effective mass of the system was 140 mg plus 60 mg/g load, if weights were used. A force transducer (Grass FT 03, Grass Instrument Co., Quincy, Mass.) was used for recording tension. For simultaneous recording of tension and length the force transducer was connected to the lever of the isotonic transducer by a chain which prevented extension of the muscle by added loads, but permitted shortening, as described by Jewell and Wilkie (7). Changes in length will be expressed as per cent of resting length.

The muscles were kept in 20 ml of oxygenated solution. The physiological solution, which will be called Ringer solution, contained, in millimoles per liter: NaCl

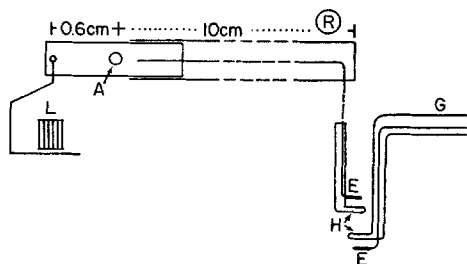


FIGURE 1

FIGURE 1. Apparatus for isotonic recording. *A*, axis of transducer to which lever is attached; *H*, holders for muscle; *E*, electrodes; *L*, load; *G*, glass tube carrying oxygen and a wire to an electrode; *R*, rod which slides horizontally and can be used to hold muscle lever.

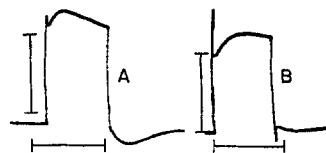


FIGURE 2

FIGURE 2. Effect of stretch and release by 12% on tension during contracture. Note delayed rise and fall of tension after change in length. (A) Muscle in 42 mM Ca; 20°C. (B) Muscle in KCl solution; 10°C. Calibration: abscissa, 1 min; ordinate, 1 g.

115, KCl 2, CaCl₂ 1, glucose 2, tris(hydroxymethyl)aminomethane chloride (Tris) 2, and had a pH of 7.2. High Ca solutions were obtained by mixing this solution with one in which all Na was replaced by 83 mM Ca. In K solution all Na was replaced by the equivalent amount of KCl.

RESULTS

1. *Effects of Rapid Changes in Length During Contractures* During contractures induced by high Ca (30–83 mM Ca) or KCl solutions, quick release caused under isometric conditions first a rapid drop of tension, then a further, much slower, diminution of tension and a slow rise. Stretch had a similar effect, but in the opposite direction (Fig 2). These effects could be obtained many times in the same preparation, but became gradually weaker, at least partly because of the gradual diminution in the strength of the contracture. Because the K-induced contractures subsided quite rapidly, effects under these conditions were generally weaker than those in high Ca solutions (Fig. 2 B).

Corresponding effects were obtained under isotonic conditions. After the passive effects produced by a change in load, further, much slower, changes in length were observed, which indicated changes in activity and which varied under different conditions. Above 15°C the muscle became shorter again, after the load was increased, then lengthened until a new stable length was established (Fig. 3 A). A decrease in load had the opposite effect. The rapid extension after an increase in the load was larger than the reverse effect induced by diminishing the load. At low temperatures the first peak of extension or shortening after changes in load were reached only after several seconds, indicating that these effects were not entirely passive.

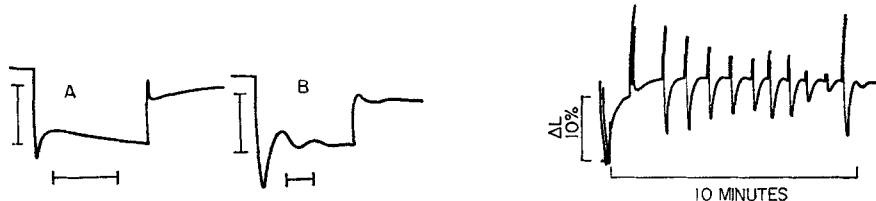


FIGURE 3

FIGURE 3. Effect of first a rapid increase then a decrease in load by 0.6 g during contracture induced by 42 mM Ca solution. (A) at high (16°C) and (B) same preparation at low (5°C) temperature. Calibration: abscissa, 1 min; ordinate, change in length by 5%.

FIGURE 4

FIGURE 4. Relaxation induced by brief passive shortening during contracture induced by immersion in 42 mM Ca solution. At the beginning of the contracture (at the left) are two twitches. The subsequent rapid upward movements are due to the passive movement of the muscle lever, allowing the muscle to shorten. Except during the rising phase of the contracture shortening was followed by extension and subsequent recovery, which gradually became oscillatory. Isotonic recording; load, 1.2g; 12°C.

In another type of experiment the muscle was allowed to shorten as much as 15% by lowering the muscle lever and then releasing it again (Fig. 4). The muscle lengthened strikingly at first, then returned slowly to its previous length. It made no difference whether the muscle stayed at the shorter length for half a second or for more than a minute. The results were also the same if the movement of the lever was controlled by a rack and pinion device which made extension slower. Noticeable effects were obtained by shortening of less than 1%; there was no threshold. No "undershoot" was observed in resting muscle.

2. *Oscillations* In the experiments just described the return to the original length was rather slow soon after the onset of the contracture. At low temperatures the rate of lengthening gradually became faster and an overshoot and finally oscillations developed (Fig. 4). At temperatures below about 15°C all changes in load induced oscillations (Fig. 3 B). An increase in load caused at first a large extension followed by damped sinusoidal oscilla-

tions of low frequency around an intermediate length. The return to the previous load produced much smaller shortening and also induced oscillations. The strongest oscillations were produced if the muscle was allowed to shorten 10–15% by depressing the muscle lever and then releasing it (Fig. 5). The initial strong extension then was followed by a contraction, usually overshooting the previous level of the contracture, and a repetition of this cycle. These effects were obtained at all Ca concentrations which induced a strong contracture, up to 83 mm, but a range of 40–50 mm was particularly favorable. Oscillations could be obtained 5–10 min after the contracture was induced, then increased gradually while their decrement decreased. When fully developed as many as six oscillations could be observed clearly after a rapid change in length. The smallest of the waves was about 0.1% of muscle

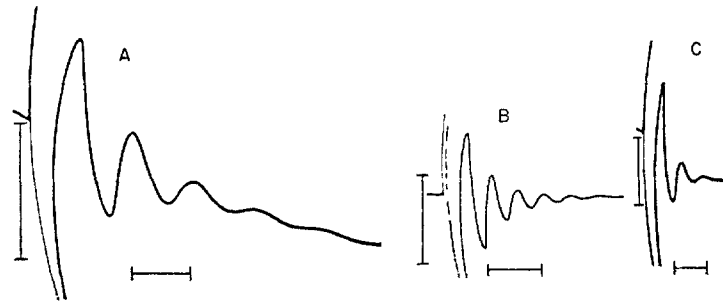


FIGURE 5. Oscillations in 42 mM Ca solution (A and B) and KCl solution (C). Temperature was 5°C in (A), 12°C in (B), and 10°C in (C). Oscillations were induced by lowering, then releasing, the muscle lever, causing the first upward stroke of the graphs, and allowing the muscle to shorten briefly. This was followed by large extension, indicated by a downward movement, and oscillations. In (A) the level of contracture dropped rapidly. Calibration: abscissa, 1 min; ordinate, change in length by 5%.

length. After immersion in high Ca solution for more than an hour the oscillations became weaker, probably because the contracture had diminished. They could be made to disappear and reappear in the same muscle by raising and lowering temperature.

The behavior of the muscle was essentially the same after immersion in KCl solution (Fig. 5 C), but the level of contracture dropped more rapidly; therefore, oscillations did not develop as fully as in high Ca solutions. In two muscles in which contractures disappeared slowly after the 42 mM Ca solution was replaced by Ringer solution, oscillations could be induced in Ringer solution for as long as 20 min, indicating that this type of activity was determined more by the level of contracture than by the nature of the medium.

Fig. 6 shows the frequency of oscillations of different preparations as a function of temperature. All values were taken when at least three cycles could be observed after a change in length. The duration of one cycle was about 75 sec at 4°C, 27 sec at 14°C, giving a Q_{10} of 2.7. Epinephrine ($5 \cdot 10^{-6}$

m) had no effect on the frequency and magnitude of oscillations; acetylcholine ($5 \cdot 10^{-5}$ M) sometimes increased oscillations slightly, probably because it also increased the contracture.

The ability to oscillate varied considerably in different preparations. All of the 26 muscles used during summer and autumn oscillated in high Ca or KCl solutions after changes in length, but the degree of damping and the number of cycles after each change in length varied. After November, however, at most two oscillations or none at all could be obtained in preparations from *Rana pipiens* after a rapid change in length. In these muscles the effects described in sections 1 and 2 were weak or absent. All preparations from the bullfrog could be made to oscillate also in winter.

3. *Modification of the Time-Course of the Isotonic Twitch* In these experiments the muscle was stimulated at a constant frequency, so that twitches were uniform. If the load was briefly removed from the muscle near the peak

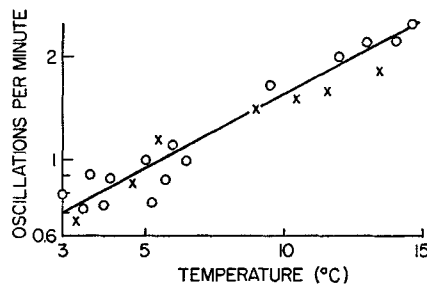


FIGURE 6. Effect of temperature on frequency of oscillations. Muscles were in high Ca (circles) or K solutions (crosses).

of a twitch, thus allowing the muscle to shorten, the reapplication of the load was followed by an abrupt relaxation (Fig. 7). The final part of relaxation generally followed the same time-course as during a normal twitch, but took place earlier. Therefore, the duration of the twitch was shortened. During an earlier phase of the twitch this procedure lowered the height of the twitch without much or any shortening of its duration (Fig. 7 C). Also in high Ca solution, the duration of the twitch remained unchanged, presumably because of the long duration of relaxation.

An inhibitory effect of shortening is also shown by the experiment illustrated in Fig. 8. At the beginning of an experiment muscles are often in a state of weak contracture, which diminishes gradually after repeated stimulation. In the presence of the contracture a twitch was followed by extension beyond the resting length. The same effect was produced by allowing the muscle to shorten passively. It should be noted that the return to the previous length follows exactly the same time-course in both cases. During contractures in high Ca solutions twitches usually had no "undershoot." Probably under

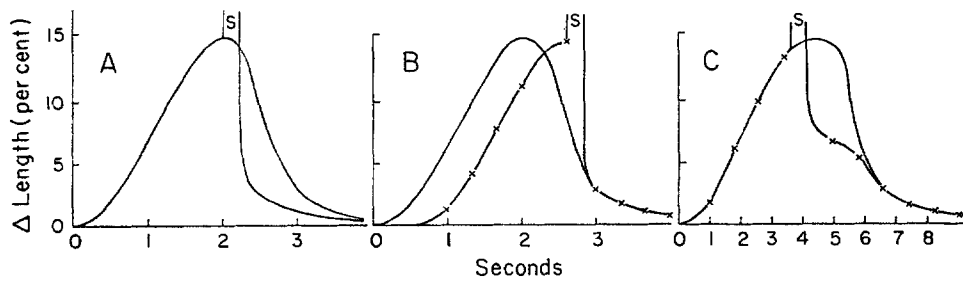


FIGURE 7. Effect of passive shortening on time-course of twitch. (A) and (B) show two successive twitches, in one of which muscle was allowed to shorten passively 10% near the peak: in (A) drawn so that the rising phase, in (B) drawn so that the last part of relaxation, is superimposed. Ringer solution; 9.5°C. (C), Two twitches superimposed; in one, marked by crosses, brief passive shortening occurs before the peak. Muscle in 3 mM Ca solution; 4°C. S, passive shortening.



FIGURE 8. Isotonic twitch in Ringer solution with "undershoot" (left) and effect of brief passive shortening produced by lowering muscle lever (right). Load, 1.2 g; 5°C.

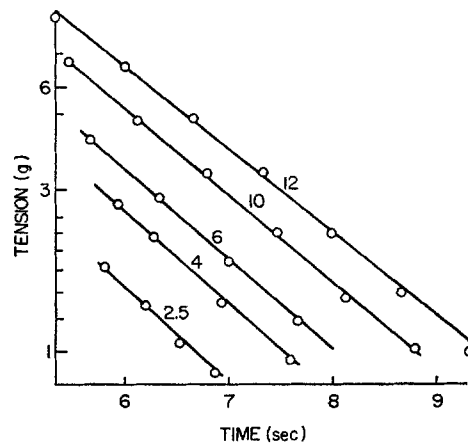


FIGURE 9. Last, isometric phase of afterloaded twitch, plotted semilogarithmically for different afterloads (given in grams above each line). With a load of 12 g the twitch was nearly isometric. Shortening at the smallest load was 31%. Abscissa, time after onset of contraction; preload, 1.5 g; 6°C.

these conditions relaxation was so slow that the inhibitory effect of shortening passed off before relaxation was complete.

4. *Effect of Shortening on the Onset of Relaxation* During an afterloaded isotonic contraction relaxation becomes isometric after tension becomes smaller than the load. This phase of the twitch was recorded by the method of Jewell and Wilkie (7) described above. Except for its beginning, isometric relaxation of the heart is exponential. In Fig. 9 this phase was plotted semilogarithmically for different afterloads. As observed previously for the sartorius, relaxation started earlier the smaller the load, that is, the more the muscle was allowed to shorten. When the load was diminished from the maximum which the muscle could lift to about one-fifth, the duration of the twitch was diminished by 22–28% of that of the isometric twitch in six experiments.

DISCUSSION

1. *Activation and Deactivation* During contractures quick changes in length cause slow and transient changes in length or tension opposite in direction to those expected. Quick release during a contracture would be expected to cause, after a rapid drop in tension, a rise to the level characteristic of the new length. However, tension actually dropped further for some seconds (Fig. 2), indicating that the shortening induced a transient diminution in activity. This result was confirmed by the observation that under isotonic conditions diminution of the load induced a transient extension (Fig. 3). This transient lowering of activity after shortening will be called deactivation, the reverse effect due to stretching activation. At low temperatures these effects combine to produce oscillations. In this type of activity, which is induced by any rapid change in length, increased length causes activation and thus shortening; this in turn leads to deactivation and extension, thereby starting a new cycle. The fact that these phenomena can be observed in depolarized muscles shows that they are due to an intracellular mechanism.

The properties of cardiac muscle just described are remarkably similar to those of insect fibrillar muscles, which oscillate during nerve stimulation in resonance with mechanical systems having a suitable range of frequencies (2, 9). These oscillations are due to the same basic processes, activation and deactivation, described here for cardiac muscle. Also glycerol-extracted insect fibrillar muscles can oscillate when immersed in a suitable solution containing adenosine triphosphate (ATP) due to the same basic properties (6). This is important because it shows that movements of Ca are not involved. That we are dealing here with phenomena of broad significance is shown by the fact that under similar conditions also glycerol-extracted vertebrate skeletal muscle shows activation and deactivation (10) and can oscillate (5, 8, 10).

In living frog skeletal muscle fibers a sudden change in load induces oscillations during a tetanic contraction (1).

It must be emphasized that the effects described here were obtained only during an "activated state," a term which is used here synonymously with "activation" as defined by Pringle (9). In living insect fibrillar muscle this state is achieved by nerve stimulation, in glycerol-extracted muscle by solutions containing ATP and a $[Ca]$ high enough to produce a weak contraction. In cardiac muscle contracture evidently also represents such an activated state, probably produced by a moderate increase in intracellular $[Ca]$.

The fact that the change in activity due to a change in length persists for a short time is essential for the initiation of oscillations and determines their frequency. That they are about 100 times slower in the heart than in insect muscle agrees with the difference in the duration of activation and deactivation, which persist for many seconds in the heart, only for about 0.1 sec in insect muscles. Another difference, the strong damping of oscillations in the heart, presumably is due to the fact that the muscle was not connected to a resonating system. That oscillations were obtained only at low temperatures probably was due to a large difference in the duration of activation and deactivation at temperatures above 15°C.

It would be important to know the precise nature of the signal which induces the changes in activity described. The new length or the change in length itself could be the determining factor. The latter of these possibilities would best agree with the transient nature of the effects. However, this does not explain why diminishing the load, then restoring it after variable periods of time, causes extension well beyond the initial length.

2. Role of Activation and Deactivation These processes are sensitive feedback mechanisms which may be expected to control activity whenever length changes. Changes in length of less than 1% had an effect on contraction. That shortening inhibits contractile activity had previously been concluded from a difference in the force produced under isometric and isotonic conditions (3), but such an effect could be due to a limitation in the available energy. It has also been reported that shortening lowers the active state (4).

More direct evidence for the assumption that the inhibitory effect of shortening comes into play during a twitch is the extension beyond the previous resting length after a twitch, if the muscle is in a state of weak contracture. Furthermore, in experiments in which length and tension were recorded simultaneously, it was found that shortening induces an earlier onset of relaxation. The same result has been obtained previously in skeletal muscle (7).

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