Mechanical and Electrical Oscillations
in Cardiac Muscle of the Turtle

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ABSTRACT  During contractures of the turtle ventricle rapid changes in length induce sinusoidal oscillations under isotonic conditions. They are due to delayed responses to stretching and release, which can be demonstrated also under isometric conditions. Oscillations of two distinct frequencies are produced under different conditions and are distinguished as high- and low-frequency oscillations. In depolarized muscles the frequency is such that the duration of one cycle is about the same as that of a normal twitch, while in high-Ca solutions the duration can be the same as in high-K solutions or about six times lower. As reported previously, twitches are followed by weak mechanical and electrical oscillations. Their frequency agrees with the high-frequency oscillations. The same effects can also be induced by stretching and release. It is suggested that the phenomena observed are due to feedback mechanisms which originate in the contractile mechanism. The high-frequency oscillations are similar to those observed previously in other muscles, particularly insect fibrillar muscle, and are not due to changes in Ca concentration. The other mechanisms involve the membrane and possibly the intracellular Ca stores.

During contractures of the frog ventricle, stretching induces a slow transient increase in activity, called activation; shortening has the opposite effect, called deactivation. These responses represent a feedback mechanism which can give rise to mechanical oscillations of low frequency (5). The work reported here was undertaken chiefly to determine whether these phenomena are related to electrical oscillations which precede or follow conducted responses of cardiac and smooth muscle (3, 4) and can initiate responses like normal, exponentially rising prepotentials. Such a relationship seemed possible because, in cases where this was tested, the ureter and the turtle ventricle, normal and oscillating prepotentials were accompanied by weak contractions. The studies on the turtle ventricle reported here agree with this suggestion and confirm a previous assumption (4) that electrical and mechanical changes have a common origin because both can be induced mechanically in active muscle. A new problem arose from the observation that oscillations of two different frequencies could be produced.
Regarding the mechanism of these contractile phenomena, it is important that oscillations and the underlying basic mechanisms can be observed in depolarized muscles, showing that they are not due to excitatory processes. The similarity with the mechanical properties of normal and glycerol-extracted insect fibrillar muscle has suggested, furthermore, that changes in internal concentration of Ca may not be involved (1, 11, 17). This conclusion is supported by studies on glycerol-extracted cardiac muscle in which activation and deactivation, and indirectly also the ability to oscillate, have been demonstrated (23).

METHODS

Unless stated otherwise the experiments reported here were carried out on the ventricle of small turtles (Chrysemys picta). The base and apex were cut off and most of the tissue inside was removed, so that a ring, about 1 mm thick and 3 mm wide was obtained. Also the atrium and ventricle of the bullfrog (Rana catesbeiana) were used in some experiments. Mechanical changes were recorded isotonically or isometrically with the apparatus described previously (5). During isotonic recording the length was changed passively by adding or removing weights or by depressing and releasing the muscle lever, thereby unloading and reloading the muscle. A Grass polygraph (Grass Instrument Co., Quincy, Mass.) with curvilinear ordinates was used. In the records upward movement indicates shortening or rise in tension.

For recording membrane potentials flexibly mounted intracellular electrodes with a resistance of about 30 MΩ were used. The muscle strips were attached in these experiments to a Grass transducer (Grass Instrument Co.) at the end of a rather flexible extension with a compliance of about 0.5 mm/g force.

Ringer solution contained in millimoles per liter: NaCl, 115; KCl, 2; CaCl₂, 1; glucose, 2; Tris, 2 and had a pH of 7.2 at 20°C. High-Ca solutions were made up by mixing this solution with one in which all Na was replaced by 83 mM Ca. In K solutions all Na of Ringer solution was replaced by K. Isometric solutions of K₂SO₄ with or without added Ca gave the same results. The solutions were gassed with O₂.

RESULTS

In most of the experiments reported here muscles were in a state of contracture induced by isosmotic K or high-Ca solutions. The results obtained with these two types of solutions will be described separately.

A. Oscillations in K Solutions

In isosmotic solutions of KCl or K₂SO₄, with or without added Ca, rapid changes in length caused damped sinusoidal oscillations under isotonic conditions (Fig. 1). The length was changed by changing the load or by depressing and releasing the muscle lever, thereby unloading and reloading the muscle. Oscillations were generally most striking after the level of the contracture had declined to about 60% of the peak, and disappeared at low levels.
The duration of one cycle was about the same as that of a twitch in Ringer solution at the same temperature, about 1 s at 25°C.

Oscillations are due to an alternation of activation and deactivation, as shown previously in experiments on the frog heart. These basic processes can be demonstrated in a simpler form by recording the changes in tension produced by stretching and releasing the muscle under isometric conditions. Without active responses tension would be expected to drop slowly to a new level after stretching and would slowly rise after release, as was observed under conditions described below (Fig. 2 C). Actually the rapid rise in tension pro-

![Figure 1](image1.png)

**Figure 1.** Effect of passive changes in length, recorded isotonically, during contracture in KCl solution. A: first increase in load from 2 to 3.5 g, causing rapid extension of muscle and oscillations, then removal of extra load. B: Same muscle. Brief unloading (upward movement) produced first large extension, then a brief contraction and oscillations. Load, 1.8 g. C: First set of oscillations was induced by diminishing load from 3 to 1.5 g. Then three records of the effect of unloading muscle briefly, with increasing intervals between unloading and reloading. Note change in the first brief contraction after loading.

![Figure 2](image2.png)

**Figure 2.** Effect of stretching and releasing on tension during contracture recorded isometrically. A: Contracture was produced by K solution in A and C, by 20 mM Ca solution in B. A and B, turtle ventricle; C, frog atrium. The rapid rise in tension is passive and is due to stretching of muscle by 8%; the rapid drop is due to rapid restoration of the previous length. The subsequent slow changes in A and B represent activation and deactivation. Note the difference in the speed of these changes and their absence in C. Calibration of ordinate, 0.5 g.
duced by stretching generally was followed by a slow, transient rise in tension, while release produced the opposite response (Fig. 2 A). Weak oscillations were sometimes also observed after stretching under isometric conditions, probably due to internal changes in length.

The decrement of the oscillations, and therefore their number after each change in length, varied widely in different animals. The ability to oscillate was diminished by prolonged storage of the animals and, in the same muscle, after several hours of experimentation, although the size of the twitches in Ringer solution was not greatly diminished. In muscles in which oscillations were weak when immersed in K solutions, the oscillations in other solutions, described below, were also weak.

Even in muscles in which no oscillations could be produced, activation and deactivation usually could be demonstrated under isometric conditions, sometimes only activation. However in the ventricle of two large turtles, in the ventricle of some frogs, and always in atria of the frog neither oscillations nor other signs of activation and deactivation were found. In these cases tension changed after stretch and release, as shown in Fig. 2 C.

When the muscle was extended by a heavy load a small spike sometimes appeared in records preceding the oscillations. This always occurred when the muscle was briefly unloaded by quickly lowering the lever and releasing it. When the interval between unloading and loading was increased the spike was smaller and appeared earlier; at a certain interval it was absent. The duration of the contraction responsible for the spike cannot be determined. However, if the moment of loading is considered the beginning, the peak was reached in 40% or less of the time to peak during a normal twitch. This brief response was sometimes also present in high-Ca solutions. That the spike is a phenomenon separate from the oscillations is evident in Fig. 1 B. In the type of record most commonly observed, this can be shown by the analysis illustrated in Fig. 3.

B. Oscillations in Ringer and High-Ca Solutions

Fresh muscles immersed in Ringer solution were usually in a state of weak contracture, indicated by an extension beyond the previous length after an isotonic twitch, as described previously for the frog ventricle (5), and also shown in Fig. 4 A. Below a temperature of about 15°C the contracture increased markedly. Then an isotonic twitch was generally followed first by a marked elongation beyond the previous length, and by damped sinusoidal oscillations with a frequency of about 1/min at 10°C. Their amplitude was increased by raising [Ca] of the medium to a level between 10 and 40 mM (Fig. 4 A). The ventricles of bullfrogs gave the same results. The duration of one cycle was about six times that of a normal twitch. Oscillations could not
be observed clearly if the contracture was weak, which was true in the turtle ventricle in 6 of a total of 26 experiments.

Oscillations of the same frequency could also be produced mechanically (Fig. 4 A). The underlying delayed effects of length changes, recorded isometrically, were much slower than in K solutions, as shown in Fig. 2, in agreement with the difference in frequency of oscillations.

Smooth muscle is present in the endocardium of the turtle atrium, and to a smaller extent in the ventricle. That this tissue is responsible for oscillations is excluded by the fact that, as mentioned above, the endocardium was removed in the preparations used. No smooth muscle activity, such as that of the atrium, was observed, and epinephrine and acetylcholine had no effect on the resting muscle.

At a temperature above about 15°C the oscillations just described could not be produced, but oscillations with a much higher frequency appeared in high-Ca solutions (Fig. 4 B) when a contracture was present. A long-lasting contracture was produced in many preparations in 20-42 mM Ca solutions. Then oscillations could be produced mechanically, by increasing or decreasing the load or by brief unloading (Fig. 4 B). Even if no sustained contracture was present in the high-Ca solutions, a slowly subsiding contracture was present after a twitch. Oscillations could then be induced mechanically as long as the contracture lasted. They had the same frequency as those in K solutions and
FIGURE 4. Oscillations in high-Ca solutions. A: low-frequency oscillations during contracture in 21 mM Ca solution induced first by brief unloading, then by a twitch (upper part not shown). Note relaxation below baseline after unloading and twitch. Load, 2.5 g. B: on left, twitch with high-frequency oscillations during relaxation. Load, 2 g. On right, effect of increasing load by from 2 to 3 g, then reducing it again; same muscle. C: Muscle was first stimulated twice. Top of twitches not shown; relaxation goes below baseline. Then two high-frequency oscillations, the first nearly reaching top of record, and slow oscillations. On right half of record low-frequency oscillations induced by brief unloading. 40 mM Ca.

will be designated as high-frequency oscillations, while the other type will be called low-frequency oscillations. The frequency of both types agreed closely in different preparations, as shown in Fig. 5, in which data from 25 muscles were plotted against temperature. The $Q_{10}$ for the high- and low-frequency oscillations was 4.0 and 3.3, respectively.

High-frequency oscillations were present also at low temperatures, but were highly damped and evident only after a series of twitches. Then they may be combined with low-frequency oscillations, as shown in Fig. 4 C. The low-frequency oscillations obtained under these conditions sometimes were very regular and nearly identical with those which were induced mechanically after a period of rest, but more often they were somewhat modified, as shown in Fig. 4 C, perhaps due to an interference between the two types of oscillations.

C. Electrical Oscillations

As shown previously, the high-frequency oscillations after twitches of the turtle ventricle are accompanied by weak electrical potential changes (4).
This result, obtained with external electrodes, was confirmed by using intracellular electrodes. In the experiment illustrated in Fig. 6 A the number and size of oscillations was small. Stronger mechanical, and probably also electrical, oscillations would have been obtained after a series of responses, but it was not possible to keep the electrodes inside for more than one twitch.

During a contracture electrical oscillations, paralleling the mechanical oscillations, could also be induced mechanically. They were present in all of 12 successful penetrations (Fig. 6 C, D), never when the muscle was relaxed (Fig. 6 B). Their magnitude did not exceed 3 mV. However, the possibility that they are artifacts can be ruled out because only very large passive movements sometimes produced immediate small electric effects. That the electrical oscillations depend on the activity of the contractile elements is shown by the fact that rapid changes in length never produced any electrical effect in the resting muscle.

**DISCUSSION**

**A. Distinction of Two Types of Oscillations**

As pointed out above, mechanical oscillations are due to a feedback mechanism in which stretching induces "activation," that is a delayed increase in activity, and shortening causes "deactivation," a delayed decrease in activity.

In the turtle ventricle-oscillations of two frequencies without intermediate values were found high-frequency oscillations, in which the duration of one cycle was similar to that of a normal twitch, and low-frequency oscillations, in
which the duration was several times this value. That they are due to different mechanisms is supported by the fact that both can coexist in the same muscle. That high-frequency oscillations do not involve the mechanism of excitation is shown by the fact that they can be induced mechanically in depolarized muscles. In the frog heart only low-frequency oscillations were found; also these responses could be induced in K solutions.

An ability to oscillate has been found in a variety of muscles: in insect fibrillar muscle (1, 11, 17), skeletal muscle (20), and cardiac muscle (5, 23), both in normal and glycerol-extracted preparations. It has been proposed, therefore, that activation and deactivation are a property of the contractile mechanism of all muscles (20). However, in the frog and turtle heart large quantitative differences in the ability to oscillate were found. In some preparations of the ventricle activation and deactivation could not be detected; they were never found during contractures of the frog atrium. Furthermore, the generalization mentioned can be applied only to the high-frequency oscillations because low-frequency oscillations have so far only been described for the living frog and turtle ventricle.

B. Relation between Mechanical and Electrical Oscillations

Prepotentials of cardiac and smooth muscle normally rise exponentially, but they can be oscillatory and are in this case often followed by oscillating after-
potentials (2, 3). In the pacemaker region of the ureter these potentials, which were oscillatory in some cases, were accompanied by weak mechanical changes (2). Similar observations were made for the oscillating after-potentials of the turtle ventricle recorded by external or internal electrodes.

It would seem reasonable to assume that the weak mechanical activity mentioned is induced by the changes in membrane potentials. However, it is well known that a contraction is induced only above the mechanical threshold (9) which in cardiac muscle has been found to be 35–40 mV in voltage-clamp experiments (8, 16). The potential change accompanying mechanical oscillations of the turtle ventricle, on the contrary, may be as small as 1 mV. It has been suggested, therefore, that prepotentials and the associated mechanical changes have a common origin within the fibers (4). The strongest argument for this assumption is the observation reported above, that mechanical oscillations which have the same frequency as the after-potentials can be induced mechanically, even in depolarized muscle and that electric oscillations can be produced by passive changes in length. The fact that these effects can be produced only in active muscle suggests that they arise in the contractile mechanism.

The existence of a link between the contractile mechanism and the surface membrane has also been concluded from entirely different types of experiments. It was observed that shortening during a twitch prolonged, stretching shortened the plateau of the action potential of the mammalian heart (22, 15, 14), and that these changes were followed by an inotropic effect on the succeeding 5–10 twitches (14). Kaufmann et al. (14) concluded that mechanical and electrical processes are linked together by a feedback mechanism. They also showed that no electric effects are produced in muscle during rest.

In mammalian cardiac muscle Reiter (18, 19) observed mechanical oscillations, which he called “Nachkontraktionen,” after a series of beats. They were observed under conditions favoring the onset of contractures, similar to those in the experiments on the turtle heart reported here. In their time relations they agree with the high-frequency oscillations, but no potential changes were observed.

C. The “Uncoupling Effect” (Brady) of Length Changes

Stretching or releasing of cardiac muscle during a response diminishes the tension below that of controls, an effect which has been called “uncoupling” by Brady (6, 7). That changes in length generally diminish and abbreviate activity has been shown strikingly in afterloaded twitches in which relaxation occurs the earlier the stronger the shortening, as has been first demonstrated for skeletal muscle by Jewell and Wilkie (12). As these authors suggested, the movement of the thin and thick filaments during changes in length hinders the formation of new crossbridges, thereby accelerating relaxation. If a moderate rate constant for the formation and breakage of these bonds is
assumed, as in the model of Huxley (10), the proportion of crossbridges attached to the thin filaments will be diminished by rapid changes in length, resulting in a loss of tension and diminution in contractile activity. As discussed by Kaufmann et al., (13) a variety of phenomena can be explained by this model.

In the experiments described by Brady and others, contractility was depressed by an increase and decrease in length, while the delayed effects underlying the oscillations are in opposite directions. The large extension produced by brief unloading could also be explained as uncoupling. It has been observed, however, that the full effect of the mechanical interventional appears with some delay. It is possible, therefore, that this effect is at least in part due to inactivation and not simply caused by slippage of the filaments.

D. Mechanism of Oscillations

Mechanically induced oscillations have been first discovered in insect fibrillar muscle (1, 17). The fact that they were found also in glycerol-extracted muscle fibers shows that neither membrane changes nor changes in internal [Ca] are involved. Changes in [Ca] were ruled out completely in these experiments by using Ca buffers and by treatment with a detergent, which inactivates nearly all enzymes. The basic properties responsible for oscillations have also been demonstrated in glycerol-extracted skeletal and cardiac muscle by recording the delayed tension changes after stretching and releasing and by an analysis of the changes in tension and length during forced oscillations of different frequencies (21, 23). At the optimal frequency the duration of one cycle varied widely in different types of muscle, but was always about the same as the duration of a normal twitch. Because the high-frequency oscillations described above follow this rule, and because of the similarity of the contractile phenomena, it appears probable that they are due to the same type of mechanism, and therefore, are not caused by changes in internal Ca.

To explain the low-frequency oscillations a second feedback mechanism must be assumed. That the contractile phenomena are the same in both types of oscillations, except for the difference in speed, may indicate that the same sensing mechanism is activated, which may also be responsible for the change in membrane potential described above.

For insect fibrillar muscle there is evidence that each oscillation is due to a single synchronized movement of the crossbridges (17, 21). It seems doubtful that this is true for the much slower oscillations of cardiac muscle, particularly for the low-frequency oscillations. However, the brief spike which may precede the oscillations may be due to synchronous activity of the crossbridges, made possible by the previous breaking of the bridges by rapid extension.
E. Conclusions

The observations presented here show that changes in length can produce direct effects on contractile activity, and indicate that all parts of the system involved in normal contractions interact so as to form a regulating system of astonishing complexity. It can be assumed that a sensing element in the contractile mechanism activates three feedback systems. One of these does not involve Ca and is assumed to be analogous to that in insect muscles. The second brings about a change in membrane potential, perhaps by altering permeability to K. This is shown by the potential changes associated with the mechanical oscillations and by a change in the plateau of the action potential. The membrane in turn can produce a delayed inotropic action, indicating a release of Ca from a Ca store. To account for the low-frequency oscillation it is furthermore tentatively assumed that the mechanical factors can influence the Ca store also more directly. This scheme is based chiefly on results obtained from the turtle heart and can also account for observations on the frog and mammalian heart, but the heart of different species and even different parts of the same heart seem to differ at least quantitatively in this respect.

Because activity is maintained continuously for some time, contractures are unusually favorable for studying how mechanical factors influence the contractile mechanism. The feedback mechanisms observed under these abnormal conditions may be expected to play a role in the control of normal cardiac activity. The mechanism responsible for the high-frequency oscillations evidently is suitable for directing the course of events during a single beat and probably is a factor determining the force-velocity relation. The low-frequency oscillations, on the other hand, indicate some action of longer duration and may be related to the inotropic effect arising from changes in the duration of the action potential, which extends over a series of several beats.

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