Effects of acidosis on Ca\(^{2+}\) sensitivity of contractile elements in intact ferret myocardium

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Komukai, Kimiaki, Tetsuya Ishikawa, and Satoshi Kurihara. Effects of acidosis on Ca\(^{2+}\) sensitivity of contractile elements in intact ferret myocardium. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H147–H154, 1998.—We investigated the effects of acidosis on the intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) and contractile properties of intact mammalian cardiac muscle during tetanic and twitch contractions. Aequorin was injected into ferret papillary muscles, and the [Ca\(^{2+}\)]\(_i\) and tension were simultaneously measured. Acidosis was attained by increasing the CO\(_2\) concentration in the bicarbonate (20 mM)-buffered Tyrode solution from 5% (pH 7.35, control) to 15% (pH 6.89, acidosis). Tetanic contraction was produced by repetitive stimulation of the preparation following treatment with 5 µM ryanodine. The relationship between [Ca\(^{2+}\)]\(_i\) and tension was measured 6 s after the onset of the stimulation and was fitted using the Hill equation. Acidosis decreased the maximal tension to 81 ± 2% of the control and shifted the [Ca\(^{2+}\)]\(_i\)-tension relationship to the right by 0.18 ± 0.01 pCa units. During twitch contraction, a quick shortening of muscle length from the length at which developed tension became maximal (L\(_{max}\)) to 92% L\(_{max}\) produced a transient change in the [Ca\(^{2+}\)]\(_i\) (extra Ca\(^{2+}\)). The magnitude of the extra Ca\(^{2+}\) was dependent on the [Ca\(^{2+}\)]\(_i\) immediately before the length change, suggesting that the extra Ca\(^{2+}\) is related to the amount of troponin-Ca complex. Acidosis decreased the normalized extra Ca\(^{2+}\) to [Ca\(^{2+}\)]\(_i\) immediately before the length change, which indicates that the amount of Ca\(^{2+}\) bound to troponin C is less when [Ca\(^{2+}\)]\(_i\) is the same as in the control. The decrease in the Ca\(^{2+}\) binding to troponin C explains the decrease in tetanic and twitch contraction, and mechanical stress applied to the preparation induced less [Ca\(^{2+}\)]\(_i\) change in acidosis.

troponin C; length change; aequorin; ventricular muscle

ISCHEMIA AND METABOLIC disorders, such as diabetes and renal dysfunction, cause extracellular and intracellular acidosis. In cardiac muscle, acidosis has long been known to produce a negative inotropic effect. However, acidosis exerts numerous and varied effects on excitation-contraction coupling in cardiac muscle. For example, acidosis inhibits the slow inward current, which should decrease the intracellular Ca\(^{2+}\) transients. Ca\(^{2+}\) uptake and Ca\(^{2+}\) release in the sarcoplasmic reticulum (SR) and the Na\(^+\)/Ca\(^{2+}\) exchanger are also inhibited by acidosis [for review, see Orchard and Kentshi (27)]. However, measurement of the intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) has revealed (3, 18, 20) that acidosis increases the peak of the Ca\(^{2+}\) transients and prolongs its time course and that, in contrast, developed tension is significantly decreased. The dissociation of the changes in the peaks of the Ca\(^{2+}\) transients and tension has been considered to be due to a decrease in maximal tension (22) and a decrease in the Ca\(^{2+}\) sensitivity of the contractile elements during acidosis (3, 18, 22, 23, 28, 30). Therefore, the contractile machinery cannot properly respond to Ca\(^{2+}\) in acidosis.

The effects of acidosis on the Ca\(^{2+}\) sensitivity of the contractile elements and the affinity of troponin C for Ca\(^{2+}\) have been extensively studied using skinned preparations (23, 28, 30) and isolated troponin C (28, 30). However, it is of interest to know how the decrease in the Ca\(^{2+}\) sensitivity of the contractile elements induced by acidosis is related to the contraction of intact preparations, because the relationship between [Ca\(^{2+}\)]\(_i\) and tension in intact preparations differs from that in skinned preparations (7, 14, 31). Therefore, in the present study, we observed the effect of acidosis on the [Ca\(^{2+}\)]\(_i\)-tension relationship in tetanized, intact ferret papillary muscles, which is a steady-state relationship. However, it is not clear that the change in the Ca\(^{2+}\) sensitivity of the contractile elements, measured at steady state, is involved in the determination of the contractile properties in twitch contraction in which the time courses of the Ca\(^{2+}\) transients and tension are different. A simple calculation of the time course of the Ca\(^{2+}\)-bound form of troponin C, using on and off rates of troponin C for Ca\(^{2+}\), indicates that a sufficient time lag between the Ca\(^{2+}\)-bound form of troponin C and tension during a twitch contraction exists (15, 29). Therefore, tension is not a simple function of [Ca\(^{2+}\)]\(_i\) and the time course of the Ca\(^{2+}\)-bound form of troponin C is closer to [Ca\(^{2+}\)]\(_i\) rather than tension.

A quick length change during a twitch contraction induces a transient increase in [Ca\(^{2+}\)]\(_i\) (extra Ca\(^{2+}\)). The magnitude of the extra Ca\(^{2+}\) is a function of [Ca\(^{2+}\)]\(_i\) immediately before length change and the magnitude of tension reduction. Therefore, the extra Ca\(^{2+}\) reflects Ca\(^{2+}\) dissociated from the Ca\(^{2+}\)-bound form of troponin C via the feedback mechanism from the cross bridges to troponin C (2, 5, 17). In the present study, we also observed the effect of acidosis on the magnitude of the extra Ca\(^{2+}\) in response to a quick shortening of muscle length to observe whether the contribution of the change in the Ca\(^{2+}\) binding to troponin C, measured at steady state, is related to the decrease in twitch tension.

The preliminary results of this study have already been presented in abstract form (11, 16).

METHODS

Preparation. Ferrets (600–1,200 g body wt) were anaesthetized with pentobarbital sodium (100 mg/kg ip) and the hearts were quickly removed. After the blood in the heart was washed with normal Tyrode solution, thin papillary muscles (diameter 0.5–1.0 mm) were dissected from the right ventricle. Both ends of the preparation were tied with silk threads. The preparation was mounted horizontally in an experimental chamber perfused with normal Tyrode solution at 30°C. One end of the preparation was connected to the

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leve of a motor (J CCX-101A, General Scanning, Watertown, CA), and the other end was connected to the arm of a tension transducer (BG-10, Kulite, Ridgefield, NJ). The motor was used to alter muscle length within 3 ms unless otherwise mentioned. A pair of platinum black electrodes was placed parallel to the preparation for electrical stimulation. The preparation was regularly stimulated at 0.2 Hz (duration, 5 ms; strength, 1.5-fold threshold) and stretched to the length at which developed tension became maximal ($L_{\text{max}}$). The mean diameter of the preparation was 0.62 ± 0.04 mm (mean ± SE, $n = 17$), and the length was 4.1 ± 0.2 mm. These parameters were measured using an eyepiece micrometer under a binocular.

Solutions. The normal Tyrode solution used for the dissection of the preparations and for the injection of aequorin was composed of the following (in mM): 135 Na+, 5 K+, 2 Ca2+, 1 Mg2+, 102 Cl−, 20 HCO3−, 1 HPO4−, 1 SO4−, 20 acetate, 10 glucose and 5 U/l insulin, pH 7.35 at 30°C when equilibrated with 5% CO2-95% O2. After the injection of aequorin, the solution was changed to phosphate-free Tyrode solution (normal Tyrode solution in which 1 mM Na2HPO4 was not included). The phosphate-free Tyrode solution (control solution) was used to avoid precipitation when the extracellular concentration of Ca2+ was increased. The phosphate-free Tyrode solution (control solution) was equilibrated with 5% CO2-95% O2. After the injection of aequorin, the solution was changed to phosphate-free Tyrode solution (normal Tyrode solution in which 1 mM NaH2PO4 was not included). The phosphate-free Tyrode solution (control solution) was used to avoid precipitation when the extracellular concentration of Ca2+ was increased. To confirm the maximal tension, BAY K 8644 (1 µM) was added to the phosphate-free Tyrode solution containing 20 mM Ca2+. When [Ca2+]i was altered, the osmotic pressure of the solution was not adjusted; CaCl2 was either simply added to or not included in the solution. For acidosis, the phosphate-free Tyrode solution was equilibrated with 15% CO2-85% O2 (pH 6.89 at 30°C). In some experiments, we tested the effects of alkalosis on the extra Ca2+. For this purpose, the phosphate-free Tyrode solution was bubbled with 2% CO2-98% O2 (pH 7.59). To modify Ca2+ handling of SR, caffeine (5 mM) was used in some experiments. The temperature of the solution was continuously monitored with a thermocouple and maintained at 30 ± 0.5°C.

Aequorin injection and measurement of light signals. Aequorin, purchased from Dr. J. R. Blinks (Friday Harbor, WA), was dissolved in 150 mM KCl and 5 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid at pH 7.0 with a final aequorin concentration of 50–100 µM. With the use of a glass micropipette, aequorin was pressure injected into 150–200 µm superficial cells of the preparation while the membrane potential was monitored. Aequorin light signals were detected with a photomultiplier (EMI 9798A, Ruislip, UK), which was mounted in a small housing (1, 2), and the aequorin light signal and tension were recorded simultaneously. All data were stored on tape (NFR-3515W, Sony Magnescale, Tokyo, J apan) and computer (PC-9801, NEC, Tokyo, J apan) for later analysis. The details of the experimental setup have been described previously (2).

Aequorin light signals were converted to [Ca2+]i using an in vitro calibration curve (6). The constants used in the present study were as follows: $n$, 3.14; $K_0$, 4.025 × 10^6; $K_r$, 114.6 [see Okazaki et al. (24) for details]. Intracellular pH (pHi) in acidosis is reported to decrease to 6.78 under the same condition as the solution used, which slightly decreases the aequorin luminescence (3). We discussed this factor for the quantitative interpretation of the [Ca2+]i in acidosis in each experiment.

Tetanic contraction. To produce tetanic contraction, the preparation was treated with 5 µM ryanodine and repetitively stimulated (duration, 40 ms; frequency, 10 Hz) for 6 s. The strength of the stimulation was adjusted to obtain a smooth contraction without ripples (12, 24, 31). During tetanic contraction, the aequorin light signal was measured through a 1-Hz low-pass filter to avoid noise. At low [Ca2+]i, two to four signals were averaged to improve the signal-to-noise ratio. [Ca2+]i, and tension, were measured 6 s after the onset of the repetitive stimulation, were plotted and fitted using the Hill equation: $T = T_{\text{max}} \times \frac{[Ca^{2+}]_i}{([Ca^{2+}]_i + K_{1/2})}$, where $T$ is measured tension, $T_{\text{max}}$ is maximal tension, $K_{1/2}$ is the [Ca2+]i that causes 50% maximal tension, and $H$ is the Hill coefficient. We also defined $pCa_{1/2}$ as $-\log K_{1/2}$.

Muscle length change. During twitch contraction, the muscle length was quickly shortened from $L_{\text{max}}$ to 92% $L_{\text{max}}$ using the electromagnetic motor. In some experiments, the magnitude of tension reduction in the control solution was measured using a storage oscilloscope (7TOTA, NEC San-ei, Tokyo, J apan). We then changed the solution to alter the peaks of the Ca2+ transients and tension and adjusted the length-change application time to produce the same magnitude of tension reduction as under the control condition. Thus we could alter the magnitude of the extra Ca2+ and observe the relationship between the extra Ca2+ and [Ca2+]i before the length change at the same tension reduction (17).

Drugs. A stock solution of ryanodine (1 mM; Agri System, PA) was made by dissolving it in warmed double-distilled water and storing at 0°C. A stock solution of BAY K 8644 (1 mM; Calbiochem, CA) was made by dissolving it in ethanol. Caffeine (Sigma Chemical, MO), with a desired concentration, was dissolved directly in the phosphate-free Tyrode solution before use.

Statistics. Measured values were expressed as means ± SE. For statistical analysis, paired Student’s t-test was employed and statistical significance was verified at $P < 0.05$ (two-tailed test).

RESULTS

Effect of acidosis on [Ca2+]i-tension relationship in intact papillary muscles. Figure 1 shows original traces of tetanic contractions and the accompanying [Ca2+]i (representative data from 6 preparations). First, [Ca2+]i and contraction at 2 mM [Ca2+]i, were recorded as a control (Fig. 1A), and then the control solution was replaced with the acidotic solution at the same [Ca2+]i. Soon after exposure to acidotic solution, [Ca2+]i slightly increased, but tension abruptly decreased (immediate effect). [Ca2+]i subsequently increased, and this was accompanied by a slight recovery in tension (slow effect). These two effects are prominent in cardiac muscles of rats compared with those of ferrets (3, 25). Several minutes later, [Ca2+]i and tension during tetanic contractions reached a steady state at which [Ca2+]i, and tension were measured (Fig. 1A). The acidotic solution was then changed to the solution containing the same [Ca2+]i, at the control extracellular pH (pH0). [Ca2+]i was then increased to 4, 6, 8, and 15 mM, and [Ca2+]i, and contractions were measured under control and acidic conditions in the same way (Fig. 1B–E). To obtain maximal tension, the preparation was treated with 20 mM Ca2+ and 1 µM BAY K 8644, which significantly increased [Ca2+]i (Fig. 1F). BAY K 8644 significantly increased tension in acidosis, but this effect was small at the control pH0. The time course of the initial phase of tetanic contraction in acidosis was significantly slower than that at the control pH0, although [Ca2+]i, at the corresponding phase in acidosis was significantly higher than that at the control pH0.
These changes suggest the decrease in the Ca$^{2+}$ sensitivity of the contractile elements in acidosis.

Acidosis decreased the maximal tension by about 20% (Table 1). In Fig. 2A, the relationships between [Ca$^{2+}$]$_i$ and tension in control and acidic solutions were plotted and the data points were fitted with the Hill equation. When the developed tension was normalized to the maximal tension under each condition, it was clear that acidosis also significantly decreased the Ca$^{2+}$ sensitivity of the contractile elements in the tetanized intact preparation (Fig. 2B). $k_{1/2}$ increased from 0.85 ± 0.07 (control) to 1.28 ± 0.09 µM (acidosis; $P < 0.01, n = 6$) (Fig. 2B and Table 1). The Hill coefficient was not significantly increased by acidosis (6.0 ± 0.7 in control and 6.9 ± 0.6 in acidosis) (Table 1).

Effect of acidosis on extra Ca$^{2+}$. The preparation was regularly stimulated at 0.2 Hz in the control solution, and the muscle length was quickly shortened from $L_{max}$ to 92% $L_{max}$ at various times after the onset of stimulation, which produced a transient increase in [Ca$^{2+}$]$_i$ (see Kurihara and Komukai (17)). We measured the magnitude of the extra Ca$^{2+}$ in the control and acidic solutions when the muscle length was altered at various times after stimulus. Figure 3 shows an example of the relationships of muscle length, the Ca$^{2+}$ transients, tension, and extra Ca$^{2+}$ when the muscle length was quickly shortened in the rising phase of contraction (corresponding to the decay phase of the Ca$^{2+}$ transients). In response to the step length change, tension was suddenly decreased and then redeveloped, and [Ca$^{2+}$]$_i$ was transiently increased; the change in [Ca$^{2+}$]$_i$ is shown in Fig. 3D as extra Ca$^{2+}$.

When the time of the length change was delayed from the onset of stimulus, the magnitude of tension reduction increased and [Ca$^{2+}$]$_i$ immediately before the length change decreased. According to Kurihara and Komukai (17), the extra Ca$^{2+}$ is dependent on 1) the magnitude of tension reduction and 2) [Ca$^{2+}$]$_i$ immediately before length change. The marked time lag between the Ca$^{2+}$ transients and tension suggests that tension development, the attachment of the cross bridges, is not a simple function of [Ca$^{2+}$], and that the interactions of contractile proteins after the Ca$^{2+}$ binding to troponin C could explain the time lag. The time course of the formation of the Ca$^{2+}$-bound form of troponin C is much closer to that of [Ca$^{2+}$] (15, 29). We hypothesized that tension reduction detaches the cross bridges from the thin filaments, and the same tension does not necessarily mean the same amount of troponin-Ca complex formed. For example, the amount of the Ca$^{2+}$-bound form of troponin C is quite different at the same tension level in the rising phase and relaxation phase in twitch contraction (15, 29). Thus tension is a final output including the processes from the Ca$^{2+}$ binding to troponin C to the attachment of the cross bridges. Therefore, if the kinetics of the cross-bridge attachment are considerably slow compared with the time course of the Ca$^{2+}$ transients, the time lag between [Ca$^{2+}$] change and tension should become considerably longer. Thus tension is sustained, although [Ca$^{2+}$] is substantially declined. Therefore, in some experiments, the length change was applied at the appropriate timing to produce the same magnitude of tension reduction. For this purpose, length change was applied at 2 mM [Ca$^{2+}$]$_o$, and the magnitude of tension reduction was measured using a storage oscilloscope as mentioned in METHODS. After the solution was changed (to a higher or lower [Ca$^{2+}$]$_o$ or acidic solutions), the time of the length change after stimulation was determined to produce the same magnitude of tension reduction as in the control solution at 2 mM [Ca$^{2+}$]$_o$.

Table 1. Effect of acidosis on Ca$^{2+}$ sensitivity of contractile elements in tetanized ferret papillary muscles

<table>
<thead>
<tr>
<th></th>
<th>$K_{1/2}$, µM</th>
<th>$\Delta$[Ca$^{2+}$]</th>
<th>Hill Coefficient</th>
<th>$T_{max}$, mN/mm$^2$</th>
<th>Relative $T_{max}$</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>0.85 ± 0.07</td>
<td>0.18 ± 0.01</td>
<td>6.0 ± 0.7</td>
<td>54.4 ± 6.4</td>
<td>0.81 ± 0.02</td>
</tr>
<tr>
<td>Acidity</td>
<td>1.28 ± 0.09*</td>
<td>6.9 ± 0.6</td>
<td>44.3 ± 6.0*</td>
<td>0.81 ± 0.02</td>
<td></td>
</tr>
</tbody>
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Values are means ± SE for 6 experiments. $K_{1/2}$, intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]$_i$) that causes 50% of maximal tension ($T_{max}$); $\Delta$[Ca$^{2+}$], change in pCa$^{2+}$, which is $-\log K_{1/2}$; Hill coefficient, steepness of [Ca$^{2+}$]-tension relationship (see RESULTS). *P < 0.01 vs. control.
solution, tension was remarkably inhibited and the comparable tension reduction to that induced in the control solution could not be applied. Therefore, we increased $[\text{Ca}^{2+}]_{\text{o}}$ in the acidotic solution to increase the developed tension. The time course of the Ca$^{2+}$ transients in acidosis was slower than that in the control, which is well known (3, 18, 27). The time of muscle length change in acidotic solution which produced the same tension reduction as in the control was later than that in the control. Thus $[\text{Ca}^{2+}]_{\text{i}}$ immediately before length change was different in the control and acidotic solutions. When the magnitude of the extra Ca$^{2+}$ was plotted against the $[\text{Ca}^{2+}]_{\text{i}}$ immediately before the length change for the same magnitude of tension reduction, the magnitude of the extra Ca$^{2+}$ in acidosis was less than that of the control at a given $[\text{Ca}^{2+}]_{\text{i}}$ (Fig. 5). At higher $[\text{Ca}^{2+}]_{\text{i}}$, the magnitude of the extra Ca$^{2+}$ did not increase further, which might be due to the saturation of the Ca$^{2+}$ binding sites of troponin C with Ca$^{2+}$.

We also plotted the magnitude of extra Ca$^{2+}$, normalized to the $[\text{Ca}^{2+}]_{\text{i}}$ immediately before the length change, which is closely related to the amount of troponin-Ca complex (15, 29), against the different magnitudes of tension reduction. Figure 6A shows that the normalized extra Ca$^{2+}$ was correlated with the magnitude of tension reduction and that acidosis decreased the normalized extra Ca$^{2+}$ (the ratio of the extra Ca$^{2+}$ to the troponin-Ca complex) for a given magnitude of tension reduction. We then also examined whether alkalosis alters the relationship between the normalized extra Ca$^{2+}$ and the magnitude of tension reduction in the opposite direction compared with that in acidosis. Alkalosis increased the normalized extra Ca$^{2+}$, although some measured points overlapped with points of the control (Fig. 6B).

Because acidosis is known to influence Ca$^{2+}$ handling mechanisms (27), the magnitude of the extra Ca$^{2+}$ might be profoundly influenced by changes in Ca$^{2+}$ removal mechanisms (SR and Na$^{+}$/Ca$^{2+}$ exchanger), particularly by SR, which is primarily responsible for removing intracellular Ca$^{2+}$ from the myoplasm in ferret ventricular muscle (25). Therefore, we treated the preparation with caffeine (5 mM), which apparently inhibits the Ca$^{2+}$ uptake in SR by enhancing Ca$^{2+}$ release. Caffeine significantly prolonged the Ca$^{2+}$ tran-

Fig. 2. Effects of acidosis on $[\text{Ca}^{2+}]_{\text{i}}$-tension relationship in tetanized ferret papillary muscle. A: relationships between $[\text{Ca}^{2+}]_{\text{i}}$ and tension for experiments shown in Fig. 1. B: developed tension was normalized to maximal tension in each case. ○, Control; ●, acidosis.

Fig. 3. Experimental schema of changes in Ca$^{2+}$ transients and tension in response to muscle length change. A: muscle length was changed (at arrow) from length at which developed tension became maximal ($L_{\text{max}}$) to 92% $L_{\text{max}}$. B: $[\text{Ca}^{2+}]_{\text{i}}$, immediately before length change. C: magnitude of tension reduction. D: magnitude of transient change in $[\text{Ca}^{2+}]_{\text{i}}$ (extra Ca$^{2+}$) as difference between $[\text{Ca}^{2+}]_{\text{i}}$ at $L_{\text{max}}$ and 92% $L_{\text{max}}$. 
sients and tension as reported previously (1, 17). In the caffeine-treated preparation, acidosis still decreased extra Ca\textsuperscript{2+} when the magnitude of tension reduction or [Ca\textsuperscript{2+}]\textsubscript{i} immediately before the length change was the same as in the control (Fig. 7), which was qualitatively similar to that observed in the absence of caffeine.

**DISCUSSION**

The observed results are likely due to intracellular rather than extracellular acidosis. The expected pH\textsubscript{i} in the present experiments was 7.06 and 6.78 in the control (5% CO\textsubscript{2}) and acidic (15% CO\textsubscript{2}) solutions, respectively (26).

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**Fig. 4.** Effects of acidosis on Ca\textsuperscript{2+} transients and tension during muscle length change. Ca\textsuperscript{2+} transients and tension were measured at L\textsubscript{max} and when muscle length was quickly shortened from L\textsubscript{max} to 92% L\textsubscript{max}. A and B: control (5% CO\textsubscript{2}). C and D: acidosis (15% CO\textsubscript{2}). [Ca\textsuperscript{2+}]\textsubscript{o} was 4 (A), 6 (B, C), and 8 (D) mM. Because developed tension was remarkably reduced by acidosis and sufficient tension reduction could not be applied, [Ca\textsuperscript{2+}]\textsubscript{i} in acidic solution was increased. Time of length change was adjusted to produce same magnitude of tension reduction in each case. Top trace: muscle length. Second trace: [Ca\textsuperscript{2+}]\textsubscript{i}. Third trace: tension. Bottom trace: difference of [Ca\textsuperscript{2+}] between 2 signals. Representative data of 4 experiments.

**Fig. 5.** Relationship between extra Ca\textsuperscript{2+} and [Ca\textsuperscript{2+}]\textsubscript{i} immediately before length change in control and acidosis. Magnitude of tension reduction was kept constant for each measured point. ○, Control; ●, acidosis. Extra Ca\textsuperscript{2+} in acidosis is less than that in control. The most rightward point in 5% CO\textsubscript{2} is near maximal, and no further increase in extra Ca\textsuperscript{2+} was observed even though [Ca\textsuperscript{2+}]\textsubscript{i} was increased. Representative data of 4 experiments.

**Fig. 6.** Relationship between extra Ca\textsuperscript{2+} normalized to [Ca\textsuperscript{2+}]\textsubscript{i} immediately before length change and magnitude of tension reduction in response to step length change. A: ○, control; ●, acidosis. Representative data of 5 experiments. B: ○, control; ●, alkalosis. pH of solution was raised by decreasing CO\textsubscript{2} concentration to 2% as mentioned in METHODS. Note different changes in normalized extra Ca\textsuperscript{2+}. Representative data of 2 experiments. Different preparations were used in A and B.

**Fig. 7.** Relationship between extra Ca\textsuperscript{2+} normalized to [Ca\textsuperscript{2+}]\textsubscript{i} immediately before length change in presence of caffeine (5 mM). Magnitude of tension reduction was kept constant for each measured point. ○, Control; ●, acidosis. Extra Ca\textsuperscript{2+} in acidosis is less than that in control. Representative data of 2 experiments.
Changes in maximal tension and Ca\(^{2+}\) sensitivity of contractile elements. The present study clearly showed that acidosis decreased the maximal tension in tetanized papillary muscles as reported in skinned trabeculae (23, 28, 30) and intact tetanized whole heart (22). At the commencement of the repetitive stimulation, tension started to increase at a faster rate and then slightly declined during the stimulation. The slight decrease in tetanic tension during stimulation might be due to the elastic components. A similar change in tetanic tension is reported (24, 31). However, some changes in the intracellular metabolites including inorganic phosphate are also candidates for explaining the slight decrease in tension during stimulation (21). Therefore, we measured [Ca\(^{2+}\)] and tension at the same time after the commencement of repetitive stimulation in the control and acidosis to avoid the influence of the metabolite changes.

The decrease in the maximal tension is considered to be due to 1) a decrease in the number of force-producing cross bridges, 2) a decrease in the force per cross bridge, and 3) a change in cross-bridge kinetics. However, Kurihara et al. (20) and Mayoux et al. (23) reported that acidosis does not alter the overall cross-bridge turnover rate. Therefore, a decrease in the number of cross bridges (10) and a decrease in the force per cross bridge are possible explanations for the decrease in the maximal tension.

To our knowledge, this is the first study that examined the effect of acidosis on the [Ca\(^{2+}\)]-tension relationship in intact tetanized cardiac muscles. The present study indicated that acidosis significantly decreased the Ca\(^{2+}\) sensitivity of the contractile elements, which was qualitatively similar to that observed in skinned preparations (23, 28, 30). This Ca\(^{2+}\)-desensitizing effect of acidosis is likely due to the apparent decrease in the affinity of troponin C for Ca\(^{2+}\) (28, 30).

However, the change in the [Ca\(^{2+}\)]-tension relationship in intact preparations in the present study was much smaller than that observed in skinned preparations. In skinned preparations, a 0.3-pH unit drop shifts the [Ca\(^{2+}\)]-tension relationship to the right by 0.3–0.4 pCa unit at half-maximal activation (K\(_{1/2}\)) (23, 28). The present results in intact preparations showed that a similar pH change (0.3 pH unit, predicted from Ref. 26) shifted the [Ca\(^{2+}\)]-tension relationship by 0.18 pCa unit.

To assess the change in the Ca\(^{2+}\) sensitivity induced by the decrease in pH, in the present study, the direct effect of pH change on the aequorin luminescence should be considered, because aequorin luminescence is slightly decreased by a drop in pH (3). If this is the case, we have underestimated the K\(_{1/2}\) during acidosis and, consequently, underestimated the change in the Ca\(^{2+}\) sensitivity induced by acidosis. A 0.3 pH drop reduces aequorin light to 84.1% at pCa 6 (3). This leads to a 5% underestimation of [Ca\(^{2+}\)], corresponding to an increase of 0.02 pCa unit in acidosis. This change is much smaller than the change in K\(_{1/2}\) induced by acidosis in the present study (0.18 pCa unit change).

On the other hand, aequorin luminescence is also affected by the intracellular free Mg\(^{2+}\) concentration ([Mg\(^{2+}\)]). However, [Mg\(^{2+}\)] is not significantly altered in acidosis (9). Therefore, the changes in the intracellular ionic environment in acidosis and, in particular, the effect of the change in pH on the aequorin light signal, do not explain the difference in the pH-dependent changes of the Ca\(^{2+}\) sensitivity of the contractile elements in intact and skinned preparations.

According to Gao et al. (7), the [Ca\(^{2+}\)]-tension relationship of the contractile elements is quite different between intact and skinned preparations, and several explanations have been considered: 1) the skinning procedure may alter the contractile elements; 2) skinning may cause a loss of intracellular proteins; 3) the solution used for the skinned preparation does not precisely mimic the physiological intracellular environment; and 4) skinning may alter the lattice spacing. Therefore, it is conceivable that these factors influence the pH-dependent changes of the Ca\(^{2+}\) sensitivity of skinned preparations. Moreover, the difference in species used and experimental temperatures might be involved in the difference of the pH-dependent change of the Ca\(^{2+}\) sensitivity between intact and skinned preparations.

Changes in extra Ca\(^{2+}\) in acidosis. The extra Ca\(^{2+}\), induced by a step length change (2, 17), reflects Ca\(^{2+}\) dissociated from the Ca\(^{2+}\)-binding site of troponin C due to the change in the affinity of troponin C for Ca\(^{2+}\). This change in the affinity of troponin C for Ca\(^{2+}\) is tension dependent rather than length dependent (8, 17, 19). Kurihara and Komukai (17) showed that the affinity of troponin C for Ca\(^{2+}\) is altered by a tension (the cross-bridge attachment-dependent) mechanism.

The present results demonstrated that acidosis significantly decreased the magnitude of the extra Ca\(^{2+}\) when the magnitude of tension reduction and the [Ca\(^{2+}\)], immediately before length change were the same as in the control (Fig. 5). The acidosis-induced change in the extra Ca\(^{2+}\) cannot be explained by the direct effect of pH on the aequorin luminescence, because the difference of the extra Ca\(^{2+}\) in the control and
in acidosis is much larger than that expected from the decrease of the aequorin luminescence due to acidosis as discussed earlier. The decrease in the extra Ca\textsuperscript{2+} is considered to be caused by 1) enhancement of the Ca\textsuperscript{2+} removal mechanism from the myoplasm; 2) a decrease in the total amount of troponin-Ca complex, which is the origin of the extra Ca\textsuperscript{2+}; and 3) inhibition of the tension-/cross-bridge-dependent feedback mechanism by acidosis. In acidosis, however, the decrease in the extracellular Ca\textsuperscript{2+} by acidosis. In acidosis, however, the decrease in the extracellular Ca\textsuperscript{2+} was observed even when the Ca\textsuperscript{2+} uptake by SR (the main Ca\textsuperscript{2+} removal mechanism in ferret ventricular muscle) was inhibited by caffeine; Ca\textsuperscript{2+} transported to SR is further released, and thus the Ca\textsuperscript{2+} uptake is apparently inhibited. Moreover, a decrease in pH inhibits the Ca\textsuperscript{2+} removal by SR and Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger (27), which should increase the extra Ca\textsuperscript{2+}. Therefore, the alteration of the Ca\textsuperscript{2+} removal mechanism is not likely the cause of the smaller extra Ca\textsuperscript{2+} in acidosis compared with that in the control.

As discussed earlier, acidosis decreased the Ca\textsuperscript{2+} sensitivity of the contractile elements, which was clearly shown in the rightward shift of the [Ca\textsuperscript{2+}] in the intact preparation (Fig. 2). Therefore, the troponin-Ca complex in acidosis should be less formed at the same [Ca\textsuperscript{2+}] compared with that in the control, which could account for the decrease in the extra Ca\textsuperscript{2+} in acidosis. However, the direct effect of acidosis on the tension-/cross-bridge-dependent feedback mechanism cannot be disregarded.

As shown in Fig. 6A, the extra Ca\textsuperscript{2+}, normalized to [Ca\textsuperscript{2+}] immediately before the length change, increased as the magnitude of tension reduction was increased. This result confirms the former report (17). Our hypothesis is as follows: 1) the time course of tension does not reflect the troponin-Ca complex; 2) the time course of the Ca\textsuperscript{2+} transient is closer to that of the troponin-Ca complex, as discussed; and 3) a quick release of muscle detaches the cross bridges, which works as a trigger for the alteration of the affinity of troponin C for Ca\textsuperscript{2+}. The normalized extra Ca\textsuperscript{2+} is the ratio of Ca\textsuperscript{2+} dissociated from the Ca\textsuperscript{2+} binding site of troponin C to the troponin-Ca complex formed at the [Ca\textsuperscript{2+}]. The direct influence of the lower pH on the calculation of the normalized extra Ca\textsuperscript{2+} can be ignored, because the extra Ca\textsuperscript{2+} and [Ca\textsuperscript{2+}] are similarly influenced by pH change. In acidosis, more Ca\textsuperscript{2+} is required to produce the same amount of troponin-Ca complex compared with that in the control. Therefore, higher [Ca\textsuperscript{2+}], required to produce the same amount of troponin-Ca complex in acidosis, decreases the normalized extra Ca\textsuperscript{2+}. Thus the relationship between the ratio of the extra Ca\textsuperscript{2+} to [Ca\textsuperscript{2+}] (normalized extra Ca\textsuperscript{2+}) and the magnitude of tension reduction, as shown in Fig. 6, reflects the apparent Ca\textsuperscript{2+} sensitivity of the contractile elements during twitch contraction. The opposite change observed in alkalosis, an increase in the normalized extra Ca\textsuperscript{2+} at the same tension reduction, further supports this hypothesis (Fig. 6B).

However, apart from this explanation, another possibility should be considered, because the relationship between [Ca\textsuperscript{2+}] and developed tension is not simple, as we mentioned in RESULTS, and protein-protein interactions after a change in [Ca\textsuperscript{2+}], (after Ca\textsuperscript{2+} binding to troponin C) are important for the process of tension development. Therefore, a large time lag between [Ca\textsuperscript{2+}] and tension exists. In cardiac muscle, the feedback mechanism from the cross bridges to troponin C (tension-dependent feedback mechanism) which influences the affinity of troponin C for Ca\textsuperscript{2+} is suggested to be involved in tension development (5, 13). Therefore, if this feedback mechanism is suppressed by acidosis, the extra Ca\textsuperscript{2+} at the same tension reduction and at the same [Ca\textsuperscript{2+}] in acidosis should be less. However, the cooperativity measured at a steady state (the Hill coefficient) which might be related to the tension-dependent feedback mechanism did not significantly alter in acidosis (Table 1 and Fig. 2B).

Acidosis decreased the maximal tension by about 20% (Table 1). If we assume that this decrease in the maximal tension in acidosis is entirely due to the decrease in the force produced per cross-bridge and that the magnitude of tension reduction is the same, then the number of detached cross bridges in the release should be 25% higher in acidosis. If we correct for the number of detached cross bridges in acidosis on the basis of this assumption, the normalized extra Ca\textsuperscript{2+} in acidosis is clearly less than that in the control (compare Fig. 8 with Fig. 6A).

In summary, acidosis decreased the Ca\textsuperscript{2+} sensitivity of the contractile elements in tetanized ferret papillary muscle. The pH-dependent change of the Ca\textsuperscript{2+} sensitivity in the intact preparation was smaller than that in the skinned preparation. Acidosis decreased [Ca\textsuperscript{2+}] change induced by a step length change, which probably reflects the less formed troponin-Ca complex at lower pH. Thus mechanical perturbations applied to cardiac muscle in acidosis induce less extra Ca\textsuperscript{2+}.

We thank Prof. C. H. Orchard for valuable comments on the first version of the manuscript, Mary Beth Sibuya for reading the manuscript, and Naoko Tomizawa for technical assistance. K. Komukai and T. Ishikawa thank Prof. Seibu Mohizuki, Dept. of Internal Medicine (IV), for continuous encouragement. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan, and a grant from The Vehicle Racing Commemorative Foundation (to S. Kurihara).

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Received 16 June 1997; accepted in final form 23 September 1997.

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