

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

R. D. VAUGHAN-JONES, D. A. EISNER, and W. J. LEDERER

From the University Laboratory of Physiology, Oxford University, Oxford OX1 3PT, England; the Department of Physiology, University College London, London WC1E 6BT, England; and the Department of Physiology, University of Maryland School of Medicine, Baltimore, Maryland 21201

ABSTRACT Intracellular pH (pH_i) was measured with a pH-sensitive micro-electrode in voltage-clamped sheep cardiac Purkinje fibers while tension was simultaneously measured. All solutions were nominally $\text{CO}_2/\text{HCO}_3^-$ free and were buffered with Tris. The addition of NH_4Cl (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 NH_4Cl resulted in a slow recovery of pH_i accompanied by a decrease of tension. Removal of NH_4Cl 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 (pH_o) was decreased, then pH_i decreased slowly. Tension also fell slowly. An increase of pH_o produced a corresponding increase of both force and pH_i . The application of strophanthidin (10 μM) increased force and produced an intracellular acidosis. The addition of NH_4Cl , 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 pH_i .

INTRODUCTION

It has been known for many years that changes of extracellular pH (pH_o) affect the contraction of cardiac muscle: acidification decreases and alkalization 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 pH_o (Vaughan Williams and Whyte, 1967) or whether they are secondary to changes in intracellular pH (pH_i) (Fry and Poole-Wilson, 1981; Allen and Orchard, 1983; Poole-Wilson and Seabrooke, 1985). Evidence for the role played by pH_i 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 Ca^{2+} : acidification reduced and alkalization increased their Ca sensitivity. However, in intact fibers, ma-

neurons that alter pH_i also modify the diastolic level of intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) (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., 1985*b*), which has been suggested to account for the observed contractile failure (Katz and Hecht, 1969; Poole-Wilson, 1978). Furthermore, many inotropic maneuvers that increase $[\text{Ca}^{2+}]_i$, and thereby directly increase contraction, also produce an intracellular acidification (Deitmer and Ellis, 1980; Vaughan-Jones et al., 1983), which would be expected to decrease contraction indirectly.

The present experiments were designed to answer three questions. (a) Can the effects of pH_o 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., 1983*a*; Vaughan-Jones et al., 1985).

METHODS

The experimental methods were similar to those described previously (Eisner and Lederer, 1979; Eisner et al., 1981, 1983*b*). 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_4Cl (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_4Cl and during the slow changes in pH_i after a change in pH_o .

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 pH_i

The first experiments were designed to investigate the effects on tension of changing pH_i under conditions where the pH of the superfusing solution (pH_o) 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 pH_i. However, during prolonged application of NH₄Cl, as in Fig. 1A, the initial alkalosis is not maintained, and pH_i 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 pH_i slowly recovers from the acidification. The addition and removal of NH₄Cl thus leads to large transient changes in pH_i. Fig. 1A shows that these changes in pH_i affect tension. The initial alkalosis caused by the addition of NH₄Cl is associated with an increase in twitch tension. However, as pH_i 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. Alkalinization 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 pH_i 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 pH_i. The experiment illustrated in Fig. 2 was designed to investigate further the effect of pH_i 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

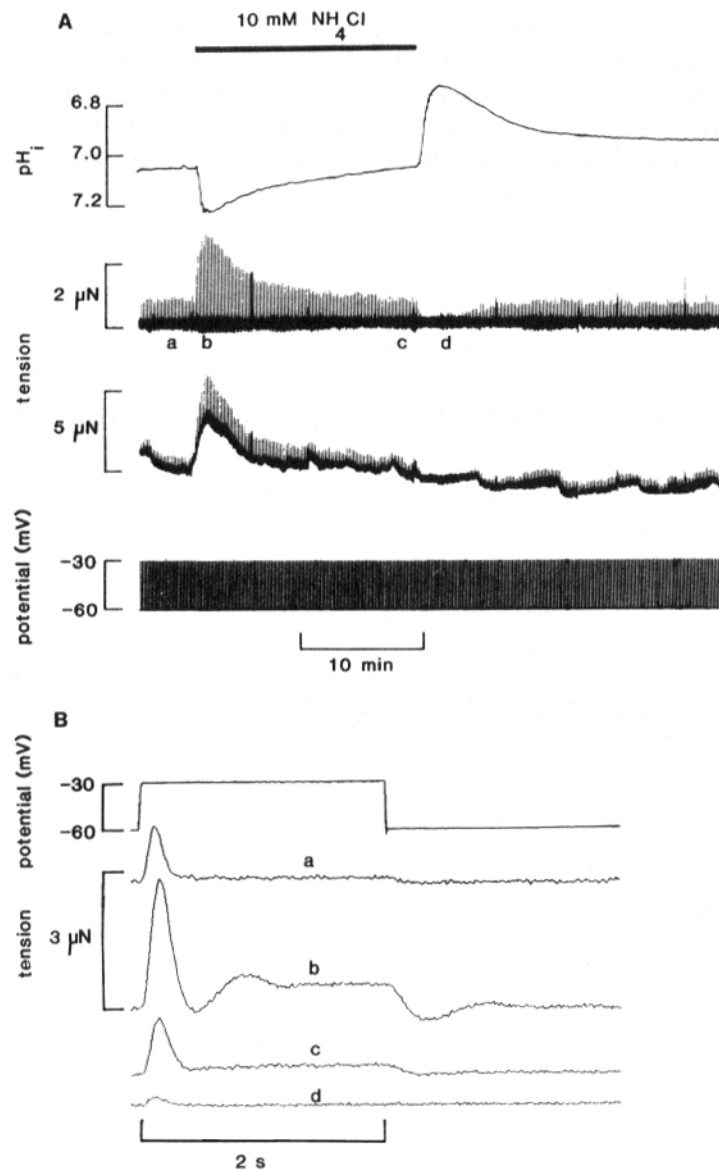


FIGURE 1. The effects of prolonged exposure to NH_4Cl on pH_i and tension. (A) Time course of effects. Traces show (from top to bottom): pH_i , tension (band-pass filtered: 0.1–10 Hz), tension (DC to 10 Hz), and membrane potential. The tension trace recorded from DC to 10 Hz illustrates the slow changes in resting tension that occur during the application of NH_4Cl . However, after removal of NH_4Cl , 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. NH_4Cl (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 pH_i 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.

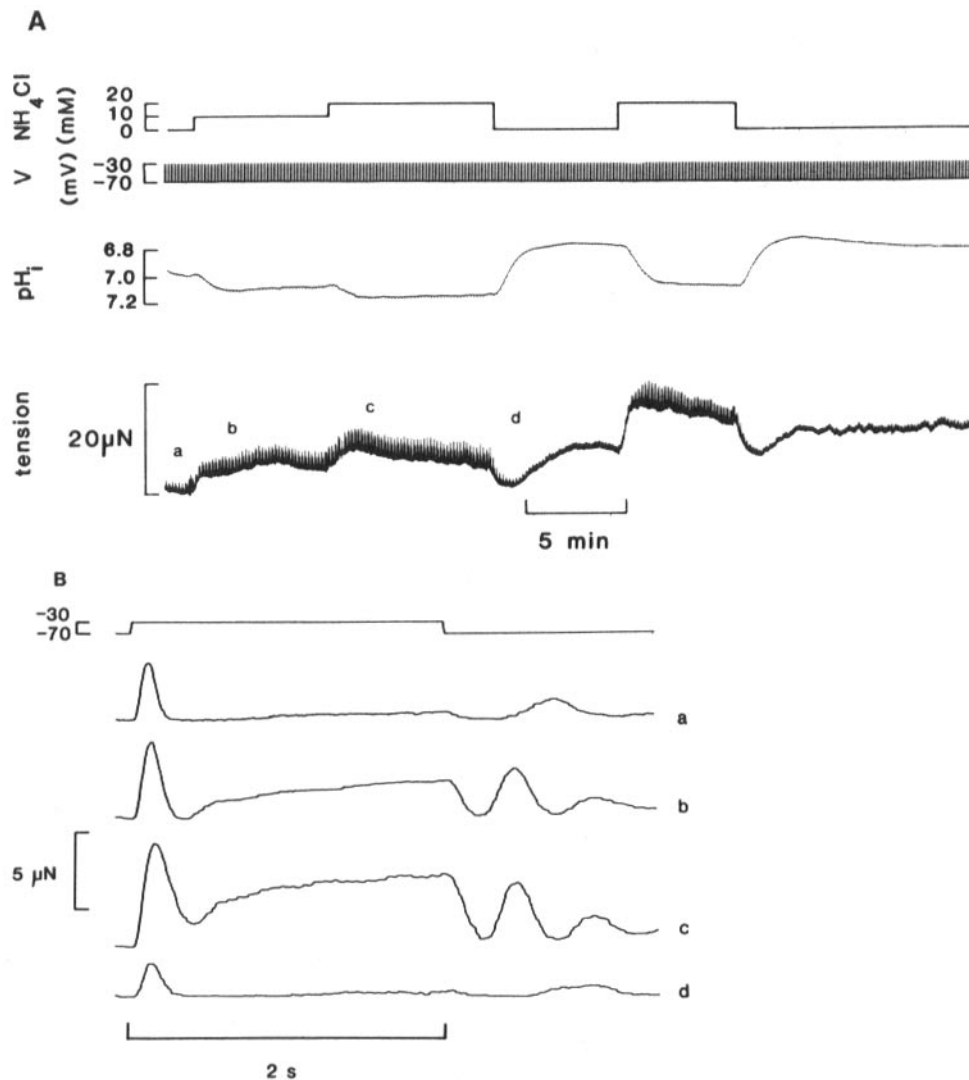


FIGURE 2. The effects of NH₄Cl in the presence of strophanthidin. (A) Time course. The traces show (top to bottom) membrane potential (*V*), pH_i, and tension. [NH₄Cl] was varied as shown above. The membrane potential was held at -70 mV and 2-s pulses were applied to -30 mV at 0.1 Hz. Strophanthidin (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 NH_4Cl are shown in Fig. 3, which plots twitch tension as a function of pH_i on linear coordinates and semilogarithmic coordinates (inset). It is apparent that changes in pH_i of ± 0.3 units produced graded changes in tension. Furthermore, in this range, the plot of logarithmic force as a function of pH_i is roughly linear, with a slope of ~ 2 . However, at values of pH_i lower than 6.8, force sometimes increased again. Such an increase of the twitch at low pH_i is illustrated in Fig. 4. In this experiment, the fiber had been exposed to 20 mM NH_4Cl for 23 min. After the removal of NH_4Cl , pH_i declined rapidly by 0.5 units to 6.7 and then recovered more slowly. The large and rapid fall in pH_i was accompanied initially by a decrease in twitch tension.

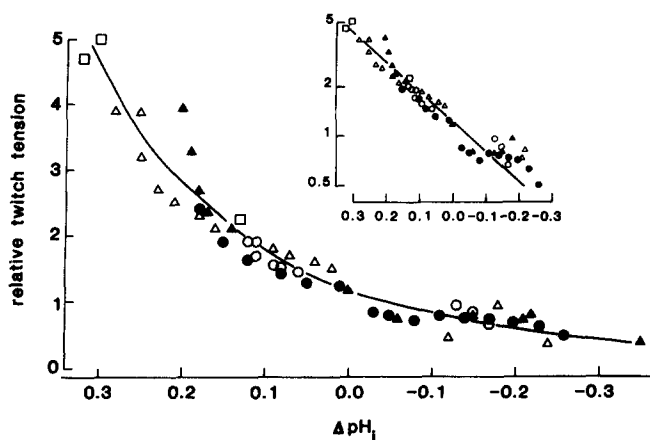


FIGURE 3. The relationship between tension and pH_i . (A) Linear scale. The ordinate shows twitch tension normalized to the level in control solution (mean $\text{pH}_i = 7.24 \pm 0.04$ SEM, $n = 7$). The abscissa is the change of pH_i from the control level during the application of NH_4Cl (positive values) or after removal of NH_4Cl (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_i were obtained during the recovery of pH_i from its most alkaline level. The points at acidic pH_i were obtained during the recovery of pH_i from its most acid level after the removal of NH_4Cl . The different symbols correspond to different preparations, except for the open squares, which were produced by a brief exposure to NH_4Cl . Each point represents a different preparation.

However, twitch tension then increased transiently, such that, at a pH_i of 6.68, it was comparable to that observed at the start of the record, when pH_i was 7.25. Such transient increases in force after the removal of NH_4Cl were observed in two out of seven fibers, but only after prolonged periods of exposure to NH_4Cl , when the removal of NH_4Cl resulted in a large acidosis. This paradoxical increase in force at low pH_i will be considered in the Discussion.

Effects on Tension of Changes in pH_i

In these experiments, pH_i 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 pH_i and twitch tension. Opposite effects on

pH_i and tension were observed after a rise in pH_o, as shown in Fig. 5B. As in the experiments with NH₄Cl, the time courses of the change in twitch tension paralleled the changes in pH_i. The quantitative relationship between tension and pH_i observed in Fig. 5 is plotted in Fig. 6. Thus, regardless of whether pH_i is altered by varying pH_o or by exposure to NH₄Cl, changes in twitch tension correlate well with changes in pH_i. It is therefore appropriate to ask whether tension can be expressed as a unique function of pH_i. To do this, we have compared the effects of changing pH_o with those of adding NH₄Cl. Fig. 7A shows that an increase of pH_o from 7.4 to 8.4 produced about the same change of pH_i as did the application of 5 mM NH₄Cl. It is apparent that the relationship between tension and pH_i was similar when pH_i was recovering from these two

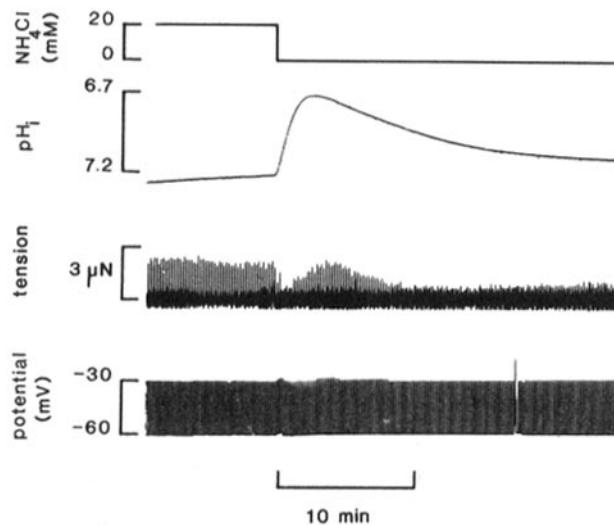


FIGURE 4. Dissociation between changes of pH_i and tension. The traces show (top to bottom) pH_i, tension (band-pass filtered at 0.1–10 Hz), membrane potential. The membrane potential was held at –60 mV and depolarized to –30 mV at 0.1 Hz. NH₄Cl (20 mM) had been present for 23 min before the record began and was removed as indicated above the record.

types of alkalosis (Fig. 7B, filled circles and open triangles). However, this relationship differs quantitatively from that seen between tension and pH_i during the onset of the effects of pH 8.4 (Fig. 7B, open circles). Such hysteresis between force and pH_i was observed consistently after reversible alterations in pH_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 pH_i, although there is usually a good qualitative correlation between pH_i and tension, the quantitative relationship depends on the direction in which pH_i was changed. Unfortunately, it was not possible to

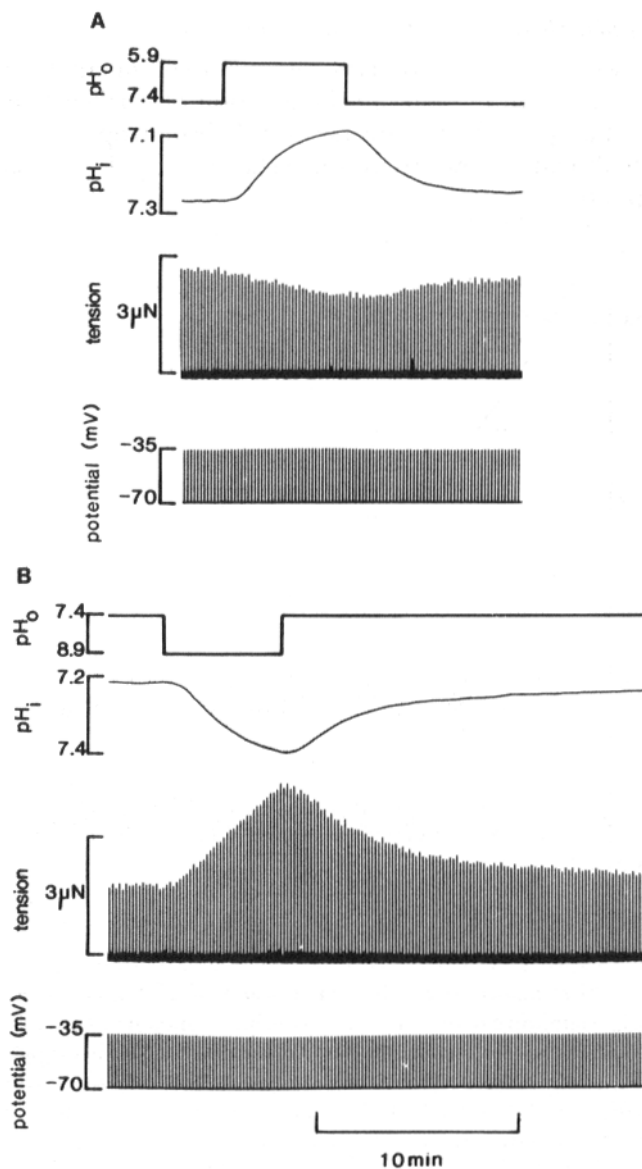


FIGURE 5. The effects on pH_i and tension of changing pH_o . In both panels, the traces show (top to bottom) pH_i , tension (band-pass filtered at 0.1 – 10 Hz), and membrane potential. (A) The effects of external acidification. pH_o was changed as indicated above the figure. The membrane potential was held at -70 mV and 2 -s depolarizing pulses were applied to -35 mV at 0.1 Hz. (B) The effects of external alkalization. Both A and B are taken from the same experiment; B was recorded 15 min after A.

discover whether a similar hysteresis between pH_i and tension occurred with NH_4Cl . This is because the rather slow response time of the pH electrode precluded accurate measurements during fast changes in pH_i (see Methods).

Changes of pH_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 $[Ca^{2+}]_o$. Both maneuvers exert their inotropic effects via changes in $[Ca^{2+}]_i$, but both maneuvers also produce changes in pH_i that may modify the contractile response.

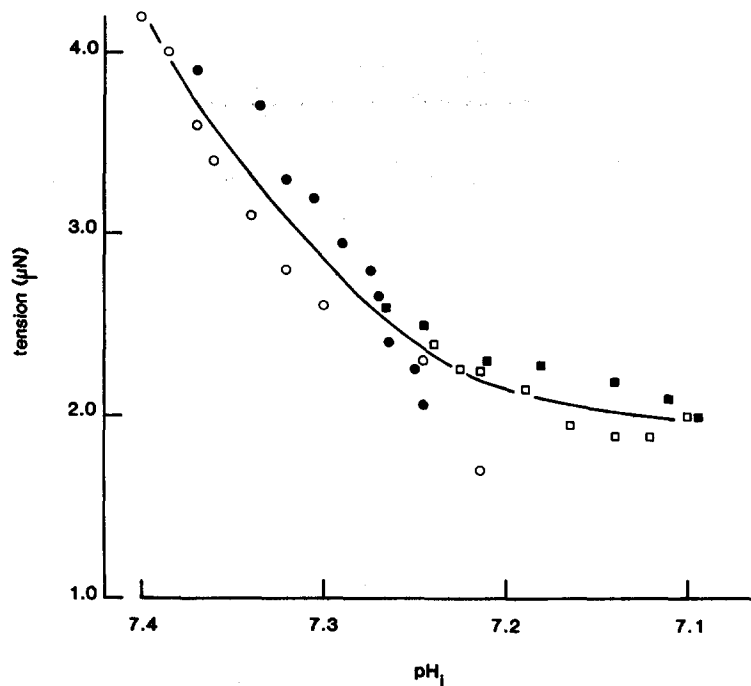


FIGURE 6. The quantitative effect on tension of a change in pH_i produced by altering pH_o . Data were taken from the experiment shown in Fig. 5. The relationship between tension and pH_i has been plotted after external acidosis (pH_o 7.4–5.9, filled squares), recovery from external acidosis (pH_o 5.9–7.4, open squares), external alkalosis (pH_o 7.4–8.9, open circles), and recovery from external alkalosis (pH_o 8.9–7.4, filled circles). The line was drawn by eye.

In the experiment of Fig. 8A, strophanthidin ($10 \mu M$) was added to the superfusing solution. It has been shown previously that this increases intracellular Na activity (a_{Na}^i), intracellular Ca activity (a_{Ca}^i), 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_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_i of this magnitude will have significant effects on contraction. This point was tested directly by adding 10 mM NH_4Cl to the superfusate after strophanthidin had been applied for 15 min. The application

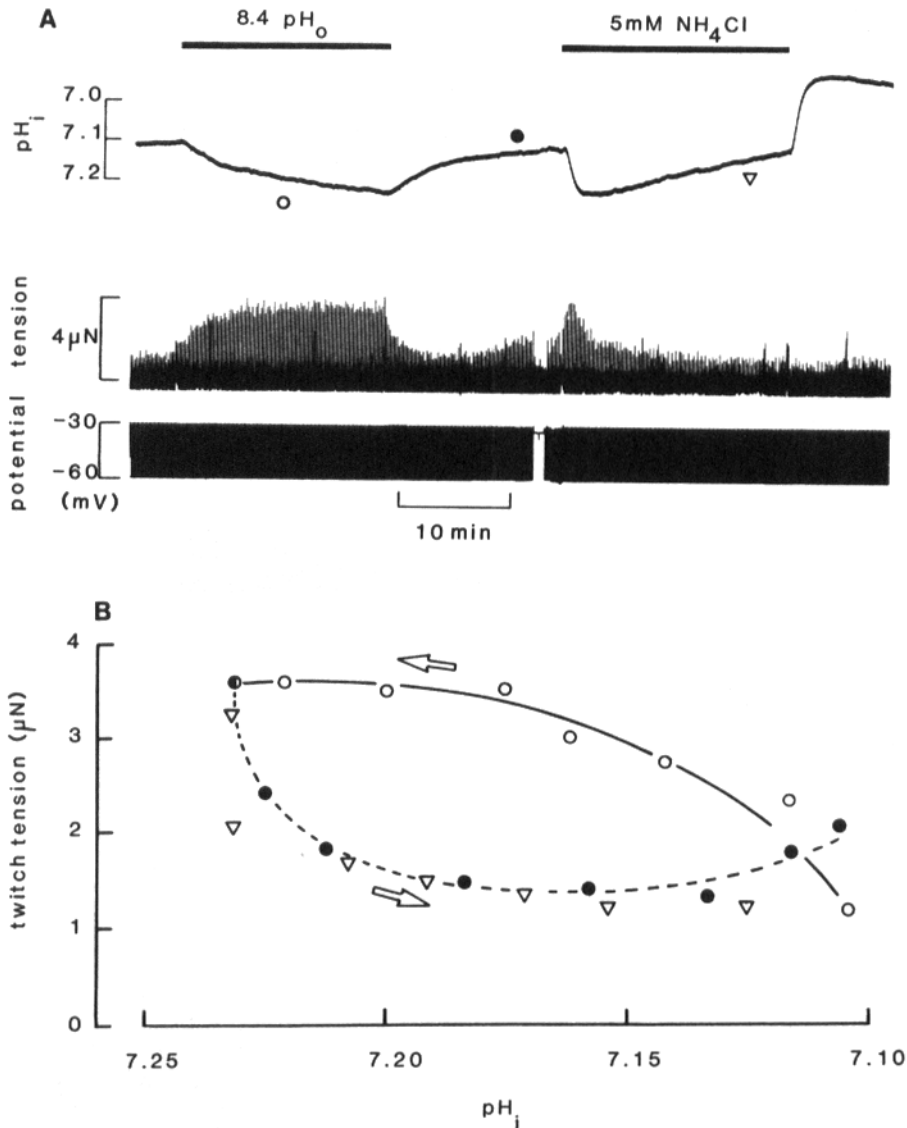


FIGURE 7. A comparison of the effects of NH_4Cl with those of changing pH_o . (*A*) Original record. The traces show (top to bottom) pH_i , tension (band-pass filtered at 0.1–10 Hz), and membrane potential. The membrane potential was held at -60 mV and 2-s depolarizing pulses were applied to -30 mV at 0.1 Hz. The control solution had a pH_o of 7.4 and, as indicated above the record, either pH_o was changed to 8.4 or 5 mM NH_4Cl was added (at constant pH_o). (*B*) Relationship between pH_i and twitch tension. The graph shows twitch tension as a function of pH_i during either the development of the effects of external alkalization (open circles), the recovery from the effects of extracellular alkalization (filled circles), and during the recovery of pH during the exposure to NH_4Cl (triangles). The symbols correspond to those shown in *A*.

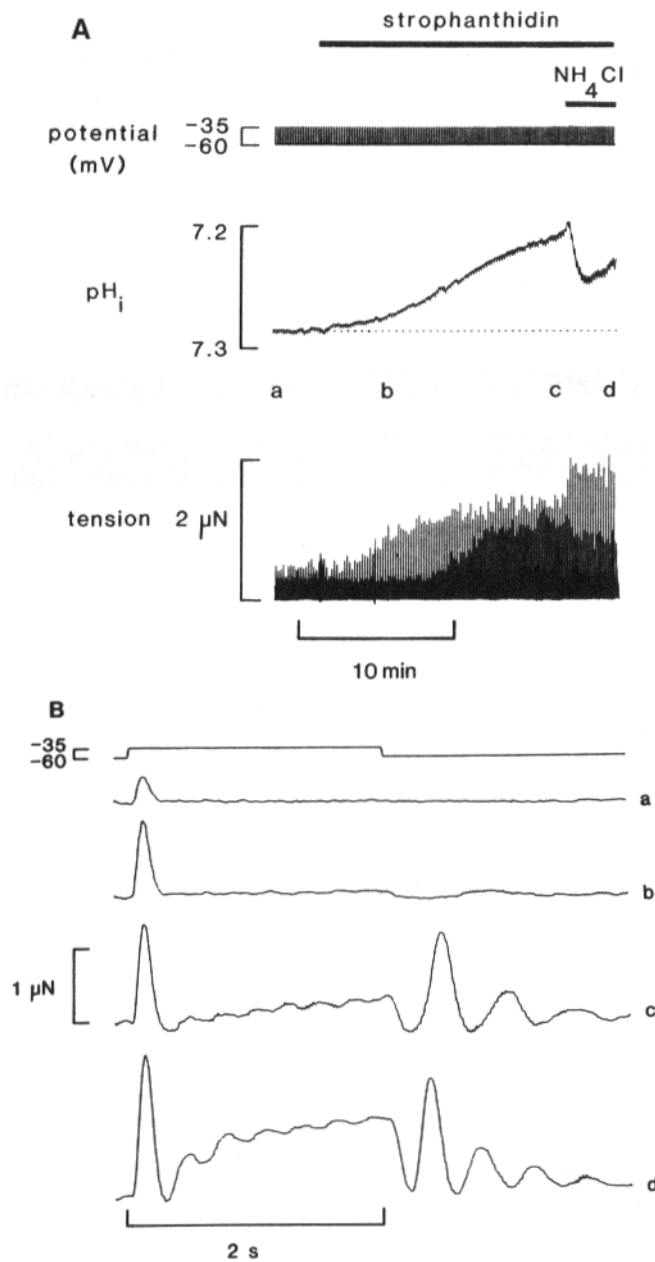


FIGURE 8. The effects of changes of pH_i on the inotropic response to Na-K pump inhibition. (A) Time course. The traces show (top to bottom) membrane potential, pH_i, and tension (band-pass filtered at 0.1–10 Hz): The membrane potential was held at -60 mV and 2-s pulses were applied at 0.1 Hz. Strophanthidin (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_4Cl 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. Alkalinization 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 $[\text{Ca}^{2+}]_o$ have been examined previously (Vaughan-Jones et al., 1983) but it is necessary to make some additional observations here. Under control conditions, raising the $[\text{Ca}^{2+}]_o$ 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 $[\text{Ca}^{2+}]_o$ 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 $[\text{Ca}^{2+}]_o$ 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 $[\text{Ca}^{2+}]_o$ from 0.5 to 5.0 mM produced a rise followed by a secondary decline (within 3 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 $[\text{Ca}^{2+}]_i$ (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 $[\text{Ca}^{2+}]_o$ 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 pH_o does not affect tension until there is a change in pH_i (Fig. 5). Furthermore, changes in tension induced with NH_4Cl , when the pH of the bulk solution is constant, also correlate well with the observed changes in pH_i . These results therefore indicate that acidification and alkalization affect tension via changes in pH_i rather than pH_o . 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., NH_4Cl , 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 pH_o by 1.0 unit has no immediate effect on force.

Quantitative Effect of pH_i on Tension

Measurements of $[\text{Ca}^{2+}]_i$ using aequorin have shown that the decrease in twitch tension during an intracellular acidosis is not associated with a decrease in the $[\text{Ca}^{2+}]_i$ transient (Allen and Orchard, 1983). Indeed, systolic $[\text{Ca}^{2+}]_i$ may even increase during acidification. Furthermore, diastolic $[\text{Ca}^{2+}]_i$ 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 $[\text{Ca}^{2+}]_i$ and must therefore be due to a decrease in the Ca^{2+} 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 $[\text{Ca}^{2+}]_i$. The data shown in Fig. 3 indicate that, when bulk pH_o is constant, a fall in resting pH_i of 0.17 units is sufficient to reduce twitch tension by 50%. Such a large effect of pH_i on force can be observed in the skinned fiber provided that the contractile proteins are <50% saturated with Ca^{2+} .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 pH_i in the intact Purkinje fiber is consistent with the data obtained from skinned fibers.

¹ In skinned cardiac fibers, the active tension-pCa curve is sigmoidal. A fall in pH_i , 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 $[\text{Ca}^{2+}]_i$. Because the tension-pCa curve is sigmoidal, the fractional depressant effect on tension of a given fall in pH_i will be smaller at low pCa values (when the myofibrils are saturated with Ca^{2+}) and larger at high pCa values (when the proteins are far from saturated with Ca^{2+}). By replottting the data presented in Fig. 1B of Fabiato and Fabiato (1978) (a tension-pCa curve determined at different pH values for skinned rat ventricular fibers), one can determine that, in skinned fibers, the mean slope of the relationship between log tension and pH_i (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 $[\text{Ca}^{2+}]_i$ 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 $[\text{Ca}^{2+}]_i$. 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 $[\text{Ca}^{2+}]_i$ 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 $[\text{Ca}^{2+}]_i$. Consequently, if the kinetics of the pH_i - $[\text{Ca}^{2+}]_i$ 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_o , 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 $[\text{Ca}^{2+}]_i$ 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 $[\text{Ca}^{2+}]_i$ 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}^i that appeared to be mediated by Na-H exchange (Deitmer and Ellis, 1980; Pivnicka-Worms et al., 1985). The transient increase in a_{Na}^i would increase $[\text{Ca}^{2+}]_i$ 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 $[\text{Ca}^{2+}]_i$, 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 $[\text{Ca}^{2+}]_i$, but, under normal conditions, the rise of $[\text{Ca}^{2+}]_i$ is too small to increase tension. Therefore, tonic tension would be seen if either $[\text{Ca}^{2+}]_i$ 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 Ca²⁺ 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}^i that acts on Na-Ca exchange to increase $[\text{Ca}^{2+}]_i$. This rise of $[\text{Ca}^{2+}]_i$ 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}^i (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 $(a_{\text{Na}}^i)^n$, 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_{Na}^i will be underestimated, and, in the absence of any pH_i change, tension will depend more steeply on a_{Na}^i , 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_i 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_i in reducing tension under these conditions.

We thank Dr. K. Kaila and Dr. C. Bountra for their comments upon earlier versions of the manuscript.

This work was supported by grants from the British Heart Foundation and Medical Research Council, U.K. (R.D.V.-J.), the National Institutes of Health (HL-25675), the American Heart Association, Maryland Affiliate (W.J.L.), the British Heart Foundation, and the Medical Research Council (D.A.E.).

Original version received 1 May 1986 and accepted version received 31 October 1986.

REFERENCES

- Allen, D. G., D. A. Eisner, P. G. Morris, J. S. Pirolo, and G. L. Smith. 1986. Metabolic consequences of increasing intracellular calcium and force production in perfused ferret hearts. *Journal of Physiology*. 376:121-141.
- Allen, D. G., D. A. Eisner, and C. H. Orchard. 1984. Characterization of oscillations of intracellular calcium concentration in ferret ventricular muscle. *Journal of Physiology*. 352:113-128.
- Allen, D. G., D. A. Eisner, J. S. Pirolo, and G. L. Smith. 1985a. The relationship between

- intracellular calcium and contraction in calcium-overloaded ferret papillary muscles. *Journal of Physiology*. 364:169-182.
- Allen, D. G., P. G. Morris, C. H. Orchard, and J. S. Pirolo. 1985*b*. A nuclear magnetic resonance study of metabolism in the ferret heart during hypoxia and inhibition of glycolysis. *Journal of Physiology*. 361:185-204.
- Allen, D. G., and C. H. Orchard. 1983. The effects of changes of pH on intracellular calcium transients in mammalian cardiac muscle. *Journal of Physiology*. 335:555-567.
- Bers, D. M., and D. Ellis. 1982. Intracellular calcium and sodium activity in sheep heart Purkinje fibres. Effects of changes of external sodium and intracellular pH. *Pflügers Archiv*. 393:171-178.
- Boron, W. F., and P. De Weer. 1976. Intracellular pH transients in squid giant axons caused by CO₂, NH₃ and metabolic inhibitors. *Journal of General Physiology*. 67:91-112.
- Cannell, M. B., R. D. Vaughan-Jones, and W. J. Lederer. 1985. Ryanodine block of calcium oscillations in heart muscle and the sodium-tension relationship. *Federation Proceedings*. 44:2964-2969.
- Chapman, R. A., and J. Tunstall. 1981. The tension-depolarization relationship of frog atrial trabeculae as determined by potassium contractures. *Journal of Physiology*. 310:97-115.
- De Hemptinne, A., R. Marrannes, and B. Vanheel. 1982. Double-barrelled intracellular pH electrode: construction and illustration of some results. In *Intracellular pH: Its Measurement, Regulation and Utilization in Cellular Functions*. R. Nuccitelli and D. W. Deamer, editors. Alan R. Liss Inc., New York. 7-19.
- Deitmer, J. W., and D. Ellis. 1980. Interactions between the regulation of the intracellular pH and sodium activity of sheep cardiac Purkinje fibres. *Journal of Physiology*. 304:471-488.
- Eisner, D. A., and W. J. Lederer. 1979. Inotropic and arrhythmogenic effects of potassium depleted solutions on mammalian cardiac muscle. *Journal of Physiology*. 294:255-277.
- Eisner, D. A., W. J. Lederer, and R. D. Vaughan-Jones. 1981. The dependence of sodium pumping and tension on intracellular sodium activity in voltage-clamped sheep cardiac Purkinje fibres. *Journal of Physiology*. 317:163-187.
- Eisner, D. A., W. J. Lederer, and R. D. Vaughan-Jones. 1983*a*. The relationship between intracellular pH and contraction in sheep cardiac Purkinje fibers. *Journal of Physiology*. 334:106P-107P. (Abstr.)
- Eisner, D. A., W. J. Lederer, and R. D. Vaughan-Jones. 1983*b*. The control of tonic tension by membrane potential and intracellular Na activity in the sheep cardiac Purkinje fibre. *Journal of Physiology*. 335:723-743.
- Eisner, D. A., W. J. Lederer, and R. D. Vaughan-Jones. 1984. The quantitative relationship between twitch tension and intracellular sodium activity in sheep cardiac Purkinje fibres. *Journal of Physiology*. 355:251-266.
- Eisner, D. A., and M. Valdeolillos. 1986. Measurement of intracellular calcium during the development and relaxation of tonic tension in sheep Purkinje fibres. *Journal of Physiology*. 375:269-281.
- Ellis, D., and K. T. MacLeod. 1985. Sodium-dependent control of intracellular pH in Purkinje fibers of sheep heart. *Journal of Physiology*. 359:81-105.
- Fabiato, A., and F. Fabiato. 1978. Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles. *Journal of Physiology*. 276:233-255.
- Fry, C. H., and P. A. Poole-Wilson. 1981. Effects of acid-base changes on excitation-contraction coupling in guinea-pig and rabbit ventricular muscle. *Journal of Physiology*. 313:141-160.
- Gaskell, W. H. 1880. On the tonicity of the heart and blood vessels. *Journal of Physiology*. 3:48-75.

- Harrison, S. M., C. Lamont, and D. J. Miller. 1985. Hysteresis in the Ca vs. tension relationship of chemically "skinned" rat cardiac muscle. *Journal of Physiology*. 364:76P. (Abstr.)
- Jacobus, W. E., I. H. Pores, S. K. Lucas, C. H. Kallman, M. L. Weisfeldt, and J. T. Flaherty. 1982. The role of intracellular pH in the control of normal and ischaemic myocardial contractility: a ^{31}P nuclear magnetic resonance and mass spectrometry study. In *Intracellular pH: Its Measurement, Regulation and Utilization in Cellular Function*. R. Nuccitelli and D. W. Deamer, editors. Alan R. Liss, Inc., New York. 537-565.
- Kaila, K., and R. D. Vaughan-Jones. 1985. The effect of amiloride on intracellular sodium, intracellular pH and tension in voltage-clamped sheep cardiac Purkinje fibres. *Journal of Physiology*. 369:78P. (Abstr.)
- Katz, A. M., and H. H. Hecht. 1969. The early "pump" failure of the ischaemic heart. *American Journal of Medicine*. 47:497-502.
- Kentish, J. C. 1986. The effects of inorganic phosphate and creatine phosphate on force production in skinned muscles from rat ventricle. *Journal of Physiology*. 370:585-604.
- Kort, A. A., and E. G. Lakatta. 1984. Calcium-dependent mechanical oscillations occur spontaneously in unstimulated mammalian cardiac tissues. *Circulation Research*. 54:396-404.
- Marranes, R., A. De Hemptinne, and I. Leusen. 1981. pH aspects of changes in conduction velocity in isolated heart fibres after partial replacement of chloride with organic anions. *Pflügers Archiv*. 389:199-209.
- Philipson, K. D., M. M. Bersohn, and A. Y. Nishimoto. 1982. Effects of pH on $\text{Na}^+\text{-Ca}^{2+}$ exchange in canine sarcolemmal vesicles. *Circulation Research*. 50:287-293.
- Piwica-Worms, D., R. Jacob, C. R. Horres, and M. Lieberman. 1985. Na/H exchange in cultured chick heart cells: pH; regulation. *Journal of General Physiology*. 85:43-64.
- Poole-Wilson, P. A. 1978. Measurement of myocardial intracellular pH in pathological states. *Journal of Molecular and Cellular Cardiology*. 10:511-526.
- Poole-Wilson, P. A., and S. R. Seabrooke. 1985. Relationship between intracellular pH and contractility in guinea-pig papillary muscles. *Journal of Physiology*. 365:63P. (Abstr.)
- Ridgway, E. B., A. M. Gordon, and D. A. Martyn. 1983. Hysteresis in the force-calcium relation in muscle. *Science*. 219:1075-1077.
- Roos, A. and W. F. Boron. 1981. Intracellular pH. *Physiological Reviews*. 61:296-434.
- Thomas, R. C. 1978. Ion-sensitive Micro-electrodes. Academic Press, Inc., New York and London.
- Valdeolmillos, M., and D. A. Eisner. 1985. The effect of ryanodine on calcium-overloaded sheep cardiac Purkinje fibres. *Circulation Research*. 56:452-456.
- Vassalle, M., and C. I. Lin. 1979. Effect of calcium on strophanthidin-induced electrical and mechanical toxicity in cardiac Purkinje fibers. *American Journal of Physiology*. H689-H697.
- Vaughan-Jones, R. D. 1979. Regulation of chloride in quiescent sheep-heart Purkinje fibres studied using intracellular chloride and pH-sensitive microelectrodes. *Journal of Physiology*. 295:111-137.
- Vaughan-Jones, R. D., D. A. Eisner, and W. J. Lederer. 1985. The effects of intracellular Na on contraction and intracellular pH in mammalian cardiac muscle. *Advances in Myocardiology*. 5:313-330.
- Vaughan-Jones, R. D., W. J. Lederer, and D. A. Eisner. 1983. Ca^{2+} ions can affect intracellular pH in mammalian cardiac muscle. *Nature*. 301:522-524.
- Vaughan Williams, E. M., and J. M. Whyte. 1967. Chemosensitivity of cardiac muscle. *Journal of Physiology*. 189:119-137.
- Waddell, W. J., and R. G. Bates. 1969. Intracellular pH. *Physiological Reviews*. 49:285-329.