Effects of Changes of Intracellular pH on Contraction in Sheep Cardiac Purkinje Fibers

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ABSTRACT Intracellular pH (pHi) was measured with a pH-sensitive micro-electrode in voltage-clamped sheep cardiac Purkinje fibers while tension was simultaneously measured. All solutions were nominally CO₂/HCO₃⁻ free and were buffered with Tris. The addition of NH₄Cl (5–20 mM) produced an initial intracellular alkalosis that was associated with an increase of twitch tension. At the same time, a component of voltage-dependent tonic tension developed. Prolonged exposure (>5 min) to NH₄Cl resulted in a slow recovery of pHi accompanied by a decrease of tension. Removal of NH₄Cl produced a transient acidosis that was accompanied by a fall of force. In some experiments, there was then a transient recovery of force. If extracellular pH (pHₑ) was decreased, then pHi decreased slowly. Tension also fell slowly. An increase of pHₑ produced a corresponding increase of both force and pHi. The application of strophanthidin (10 µM) increased force and produced an intracellular acidosis. The addition of NH₄Cl, to remove this acidosis partially, produced a significant increase of force. The above results show that contraction is sensitive to changes of intracellular but not extracellular pH. This pH dependence will therefore modify the contractile response to inotropic maneuvers that also affect pHₑ.

INTRODUCTION

It has been known for many years that changes of extracellular pH (pHₑ) affect the contraction of cardiac muscle: acidification decreases and alkalinization increases the force of contraction (cf. Gaskell, 1880). More recent work has attempted to determine whether these effects are a direct consequence of changes in pHₑ (Vaughan Williams and Whyte, 1967) or whether they are secondary to changes in intracellular pH (pHi) (Fry and Poole-Wilson, 1981; Allen and Orchard, 1983; Poole-Wilson and Seabrooke, 1985). Evidence for the role played by pHₑ has come from the work of Fabiato and Fabiato (1978) on skinned cardiac fibers. These authors showed that changing the pH of the bathing solution modified the sensitivity of the contractile proteins to Ca²⁺: acidification reduced and alkalinization increased their Ca sensitivity. However, in intact fibers, ma-
neuvers that alter pH, also modify the diastolic level of intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\(i\)) (Bers and Ellis, 1982; Allen et al., 1984) and the magnitude of the Ca\(^{2+}\) transient that underlies the twitch (Allen and Orchard, 1983). Hence, in intact cells, a change in pH\(i\) is likely to affect contraction via more than one mechanism.

pH\(i\) is changed by many experimental maneuvers and may have significant effects on contraction. For example, ischemia or hypoxia produce an intracellular acidification (e.g., De Hemptinne et al., 1982; Allen et al., 1985b), which has been suggested to account for the observed contractile failure (Katz and Hecht, 1969; Poole-Wilson, 1978). Furthermore, many inotropic maneuvers that increase [Ca\(^{2+}\)]\(i\), and thereby directly increase contraction, also produce an intracellular acidification (Deitmer and Ellis, 1980; Vaughan-Jones et al., 1985), which would be expected to decrease contraction indirectly.

The present experiments were designed to answer three questions. (a) Can the effects of pH\(i\) on contraction in cardiac fibers be accounted for by the subsequent changes of pH\(i\)? (b) What is the quantitative relationship between pH\(i\) and contraction in intact fibers? (c) To what extent do changes of pH\(i\) modify the inotropic actions of other experimental maneuvers?

The experiments were carried out on Purkinje fibers isolated from sheep hearts. pH\(i\) was measured directly with a recessed-tip, pH-sensitive glass microelectrode (Thomas, 1978) and tension was recorded simultaneously. Twitch and tonic tension in this tissue are voltage dependent (e.g., Eisner and Lederer, 1979). Consequently, it was necessary to control the membrane potential using a voltage-clamp apparatus so that depolarizing pulses of constant amplitude and duration could be used to elicit force development. Preliminary accounts of some of these results have appeared already (Eisner et al., 1983a; Vaughan-Jones et al., 1985).

M E T H O D S

The experimental methods were similar to those described previously (Eisner and Lederer, 1979; Eisner et al., 1981, 1983b). Briefly, sheep hearts were obtained from a local abattoir. Purkinje fibers were isolated, shortened to 1–2 mm in length, and attached to a tension transducer in the experimental bath. Voltage-clamp control was maintained with a two-microelectrode clamp. pH\(i\) was measured with a recessed-tip, pH-sensitive glass microelectrode (see Thomas, 1978; Vaughan-Jones, 1979). The pH electrodes were calibrated in the experimental bath before and after each experiment using a modified Tyrode’s solution with the pH adjusted to 7.4 and 6.4 at 37°C. Acceptable electrodes displayed a response of 54–60 mV per pH unit, with a 90% response occurring within 20 s of changing the pH of the bathing solution. Because of this rather slow response time, the relationship between tension and pH\(i\) could not be quantified during rapid changes in pH\(i\), such as were produced immediately after the addition or removal of external NH\(_4\)Cl (see, e.g., Fig. 1). Slower changes of pH\(i\) (over several minutes) can be measured accurately. Consequently, the quantitative effect of pH\(i\) upon tension was measured during the slow secondary changes in pH\(i\) that occur after the addition and removal of NH\(_4\)Cl and during the slow changes in pH\(i\) after a change in pH\(i\).

In several of the figures, the tension traces have been high-pass filtered (as indicated in the legends) to remove changes of baseline tension in order to resolve changes in twitch tension.
Solutions

The modified Tyrode's solution consisted of (mM): 145 NaCl, 4 KCl, 5 CaCl₂, 1 MgCl₂, 10 glucose, 10 Tris-HCl. The pH was adjusted to 7.4 at 37°C. All solutions were bubbled with air.

RESULTS

Effects on Tension of Changes of pHᵢ

The first experiments were designed to investigate the effects on tension of changing pHᵢ under conditions where the pH of the superfusing solution (pHₑ) was constant. This was achieved by exposing the preparation to NH₄Cl. Fig. 1A shows the effects of an 18-min exposure to NH₄Cl (20 mM). The application of NH₄Cl initially produced an intracellular alkalization because the highly permeant NH₃ crosses the membrane more quickly than does NH₄⁺ (e.g., Boron and De Weer, 1976). Once inside the cell, NH₃ combines with H⁺ ions to form NH₄⁺, thus raising pHᵢ. However, during prolonged application of NH₄Cl, as in Fig. 1A, the initial alkalosis is not maintained, and pHᵢ gradually recovers to a less alkaline level. This is caused by the slow entry of NH₄⁺ ions into the cell (see Roos and Boron, 1981, for a discussion of this process). The eventual removal of external NH₄Cl leads to a large intracellular acidification and this occurs because NH₄⁺ ions that have accumulated intracellularly now convert to NH₃, which escapes from the cell, leaving behind H⁺ ions. Finally, these H⁺ ions are extruded from the cell via Na-H exchange (Deitmer and Ellis, 1980), so that pHᵢ slowly recovers from the acidification. The addition and removal of NH₄Cl thus leads to large transient changes in pHᵢ. Fig. 1A shows that these changes in pHᵢ affect tension. The initial alkalosis caused by the addition of NH₄Cl is associated with an increase in twitch tension. However, as pHᵢ becomes less alkaline again on prolonged exposure to NH₄Cl, twitch tension also declines. Similarly, the transient acidification on removal of NH₄Cl is accompanied by a transient decrease of twitch tension. Specimen tension records are shown on an expanded time scale in Fig. 1B. Alkalization increased not only the twitch component of tension but also revealed a component of voltage-dependent tonic tension that was maintained throughout the depolarizing voltage-clamp step. Conversely, intracellular acidification, as well as reducing twitch tension, also abolished this voltage-dependent component of tonic tension.

A similar effect of pHᵢ upon tonic tension was also evident at the holding potential, which, in Fig. 1A, was -60 mV. It is clear that after the addition of NH₄Cl, there was a transient increase in resting force, which, like that seen for the twitch, followed the transient rise in pHᵢ. The experiment illustrated in Fig. 2 was designed to investigate further the effect of pHᵢ on the voltage-dependent component of tonic tension. In order to increase this component of tension, the preparation was exposed to strophanthidin (10 µM; see Eisner et al., 1983). The specimen record (Fig. 2B, trace a) shows that under these conditions, depolarization produced a tonic component of tension as well as an aftercontraction. Next, NH₄Cl was added, first at a concentration of 10 mM and then at 20 mM. This produced a graded intracellular alkalization (Fig. 2A) accompanied by an
FIGURE 1. The effects of prolonged exposure to NH₄Cl on pHᵢ and tension. (A) Time course of effects. Traces show (from top to bottom): pHᵢ, tension (band-pass filtered: 0.1–10 Hz), tension (DC to 10 Hz), and membrane potential. The tension trace recorded from DC to 10 Hz illustrates the slow changes in resting tension that occur during the application of NH₄Cl. However, after removal of NH₄Cl, disturbances in the solution flow produced artifactual disturbances of resting tension, which are evident as spontaneous, downward baseline displacements. The membrane potential was held at −60 mV and 2-s pulses were applied to −30 mV. NH₄Cl (10 mM) was applied for the period shown above. (B) Specimen records displayed on a faster time base, obtained at the points indicated by the letters in A. Note the appearance of voltage-dependent tonic tension when pHᵢ is alkaline.
increase of tonic tension, twitch tension, and the aftercontraction (Fig. 2B, traces b and c). Finally, the removal of NH₄Cl produced an intracellular acidosis and a corresponding decrease of all components of tension (Fig. 2B, trace d). Therefore, an intracellular alkalosis increased and an intracellular acidosis decreased twitch tension, resting tonic tension, voltage-dependent tonic tension, and, when present, the aftercontraction.

**FIGURE 2.** The effects of NH₄Cl in the presence of strophanthidin. (A) Time course. The traces show (top to bottom) membrane potential (V), pHᵢ, and tension. [NH₄Cl] was varied as shown above. The membrane potential was held at −70 mV and 2-s pulses were applied to −30 mV at 0.1 Hz. Strophanthidin (10 μM) had been present for 20 min before the record began. (B) Specimen records obtained at the points shown in A.
The data from seven experiments with NH₄Cl are shown in Fig. 3, which plots twitch tension as a function of pHᵢ on linear coordinates and semilogarithmic coordinates (inset). It is apparent that changes in pHᵢ of ±0.3 units produced graded changes in tension. Furthermore, in this range, the plot of logarithmic force as a function of pHᵢ is roughly linear, with a slope of ~2. However, at values of pHᵢ lower than 6.8, force sometimes increased again. Such an increase of the twitch at low pHᵢ is illustrated in Fig. 4. In this experiment, the fiber had been exposed to 20 mM NH₄Cl for 23 min. After the removal of NH₄Cl, pHᵢ declined rapidly by 0.5 unit to 6.7 and then recovered more slowly. The large and rapid fall in pHᵢ was accompanied initially by a decrease in twitch tension.

![Figure 3](image-url)  
**Figure 3.** The relationship between tension and pHᵢ. (A) Linear scale. The ordinate shows twitch tension normalized to the level in control solution (mean pHᵢ = 7.24 ± 0.04 SEM, n = 7). The abscissa is the change of pHᵢ from the control level during the application of NH₄Cl (positive values) or after removal of NH₄Cl (negative, acidic values). No attempt was made to measure the relationship during the rapid development of the alkalosis (because of the slow response time of the pH electrode; see Methods). The points at alkaline pH were obtained during the recovery of pHᵢ from its most alkaline level. The points at acidic pH were obtained during the recovery of pHᵢ from its most acid level after the removal of NH₄Cl. The different symbols correspond to different preparations, except for the open squares, which were produced by a brief exposure to NH₄Cl. Each point represents a different preparation.

However, twitch tension then increased transiently, such that, at a pHᵢ of 6.68, it was comparable to that observed at the start of the record, when pHᵢ was 7.25. Such transient increases in force after the removal of NH₄Cl were observed in two out of seven fibers, but only after prolonged periods of exposure to NH₄Cl, when the removal of NH₄Cl resulted in a large acidosis. This paradoxical increase in force at low pHᵢ will be considered in the Discussion.

**Effects on Tension of Changes in pHᵢ**

In these experiments, pHᵢ was changed by altering the pH of the superfusing solution. Fig. 5A shows that an external acidification (from pH 7.4 to 5.9) produced a slow reversible fall in pHᵢ and twitch tension. Opposite effects on
\( \text{pH}_\text{i} \) and tension were observed after a rise in \( \text{pH}_\text{o} \), as shown in Fig. 5B. As in the experiments with \( \text{NH}_4\text{Cl} \), the time courses of the change in twitch tension paralleled the changes in \( \text{pH}_\text{i} \). The quantitative relationship between tension and \( \text{pH}_\text{i} \) observed in Fig. 5 is plotted in Fig. 6. Thus, regardless of whether \( \text{pH}_\text{i} \) is altered by varying \( \text{pH}_\text{o} \) or by exposure to \( \text{NH}_4\text{Cl} \), changes in twitch tension correlate well with changes in \( \text{pH}_\text{i} \). It is therefore appropriate to ask whether tension can be expressed as a unique function of \( \text{pH}_\text{i} \). To do this, we have compared the effects of changing \( \text{pH}_\text{o} \) with those of adding \( \text{NH}_4\text{Cl} \). Fig. 7A shows that an increase of \( \text{pH}_\text{o} \) from 7.4 to 8.4 produced about the same change of \( \text{pH}_\text{i} \) as did the application of 5 mM \( \text{NH}_4\text{Cl} \). It is apparent that the relationship between tension and \( \text{pH}_\text{i} \) was similar when \( \text{pH}_\text{i} \) was recovering from these two types of alkalosis (Fig. 7B, filled circles and open triangles). However, this relationship differs quantitatively from that seen between tension and \( \text{pH}_\text{i} \) during the onset of the effects of \( \text{pH} 8.4 \) (Fig. 7B, open circles). Such hysteresis between force and \( \text{pH}_\text{i} \) was observed consistently after reversible alterations in \( \text{pH}_\text{o} \) (six experiments on four fibers). For example, inspection of the data shown in Fig. 6 also reveals a hysteresis, although in this case it is less pronounced than that seen in Fig. 7. It should also be noted that hysteresis did not occur consistently in a given direction; e.g., that shown in Fig. 6 is in the opposite direction to that shown in Fig. 7B. The conclusion we draw from these experiments, therefore, is that after a change of \( \text{pH}_\text{i} \), although there is usually a good qualitative correlation between \( \text{pH}_\text{i} \) and tension, the quantitative relationship depends on the direction in which \( \text{pH}_\text{i} \) was changed. Unfortunately, it was not possible to
Figure 5. The effects on $\mathrm{pH}_i$ and tension of changing $\mathrm{pH}_o$. In both panels, the traces show (top to bottom) $\mathrm{pH}_i$, tension (band-pass filtered at 0.1–10 Hz), and membrane potential. (A) The effects of external acidification. $\mathrm{pH}_o$ was changed as indicated above the figure. The membrane potential was held at $-70 \text{ mV}$ and 2-s depolarizing pulses were applied to $-35 \text{ mV}$ at 0.1 Hz. (B) The effects of external alkalinization. Both A and B are taken from the same experiment; B was recorded 15 min after A.

Discover whether a similar hysteresis between $\mathrm{pH}_i$ and tension occurred with $\mathrm{NH}_4\mathrm{Cl}$. This is because the rather slow response time of the pH electrode precluded accurate measurements during fast changes in $\mathrm{pH}_i$ (see Methods).
Changes of pH\textsubscript{i} Modify Other Inotropic Maneuvers

In this section, we consider briefly two common experimental maneuvers that result in large changes of tension: (a) the addition of cardioactive steroids and (b) variations in [Ca\textsuperscript{2+}]\textsubscript{o}. Both maneuvers exert their inotropic effects via changes in [Ca\textsuperscript{2+}]\textsubscript{i}, but both maneuvers also produce changes in pH\textsubscript{i} that may modify the contractile response.

![Graph showing the quantitative effect on tension of a change in pH\textsubscript{i} produced by altering pH\textsubscript{o}. Data were taken from the experiment shown in Fig. 5. The relationship between tension and pH\textsubscript{i} has been plotted after external acidosis (pH\textsubscript{o} 7.4–5.9, filled squares), recovery from external acidosis (pH\textsubscript{o} 5.9–7.4, open squares), external alkalosis (pH\textsubscript{o} 7.4–8.9, open circles), and recovery from external alkalosis (pH\textsubscript{o} 8.9–7.4, filled circles). The line was drawn by eye.]

In the experiment of Fig. 8A, strophanthidin (10 μM) was added to the superfusing solution. It has been shown previously that this increases intracellular Na activity (a\textsubscript{Na}), intracellular Ca activity (a\textsubscript{Ca}), and the various components of tension, including the appearance of an oscillatory aftercontraction and a transient inward current, both of which occur after repolarization (e.g., Eisner et al., 1983b). Fig. 8A also shows that pH\textsubscript{i} decreased by 0.1 unit (cf. Deitmer and Ellis, 1980; Vaughan-Jones et al., 1983). The results of the experiments of Figs. 1–7 suggest that a change in pH\textsubscript{i} of this magnitude will have significant effects on contraction. This point was tested directly by adding 10 mM NH\textsubscript{4}Cl to the superfusate after strophanthidin had been applied for 15 min. The application
FIGURE 7. A comparison of the effects of NH₄Cl with those of changing pHₐ. (A) Original record. The traces show (top to bottom) pHₐ, tension (band-pass filtered at 0.1–10 Hz), and membrane potential. The membrane potential was held at −60 mV and 2-s depolarizing pulses were applied to −30 mV at 0.1 Hz. The control solution had a pHₐ of 7.4 and, as indicated above the record, either pHₐ was changed to 8.4 or 5 mM NH₄Cl was added (at constant pHₐ). (B) Relationship between pHₐ and twitch tension. The graph shows twitch tension as a function of pHₐ during either the development of the effects of external alkalinization (open circles), the recovery from the effects of extracellular alkalinization (filled circles), and during the recovery of pH during the exposure to NH₄Cl (triangles). The symbols correspond to those shown in A.
FIGURE 8. The effects of changes of pH; on the inotropic response to Na-K pump inhibition. (A) Time course. The traces show (top to bottom) membrane potential, pH; and tension (band-pass filtered at 0.1-10 Hz): The membrane potential was held at -60 mV and 2-s pulses were applied at 0.1 Hz. Strophanthidin (10 μM) and NH₄Cl (5 mM) were applied for the periods indicated above the record. (B) Specimen records of membrane potential and tension (DC to 10 Hz) obtained at the points indicated in A.
of NH$_4$Cl transiently returned pH$_i$ to a less acid level and simultaneously produced a large increase of tension. The effects on tension are shown on an expanded time scale in Fig. 8B. Alkalization increased all components of tension (the increases in Fig. 8B are 50% for twitch, 120% for voltage-dependent tonic tension, and 21% for the aftercontraction). These results therefore make it clear that the acidification produced by strophanthidin will have greatly attenuated the positive inotropic effects.

The effects on pH$_i$ and tension of elevating [Ca$^{2+}$]$_o$ have been examined previously (Vaughan-Jones et al., 1983) but it is necessary to make some additional observations here. Under control conditions, raising the [Ca$^{2+}$]$_o$ from 0.5 to 5.0 mM produces a rise in twitch tension but has little effect on pH$_i$ (not shown). However, after prolonged inhibition of the Na-K pump, the same rise in [Ca$^{2+}$]$_o$ leads to a large rise and then, over the next few minutes, a secondary decline in all the components of tension (twitch tension, voltage-dependent tonic tension, and the aftercontraction; Eisner et al., 1983; Vaughan-Jones et al., 1983), and it is notable that this secondary decline in tension is associated with an intracellular acidosis of 0.1-0.2 units. We have observed these effects of [Ca$^{2+}$]$_o$ on pH$_i$ and tension in several fibers when the Na-K pump is inhibited with either a cardioactive steroid (10$^{-5}$ M strophanthidin for 15-30 min) or by superfusion with a K-free solution. Although not illustrated here, the effects can be seen clearly in Fig. 3 of Vaughan-Jones et al. (1983) (Na-K pump inhibited in K-free solution). In that figure, raising [Ca$^{2+}$]$_o$ from 0.5 to 5.0 mM produced a rise followed by a secondary decline (within 5 min) of tonic tension to ~60% of its peak value and this was associated with an intracellular acidosis of 0.15 units. The fall in pH$_i$ has been shown to be secondary to a rise in [Ca$^{2+}$]$_o$ (Vaughan-Jones et al., 1983). It is now evident that the intracellular acidosis of 0.15 units can account for much of the secondary decline in tension when reference is made to the effects of pH$_i$ on tension seen in the present work. Although other factors also contribute to the secondary decline of tension when [Ca$^{2+}$]$_o$ is raised (see Discussion), the present work emphasizes that the effects of changes in pH$_i$ cannot be ignored.

**DISCUSSION**

**Effects on Tension of Changes in pH$_i$ and pH$_o$**

The present work provides direct measurements of pH$_i$, coupled with simultaneous measurements of twitch and tonic tension in an isolated cardiac preparation. Most previous studies on the effects of pH on contraction have measured pH$_i$ in intact hearts using either nuclear magnetic resonance (Jacobus et al., 1982) or dye distribution techniques (Waddell and Bates, 1969). These techniques suffer, however, from very limited time resolution in comparison with the pH-sensitive electrodes used in the present study (the recessed-tip pH electrodes used in the present work gave a 90% response to a pH change within 20 s; see Methods).

Under most circumstances, we find that an intracellular acidosis is associated with a fall in twitch and tonic tension. In addition, the time course of change in tension follows, at least qualitatively, the change in pH$_i$. It is important to note
that altering pHo does not affect tension until there is a change in pHi (Fig. 5). Furthermore, changes in tension induced with NH4Cl, when the pH of the bulk solution is constant, also correlate well with the observed changes in pHi. These results therefore indicate that acidification and alkalinization affect tension via changes in pHi rather than pHo. De Marranes et al. (1981) have shown that the addition of external weak acids produces a small transient alkalosis at the extracellular surface of a Purkinje fiber. Similarly, superfusion of an external weak base, e.g., NH4Cl, produces a transient acidosis at the surface (Kaila, K., and R. D. Vaughan-Jones, unpublished observations). While we cannot exclude entirely some influence on tension from alterations in surface pH, we conclude that this effect will be small, simply because changing the bulk pHo by 1.0 unit has no immediate effect on force.

**Quantitative Effect of pHi on Tension**

Measurements of [Ca2+]i using aequorin have shown that the decrease in twitch tension during an intracellular acidosis is not associated with a decrease in the [Ca2+]i transient (Allen and Orchard, 1983). Indeed, systolic [Ca2+]i may even increase during acidification. Furthermore, diastolic [Ca2+]i also rises rather than falls during an intracellular acidosis (Bers and Ellis, 1983; Allen et al., 1984; but compare Hess and Weingart, 1982). It therefore appears that the fall in twitch and tonic tension during an intracellular acidosis is not due to a fall in either systolic or diastolic [Ca2+]i, and must therefore be due to a decrease in the Ca2+ sensitivity of the contractile proteins. Direct evidence for this is provided by work on skinned cardiac muscle: acidification shifts the tension-pCa curve to higher Ca concentrations (Fabiato and Fabiato, 1978). However, a quantitative comparison of our results with those observed in skinned fibers is complicated by the fact that we have not measured [Ca2+]i. The data shown in Fig. 3 indicate that, when bulk pHo is constant, a fall in resting pHi of 0.17 units is sufficient to reduce twitch tension by 50%. Such a large effect of pHi on force can be observed in the skinned fiber provided that the contractile proteins are <50% saturated with Ca2+.1 That this is likely to be the case in the present experiments is indicated by the fact that under control conditions, twitch tension in a Purkinje fiber is normally a small fraction of that which can be produced after, say, exposure to high concentrations of cardiac glycosides (see Eisner et al., 1983b, 1984). Hence, the relatively high sensitivity of tension to variations in pHi in the intact Purkinje fiber is consistent with the data obtained from skinned fibers.

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1 In skinned cardiac fibers, the active tension-pCa curve is sigmoidal. A fall in pHi, in the range 7.4—6.6, produces a shift of this curve along the pCa axis to lower pCa values, i.e., to a higher [Ca2+]. Because the tension-pCa curve is sigmoidal, the fractional depressant effect on tension of a given fall in pHi will be smaller at low pCa values (when the myofibrils are saturated with Ca2+) and larger at high pCa values (when the proteins are far from saturated with Ca2+). By replotting the data presented in Fig. 1B of Fabiato and Fabiato (1978) (a tension-pCa curve determined at different pHi values for skinned rat ventricular fibers), one can determine that, in skinned fibers, the mean slope of the relationship between log tension and pHi (at a constant pCa) is close to 2.0, provided that tension is <50% of maximum tension (this slope of 2.0 should be compared with the similar slope observed in Fig. 3B of the present work). However, as expected, at higher levels of tension, this slope in skinned fibers can decrease considerably.
However, a quantitative analysis of the present work shows that the exact relationship between $pH_i$ and tension depends on the way in which $pH_i$ is changed, a finding that is clearly at variance with the work on skinned fibers, where a unique relationship was observed.

The reason for the difference between the $pH$ sensitivity of skinned and intact fibers is not known, but it seems likely that a change of $pH_i$ in intact fibers has an effect not only on the $Ca^{2+}$ sensitivity of the myofilaments, as in skinned fibers, but also on the processes that regulate $[Ca^{2+}]_i$ and deliver $Ca^{2+}$ to the myofilaments. For example, a change in $pH_i$ could influence (a) $Ca^{2+}$ release and reuptake at internal stores such as the sarcoplasmic reticulum or other intracellular "buffer sites" (Fabiato and Fabiato, 1978), (b) $Ca^{2+}$ influx via membrane channels (Fry and Poole-Wilson, 1981), and (c) $Ca^{2+}$ efflux and/or influx via Na-Ca exchange (Philipson et al., 1982). Such additional influences of $pH_i$ could then contribute to the observation, mentioned above, that altering $pH_i$ in intact fibers also changes $[Ca^{2+}]_i$. The difference between the $pH_i$ sensitivity of force in skinned and intact fibers may therefore be due to the additional influence of $pH_i$ on $[Ca^{2+}]_i$ in intact fibers.

Another observation in the present work is that in an intact fiber, the quantitative relationship between tension and $pH_i$ can depend on the direction in which $pH_i$ changes. Such hysteresis between force and $pH_i$ has not, as yet, been reported for either skinned or intact fibers. Its origin remains unknown, but there are at least three possible contributing factors. (a) As mentioned above, a change in $pH_i$ can affect $[Ca^{2+}]_i$. Consequently, if the kinetics of the $pH_i$-$[Ca^{2+}]_i$ interaction were to depend on the direction in which $pH_i$ was changed, this would generate a hysteresis between force and $pH_i$. (b) We cannot exclude the possibility that, for example, after a change in $pH_i$, there is for a few minutes a distribution of cells within the Purkinje strand that possess different values of $pH_i$. Since $pH_i$ in the present work was measured in a single cell, whereas force development was measured from the whole tissue, it is possible that a nonhomogeneity of $pH_i$ under some non-steady state conditions could contribute to the hysteresis between force and $pH_i$. (c) Finally, hysteresis has been reported between $[Ca^{2+}]_i$ and force development in both cardiac muscle (Harrison et al., 1985) and skeletal muscle (Ridgway et al., 1983), and this phenomenon will further complicate the $pH_i$ dependence of force. However, the latter effect would be expected to influence both skinned and intact fibers equally.

One puzzling observation in the present work is that, on removal of external $NH_4Cl$, after the initial rapid fall of tension, there was sometimes a secondary redevelopment of force. This secondary rise of tension coincided with the intracellular acidosis and seemed to occur when this acidosis was large. It is possible that, under these conditions, the intracellular acidosis promoted a rise in $[Ca^{2+}]$, that was sufficiently large to increase developed tension despite the fact that $pH_i$ had decreased. It is interesting to note that the peak of the acidosis after $NH_4Cl$ removal coincided with a transient rise in $a_{Na}$ that appeared to be mediated by Na-H exchange (Deitmer and Ellis, 1980; Piwnica-Worms et al., 1985). The transient increase in $a_{Na}$ would increase $[Ca^{2+}]_i$ via Na-Ca exchange and could account for the rise of tension at low $pH_i$. 
Simultaneous measurements of pH_i and contraction have recently been reported for guinea pig papillary muscle (Poole-Wilson and Seabrooke, 1985). Unlike the results reported here, an intracellular acidosis was always associated with a decrease in tension. Furthermore, a linear relationship between pH_i and tension was found and no evidence of hysteresis between pH_i and force was reported. A linear dependence of developed pressure on pH_i has also been reported for intact rabbit heart (Jacobus et al., 1982). It may therefore seem surprising that a steeper, nonlinear dependence of force on pH_i can be observed in the Purkinje fiber (e.g., Figs. 3 and 6). However, since the present work shows that the quantitative dependence of force on pH_i can depend upon the way in which pH_i is varied, it would be premature to conclude that the differences between our results and those reported previously are due to real differences in the pH_i sensitivity of contraction in different cardiac tissues.

**Effect of a Change in pH_i on Tonic Tension**

Our experiments show that a change in pH_i affected not only twitch tension but also resting tension and voltage-dependent tonic tension (see Fig. 1). However, these components of tonic tension are not normally observed in the Purkinje fiber unless a_{Na}^i, and hence [Ca^{2+}]_i, has been elevated (Eisner et al., 1983b). It is therefore interesting to note the tension records in alkaline conditions. Not only was there the appearance of a tonic contracture at the holding potential (Fig. 1), but there was also a component of voltage-dependent tonic tension. These results can be explained if depolarization always produces a tonic increase of [Ca^{2+}]_i, but, under normal conditions, the rise of [Ca^{2+}]_i is too small to increase tension. Therefore, tonic tension would be seen if either [Ca^{2+}]_i was increased or the Ca sensitivity of the contractile proteins was increased.

An alternative explanation for the appearance of voltage-dependent tonic tension at high pH_i may be given by the observation that a low pH_i inhibits Na-Ca exchange in cardiac muscle (Philipson et al., 1982). It has been argued that, in cardiac muscle, the rise in tonic tension upon depolarization is controlled by a Ca^{2+} influx via Na-Ca exchange (Chapman and Tunstall, 1981; Eisner et al., 1983b). Hence, a larger tonic force at a more alkaline pH_i could represent a release from inhibition of this exchange mechanism.

**Effects of pH_i during Inotropic Interventions**

It is well established that the positive inotropic effects of Na-K pump inhibition are produced by an increase in a_{Na} that acts on Na-Ca exchange to increase [Ca^{2+}]. This rise of [Ca^{2+}]_i is, however, accompanied by an intracellular acidification, and we have suggested previously that this acidification may attenuate the rise of tension produced by Na-K pump inhibition and will thereby decrease the steepness of the relationship between tension and a_{Na} (Eisner et al., 1983b, 1984). This is confirmed in the present experiments, which show that when the acidification was reversed, a large increase in all the components of tension was produced (cf. Fig. 8B, traces c and d). In previous work, we found that tension varied with (a_{Na})^n, where n, on average, is ~3 (Eisner et al., 1983b, 1984). The present results show that pH_i fell by ~0.1 unit after inhibition of the Na-K pump.
(Fig. 8; see also Vaughan-Jones et al., 1983; Kaila and Vaughan-Jones, 1985). Therefore, the dependence of tension on $a_{\text{Na}}$ will be underestimated, and, in the absence of any pH change, tension will depend more steeply on $a_{\text{Na}}$, making $n$ closer to 4 than to 3.

Similar arguments apply to the effects of elevating $[\text{Ca}^{2+}]_o$. After inhibition of the Na-pump, elevating $[\text{Ca}^{2+}]_o$ produces an increase in twitch and tonic tension, which then declines back toward control levels (Vaughan-Jones et al., 1983; Allen et al., 1984). The transient component of the response may be accounted for partly by the observation that increasing $[\text{Ca}^{2+}]_o$ produces only a transient rise in $[\text{Ca}^{2+}]_i$ (Allen et al., 1984; Eisner and Valdeolmillos, 1986; Kaila, K., J. Voipio, and R. D. Vaughan-Jones, manuscript in preparation). However, the present work indicates that another contributing factor is likely to be the intracellular acidification that occurs after the elevation of $[\text{Ca}^{2+}]_o$.

A decrease in pH may be relevant to two related observations in the literature. (a) If the Na-K pump is inhibited for a prolonged period, then after the initial rise in force, developed tension gradually decreases. (b) When the Na-K pump is inhibited, increasing $[\text{Ca}^{2+}]_o$ can actually decrease developed tension. Both observations have been attributed to the phenomenon of "Ca overload" (Vassalle and Lin, 1979; Allen et al., 1985a). Two explanations have already been presented for this Ca overload. (a) The fall in twitch tension under these conditions may be produced by spontaneous oscillatory release of Ca$^{2+}$ from the sarcoplasmic reticulum (Kort and Lakatta, 1984; Cannell et al., 1985; Valdeolmillos and Eisner, 1985; Allen et al., 1985a). This release occurs most prominently at high mean levels of $[\text{Ca}^{2+}]_i$ (Allen et al., 1985a). When such $[\text{Ca}^{2+}]_i$ oscillations precede the stimulation of a twitch via depolarization, then both the $[\text{Ca}^{2+}]_i$ transient and twitch tension are reduced (Allen et al., 1985a). (b) The fall of tension is accompanied by an increase of the intracellular inorganic phosphate concentration (Allen et al., 1986). An increase of inorganic phosphate decreases the Ca sensitivity of the contractile apparatus (Kentish, 1986) and could account for part of the fall of force. While the present data do not conflict with these interpretations of the Ca overload phenomenon, they nevertheless point to a prominent role of changes in pH in reducing tension under these conditions.

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