Maximal Ca$^{2+}$-activated Force and Myofilament Ca$^{2+}$ Sensitivity in Intact Mammalian Hearts

Differential Effects of Inorganic Phosphate and Hydrogen Ions

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ABSTRACT Myofilament Ca$^{2+}$ sensitivity and maximal Ca$^{2+}$-activated force are fundamental properties of the contractile proteins in the heart. Although these properties can be evaluated directly in skinned preparations, they have remained elusive in intact tissue. A novel approach is described that allows maximal Ca$^{2+}$-activated force to be measured and myofilament Ca$^{2+}$ sensitivity to be deduced from isovolumic pressure in intact perfused ferret hearts. Phosphorus nuclear magnetic resonance spectra are obtained sequentially to measure the intracellular inorganic phosphate (Pi) and hydrogen ion (H$^+$) concentrations. After a period of perfusion with oxygenated, HEPES-buffered Tyrode solution, hypoxia is induced as a means of elevating [Pi]. The decline in twitch pressure can then be related to the measured increase in [Pi]. After recovery, hearts are perfused with ryanodine to enable tetanization and the measurement of maximal Ca$^{2+}$-activated pressure. Hypoxia is induced once again, and maximal pressure is correlated with [Pi]. We then compare the relations between [Pi] and maximal pressure on the one hand, and [Pi] and twitch pressure on the other. If the two relations differ only by a constant scaling factor, then the decline in twitch pressure can be attributed solely to a decline in maximal pressure, with no change in myofilament sensitivity. We obtained such a result during hypoxia, which indicated that Pi accumulation decreases maximal force but does not change myofilament sensitivity. We compared these results with acidosis (induced by bubbling with 5% CO$_2$). In contrast with Pi, the accumulation of H$^+$ decreases twitch force primarily by shifting myofilament Ca$^{2+}$ sensitivity. This approach in intact tissue has strengths and limitations complementary to those of skinned muscle experiments.

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INTRODUCTION

Inorganic phosphate (Pi) and hydrogen ions (H+) have long been recognized as two major endogenous modulators of contractile force in mammalian heart muscle (Katz and Hecht, 1969; Herzig and Ruegg, 1977). Nevertheless, there remains considerable uncertainty as to how these metabolites decrease twitch force. Measurements of the free intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) have not shown a decrease in activator Ca\(^{2+}\) during hypoxia (which rapidly raises [Pi]) or during acidosis (Allen and Orchard, 1983a, b). Conversely, results from skinned heart muscle preparations indicate that both Pi (Solaro et al., 1980; Kentish, 1986; Godt and Nosek, 1986) and H+ (Fabiato and Fabiato, 1978; Donaldson and Hermansen, 1978) decrease the efficacy of Ca\(^{2+}\) in activating contraction, by decreasing either the maximal force-generating ability in response to Ca\(^{2+}\), or the sensitivity to Ca\(^{2+}\) (i.e., the normalized relation between [Ca\(^{2+}\)]\(_i\) and force). Although the two possibilities have different mechanistic implications, the relative importance of changes in maximal Ca\(^{2+}\)-activated force and in myofilament Ca\(^{2+}\) sensitivity remains controversial. In particular, there is no consensus regarding Pi: initial studies found only a decrease in maximal Ca\(^{2+}\)-activated force (Solaro et al., 1980), but recently a change in myofilament Ca\(^{2+}\) sensitivity has also been reported (Kentish, 1986; Godt and Nosek, 1986).

We describe here a new experimental approach that enables us to measure maximal Ca\(^{2+}\)-activated force and to deduce myofilament Ca\(^{2+}\) sensitivity in intact perfused mammalian hearts, while allowing the simultaneous determination of [H\(^+\)] and [Pi]. Maximal Ca\(^{2+}\)-activated force is inferred from the saturation of tetanic pressure with respect to [Ca\(_o\)] in hearts treated with ryanodine (Marban et al., 1986), following upon the previous characterization of this approach in aequorin-injected ferret ventricular muscle (Yue et al., 1986). For the measurements of [H\(^+\)] and [Pi], we use phosphorus nuclear magnetic resonance (NMR), a nondestructive method for studying metabolism in intact functioning organs that takes advantage of the magnetic dipole of \(^{31}\)P to obtain spectroscopic signals (Ingwall, 1982; Gadian, 1983). The results imply that H\(^+\) and Pi act in quite different ways on the contractile machinery. A preliminary report has appeared (Marban and Kusuoka, 1987).

METHODS

Many of the methods have already been described in detail (Marban et al., 1986; Kusuoka et al., 1986). Briefly, hearts were removed from 10–14-wk-old ferrets that had been anesthetized with sodium pentobarbital (80 mg/kg i.p.). The aorta was cannulated rapidly, and each heart was perfused at a pressure of 65 mmHg with 100% O\(_2\)-bubbled solution of the following composition (millimolar): 108 NaCl, 5 KCl, 1 MgCl\(_2\), 5 HEPES (pH 7.4), 20 Na acetate, 10 glucose, 2 CaCl\(_2\), at 30\(^\circ\)C. The left ventricle was filled with a balloon containing 15 mM Mg trimetaphosphate as a standard for the NMR experiments, and the heart was paced at 1.8–2 Hz. Isovolumic left ventricular pressure was recorded continuously on a chart recorder. In several experiments performed to illustrate the protocol (Fig. 1), a magnetic tape recorder was also used, but this precluded simultaneous NMR measurements because of the technical difficulty of tape recording in a strong magnetic field.
Phosphorus NMR Measurements

After 10–20 min of equilibration, the heart was lowered into the bore of a 4.2-T magnet and NMR spectra were collected every 2 min. Spectra were obtained on a spectrometer (WH-180, Bruker Instruments, Inc., Billerica, MA; \(^{31}\)P resonance frequency = 72.89 MHz) operated in the pulsed Fourier transform mode, and the data were analyzed using a computer (1280, Nicolet Instrument Corp., Madison, WI). Intramyocardial concentrations of \(\text{P}^2\) and high-energy phosphate compounds were calculated from the areas under their respective peaks in the phosphorus NMR spectra (Kusuoka et al., 1986). The \(\text{H}^+\) concentration was determined from the shift between the peaks for \(\text{P}^2\) and phosphocreatine, as described previously (Flaherty et al., 1982). Although the peak for \(\text{P}^2\), is not large in well-oxygenated myocardium (e.g., Fig. 2A), its chemical shift (4.7–5.2 ppm) is quite distinct from that of the neighboring phosphodiester (~5.5 ppm) and sugar phosphate (>6 ppm) peaks, so that \(\text{[H}^+]\) can generally be estimated reliably (Burt et al., 1979). The heart was weighed at the conclusion of each experiment.

Experimental Strategy for Measurement of Maximal Pressure and Deduction of Myofilament \(\text{Ca}^{2+}\) Sensitivity

To increase \([\text{P}^2]\), hypoxia was induced for sufficiently brief periods to avoid significant intracellular acidification. This was accomplished by bubbling the perfusate with room air or with 100% \(\text{N}^2\). The experimental strategy consisted of two stages. First, isovolumic pressure was measured during twitch contractions in 2 mM \([\text{Ca}]_o\), and the hearts were transiently subjected to hypoxia. After a recovery period, ryanodine (1 \(\mu\)M) was added to the perfusate to enable tetanization and the measurement of maximal \(\text{Ca}^{2+}\)-activated pressure in 15 mM \([\text{Ca}]_o\), as previously described (Marban et al., 1986). To determine the effects of \(\text{P}^2\), on maximal \(\text{Ca}^{2+}\)-activated pressure, hypoxia was then repeated. Each heart thus yielded the data required to determine the relations between \([\text{P}^2]\) and developed pressure during both submaximal and maximal activation.

Myofilament sensitivity was inferred by comparing the changes in twitch contraction as a function of \([\text{P}^2]\) with the corresponding changes in maximal \(\text{Ca}^{2+}\)-activated pressure as a function of \([\text{P}^2]\). If a decrease in maximal \(\text{Ca}^{2+}\)-activated force were the sole cause of the decrease in twitch force, then the relation between \([\text{P}^2]\) and twitch pressure should simply be a scaled version of that between \([\text{P}^2]\) and maximal \(\text{Ca}^{2+}\)-activated pressure. Any difference in the relations that could not be reconciled by a constant scaling factor would signal a change in myofilament \(\text{Ca}^{2+}\) sensitivity. It is assumed that the \([\text{Ca}^{2+}]\), transients underlying twitches do not change significantly during hypoxia (as supported by aequorin luminescence measurements in ferret papillary muscles; Allen and Orchard, 1983a), and that the \([\text{Ca}^{2+}]\)-tension relation during twitches reflects that at steady state in a simple way (as found by Fabiato, 1981; but cf. Yue et al., 1986). These assumptions are analyzed critically in the Discussion.

The experimental strategy for determining the effects of \(\text{H}^+\) was identical, except that periods of acidosis (included by bubbling the perfusate with 5% \(\text{CO}_2/95\%\ \text{O}_2\) were substituted for hypoxia. In this protocol, the acquisition time for each \(^{31}\)P-NMR spectrum was increased to 4 min to improve the accuracy of the measurements of the chemical shift between \(\text{P}^2\), and phosphocreatine. We have consistently expressed the results as \([\text{H}^+]\) rather than \(\text{pH}\) to facilitate the comparison with \([\text{P}^2]\] using linear regression analysis.

RESULTS

\(\text{P}^2\) Elevation during Hypoxia

Fig. 1 illustrates the two-stage experimental protocol used to investigate the
effects of P. Panel A shows twitch contractions in 2 mM $[\text{Ca}]_o$ before, during, and after hypoxia induced by bubbling with solutions of decreased oxygen concentration. The slight transient increase in force during early hypoxia has been described previously and is attributed to a short-lived intracellular alkalin-

![Graph showing twitch pressure and maximal Ca$^{2+}$-activated pressure during hypoxia.](image)

**Figure 1.** Depression of twitch pressure (A) and maximal Ca$^{2+}$-activated pressure (B) during hypoxia in a perfused ferret heart. Isovolumic pressure is shown before, during, and after hypoxia (A). The perfusate was then re-equilibrated with 100% O$_2$, and ryanodine (1 μM) was added to enable tetanization. [Ca$_o$] was increased to 15 mM, maximal Ca$^{2+}$-activated pressure was sampled by inducing tetanus (i.e., pacing at 10–12 Hz) every 60 s, and hypoxia was repeated (B).

... which we have confirmed (see below). Thereafter, the effects of P$_o$ appear to predominate, with a progressive decline in twitch force that is clearly evident in the records displayed on an expanded time base (lower row). Twitch pressure recovered completely after several minutes of reoxygen-
Maximal Force and Myofilament Sensitivity in the Heart

Panel B shows the second stage of the experiment. Tetanic pressure was measured in 15 mM [Ca], before, during, and after hypoxia. The tall spikes of pressure in the upper row correspond to tetani, shown underneath on an expanded time base. These tetani report the maximal Ca\textsuperscript{2+}-activated pressure, which decreases progressively during hypoxia (Kusuoka et al., 1986) and recovers quickly afterward.

NMR spectra were collected sequentially every 2 min throughout the experiments to measure [P\textsubscript{i}] and [H\textsuperscript{+}]. Fig. 2 shows typical \textsuperscript{31}P-NMR spectra obtained before (A) and during (B) hypoxia in a ferret heart undergoing twitch contractions. The spectra reveal a clearcut increase in P\textsubscript{i} (the leftmost peak in each spectrum) during hypoxia, but no significant change in [H\textsuperscript{+}] (indicated above each spectrum).

The time courses of the changes in twitch pressure, [P\textsubscript{i}], and [H\textsuperscript{+}] during a representative experiment are shown in Fig. 3A. The top row shows developed twitch pressure (as percent of the control pressure of 110 mmHg), while [P\textsubscript{i}] and [H\textsuperscript{+}] are plotted in the middle and bottom rows. Hypoxia was induced during...
the period between 4 and 16 min. The first observable change was a slight alkalosis associated with a small increase in twitch pressure, as reported previously (Allen et al., 1985). Thereafter, [H+] changed little during the hypoxic period. In contrast, [Pi] increased markedly from 0.30 to 7.11 μmol/g wet wt. Developed pressure decreased as [Pi] increased, consistent with the hypothesis that Pi accumulation causes the early contractile failure of hypoxia (Herzig and Ruegg, 1977; Kubler and Katz, 1977; Allen et al., 1985; Kusuoka et al., 1986). [Pi] and pressure promptly recover to baseline levels upon reoxygenation. The large reversible changes in [Pi], with only minor variations in [H+], are representative of the results with brief hypoxia in a total of six hearts.

Tetani were then induced in 15 mM [Ca]o to observe the changes in maximal Ca2+-activated pressure during hypoxia (Fig. 3B). Tetanic pressure, plotted as percent of the maximal tetanic pressure (318 mmHg), decreased during the

**FIGURE 3.** Time course of changes in twitch and tetanic developed pressure (DP), [Pi] (Δ), and [H+] (○) during hypoxia. Each value is plotted at the time corresponding to acquisition of the NMR spectra. (A) Normalized developed twitch pressure (●), plotted as percent of the control twitch pressure in oxygenated solution before hypoxia (110 mmHg). Hypoxia was induced during the 4–16 min period. (B) Normalized developed pressure during tetani in 15 mM [Ca]o (●), as percent of the maximal tetanic pressure measured in oxygenated solution (318 mmHg). Hypoxia was induced during the 42–58 min period.
hypoxic period (42–58 min), concomitant with an increase in \( [P_i] \). \([H^+]\) remained fairly constant throughout hypoxia. The depression of force at any given \( [P_i] \) appeared to be quite similar for the twitch (A) and the tetanus (B); a more direct means of comparing the data from the two stages of these experiments is presented in the following section.

![Diagram](https://via.placeholder.com/150)

**Figure 4.** Relations between \( [P_i] \) and developed pressure during twitches (C) and tetani (A), determined from an experiment like that illustrated in Fig. 1. \( [P_i] \) was correlated with the developed pressure measured during the collection of each NMR spectrum to yield the points in each panel. (A) Absolute values for twitch and tetanic pressure in control and during hypoxia. (B) Data in A normalized by the twitch or tetanic pressure in the control and plotted as percent control developed pressure. The regression lines are the best fits for twitches (solid line) or tetani (dashed line) determined by a least-squares algorithm; for slopes see Table I, A (experiment 6).

**Relations between \([P_i]\) and Isovolumic Pressure during Twitches and Tetani**

The values for \( [P_i] \) can be correlated with the simultaneous isovolumic pressure data to yield \( [P_i]\)-pressure relations, as shown for one heart in Fig. 4A. The open squares plot twitch force against \( [P_i] \) as they vary during hypoxia, while the filled
triangles represent the maximal Ca\textsuperscript{2+}-activated pressure as a function of [P\textsubscript{i}]. Qualitatively, it is apparent that twitch pressure and maximal Ca\textsuperscript{2+}-activated pressure both decline as [P\textsubscript{i}] increases.

An explicit comparison between the two relations is facilitated by normalizing the pressure data and expressing them as percent of control (Fig. 4B). If a decrease in the maximal Ca\textsuperscript{2+}-activated force were the sole cause of the decrease in twitch force due to P\textsubscript{i}, then there should be no significant difference between the normalized relations for twitches and for maximal Ca\textsuperscript{2+}-activated pressure. Indeed, such is the case. Each set of data is well fitted by linear least-squares regression, and the slopes of the two regression lines are not significantly different (P > 0.05). This result, which was observed consistently in all six hearts devoted to this protocol (Table I, A), agrees with the lack of a decrease in myofilament Ca\textsuperscript{2+} sensitivity with increased [P\textsubscript{i}] noted by Solaro et al. (1980), but not with the marked decrease in Ca\textsuperscript{2+} sensitivity reported by other investigators (Kentish, 1986; Godt and Nosek, 1986). The origin of the discrepancy among these studies, all of which were in skinned preparations, is not clear.

We ascertained that the effects on force during hypoxia are indeed closely correlated with the measured changes in P\textsubscript{i}, as would be required if the decrease in force were attributable to the increase in [P\textsubscript{i}]. In all six hearts, there was a highly significant (P < 0.001) correlation between [P\textsubscript{i}] and developed pressure during hypoxia. Each experiment was also checked to ascertain whether changes in [H\textsuperscript{+}] during hypoxia might be sufficiently great to complicate the interpretation of hypoxia solely in terms of [P\textsubscript{i}]. In four of six experiments, no significant correlation (P > 0.05) was found between [H\textsuperscript{+}] and developed pressure. The remaining two experiments exhibited a statistically significant correlation between pressure and [H\textsuperscript{+}], but the coefficients of correlation were weaker than

### Table I

**Coefficients of Slope in Regression Lines**

<table>
<thead>
<tr>
<th>(A) Hypoxia</th>
<th>Twitches</th>
<th>Tetani</th>
<th>%DP/\muM/g wet wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>d(d(%OP)/d([P\textsubscript{i}]))</td>
<td>%DP/\muM/g wet wt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-13.19</td>
<td>-21.40*</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>-14.00</td>
<td>-14.72*</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>-5.82</td>
<td>-7.56*</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>-9.76</td>
<td>-9.54*</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>-10.41</td>
<td>-11.67*</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>-10.78</td>
<td>-13.08*</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(B) Acidosis</th>
<th>Twitches</th>
<th>Tetani</th>
<th>%DP/\muM</th>
</tr>
</thead>
<tbody>
<tr>
<td>d(d(%DP)/d([H\textsuperscript{+}]))</td>
<td>%DP/\muM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-758.4</td>
<td>-329.5*</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>-589.5</td>
<td>-133.5*</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>-467.0</td>
<td>-125.2*</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>-1,103.5</td>
<td>-541.5*</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>-591.0</td>
<td>-244.4*</td>
<td></td>
</tr>
</tbody>
</table>

Significance levels for the differences in slopes between twitches and tetani: * not significant; *P < 0.001; *P < 0.05. DP, developed pressure.
those with \([P_i]\); the results of these experiments (numbers 1 and 5, Table I, A), in terms of the present analysis, were indistinguishable from those in the other hearts. These findings are consistent with the idea that \([P_i]\) is the primary cause of the negative inotropic effect of hypoxia (Kusuoka et al., 1986).

**Intracellular Acidification: Change in Myofilament Ca\(^{2+}\) Sensitivity?**

A conceptually identical approach was used to determine the effects of acidosis on the contractile machinery. We induced acidosis by bubbling the perfusate with 5% CO\(_2\)/95% O\(_2\) rather than 100% O\(_2\), and we observed the gradual changes in \([H^+]\) during equilibration with the new gas mixture. Fig. 5 shows representative NMR spectra collected before (A) and during (B) perfusion with CO\(_2\)-bubbled solution. These demonstrate an increase in \([H^+]\), with little change otherwise.

Previous investigation of the \([H^+]\) dependence of maximal Ca\(^{2+}\)-activated pressure in intact hearts (Kusuoka et al., 1986) revealed little effect except at markedly elevated \([H^+]\) (>0.16 \(\mu M\)), consistent with results from a variety of skinned muscle preparations (Fabiato and Fabiato, 1978; Donaldson and Hermansen, 1978). Nevertheless, acidosis exerts a profound negative inotropic effect
on twitch contractions (Jacobus et al., 1982), which suggests that a decrease in
myofilament sensitivity may feature prominently at submaximal levels of con-
tractile activation. Fig. 6A shows the time course of twitch pressure, \([H^+]\), and
\([P_i]\) in one heart before, during, and after acidosis. Isovolumic pressure declined
by 50% during perfusion with CO\(_2\)-bubbled solution, while \([H^+]\) rose from 83 to
162 nM. Twitch pressure and intramyocardial metabolites recovered completely
upon removal of acidosis. Panel B shows the second stage of the experiment, in

![Diagram](FIGURE 6. Time course of changes in twitch and tetanic pressure, \([H^+]\) (○), and \([P_i]\) (△) during acidosis. Each value is plotted at the time corresponding to acquisition of the NMR spectra. (A) Normalized developed twitch pressure (●) as percent of control (114 mmHg). Acidosis was induced in the 12–40-min period. (B) Normalized tetanic pressure (■) as percent of maximal tetanic pressure (262 mmHg). Acidosis was induced in the 68–96-min period.

which tetanic pressure was measured and acidosis was re-induced. Maximal Ca\(^{2+}\)-
activated pressure decreased by only 30% during acidosis, although \([H^+]\) reached
166 nM, comparable to the level attained during twitches. The discrepancy
between the extent of depression of twitch contractions on the one hand and
maximal Ca\(^{2+}\)-activated pressure on the other hints at a decrease in myofilament
Ca\(^{2+}\) sensitivity accompanying acidosis.

We verified this by examining the relations between \([H^+]\) and developed
pressure during acidosis, as illustrated in Fig. 7 for the same heart as in Fig. 6. Panel A shows the data before normalization, and B shows the normalized [H\(^+\)]-pressure relations. In striking contrast to the findings with hypoxia and Pi\(_o\), the slopes of the best-fit regression lines for twitches (solid line) and tetani (dashed line) are quite different, that for twitches being significantly steeper (\(P < 0.001\)). This finding, which was verified in four other hearts (Table I, B), is just what would be expected with a decrease in myofilament Ca\(^{2+}\) sensitivity superimposed on a lesser decrease in maximal Ca\(^{2+}\)-activated force. These results cannot be
explained by known changes in $[\text{Ca}^{2+}]$: the slight increase in the $[\text{Ca}^{2+}]$ transient that accompanies acidosis (Allen and Orchard, 1983b) would, if anything, tend to minimize the effects of a concomitant decrease in myofilament $\text{Ca}^{2+}$ sensitivity.

We ascertained that the changes in function and metabolism in these hearts were reversible, given the sequential nature of the experimental protocol. Figs. 3 and 6 show that the effects of both hypoxia and acidosis can be quite reversible. This positive feature was borne out in all hearts studied: the mean twitch pressure was not significantly different by paired t tests before and after hypoxia (108 vs. 104 mmHg, $n = 6, P > 0.50$) or acidosis (106 vs. 127 mmHg, $n = 5, P > 0.10$). Similarly, the changes in intramyocardial metabolites were consistently reversible.

**DISCUSSION**

It is important to consider the assumptions that underlie our analysis and to question their validity. Interpretation of the results on maximal $\text{Ca}^{2+}$-activated pressure requires only the assumption that saturation of the contractile machinery with respect to $[\text{Ca}^{2+}]$ is reached during tetani in high $[\text{Ca}]$. This has been examined critically in previous articles (Yue et al., 1986; Marban et al., 1986) and appears to be justified. Increasing $[\text{Ca}]$ causes no further increase in tetanic force during steady state hypoxia, which suggests that saturation is still achieved (Kusuoka et al., 1986). Nevertheless, if saturation were not reached during advanced hypoxia or acidosis, this would tend to underestimate the magnitude of any superimposed decrease in myofilament $\text{Ca}^{2+}$ sensitivity. Such an effect would not invalidate our conclusion that $\text{Ca}^{2+}$ sensitivity is decreased by $\text{H}^{+}$, but it would tend to obscure any real effect of $\text{Pi}$ on $\text{Ca}^{2+}$ sensitivity.

In order to interpret normalized twitch force as an indication of myofilament sensitivity, two other assumptions are required: (a) $[\text{Ca}^{2+}]$ transients do not change during the maneuver in question, and (b) the $[\text{Ca}^{2+}]$-tension relation during twitches is scaled by maximal force in the same manner as is the steady state $[\text{Ca}^{2+}]$-tension relation. The first assumption appears reasonable given previous measurements of $[\text{Ca}^{2+}]$ in ventricular muscle of the same species, under virtually identical ionic conditions. $[\text{Ca}^{2+}]$ transients did not change during hypoxia (Allen and Orchard, 1983a), and increased slightly during acidosis (Allen and Orchard, 1983b). The second assumption requires that a change in maximal force linearly scale the relationship between peak $[\text{Ca}^{2+}]$, and peak force during twitch contractions. This condition would certainly be satisfied if the steady state $[\text{Ca}^{2+}]$-tension relationship were identical to that between peak force and peak $[\text{Ca}^{2+}]$, as has been demonstrated during very slow twitches (Fabiato, 1981). During faster physiologic contractions, however, there may be a dissociation between the steady state and twitch $[\text{Ca}^{2+}]$-force relationships (Yue et al., 1986). In such a case, there is less certainty that the twitch $[\text{Ca}^{2+}]$-force relationship would be scaled precisely by maximal force.

**Comparison with Skinned Muscle Experiments**

The present approach allows observations from direct studies of the contractile proteins in skinned preparations to be tested rigorously in intact hearts. Indeed,
the strengths and limitations of the two approaches are complementary. Skinning
allows direct access to the myofilaments, so that ionic composition and other
factors can be manipulated over a broad range. This necessarily introduces
perturbation of the physiologic intracellular environment. We are now able to
measure maximal Ca^{2+}-activated pressure in the intact heart, but we are still
challenged by the need to devise interventions that influence the intracellular
environment indirectly but specifically. This requires that the factor of interest
in the intracellular environment (and other possible confounding factors) be
monitored throughout the experiment. Fortunately, this is possible for [P_i] and
[H^+] using 31P-NMR.

The intact heart is ideally suited for testing the physiological importance of
predictions from skinned muscle, particularly when the results from various
skinned muscle preparations are discrepant. Our acidosis experiments confirm
the predictions from skinned muscle, where there has been general agreement
that myofilament sensitivity changes are more prominent than the decrease in
maximal force induced by H^+. There is no such consensus for P_i; Solaro and co-
workers (1980) reported no shift in sensitivity, whereas Kentish (1986) found
large rightward shifts in the normalized Ca^{2+}-tension relation of skinned rat
ventricular muscles as [P_i] increased (see also Godt and Nosek, 1986). A similar
decline in Ca^{2+} sensitivity had been reported previously by Brandt and co-
workers (1982) in skeletal muscle. Our own results favor the idea that myofil-
ament Ca^{2+} sensitivity is not importantly altered by P_i in the intact heart. The
origin of the discrepancy among the studies in skinned muscle is not addressed
by our work, although Kentish (1986) has been careful to point out that his
results are quite sensitive to the diameter of the individual skinned muscle
preparation: P_i has a much greater depressant effect on both maximal force and
Ca^{2+} sensitivity in thinner preparations (his Fig. 2), which hints at an important
technical factor that may underlie some of the variability in the published data.

Mechanistic Implications
While maximal Ca^{2+}-activated pressure represents an unequivocal endpoint for
comparison with data from skinned muscle (Marban et al., 1986), the physiolog-
ically important twitch contraction requires that myofilament Ca^{2+} sensitivity
also be taken into consideration. It is clear from this work that myofilament
sensitivity changes figure prominently in the inotropic effects of acidosis, al-
though not in those of P_i. Using this conceptual framework, we can rationalize
why acidosis exerts a profound negative inotropic effect on twitch con-
tractions without decreasing [Ca^{2+}], and without a proportionate decrease in
maximal Ca^{2+}-activated force. Our physiological findings also point to important
differences between the molecular mechanisms of action of P_i and H^+ on the contractile
machinery. Depression of maximal Ca^{2+}-activated force suggests a decrease in
the maximal number of force-generating cross-bridges. Such a result is not
unexpected with P_i accumulation, given recent evidence from skeletal muscle
that P_i release is coupled to the formation of the dominant force-generating state
in a reaction that is readily reversible (Hibberd et al., 1985). On the other hand,
a shift in myofilament Ca^{2+} sensitivity suggests a decrease in the Ca^{2+}-binding
affinity of the myofibrils, which may be mediated by interaction of H+ with the thin filaments of adult ventricular muscle (Solaro et al., 1986).

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REFERENCES


Fabiato, A. 1981. Myoplasmic free calcium concentration reached during the twitch of an intact isolated cardiac cell and during calcium-induced release of calcium from the sarcoplasmic reticulum of a skinned cardiac cell from the adult rat or rabbit ventricle. *Journal of General Physiology*. 78:457–497.


