Acidosis Facilitates Spontaneous Sarcoplasmic Reticulum Ca\(^{2+}\) Release in Rat Myocardium


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ABSTRACT Previous studies have shown that acidosis increases myoplasmic [Ca\(^{2+}\)] (Ca\(_i\)). We have investigated whether this facilitates spontaneous sarcoplasmic reticulum (SR) Ca\(^{2+}\) release and its functional sequelae. In unstimulated rat papillary muscles, exposure to an acid solution (produced by increasing the [CO\(_2\)] of the perfusate from 5 to 20%) caused a rapid increase in the mean tissue Ca\(_i\), as measured by the photoprotein aequorin. This was paralleled by an increase in spontaneous microscopic tissue motion caused by localized Ca\(^{2+}\) myofilament interactions, as monitored in fluctuations in the intensity of laser light scattered by the muscle. In regularly stimulated muscles, acidosis increased the size of the Ca\(^{2+}\) transient associated with each contraction and caused the appearance of Ca\(_i\) oscillations in the diastolic period. In unstimulated single myocytes, acidosis depolarized the resting membrane potential by ~5 mV and enhanced the frequency of spontaneous contractile waves. The small sarcolemmal depolarization associated with each contractile wave increased and occasionally initiated spontaneous action potentials. In regularly stimulated myocytes, acidosis caused de novo spontaneous contractile waves between twitches; these waves were associated with a decrease in the amplitude of the subsequent stimulated twitch. Ryanodine (2 \(\mu\)M) abolished all evidence of spontaneous Ca\(^{2+}\) release during acidosis, markedly reduced the acidosis-induced increase in aequorin light, and reduced resting tension. We conclude that acidosis increases the likelihood for the occurrence of spontaneous SR Ca\(^{2+}\) release, which can (a) cause spontaneous action potentials, (b) increase resting tension, and (c) negatively affect twitch tension.

INTRODUCTION

Acidosis decreases twitch tension in mammalian heart, but increases the size of the Ca\(^{2+}\) transient that initiates contraction (Allen and Orchard, 1983). An

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increase in resting cytosolic \([\text{Ca}^{2+}]\) (\(\text{Ca}_i\)) has also been observed during acidosis (Lea and Ashley, 1978; Bers and Ellis, 1982). An increase of \(\text{Ca}_i\) can lead to spontaneous releases of \(\text{Ca}^{2+}\) from the sarcoplasmic reticulum (SR) (Orchard et al., 1983; Wier et al., 1983; Allen et al., 1984b; Kort et al., 1985b; Capogrossi and Lakatta, 1985), which may produce (a) a transient depolarization of the cell membrane, which, if large enough, can trigger an action potential (Bahinski et al., 1986); (b) an altered diastolic tone (Lakatta and Lappe, 1981; Stern et al., 1983; Kort et al., 1985a, b); and (c) an altered release of \(\text{Ca}^{2+}\) during a subsequent stimulated twitch (Suarez-Isla et al., 1984; Kort and Lakatta, 1984; Capogrossi and Lakatta, 1985; Allen et al., 1985; Valdeolmillos and Eisner, 1985). It seemed possible that these effects may play some role in the changes of twitch tension observed during acidosis.

The direct effect of acidosis on the SR is to decrease \(\text{Ca}^{2+}\) uptake and thus subsequent release (Fabiato and Fabiato, 1978; Inesi and Hill, 1983; Fabiato, 1985a). It cannot be predicted, therefore, whether during acidosis an increase of \(\text{Ca}_i\) will enhance spontaneous SR \(\text{Ca}^{2+}\) release or whether the direct depression of the SR function might inhibit or abolish it in spite of a raised \(\text{Ca}_i\). There is evidence that the former is the case in preparations bathed in low [\(\text{Na}^+\)] (Allen et al., 1984a).

In the present experiments, intact cardiac muscle and single cardiac myocytes from rat myocardium were used to examine the effect of acidosis on spontaneous SR \(\text{Ca}^{2+}\) release. Rat myocardium is particularly prone to such \(\text{Ca}^{2+}\) oscillations since they occur at relatively low perfusate \([\text{Ca}^{2+}]\) (Stern et al., 1983; Lakatta et al., 1985b; Kort et al., 1985a; Capogrossi et al., 1986a). These spontaneous \(\text{Ca}^{2+}\) releases result in mechanical oscillations that are accompanied by a small (1–3 mV) depolarization of the cell membrane (Suarez-Isla et al., 1984) and are abolished by inhibitors of SR function (Lakatta et al., 1985b).

These experiments showed that in unstimulated preparations, acidosis enhanced the frequency of these spontaneous oscillations. The magnitude of the membrane depolarization associated with each oscillation also increased and on occasion became large enough to trigger spontaneous action potentials. Ryanodine (2 \(\mu\)M) or caffeine (10 mM) abolished the spontaneous mechanical and electrical oscillations both before and during acidosis. In stimulated preparations, acidosis caused spontaneous release in the diastolic period. When this occurred in single myocytes, the diastolic length transiently decreased, the membrane potential transiently depolarized, and the amplitude of the next twitch was reduced. These results demonstrate that acidosis exacerbates spontaneous \(\text{Ca}^{2+}\) release in unstimulated rat myocardium, and that such release may modulate the response of tension to acidosis and may be arrhythmogenic.

**METHODS**

**Papillary Muscle Experiments**

Rats were killed using either chloroform or cervical dislocation. A thin (\(\leq 0.9\) mm; range, 0.3–0.9 mm), uniform papillary muscle was dissected from the right ventricle and mounted horizontally in a muscle bath (0.3 ml vol) between a fixed hook and a tension transducer (UC2, Statham, Inc., Oxnard, CA). The muscle was superfused with Tyrode solution at
a rate of 12 ml/min, and maintained at 30°C. Two series of experiments were carried out using papillary muscles. In the first, tension and microscopic tissue motion were monitored. In the second, tension and Ca\textsuperscript{2+} were monitored.

**Measurements of Tissue Motion**

Spontaneous Ca\textsuperscript{2+} release causes spontaneous myofilament motion, which propagates longitudinally as a contractile band (Kort and Lakatta, 1984; Kort et al., 1985). The presence of motion caused by contractile waves within papillary muscles can be detected by monitoring fluctuations in the intensity of laser light, which has been scattered by the muscle (Stern et al., 1983; Kort and Lakatta, 1984). The methods used for their detection have been described previously (Stern et al., 1983; Kort and Lakatta, 1984). Briefly, the light scattered at 30° from an incident HeNe laser was collected on a photomultiplier tube, the output of which was analyzed using an autocorrelator. In the present study, the normalized autocorrelation function (which is related to the product of the frequency and amplitude of the motion of light scatterers) was characterized by its half-time of decay as in previous studies (Stern et al., 1983).

**Measurement of Ca\textsuperscript{i}**

Ca\textsuperscript{i} was monitored using the photoprotein aequorin. The methods were essentially the same as those described by Orchard and Lakatta (1985). Briefly, the photoprotein aequorin was microinjected into 50–100 superficial cells of the papillary muscle. Aequorin light was monitored using a photomultiplier tube (9893B/350, Thorn-EMI Gencom, Inc., Plainview, NY). The output from the photomultiplier tube, along with tension and a stimulus marker, was displayed on a four-channel pen recorder (2400S, Gould Inc., Cleveland, OH). Since the aequorin light signal is intrinsically noisy, in stimulated muscles it is necessary to average the responses to several stimuli to achieve an adequate signal-to-noise ratio. This was achieved using an MNC-II microcomputer (Digital Equipment Corp., Maynard, MA), sampling at 1 kHz, or a Compaq microcomputer, sampling at 3 kHz. All signals were also recorded on an FM tape recorder (3968A, Hewlett-Packard Co., Palo Alto, CA) for later analysis. The use of aequorin as an indicator of Ca\textsuperscript{i} in heart muscle is now well established (for a recent review, see Blinks et al., 1982). For the purposes of the present experiments, three points should be noted. (a) The amount of light emitted by aequorin is proportional to [Ca\textsuperscript{2+}] raised to the power of 2.5; gradients of Ca\textsuperscript{i} will lead to disproportionately large increases of light and thus the estimates of Ca\textsuperscript{i} given should be regarded as upper limits. (b) The effect of acidosis on aequorin light emission is to decrease slightly the amount of light emitted at a given pCa (see Allen and Orchard, 1983). (c) Aequorin light was converted to [Ca\textsuperscript{2+}] as described previously (Orchard and Lakatta, 1985) using the method of Allen and Blinks (1979). However, in the presence of inhomogeneity of [Ca\textsuperscript{2+}] within and among cells owing to the presence Ca\textsuperscript{2+} oscillations, the [Ca\textsuperscript{2+}] reported represents a spatiotemporal-averaged tissue value, which is not interpreted as the average [Ca\textsuperscript{2+}] within a given myocyte (Orchard et al., 1983; Kort et al., 1985).

**Single-Cell Experiments**

Single myocytes were obtained by enzymatic dissociation of rat hearts, as described previously (Capogrossi and Lakatta, 1985). Approximately 70% of the cells obtained in this way are rod-shaped, have a clear structure and a normal resting membrane potential, and respond to electrical stimulation with a twitch contraction. Cells showing these characteristics were observed and monitored as described previously (Capogrossi and Lakatta, 1985). Briefly, the cells were placed in a chamber perfused with Tyrode solution (see below) on the stage of a microscope (E. Leitz, Inc., Rockleigh, NJ). A video camera
on one port of the microscope allowed the image to be recorded on tape. The passage of a spontaneous contractile wave or an electrically stimulated twitch causes a displacement of the cell edge. The magnitude of this displacement was monitored on the video image using an edge motion detector (model 303, Instrumentation for Physiology and Medicine, Inc., San Diego, CA). The output from the edge detector, along with a stimulus marker, was displayed on a two-channel pen recorder.

In some experiments, membrane potential was monitored in these cells as described previously (Houser et al., 1985). Briefly, monocytes were placed in an experimental chamber mounted on the stage of an inverted (Nikon Diaphot) microscope. The bath (37°C) was continuously superfused with Tyrode solution (pH 7.35). Single myocytes were impaled with standard microelectrodes (30–60 MΩ) filled with 5 M KCl. Microelectrodes were connected to a high-impedance negative capacitance amplifier (Axoclamp 1A, Axon Instruments, Burlingame, CA) for faithful measurement of transmembrane potential.

Solutions
All experiments were carried out in Tyrode solution containing (mmol/liter): 135 Na+, 5 K+, 1 Mg2+, 104 Cl−, 1 HCO3−, 1 HPO42−, 20 acetate, 10 glucose, 4 × 10−5 insulin, and 5% CO2/95% O2 to give a pH of 7.35. Perfusate [Ca2+] (Ca0) was varied between 2 and 5 nM by additions of 1 M CaCl2. Unless otherwise stated, Ca0 was 4 mM. Ouabain and ryanodine were kept as concentrated stock solutions of 10 and 1 mM, respectively, and diluted to their final concentrations before use. Solutions containing caffeine were made up fresh for each experiment. To produce acidosis, the Tyrode solution was equilibrated with 20% CO2/80% O2 to give a final solution pH of 6.8. Preparations were exposed to this solution for 20 min.

RESULTS
Effect of Acidosis on Unstimulated or Very Slowly Stimulated Preparations

Papillary muscle experiments. Fig. 1A shows the effect of increasing the [CO2] of the superfusate on the spontaneous, microscopic tissue motion of a rat papillary muscle in the absence of stimulation or at very low (<0.05 Hz) stimulation rates. On exposure to the acid solution, there was an immediate decrease in this motion, as indicated by a decrease in the scattered light intensity fluctuations (SLIF), quantitated as f0, (see figure legend). Over the next 5–10 min, the SLIF increased to ~140% of control and then subsequently declined again toward control values. This result shows that there are three phases in the changes of microscopic tissue motion during acidosis: a rapid decrease, a subsequent increase with an overshoot, and then a final return toward control values. SLIF depend on the degree of Ca2+ loading of the cell, SR function (Stern et al., 1983; Kort and Lakatta, 1984), and the myofilament response to Ca2+ (Lakatta and Lappe, 1981). We have used the photoprotein aequorin to determine whether the observed changes of SLIF could be ascribed to changes of Ca0.

In unstimulated muscles in normal Tyrode solution, we could not detect resting Ca0 with aequorin, or a change in resting Ca0 owing to the acidosis, which is in agreement with a previous study (Allen and Orchard, 1983). However, if Ca0 was raised to 5 mM in the presence of 100 μM ouabain, acidosis produced a detectable increase of Ca0 (Fig. 2A). Ca0 increased to a peak after 5–10 min,
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before declining toward control values. The time course of this increase in \( \text{Ca}_i \) during acidosis was very similar to the increasing phase of SLIF (compare Figs. 1A and 2A). Fig. 2A also shows that resting tension, like SLIF, initially decreased on exposure to the acid solution, recovered slightly, and then fell gradually.

Fig. 2B shows that the addition to the perfusate during exposure to the acid solution of \( 2 \mu \text{M} \) ryanodine, which inhibits SR \( \text{Ca}^{2+} \) release (Fabiato, 1985b), led to a decrease of acouorin light and a parallel decrease of resting tension. Fig. 2C shows that when the muscle was subsequently exposed to the acid solution, still in the presence of ryanodine, the increase in \( \text{Ca}_i \) was barely detectable, and resting tension declined monophasically. Similar effects of acidosis on \( \text{Ca}_i \) and of ryanodine were observed in ferret muscles (Orchard, 1986) with the exception.

**Figure 1.** (A) The effect of acidosis on mean (± SEM) tissue motion in rat papillary muscles (\( n = 3 \)) before, during, and after acidosis. Tissue motion was monitored by monitoring fluctuations in laser light scattered by the muscle (see Methods). These were quantified as \( f_n = \frac{1}{2} \pi \cdot t_n \), where \( t_n \) is the half-time of decay of the normalized autocorrelation function of the scattered light. The period of exposure to acidosis is shown above the record. (B) Developed tension in response to an electrical stimulation every 30 s.

that, even in the presence of ryanodine, acidosis always caused a measurable increase in acouorin light. It appears most likely, therefore, that the decrease of resting tension during acidosis in ryanodine, which does not alter myofilament \( \text{Ca}^{2+} \) sensitivity (Fabiato, 1985b), is due to acidosis decreasing myofilament \( \text{Ca}^{2+} \) sensitivity (Fabiato and Fabiato, 1978; Blanchard and Solaro, 1984). The effect of ryanodine on resting tension (C vs. A), and on acouorin luminescence in Fig. 2, B and C, can be attributed to the inhibition of the spontaneous \( \text{Ca}^{2+} \) oscillations by ryanodine (Wier et al., 1983; Orchard et al., 1983). Thus, exaggeration of periodic \( \text{Ca}^{2+} \) oscillations during acidosis produces an increase in \( \text{Ca}_i \), averaged...
throughout the tissue, which increases resting tension (Kort et al., 1985a) and hence offsets the acidosis-induced decrease in myofilament Ca\(^{2+}\) sensitivity. This effect of acidosis on spontaneous Ca\(^{2+}\) oscillations could be due to an increase in their frequency or amplitude or both. Direct observation of the myofilament response to spontaneous Ca\(^{2+}\) release in single myocytes permits differentiation between these possibilities (Kort et al., 1985a).

**Figure 2.** The effect of acidosis and ryanodine on Ca\(_i\) and resting tension in an unstimulated papillary muscle (see text). In each panel, the top record shows aequorin light filtered at 0.1 Hz, and the lower panel shows resting tension. The period of acidosis is shown above the record. Before acidosis, the light is the dark current of the photomultiplier tube and resting tension is 10.4 mN/mm\(^2\). (A) The effect of exposure to acidosis on aequorin light and resting tension. (B) The effect of addition of 2 \(\mu\)M ryanodine to the perfusate (arrow) on the increase of aequorin light produced by acidosis. (C) Subsequent exposure to acidosis in the presence of 2 \(\mu\)M ryanodine. All solutions contained 5 mM Ca\(^{2+}\) and 100 \(\mu\)M ouabain. The aequorin light record is calibrated as fractional luminescence (FL). An FL of 10\(^{-5}\) is equivalent to a Ca\(_i\) of 5.7 \(\times\) 10\(^{-7}\) M. Because of the spatiotemporal inhomogeneity in Ca\(_i\) caused by spontaneous oscillatory Ca\(^{2+}\) release (Kort et al., 1985b), this value represents an upper limit for cell [Ca\(^{2+}\)].

**Single-cell experiments.** Before exposure to acidosis, unstimulated rat cells, like muscles, exhibit spontaneous contractile waves. Fig. 3A shows the effect of acidosis on spontaneous contractile waves in a representative single myocyte. On exposure to the acid solution, there was an immediate and rapid decrease in the frequency of the spontaneous contractile waves. Frequency then increased above the control level before declining again toward control (Fig. 4A). The time
course of the effect of acidosis on contractile wave frequency parallels its effect on SLIF and resting tension. The amplitude of the cell displacement owing to the passage of a wave also increased, but this was small compared with the frequency effect (Fig. 4B). The spontaneous contractile waves during acidosis were reversibly abolished by caffeine (Fig. 3B), which causes a net release of \( \text{Ca}^{2+} \) from the SR, leading to its \( \text{Ca}^{2+} \) depletion. It is notable that as the wave frequency increased during acidosis, the resting cell length decreased (this can be seen in Fig. 3B as an upward shift in the baseline as the waves recur after removal of caffeine during acidosis).

Fig. 5A shows the membrane potential of an isolated myocyte before, during,
and after exposure to acid solution. Before acidosis (left side), the membrane potential transiently depolarized during the passage of a contractile wave (cf. Suarez-Isla et al., 1984). In four cells, the control resting membrane potential was \(-79 \pm 1.0\) mV and the transient depolarization associated with these waves was \(3.05 \pm 0.7\) mV. Exposure to the acid solution led to (a) a decrease of resting membrane potential (Fig. 5, A and C) and (b) an increase in the amplitude of the transient depolarization associated with each contractile wave (Fig. 4B). The augmented transient depolarization that accompanied the contractile wave during acidosis was sometimes of sufficient amplitude to trigger an action potential (large deflections in Fig. 5A). Fig. 5B shows, on an expanded time base, a spontaneous action potential arising from a spontaneous transient depolarization that accompanied the contractile wave. The right-hand tracing illustrates an electrically stimulated action potential for comparison. It is clear that the spontaneous action potential has a smaller Na\(^{+}\) spike, presumably because of the initial slow depolarization, and is longer than the control action potential. When the acid solution was removed, these changes were reversed on returning to normal pH.

In the above experiments in myocytes, the bathing [Ca\(^{2+}\)] was 4.0 mM. The issue arises whether acidosis will have similar effects at lower bathing [Ca\(^{2+}\)] and thus at lower Ca\(_i\). We addressed this possibility in the experiments depicted in
Figs. 6 and 7. Fig. 6 shows that in cells bathed in \([\text{Ca}^{2+}]\) of 2.0 mM, effects of acidosis were observed on resting membrane potential, electrical oscillation amplitude, and oscillation frequency that were similar to those observed in higher bathing \([\text{Ca}^{2+}]\). However, the magnitude of the change in resting potential and the overshoot in the electrical amplitude and frequency of the oscillation are smaller in the lower than in the higher bathing \([\text{Ca}^{2+}]\). A sufficient reduction in bathing \([\text{Ca}^{2+}]\) can abolish the spontaneous waves in rat myocytes (Capogrossi et al., 1986a). The effect of acidosis in rat myocytes bathed in a \([\text{Ca}^{2+}]\) sufficiently...
low to abolish spontaneous contractile waves before acidosis is shown in Fig. 7. Even under this condition, acidosis could cause de novo spontaneous Ca\textsuperscript{2+} oscillations that occasionally were sufficient to initiate spontaneous twitches.

In unstimulated preparations, therefore, acidosis caused a triphasic effect. (a)

**Figure 6.** The average effect of acidosis on resting membrane potential (A) and on the frequency (B) and amplitude (C) of oscillations in membrane potential associated with contractile waves in three cardiac myocytes bathed in 2.0 mM Ca\textsuperscript{2+}. The dotted lines are the results from Figs. 4 and 5 C, i.e., in cells bathed in 4.0 mM Ca\textsuperscript{2+}.

There was an initial rapid decrease of resting tension and oscillation frequency, with either no detectable change or a small increase of Ca\textsubscript{i}. Resting membrane potential depolarized slightly, and the amplitude of the transient depolarization associated with each contractile wave increased slightly. (b) There was a secondary
increase of resting tension, oscillation frequency, mechanical amplitude, and average tissue Ca, with a relatively larger increase in the amplitude of the transient depolarization. (c) There was a return of these variables toward control.

Effect of Acidosis on Electrically Stimulated Preparations

Papillary muscle experiments. It has previously been shown (Lakatta et al., 1985a) that periodic spontaneous Ca²⁺ oscillations in unstimulated preparations can be abolished by regular electrical stimulation at rates greater than the spontaneous oscillation frequency. A subsequent increase in the spontaneous oscillation frequency caused by an increase in cell Ca²⁺ loading will then cause
the oscillations to appear in the diastolic interval during stimulation (Capogrossi and Lakatta, 1985). Since the results obtained in unstimulated preparations indicate that acidosis could increase spontaneous Ca\(^{2+}\) release, we investigated whether acidosis could produce oscillations in the diastolic interval in stimulated preparations.

Fig. 8A shows chart recordings of aequorin light and developed tension from a rat papillary muscle that was being stimulated at 0.33 Hz. As in unstimulated preparations, it was necessary to increase Ca\(_o\) and to add ouabain in order to measure diastolic Ca\(_i\) using aequorin. However, the response of the muscle to acidosis was similar to that published previously at a lower bathing [Ca\(^{2+}\)] (Fig. 2 in Allen and Orchard, 1983). On exposure to the acid solution, there was an initial rapid decrease of developed tension, which was followed by a partial recovery. Fig. 8B shows records of aequorin light and tension averaged during the periods shown in panel A. This shows that the size of the Ca\(^{2+}\) transient, which accompanies contraction, was increased during the acid period. The peak increase occurred within 5 min. The size of the light transient then decreased but remained higher than control. Thus, the time course of the effect of acidosis on the aequorin light transient parallels its effect on resting light (Fig. 2). It is

![Figure 8](https://jgp.rupress.org/content/90/1/156/F2.large.jpg)
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clear from the high-gain light records that oscillations of Ca\textsubscript{i} were present during the diastolic period in the acid solution, at about the time when the Ca\textsubscript{i} was highest. It is also of interest that while spontaneous oscillations of Ca\textsuperscript{2+} were quite marked, the corresponding “aftercontraction” was relatively small. This is similar to the relatively minor effect of acidosis on the amplitude of the spontaneous contractile waves (Fig. 4) and may be due to a decrease of myofilament Ca\textsuperscript{2+} sensitivity during acidosis (Donaldson et al., 1978; Fabiato and Fabiato, 1978; Blanchard and Solaro, 1984). With further time in acidosis, twitch tension continued to increase toward the control level, but Ca\textsubscript{i} decreased. During this time, the diastolic Ca\textsuperscript{2+} oscillations also decreased, and this could be a cause of the divergence between systolic Ca\textsubscript{i} and twitch tension (Allen et al., 1985).

Single-cell experiments. Spontaneous Ca\textsuperscript{2+} release in the diastolic period has previously been shown to decrease the twitch amplitude in response to a subsequent stimulus (Suarez-Isla et al., 1984). A similar effect occurred during acidosis. Fig. 9A shows a record from a single rat myocyte, stimulated at 1 Hz, before and during exposure to the acid solution. Before exposure to the acid solution, the amplitude of shortening during the twitch was relatively constant in response to each stimulus. However, when the cell was exposed to the acid solution, the twitch height became less uniform. Fig. 9B shows parts of this record on a faster time base and suggests a reason for this nonuniformity. Under control conditions, there were no spontaneous mechanical oscillations during the diastolic period. However, during exposure to the acid solution, spontaneous contractile waves
occurred in many diastolic intervals. It is apparent that the size of a given twitch was reduced if there had been spontaneous contractile activity in the diastolic period preceding it. The most likely explanation of this result is that if the SR of the cell has released Ca\(^{2+}\) spontaneously during the diastolic period, then there is less Ca\(^{2+}\) available for release in response to the next stimulus or the trigger for release is still partially inactivated (Suarez-Isla et al., 1984). A transient membrane depolarization accompanied the contractile wave when it occurred during diastole (not shown).

Since spontaneous Ca\(^{2+}\) releases may modulate the response of whole muscles to acidosis, we investigated the responses of papillary muscles to acidosis in the presence of ryanodine, which abolishes spontaneous Ca\(^{2+}\) release (Wier et al., 1983). Fig. 10 shows the effect of ryanodine on aequorin light and tension from a rat papillary muscle, before and during exposure to the acid solution. Before acidosis, 2 μM ryanodine (lower tracing) markedly decreased twitch tension. Acidosis in ryanodine still produced an immediate marked decrease of developed tension, followed by a gradual increase that was much smaller than that in the absence of drug. It is also of interest that there was a marked decrease of resting tension in the presence of ryanodine on exposure to the acid solution, similar to that observed in unstimulated papillary muscles, which was reversed on returning to normal pH. Caffeine (10 mM) in a perfusate [Ca\(^{2+}\)] of 2 mM produced a qualitatively similar result (not shown).

**DISCUSSION**

It has been shown previously (Fig. 3, Ellis and Thomas, 1976) that the intracellular pH in response to an increase in perfusate [CO\(_2\)] decreases monotonically,
showing no recovery for at least 14 min. The present result show that this type of acidosis causes an increase in the frequency of spontaneous Ca$^{2+}$ oscillations in both unstimulated and stimulated preparations. These oscillations of Ca$^{2+}$ result in a localized Ca$^{2+}$ of 1–7 μM (Cobbold and Bourne, 1984; Kort et al., 1985b; McIvor et al., 1986), and so may be expected to have marked effects. The response to acidosis in intact preparations will depend not only on the mean Ca$^{2+}$ and a reduction in the affinity of the myofilaments for Ca$^{2+}$, but also on the effect of acidosis on spontaneous oscillations of Ca$^{2+}$. When the SR Ca$^{2+}$ release is disabled by ryanodine or its Ca$^{2+}$ loading is decreased by high concentrations of caffeine, the spontaneous periodic oscillations of Ca$^{2+}$ are abolished. Since these oscillations are dependent on SR function, the present results suggest that the increase of Ca$^{2+}$ produced by acidosis can overcome the direct depressant effect of acidosis on the SR (Fabiato, 1985a, b). The source of an increase in Ca$^{2+}$ that exacerbates the oscillations during acidosis is not addressed in the present study. However, a change in pH is known to affect both the Na-Ca and Na-H exchangers (Philipson et al., 1982; Piwnica-Worms et al., 1985), which may alter net transsarcolemmal Ca$^{2+}$ flux. Intracellular Ca$^{2+}$ buffering by proteins, e.g., troponin, or by organelles, e.g., mitochondria (Fry and Poole-Wilson, 1981), is also pH dependent.

The effect of acidosis on spontaneous oscillatory Ca$^{2+}$ release was not constant during acidosis caused by such an increase in perfusate CO$_2$ but varied with time in a triphasic manner.

The Initial Rapid Decrease in Oscillation Frequency

In resting preparations. A decrease in the frequency of spontaneous Ca$^{2+}$ release occurred, as evidenced by the decrease of wave frequency in the unstimulated single myocytes. Since there was no detectable change in the amplitude of spontaneous motion in the single cells, the initial decrease in SLIF in the unstimulated papillary muscles was probably due to a decrease in oscillation frequency. While a decrease of oscillation frequency should decrease aequorin light (Kort et al., 1985b; Eisner and Valdeolmillos, 1986), this did not change during this phase. This suggests that (a) aequorin light is less sensitive in detecting low-grade cellular Ca$^{2+}$ oscillations than SLIF, or (b) the amplitude of the spontaneous Ca$^{2+}$ releases increased.

The decrease in oscillation frequency may be due to the direct inhibitory effect of acidosis on the SR. In support of this suggestion, the early decrease of pH, when superfusate CO$_2$ is increased has a time course similar to the initial decrease of frequency observed in the present experiments (Orchard, 1985; Poole-Wilson and Seabrooke, 1985). The early decline in resting tension may be due to: (a) a decrease in the sensitivity of the myofilaments to Ca$^{2+}$, and/or (b) a decrease in the frequency of the Ca$^{2+}$ oscillations, since when the mean Ca$^{2+}$ is low, these oscillations may increase resting tension (Stern et al., 1983; Kort et al., 1985b; Cannell et al., 1985). A decrease in either the frequency or amplitude of the oscillations would therefore tend to decrease resting tension.

When the spontaneous Ca$^{2+}$ oscillations were abolished by ryanodine during this early period (Fig. 2 C), acidosis had a relatively small effect on aequorin light.
This is the case because the average resting Ca, is usually too low to be detected by this method (Fig. 2C). In the absence of the spontaneous releases of Ca, the true effect of acidosis on the Ca²⁺-myofilament interaction (Donaldson et al., 1978; Fabiato and Fabiato, 1978; Blanchard and Solaro, 1984) is observed as a substained decrease in resting tension occurs (Figs. 2C and 8B), which suggests that the decrease of resting tension is due to the effect of acidosis on the Ca²⁺ sensitivity of the myofilaments.

In stimulated preparations. In stimulated preparations, the initial decline in twitch tension may be attributed to both an H⁺-dependent depression of SR function (Fabiato, 1985a) and a decrease in myofilament sensitivity to Ca²⁺ (Donaldson et al., 1978; Fabiato and Fabiato, 1978; Allen and Orchard, 1983; Blanchard and Solaro, 1984).

The Secondary Increase in the Frequency of Spontaneous Ca²⁺ Release

In unstimulated preparations. Glycosides were required to demonstrate that there was an increase in aequorin light in muscles during this phase, and that the magnitude of the secondary increase in oscillation frequency and amplitude in myocytes depended on Ca, which suggests that events that occur during this phase depend upon cell Ca²⁺ loading. During this period, SLIF and resting tension in muscle increased in parallel with the increase in aequorin light. That the marked increase in aequorin light during this period is essentially abolished by ryanodine (Fig. 2B) suggests that it arises entirely from a spatial average among cells of the increase in Ca, owing to asynchronous spontaneous SR Ca²⁺ release. In the isolated myocytes, the frequency of spontaneous contractile waves increased during this phase. Since H⁺ is thought to depress mammalian SR (Fabiato and Fabiato, 1978; Fabiato, 1985a), the secondary increase in the frequency of spontaneous SR Ca²⁺ release suggests that an increase in Ca during acidosis can overcome the depressant effect of H⁺ on SR Ca²⁺ function (Inesi and Hill, 1983). When oscillations are inhibited in the bulk muscle (Fig. 2B), there is a decrease in resting tension (Fig. 2C) and an increase in the resting length in cells (Fig. 3B). This implies that the increase in resting tension (or the decrease in cell length) during this phase in the absence of ryanodine is attributable to the increase in frequency of SR Ca²⁺ oscillations. This effect, then, overcomes the direct effect of H⁺ on myofilament Ca²⁺ sensitivity.

Although it is clear that the frequency of spontaneous Ca²⁺ release increases during acidosis, the effect of acidosis on the amplitude of this release is not as clear. The contractile wave amplitude measured in the present study is for waves that originate at the myocyte end and propagate the entire cell length, i.e., “unifocal” waves (Capogrossi and Lakatta, 1985). Cell edge displacement caused by this type of contractile wave increased slightly, despite the presence of an H⁺-induced diminution in the Ca²⁺ affinity of the myofilaments, and in the presence of a decrease in the interwave interval. Since both of these would tend to decrease the amplitude of motion, the observed moderate increase in amplitude suggests that the amount of Ca²⁺ released in each oscillation must have increased.

The acidosis-induced increase in the amplitude of the oscillation-induced transient depolarization during this phase could be attributed to Ca, modulation
of a nonspecific cation conductance (Colquhoun et al., 1981) or Ca\(^{2+}\) efflux via the Na-Ca exchanger. The decrease in the resting membrane potential could also be due to an increase of Ca\(^{2+}\) affecting these mechanisms. However, the larger spontaneous depolarization need not be paralleled by an increase in inward current owing to the Ca\(^{2+}\) release. A similar increase in the amplitude of the transient depolarization during a wave might also result from a reduction in outward background current or from a decreased K\(^{+}\) conductance, which the increased "tonic" depolarization could represent (Fig. 5C). In this regard, both the tonic depolarization and increased resistance could result from H\(^{+}\) block of K\(^{+}\) conductance (Moody, 1984), which could also explain the reduced K\(^{+}\) efflux previously demonstrated during acidosis (Poole-Wilson and Langer, 1975). Whatever the mechanism for its augmentation, the enhanced transient depolarization associated with the spontaneous Ca\(^{2+}\) release during acidosis was occasionally sufficient to trigger action potentials in unstimulated preparations during this phase. Thus, the present results clearly show that acidosis may be arrhythmogenic.

**Stimulated preparations.** Previous studies have shown that electrical stimulation suppresses spontaneous oscillations that occur in non--Ca\(^{2+}\)-overloaded rat myocytes or papillary muscles (Lakatta et al., 1985a; Capogrossi and Lakatta, 1985). An additional Ca\(^{2+}\) load placed upon these preparations, however, decreases the interval for a spontaneous oscillation to occur after a previous twitch. The present results show that this interval was decreased sufficiently during acidosis to permit the oscillations to occur in the interval between twitches. In the present study, the early recovery in twitch tension during acidosis was accompanied by an increase in the size of the Ca\(^{2+}\) transient (Fig. 8B, ii). This increase in Ca\(^{2+}\) release during a twitch suggests that SR Ca\(^{2+}\) loading increased during acidosis and that this mechanism increased the oscillation frequency sufficiently to permit Ca\(^{2+}\) oscillations to appear in the diastolic interval during regular electrical stimulation. Spontaneous diastolic sarcomere oscillations have also been reported to occur in stimulated preparations during acidosis under conditions similar to those employed in the present study (Ricciardi et al., 1986).

When such diastolic oscillations occurred in single myocytes, less twitch shortening was observed in response to the subsequent excitation (Fig. 9). This effect of the diastolic oscillation in the single myocyte has been observed even in the absence of acidosis (Capogrossi et al., 1986b). In the intact muscle, twitch force reflects the average SR Ca\(^{2+}\) release throughout the tissue by an action potential. Perturbations that increase the SR Ca\(^{2+}\) load effect a net increase in SR Ca\(^{2+}\) release in response to an action potential. The secondary increase in twitch force and the aequorin transient during acidosis indicate that the average SR Ca\(^{2+}\) release is increased. However, in the intact muscle, spontaneous diastolic Ca\(^{2+}\) release does not occur in all cells (Stern et al., 1983). In those cells, throughout the tissue in which it does occur, it would be expected to decrease the twitch amplitude in the ensuing systole, as shown by studies in individual myocytes (Capogrossi et al., 1986a) and in Fig. 9 of the present study. These cells contribute less to tissue tension than cells that do not have spontaneous diastolic release. Additionally, these cells with decreased tension increase the compliance against
which the other cells will contract (Kort and Lakatta, 1984; Kort et al., 1985b). Thus, the de novo appearance or exacerbation of spontaneous Ca
2+
 release during this phase of acidosis will limit the extent of the recovery of developed tension. However, the primary effect of enhanced SR Ca
2+
 loading of all cells within intact tissue during this phase of acidosis outweighs the secondary effect of the Ca
2+
 loading itself to cause spontaneous Ca
2+
 release between twitches in some of the cells within the tissue, as a secondary increase in tissue twitch force does occur.

The Tertiary Decrease of Spontaneous Ca
2+
 Release During Continued Acidosis

The changes seen during this period are exactly the opposite of those observed during the secondary increase described above, and are most probably due, therefore, to the measured decrease in Ca
2+
 producing effects opposite to those described above for an increase in Ca
2+
. The continued increase in twitch tension during this period, in spite of a decreasing Ca
2+
 transient in response to electrical stimulation (Fig. 8B, iii, and 10A), is explained by a decrease in oscillation frequency and thus a reduction in the deleterious effects of spontaneous Ca
2+
 release on twitch tension in intact muscles, as discussed above.

Conclusions

The present study shows that spontaneous releases of Ca
2+
 have many effects that may be important in modulating the response of heart muscle to acidosis. In particular, these releases may affect: (a) Cardiac rhythmicity. Since acidosis can lead to the appearance of spontaneous action potentials in both unstimulated and stimulated preparations, it is clear that acidosis is potentially arrhythmogenic, and is likely to be so for several minutes after its onset. (b) Resting tension. Although the initial decrease of resting tension observed in the present study in unstimulated preparations is probably due to changes in myofilament sensitivity to Ca
2+
, since it occurs in the presence of ryanodine, it seems clear that the subsequent increase and decline of resting tension is due to changes in spontaneous Ca
2+
 release. It also seems possible that these oscillations help maintain resting tension in stimulated preparations (Fig. 10). (c) Developed (twitch) tension. An exacerbation of spontaneous Ca
2+
 release cannot cause the initial decrease of developed tension observed during acidosis, since this is still present when spontaneous release has been inhibited using ryanodine. It is likely, however, that spontaneous Ca
2+
 release modulates developed tension. In particular, it is possible that the presence of diastolic oscillations limits the extent of recovery of developed tension midway through the acidosis, i.e., at the time when the Ca
2+
 transient is maximal. Additionally, it is possible that the decline of the oscillation frequency late in acidosis is responsible for some of the recovery of developed tension at a time when the size of the Ca
2+
 transient is decreasing.

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