Spontaneous Mechanical Activity in Depolarized Frog Ventricle

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ABSTRACT Spontaneous mechanical activity can be produced in depolarized frog ventricle by bathing the tissue in a solution with low Na, low Ca, and high K⁺. The contractions can be inhibited by depleting the tissue of Ca first, but they are relatively insensitive to changes in either extracellular \([\text{Ca}^{++}]\) or \([\text{Ca}^{++}]/[\text{Na}^{+}]^2\). They are terminated very rapidly by raising \([\text{Na}^{+}]\) to 40 mM. Local anesthetics enhance the spontaneous activity in proportion to the concentration of their free base form. These contractions occur relatively rhythmically for several hours. Since the preparation is multicellular, this suggests a mechanism for intercellular communication without change in membrane potential.

INTRODUCTION

Although it is generally believed that depolarization of the surface membrane of an amphibian heart initiates the process of contraction and that direct activation of the contractile proteins is produced by saturation of Ca binding sites on troponin, the source of this calcium and the relationship of its release to depolarization are not known. In view of the fine control of tension by the membrane potential, Morad and Orkand (1971) have suggested that the activator calcium comes either from the membrane or through it. Tension, however, does not rise until 25-100 ms after the onset of depolarization except when the muscle has been previously depolarized to a suprathreshold level. Apparently some cellular processes must be initiated before a substantial increase in sarcoplasmic Ca can occur. The delay could be due to the time required for the concentration of sarcoplasmic calcium to rise to threshold level, but the early rate of rise of tension would indicate that the concentration of calcium is increasing too rapidly to require so long a period to reach threshold. Radiocalcium measurements (Niedergerke, 1963) of the uptake during contraction of frog heart do not provide decisive information about the adequacy of a transmembrane flux for activation of contraction, for although the influx is small compared with the amount necessary to produce the measured tension, the flux technique may underestimate the true movement of calcium. On the other hand, in turtle heart extracellular calcium does appear to move from the extracellular space to the contractile proteins during a cardiac cycle (Weidmann, 1959).

Intracellular stores of calcium capable of release and activation of contraction
have been demonstrated in frog heart (Winegrad, 1973), and total tissue calcium increases in proportion to the increased contractility as a function of the rate of stimulation (Sands and Winegrad, 1970). It is not clear, however, whether these pools are functional during normal contractions.

Spontaneous contractions can be produced in cardiac muscle under the appropriate conditions, and these contractions have been studied for the insights they can provide about calcium and excitation-contraction coupling in frog heart. A preliminary description of this work was given to The Physiological Society in 1972 (Winegrad, 1972).

METHODS

A ring of tissue about 1 mm thick was cut from the ventricle of *Rana pipiens* and then opened up to form a strip 5-10 mm in length. The strips were suspended in a bath at room temperature, and their tensions continuously recorded in a manner which has already been described (Winegrad, 1973). The solutions which were used in the experiments are given in Table I. The pH of all solutions was carefully checked after the addition of any drugs; it was normally 7.0.

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>SOLUTIONS USED IN EXPERIMENTS</th>
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<tr>
<td>Solution</td>
<td>NaCl (mM)</td>
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<tr>
<td>Ringer</td>
<td>117</td>
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<tr>
<td>I</td>
<td>3 × 10^-2</td>
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<tr>
<td>II</td>
<td>3 × 10^-3</td>
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<td>III</td>
<td>3 × 10^-3</td>
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<td>IV</td>
<td>10</td>
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<tr>
<td>V</td>
<td>3 × 10^-4</td>
</tr>
<tr>
<td>VI</td>
<td>40</td>
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The total amount of sodium and calcium contamination in solutions to which no sodium or calcium had been added was measured by atomic absorption spectrometry and found to be consistently about 3 μM for sodium and 5 μM for calcium. In solutions I–VI the presence of either sodium or calcium in micromolar concentrations indicates that no sodium or calcium was added, but a small amount of contamination existed.

Transmembrane potentials were measured with microelectrodes filled with KCl and displayed on both a Tektronix 565 oscilloscope (Tektronix, Inc., Beaverton, Ore.) and a Brush 220 ink recorder (Gould Inc., Chicago, Ill.). The microelectrodes had resistances of 8–10 MΩ and tip potentials of no more than 5 mV.

In some experiments the tissue was observed with differential interference optics in order to correlate the sarcomere pattern in different regions of the tissue with the mechanical performance of the tissue. A water immersion lens (40 × numerical aperture 0.75) was used with an illuminating cone of about 0.6 to permit relatively thin optical sectioning of the tissue.

RESULTS

Spontaneous Mechanical Activity

Spontaneous changes in tension (which will arbitrarily be called contractions in spite of their long time course) were seen in frog ventricles several minutes after
the Ringer's solution bathing the tissue had been replaced by a solution containing 140 mM K⁺ but no added Ca⁺ or Na⁺ (Fig. 1). This phenomenon was observed in about 90% of the approximately 60 experiments during which the spontaneous activity was studied. The contractions varied in amplitude from a small fraction of twitch tension to significantly greater than twitch tension, and in duration from about a minute to as long as several minutes. The rate of relaxation from a spontaneous contraction was almost always slower than the rate of rise in tension, and frequently small contractions were superimposed on larger ones. In some preparations the intermittent activity lasted as long as 12 h after the beginning of the exposure to the high K⁺ solution (Fig. 2). In most preparations large spontaneous contractions occurred about every 30 min, but occasionally the frequency was double that value. Although the spontaneous activity appeared in 140 mM KCl, 70 mM K₂SO₄ was used more often because the nature of the major anion appeared to make no difference, and cells would be
expected to swell considerably during prolonged exposure to a high concentration of KCl (Boyle and Conway, 1941).

The length of the interval between the beginning of the soak in high K+ and the first moderately large spontaneous contraction (excluding the contracture produced by the initial depolarization from the elevated K+ concentration) was dependent on both the state of the muscle before the K+ depolarization and the constituents of the depolarizing solution. The average interval between the transfer to 70 mM K₂SO₄ (solution II) and the first large spontaneous contraction was 20-30 min (Fig. 1 B). The addition of 5 mM EGTA to the depolarizing solution (solution III) for 5 min (Fig. 1 C) or 1 mM EGTA to the depolarizing solution for the entire period of exposure (Fig. 1 D) shortened the time to the first contraction, decreased both the frequency and the amplitude of spontaneous contractions, and resulted in the development of a moderate contracture instead of intermittent contractile activity.

There were three basic requirements for the production of the spontaneous contractions: (a) extracellular Na of less than 15 mM, (b) extracellular K of at least 80 mM, and (c) an extracellular Ca greater than 10⁻⁸ and less than 5 × 10⁻⁴ M. The anionic content of the solution did not influence the appearance of the spontaneous activity (compare Fig. 1 A and 1 B) nor did the modest changes in osmotic pressure associated with the substitution of 70 mM K₂SO₄ for 140 mM KCl. The critical concentration range for each of the cations was ascertained by determining the upper and/or lower concentration limits which did not inhibit established spontaneous activity. Sustained relaxation or a maintained low level of tension resulted with higher Na concentrations and either lower or higher calcium concentrations. The sensitivity to extracellular Na was the most pronounced (Fig. 3). A spontaneous contraction relaxed very rapidly with a time

FIGURE 2. Spontaneous contractions over several hours in ventricle soaked in 0.1 mM tetracaine, 65 mM K₂SO₄, 10 mM NaCl, 5 μM Ca, and 10 mM Tris buffered at pH 6.5. Note periodicity of about 25 min for contractions.
course consistent with the rate of diffusion through the extracellular space when extracellular Na was abruptly increased from 10 to 40 mM. The spontaneous activity returned when the Na concentration was lowered again, often in as short a time as 3 min. The contractions were quickly terminated even when the extracellular Ca concentration was elevated at the same time as the Na concent-

![Graphs showing the rate at which changes in Na, K, and EGTA concentrations inhibit spontaneous contractions.](image)

**Figure 3.** The rate at which changes in Na, K, and EGTA concentrations inhibit spontaneous contractions. All solutions contain 0.1 mM tetracaine buffered with 10 mM Tris at pH 6.5. (A) Increase of Na from 10 to 40 rapidly inhibits contraction. (B) The rate at which an increase in Na from 10 to 40 inhibits contraction is not altered by changing Ca²⁺ concentration to keep [Ca⁺⁺]/[Na⁺]² constant. (C) The addition of 1 mM EGTA inhibits slowly. A small spontaneous contraction occurs 3 min after addition of EGTA. (D) 5 mM EGTA inhibits more rapidly than 1 mM but more slowly than 40 mM Na⁺. Note difference in shape of relaxation after addition of EGTA from that after increase of Na. (E) Very slow effect of decreasing K⁺. (F) Failure of 0.1 mM Ca⁺⁺ to inhibit spontaneous contraction.

tration to maintain constant [Ca⁺⁺]/[Na⁺]² in order to eliminate the effect of Na on Ca exchange across the cell membrane (Lüttgau and Neidergerke, 1958). The response to a change in extracellular Ca in either direction was nowhere near as dramatic. The addition of Ca during a contraction caused only a questionable increase in the force, and the addition of EGTA only slowly inhibited the spontaneous mechanical activity. Replacement of most or all of the K by Li was followed by a similarly slow disappearance of the spontaneous contractions.
After the contractions had disappeared, restoration of 130 mM K to the extracellular fluid produced a large contraction, presumably due to the depolarization of the membrane which had repolarized in the low K, and then within 10 min a return of spontaneous activity.

**Response to Drugs**

Although the spontaneous mechanical activity was not changed by the addition of 10^{-3} M propranolol, 10^{-4} M atropine, or 0.1 mM ouabain, the amplitude and the rhythm of the contractions were significantly enhanced by the local anesthetics tetracaine and procaine (only concentrations between 10^{-5} and 10^{-4} M were tested). The former was more potent but the response produced by each was sensitive to extracellular pH (Fig. 4). Although a pH change between 6.0 and 8.0 had no effect itself on the spontaneous contractions greater alkalinity enhanced the effect of the anesthetics. Procaine with a pK_a of 9.0 did not alter the contractions at pH 6.5, but it enhanced their frequency and the amplitude at pH greater than 7.4. On the other hand, tetracaine, which has a pK_a of 8.2, had a marked effect on the contractions at pH 6.5, and at pH 7.4 the oscillations in tension gradually merged into a strong, maintained contracture. Since local anesthetics enter the cell primarily in the neutral form (Bianchi and Bolton, 1967) this pH dependency is consistent with an intracellular effect of the drug.

The addition of 3 mg/ml of the metabolic inhibitor oligomycin markedly decreased the amplitude of the spontaneous contractions and increased the
base-line tension at the same time. Addition of an equal amount of the solvent ethanol without the drug had no effect on the oscillations and produced only a questionable increase in base-line tension.

Source of Activation

The spontaneous contractions appeared to involve a slowly exchanging pool of cellular Ca. When the tissue had been moderately depleted of Ca by stimulating it in Ca-free Ringer's until the contraction height had fallen to about 5% of its initial value, the spontaneous contractions that appeared in 140 mM K, 0 or 10 mM Na solution were unchanged (Fig. 5). If, however, the muscle had been severely depleted of Ca by stimulating it in Ca-free Ringer's until the response had completely disappeared, the tissue remained quiescent for a long time in high K, Na-free solution. Spontaneous activity did not develop even after several hours when the severely Ca-depleted muscle was bathed in a solution containing 140 mM K and Ca and Na in a ratio which was lower than normal as a result of the addition of 12 mM Na, but if the [Ca]/[Na]^2 ratio exceeded that in normal Ringer's the spontaneous contractions appeared and became larger with time (Fig. 6).

Coordination of Spontaneous Activity

In spite of the multicellular nature of the ventricular tissue and the maintained depolarization of the cells from the high K^+, which prevented electrical conduction, a large percentage of the cells periodically contracted in phase for as long as 12 h. Maintained synchrony of that duration would have been unlikely in the absence of some kind of communication among the depolarized cells. During the period of elevated but relatively constant tension between the spontaneous contractions, all muscle fibers had essentially the same sarcomere lengths except for those at the damaged edges. There were no wavy myofibrils that would have been indicative of passive shortening and no high frequency movements. Activations...
Figure 6. The effect of severe depletion of Ca on the appearance of spontaneous contractions. The ventricular strip was stimulated in Ringer's solution with no added Ca$^{++}$ until the contractile response to electrical stimulation disappeared completely. The solution was then replaced at the arrow by one containing 140 mM KCl, 0.1 mM tetracaine, 5 μM Na, and 5 μM Ca. Notice the long lag before spontaneous contractions begin.
tion was probably relatively uniform among all the cells during the sustained tension between the spontaneous contractions. As the spontaneous contractions did not always begin in the same cells, it was not technically possible to catch the onset of increased force development and to determine the size of the initial contracting unit, but a large number of shortening cells was seen during the rising phase of a spontaneous contraction. The shortening gradually spread without any indication of localized relaxation, and the decline in tension was associated with a general increase in sarcomere length. A quick stretch of the tissue between spontaneous contractions did not initiate any mechanical activity, and a small, quick release during a spontaneous contraction was followed by redevelopment of tension.

The addition of 4 mM Ni\(^{++}\) to the high K, low Na solution had no effect, but 4 mM Mn\(^{++}\), which inhibits transmembrane movements of Ca (Hagiwara and Nakajima, 1966), completely blocked the spontaneous contractions and produced an increased level of maintained tension (Fig. 7). In the presence of Mn\(^{++}\) no localized mechanical activity was observed through the microscope, but after the removal of the Mn\(^{++}\) spontaneous contractions of smaller amplitude reappeared.

**Electrophysiology**

Elevation of the extracellular concentration of K\(^+\) to 140 mM always depolarized the cells to a potential between -5 and -10 mV and in three preparations where the potential was measured during several spontaneous contractions no change occurred in either the superficial or the deeper fibers.

In view of the observations of Wiggins and Cranefield (1974) that cardiac Purkinje cells were repolarized by raising extracellular Na after a period of superfusion with Na-free or low Na solutions, the response of the membrane potential in the contracting ventricular strip to an abrupt increase in extracellular Na sufficient to terminate spontaneous mechanical activity was measured. When the concentration of Na was raised from 10 to 40 mM and the K concentration decreased from 130 to 100 mM, force abruptly declined and the transmembrane potential increased from -5 to the range of -40 to -45 mV. This repolarization occurred each time the Na was raised and then remained as long as the Na was 40 mM, for as long as 40 min. Depolarization occurred rapidly with the withdrawal of Na.
DISCUSSION

Spontaneous contractions in mammalian cardiac muscle have been described by Bloom (1970), Fabiato and Fabiato (1973), and Glitsch and Pott (1975), but neither Bloom nor Fabiato and Fabiato were able to find the same phenomenon in frog ventricle. Although the spontaneous activity in the mammalian heart had a sensitivity to extracellular Na and appeared to be related to an intracellular release of Ca\(^{++}\) in the absence of change in the potential difference across the surface membrane, it differed in some fundamental ways from what is reported here for frog ventricle. The former had a higher frequency, a much shorter duration, and a much smaller amplitude than those observed in the frog. They could easily have been due to changes in individual cells or in small groups of cells in which the surface membranes had been purposely disrupted mechanically. The amplitude of the spontaneous contractions in the frog ventricle sometimes equaled 150% of the maximum tension which could be produced by the tissue with conventional stimulation and consequently must have represented a coordinated activity of many cells.

Source of Activation

The activator responsible for the cyclical production of spontaneous activity was probably calcium since it alone of the various physiological substances which can rapidly alter the state of the contractile proteins is stored in the cell in a manner permitting rapid release and reaccumulation (Winegrad, 1965; Jöbsis and O'Connor, 1966; Ashley and Ridgway, 1968). It is unlikely that the tension was due to low intracellular MgATP in view of the rapid relaxation in response to an increase in Na. The decline in the amplitude of the contractions with depletion of tissue calcium and the lack of a rapid response of the spontaneous activity to changes in either extracellular Ca\(^{++}\) or the ratio of \([\text{Ca}^{++}] / [\text{Na}^{+}]\) both favor an intracellular over an extracellular source. The existence of a sizable pool of exchangeable calcium in resting frog heart cells has already been demonstrated (Winegrad, 1973).

The sarcoplasmic reticulum is the most likely intracellular source of the activator calcium for several reasons: (a) the involved calcium is slowly exchanging; (b) oligomycin seems to inhibit its storage; this drug would block Ca uptake by sarcoplasmic reticulum but in the presence of O\(_2\) and substrate mitochondrial Ca uptake would still be supported by the "phosphorylated intermediate" (Chance, 1965); and (c) local anesthetics, which have been shown to increase Ca leakage from the sarcoplasmic reticulum (Thorpe and Seeman, 1971), enhance the spontaneous activity, probably due to an intracellular action, as their effect is proportional to the extracellular concentration of the more permeant or uncharged form of the drug (Bianchi, 1968; Bianchi and Bolton, 1967).

In a large percentage of experiments where spontaneous contractions were observed some elevation in tension was maintained between the contractions, and the spontaneous contractions themselves could be reversibly converted to a sustained force by changes in the bathing solution such as the addition of Mn\(^{++}\) or the elevation of pH in the presence of tetracaine. Although there was no conclusive evidence the similar persistence of the sustained and the periodic
force in a very low extracellular concentration of Ca suggests that they may be manifestations of a similar kind of release of intracellular Ca, one continuous and the other intermittent.

Nature of Activation Process
Although the mechanism of the activation process is not clear, the requirement for elevated K⁺ and the rapid drop in tension as the membrane is repolarized from an increase in extracellular Na indicate its sensitivity to the transmembrane potential difference. This in itself is of some interest as most discussions of the transmembrane potential difference in amphibian heart have been concerned with its effect on events localized entirely to the sarcolemma. Some loss of cellular calcium is probably required for the initiation of the contractions in view of the shortening of the time for onset by the presence of EGTA in the bathing solution. Additional factors must be involved, however, possibly at the level of the sarcoplasmic reticulum rather than the surface membrane, to account for the initial need for low Na and the cyclical nature of the spontaneous activity.

The repolarization which was produced by increasing extracellular Na was similar to what has been reported by Wiggins and Cranefield (1974) in cardiac Purkinje fibers, but it occurred in spite of an extracellular concentration of K of 100 mM. Their suggestion that the repolarization is produced by an electrogenic extrusion of Na may be applicable in these experiments, but the very rapid response to the increase in Na and the relatively constant membrane potential over a period of 45 min after the elevation of Na, when intracellular Na must have been changing, argues more in favor of an extracellular role for the Na. An electrogenic Ca-Na exchange is one possibility.

Intercellular Communication
The synchrony of the spontaneous contractions over many hours in a population of depolarized cells indicates that intercellular communication can probably occur without changes in membrane potential. The low resistance junctions between cells (Weidmann, 1970) provide one mechanism, and Ca diffusion from active cells could trigger a release of additional calcium from stores in adjacent cells (Ford and Podolsky, 1972; Endo et al., 1970; Fabiato and Fabiato, 1973). In view of the relative insensitivity of the spontaneously contracting tissue to abrupt increases in the extracellular concentration of Ca it is unlikely that coupling between cells involves Ca diffusion out of one cell into the extracellular space before diffusion into the adjacent cells. Since the intracellular concentration of Ca which raises the resistance of intercellular junctions is higher than what is necessary to produce tension or Ca-triggered Ca release in heart muscle (Loewenstein et al., 1967; Fabiato and Fabiato, 1973), alteration of the low resistance pathway during the activation should not have occurred. The reversible depression of coordinated spontaneous contractions by Mn⁴⁺ could have been the result of the ions' inhibition of transmembrane movements of Ca (Hagiwara and Nakajima, 1965).

In summary, the experiments described above indicate that in frog ventricle there is a cellular pool of Ca which becomes labile when the bathing solution...
contains low Na, high K, and low Ca, and that this pool may be the sarcoplasmic reticulum. Contractions may be propagated from cell to cell by the diffusion of Ca across permeable junctions.

Supported by Research Grants from the U.S.P.H.S. (NB 04409) and the American Heart Association (73-686).

Received for publication 29 September 1975.

REFERENCES


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