Effects of Hypoxia and Hypercapnia on the Force–Velocity Relation of Rabbit Myocardium

Keith R. Walley, Lincoln E. Ford, and L.D.H. Wood

The separate effects of hypoxia and hypercapnia on the force–velocity relation of rabbit myocardium were compared in 10 papillary or trabecular muscles superfused using control (95% O₂–5% CO₂), hypoxic (18% O₂), and hypercapnic (20% CO₂) physiological salt solutions. This level of hypoxia did not irreversibly damage the muscles and reduced peak isometric force by 53±11%. The level of hypercapnia was chosen to match the force depression (50±12%) produced by hypoxia. Multiple force–velocity points were measured by applying critically damped isotonic force steps at 90% of the time to peak isometric force and at the time to 50% peak isometric force. These points defined the force–velocity relation and maximum velocity of shortening, the extrapolated isometric force, and the maximum power of nonpotentiated and postextrasystolic potentiated contractions. Hypoxia and hypercapnia reduced maximum force and maximum power nearly equally. Maximum velocity of shortening decreased more during hypoxia (21±12%) than during hypercapnia (12±9%) (p<0.01). Postextrasystolic potentiation completely reversed the reduction of maximum velocity of shortening during hypercapnia but not during hypoxia. A 6% internal load could account for the reduction in maximum velocity of shortening during hypercapnia and all but 9% of the reduction in maximum velocity of shortening during hypoxia. The relative time course of the force–velocity relation was not altered by either hypoxia or hypercapnia. We conclude that hypercapnia reduces the effect of activation because increased activation (by postextrasystolic potentiation) restored the force–velocity relation and maximum velocity of shortening to control values. In contrast, hypoxia results in a small but significant additional reduction of maximum velocity of shortening. This suggests an additional effect of hypoxia on the contractile proteins, indicating decreased maximum rate of actomyosin ATPase cycling. Maximum power was decreased only by the force effects and not by the velocity effects of hypoxia and hypercapnia. (Circulation Research 1991;69:1616–1625)

Both hypoxia¹,² and hypercapnia³,⁴ depress myocardial contractility in acute respiratory failure and may lead to cardiogenic shock. To better understand this, previous studies have examined the effect of hypoxia⁵,⁶ and hypercapnia⁷,⁸ on force–velocity relations of cardiac muscle. Substantial developments in two areas since the publication of these studies imply that previous results are incomplete and possibly erroneous, prompting us to reexamine these important issues.

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The first development is technical. Earlier studies were performed using inertial levers that were incapable of making critically damped quick releases to isotonic loads. To avoid such rapid steps, most of the earlier studies measured force–velocity points during afterloaded contractions, and the velocity at each force was measured at a different time in the twitch. Because the series elastic elements become stretched during the rise of force, each force–velocity point was also measured at a different contractile element length. It is now clear that the myocardial force–velocity relation changes both with time in the twitch and with contractile element length.⁹,¹⁰ More recent studies have shown that the experiments using quick releases applied by a rapid servo system give results very different from those obtained with afterloaded contractions.¹¹ Therefore, we have reexamined the separate effects of hypoxia and hypercapnia on cardiac muscle using a servo system to apply critically
damped quick releases to different loads at the same time in the twitch.

The second development has been in our understanding of the effects of activation on the contractile properties of heart muscle. A change in activation causes a change in the number of force-generating cross-bridges in the muscle, with no change in individual cross-bridge kinetics. In skeletal muscle, changes in activation cause no change in maximum velocity of shortening,\textsuperscript{12,13} and in cardiac muscle, there is only a small change that can be explained quantitatively by a small internal load.\textsuperscript{9} Several different types of positive inotropic agents have been shown to have effects on the force–velocity relation that are indistinguishable from each other and from an increase in activation.\textsuperscript{14} However, the effects of negative inotropic interventions are not known. Therefore, we have studied hypoxia and hypercapnia to determine whether their effects can be accounted for entirely by changes in activation.

We have previously advocated the use of instantaneous maximum power (determined as the maximum in the product of force times velocity) as an index of changes in the force–velocity relation.\textsuperscript{9,11,14} In normally functioning muscle, this parameter varies in direct proportion to changes in extrapolated isometric force\textsuperscript{9} and varies with changes in activation.\textsuperscript{11} Because it is determined by interpolation among the data points rather than by extrapolation, it has much lower standard errors.\textsuperscript{13} In addition, it should be doubly sensitive to changes that alter cross-bridge kinetics, since it is sensitive both to changes in force and velocity. Finally, it should be especially sensitive to changes in substrate or reaction product concentrations when these concentrations are rate limiting, since it is measured under conditions where substrate utilization is near its maximum. With these considerations in mind, we have paid particular attention to changes in maximum power in our analysis of the force–velocity properties.

Our findings for hypoxia and hypercapnia are consistent with recent studies of positive inotropic interventions that demonstrate little or no change in the maximum velocity of shortening during changes in activation and, therefore, support the view that changes in activation do not alter cross-bridge kinetics. However, our findings are considerably different from previous studies of hypoxia and hypercapnia that report changes in the maximum velocity of shortening.\textsuperscript{5–8} These differences suggest that the current understanding of the mechanism of force, velocity, and power reduction during hypoxia and hypercapnia must be modified.

Materials and Methods

Muscle Preparation

Ten young adult rabbits weighing 2.0–2.5 kg were anesthetized with 100 mg ketamine and 40 mg xylazine, and their hearts were removed. Right ventricular papillary muscles (n=7) or trabeculae (n=3) having cross-sectional areas <0.55 mm\textsuperscript{2} (0.38±0.17 mm\textsuperscript{2}) were dissected free and mounted in platinum foil clips that were then hooked to the apparatus.\textsuperscript{15} The muscle was electrically stimulated every 1.2–1.5 seconds. Throughout the initial dissection procedure, the hearts and isolated muscles were kept at 37°C.\textsuperscript{14} Once attached to the apparatus, the muscles were gradually cooled to 23–25°C. After a 1-hour period of stabilization, the length–tension relation was measured to determine the length at which maximum active isometric force was developed (L\textsubscript{max}). The muscle length was then set at 90% L\textsubscript{max} for all subsequent measurements to minimize the effects of rest force. At the end of the experiment, the muscles were removed from the apparatus, cut free of the clips, blotted dry, and weighed. Cross-sectional areas were determined as the weight divided by length and the specific gravity of the muscle (taken as 1.05).

Solutions

Experiments were done in a physiological salt solution containing (mM) NaCl 118, KCl 5, NaH\textsubscript{2}PO\textsubscript{4} 1.2, NaHCO\textsubscript{3} 22.5, MgSO\textsubscript{4} 2, CaCl\textsubscript{2} 1, and HEPES 5, adjusted to pH 7.3–7.4 with NaOH after being equilibrated with 95% O\textsubscript{2}–5% CO\textsubscript{2}. This solution was then kept in three 500-ml bottles that were continuously bubbled with either 95% O\textsubscript{2}–5% CO\textsubscript{2} (control), 18% O\textsubscript{2}–77% N\textsubscript{2}–5% CO\textsubscript{2} (hypoxia), or 80% O\textsubscript{2}–20% CO\textsubscript{2} (hypercapnia). The gas partial pressures in the solutions as they entered the muscle chamber, which were measured using a blood gas machine (Radiometer, Copenhagen), were as follows: for control, PO\textsubscript{2}=681 mm Hg, PCO\textsubscript{2}=46 mm Hg, pH 7.30; for hypoxia, PO\textsubscript{2}=137 mm Hg, PCO\textsubscript{2}=44 mm Hg, pH 7.32; for hypercapnia, PO\textsubscript{2}=556 mm Hg, PCO\textsubscript{2}=164 mm Hg, pH 6.81. Curiously, we found that the solution was only fully equilibrated with the gas when the top of the reagent bottle was closed using a stopper pierced by a narrow (4-mm i.d.) 25-cm-long glass tube. If the top of the bottle was left unstoppered, with a 20-mm opening at its top, the concentrations of O\textsubscript{2} and CO\textsubscript{2} in the solution reaching the muscle were substantially less than those in the gas mixture.

Apparatus

The muscle was held horizontally in a covered chamber measuring 2×2×15 mm. A short rod projected from a servo motor through a seal to the closed, upstream end of the chamber. A horizontal stainless steel hook projected from the vertical arm of a force transducer under the chamber cover glass at the downstream end. Solution from one of the three bubbled bottles flowed continuously at the rate of ~1.5 cm/sec (3–5 ml/min) through the chamber.

The servo system for producing isotonic steps has been described in detail previously.\textsuperscript{14,15} Briefly, one end of the muscle was attached to a photoelectric force transducer having a resonant frequency of ~5 kHz.\textsuperscript{16} The other end of the muscle was attached to a linear (loudspeaker-type) servo motor that could be controlled either from its own internal position sen-
sor (length control) or from the force transducer signal (force control). Switching between the two types of control was accomplished by a diode switching network. An IBM PC computer equipped with a Tecmar Labmaster interface board was used to digitize records of force and length and to control the timing of experimental events. The SALT programming language was used to control the interface board. An external timing device (BRS/LVS, Beltsville, Md.) was used to stimulate the muscle.

Isotonic steps were applied to every eighth contraction, with the seven intervening isometric contractions used for recovery. An extrasystole was applied immediately before the contraction preceding every other isotonic contraction (every 16th stimulus) to produce a postextrasystolic potentiated contraction.

**Force–Velocity Measurements**

For each experimental condition, digital recordings of isometric contractions were made to determine the time of peak isometric force. Isotonic steps were applied at 90% of the time to peak isometric force. This time was chosen because of the suggestion that activation reaches its peak shortly before the time of peak isometric force and because force has very nearly reached its maximum at this time. In addition, isotonic steps were applied at the time when developed twitch force had reached 50% of its full value in control contractions. Critically damped isotonic force steps to a predetermined force were accomplished in 2–4 msec by the servo-controlled linear motor (Figure 1). The rise of twitch force was sampled at 5-msec intervals until 10 msec before the isotonic force step. Force and length recordings were then sampled at 0.2-msec intervals for 30 msec while the muscle shortened isotonically, after which the longer (5-msec) interval was used to sample the remainder of the contraction. Velocity was determined by fitting a least-squares linear regression to the length recording over the 6–10-msec interval after the release (between the dashed lines in Figure 1). Isotonic force was averaged over the same 4-msec interval.

The servo system and data sampling were automated to collect 14–16 force–velocity points spanning the entire force range (Figure 2); one point was collected for each isotonic force step. The alternation of postextrasystolic potentiated contractions with nonpotentiated contractions ensured that the force–velocity curves from the two types of contractions were obtained under matched conditions. Peak isometric force was measured as the average of the forces measured immediately before each of the isotonic steps used to generate the curves.

The force–velocity points were fitted to the Hill hyperbola \([\text{force} + a] \cdot [\text{velocity} + b] = c\), where \(a\), \(b\), and \(c\) are the fitted parameters) to determine force–velocity relations for potentiated and nonpotentiated states using a Newton-Gauss nonlinear least-squares fitting routine that minimized the variance in velocity weighted for power (Figure 2).

![Figure 1](http://circres.ahajournals.org/)

**FIGURE 1.** Top panel: Development of force plotted against time for a single contraction. Between the vertical dotted lines the time scale is expanded 25 times to illustrate the rapid isotonic force step (complete in 3 msec) that was imposed at 90% of the time to peak isometric force. Bottom panel: Velocity of shortening measured as the slope of the length vs. time relation. \(L_{\text{max}}\), length at which maximum active isometric force was developed. Force and velocity were averaged from 6 to 10 msec after the start of the isotonic force step (indicated by the short vertical dashed lines) to obtain one data point on the force–velocity relation shown in Figure 2.

Maximum velocity of shortening was calculated as \(c/a - b\), maximum extrapolated isometric force was calculated as \(c/b - a\), and maximum power was calculated as \(a \cdot b + c = 2\sqrt{abc}\). This same procedure and analysis was repeated with the isotonic steps occurring at 50% of peak isometric force so that the time course of activation could be assessed.

**Protocol**

Data were collected in the following order: in control, hypoxia, control, hypercapnia, and control conditions in five muscles and in reverse order in the other five muscles. Collection of all 16 data points for both nonpotentiated and potentiated contractions took ~5 minutes. Muscles were allowed to stabilize for a minimum of 15 minutes in each new superfusing solution before measurements were made. Recovery of force and maximum velocity of shortening after hypoxia was slow (~10–15 minutes). Therefore, 90 minutes of recovery after hypoxia was allowed before the next control data set was collected.

**Statistical Analysis**

We tested for differences in measured variables using a repeated-measures analysis of variance. When \(p < 0.05\), a Bonferroni-corrected \(t\) test for multiple comparisons identified specific differences between interventions (hypoxia and hypercapnia) and
the control set immediately before the intervention. Data are reported as mean±SD.

Results

Preliminary experiments using different concentrations of oxygen were performed to determine the degree of hypoxia that the muscle could tolerate without damage. When muscles were stimulated to contract in oxygen concentrations <18%, the rest tension rose, and the developed force did not recover fully when 95% oxygen was restored. Because these findings suggested irreversible damage, oxygen concentrations of 18% were used in the final series of experiments described here. This level of hypoxia decreased peak isometric force by 53% of the control level. The level of hypercapnia (20% CO₂) was chosen to match the degree of force depression obtained during hypoxia. Under these conditions, rest tension did not change during the experiment, and the developed force returned to the expected control levels after exposure to hypoxia and hypercapnia.

Table 1 summarizes characteristics of the 10 muscles used in the final series of experiments. At 90% of L_max, rest force was 4.6±0.4% of peak isometric force of nonpotentiated control contractions. The effects of the hypoxia and hypercapnia on peak isometric force were approximately the same, causing a 53±11% and 50±12% reduction, respectively (Table 2). The onset of force reduction during hypercapnia was rapid, with peak isometric force reaching a minimum in 2–3 minutes, followed by partial recovery at 15 minutes (Figure 3). The force reduction caused by hypoxia was much slower, requiring 10–15 minutes to achieve the full effect, and there was no partial force recovery or further reduction during continued exposure to hypoxia. The recovery from hypercapnia was similarly rapid, having a transient overshoot in peak isometric force. Recovery from hypoxia was slower, and there was no overshoot (Figure 3). In contrast to the nearly equal effects on nonpotentiated peak isometric force, hypoxia produced more reduction of potentiated peak isometric force than hypercapnia (Figure 3 and Table 2), but

### Table 1. Characteristics of the 10 Muscles Studied

<table>
<thead>
<tr>
<th>Muscle No.</th>
<th>L_max (mm)</th>
<th>Weight (mg)</th>
<th>Area (mm²)</th>
<th>Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.1</td>
<td>1.36</td>
<td>0.44</td>
<td>0.75</td>
</tr>
<tr>
<td>2</td>
<td>4.2</td>
<td>2.32</td>
<td>0.55</td>
<td>0.84</td>
</tr>
<tr>
<td>3</td>
<td>2.8</td>
<td>1.30</td>
<td>0.46</td>
<td>0.77</td>
</tr>
<tr>
<td>4</td>
<td>4.4</td>
<td>0.96</td>
<td>0.22</td>
<td>0.53</td>
</tr>
<tr>
<td>5</td>
<td>4.2</td>
<td>2.06</td>
<td>0.49</td>
<td>0.79</td>
</tr>
<tr>
<td>6</td>
<td>3.7</td>
<td>1.50</td>
<td>0.41</td>
<td>0.72</td>
</tr>
<tr>
<td>7</td>
<td>2.6</td>
<td>1.13</td>
<td>0.43</td>
<td>0.74</td>
</tr>
<tr>
<td>8</td>
<td>4.8</td>
<td>0.40</td>
<td>0.08</td>
<td>0.33</td>
</tr>
<tr>
<td>9</td>
<td>4.0</td>
<td>2.20</td>
<td>0.55</td>
<td>0.84</td>
</tr>
<tr>
<td>10</td>
<td>3.3</td>
<td>0.45</td>
<td>0.14</td>
<td>0.42</td>
</tr>
</tbody>
</table>

Mean±SD 3.7±0.7 1.37±0.68 0.38±0.17 0.67±0.18

L_max length at which maximum active isometric force was developed.

### Table 2. Effects of Hypoxia and Hypercapnia on Isometric Contractions in Rabbit Myocardium

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Hypoxia</th>
<th>Hypercapnia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest force (mN/mm²)</td>
<td>1.5±1.3</td>
<td>1.4±0.9</td>
<td>1.4±1.1</td>
</tr>
<tr>
<td>Nonpotentiated peak isometric force (mN/mm²)</td>
<td>32.4±15.6</td>
<td>15.3±11.1*</td>
<td>16.2±12.9*</td>
</tr>
<tr>
<td>Nonpotentiated time to peak isometric force (msec)</td>
<td>244±42</td>
<td>204±50*</td>
<td>202±44*</td>
</tr>
<tr>
<td>Potentiated peak isometric force (mN/mm²)</td>
<td>45.2±17.4</td>
<td>26.5±18.3*</td>
<td>30.6±13.7*</td>
</tr>
<tr>
<td>Potentiated time to peak isometric force (msec)</td>
<td>236±31</td>
<td>209±45†</td>
<td>215±42†</td>
</tr>
</tbody>
</table>

Values are mean±SD; n=10 muscles.

* p<0.01 and † p<0.05 vs. control.
the relative reduction of potentiated peak isometric force (hypoxia, 41%; hypercapnia, 32%) was less than the reduction of nonpotentiated peak isometric force (hypoxia, 53%; hypercapnia, 50%) for both interventions.

Hypoxia and hypercapnia shortened time to peak isometric force by 11% and 9%, respectively (Table 2), possibly related to decreased force production. As shown in Figure 4, hypercapnia abbreviated the duration of contraction, whereas hypoxia caused a decrease in force without much change in duration because relaxation was prolonged.

**Figure 4.** Graph showing the time course of force development for control, hypoxic, and hypercapnic contractions for one representative muscle. Both hypoxia and hypercapnia depress peak isometric force. Hypoxia also slows relaxation.

**Figure 3.** Recordings of twitch force vs. time illustrated for a single muscle. The depression in force generation here is somewhat greater than average (Table 2). Postextrasystolic potentiated contractions can be seen to occur every 16th contraction. The middle recording during hypoxia is after 3 minutes, and the right recordings for hypercapnia and hypoxia are steady-state recordings after 10 minutes. See text for discussion.

**Force–Velocity Relations**

The major effect of both hypoxia and hypercapnia on the force–velocity relation was to decrease peak isometric force, extrapolated isometric force, and maximum power by approximately half (Table 3 and Figure 5). The effects on maximum velocity of shortening were less (Table 3). At 90% of the time to peak isometric force, extrapolated isometric force was ~9% greater than peak isometric force under all experimental conditions. The reductions in maximum power during hypoxia (55±7%) and hypercapnia (48±10%) were equal to the reduction in peak isometric force during hypoxia (53±11%) and hypercapnia (50±12%). Therefore, the small reduction in maximum velocity of shortening did not seem to be associated with an additional decrease in maximum power for either hypoxia or hypercapnia.

Postextrasystolic potentiation increased peak isometric force and maximum power approximately equally under control conditions. Maximum velocity of shortening did not increase substantially in control potentiated beats, in agreement with previous reports. Postextrasystolic potentiation approximately reversed the effect of hypercapnia on the force–velocity relation by increasing maximum velocity of shortening, peak isometric force, and maximum power to approximately the value of control nonpotentiated beats (Table 3). Postextrasystolic potentiation increased all of these parameters to a lesser extent during hypoxia.
At the time to 50% peak isometric force in the control beats, maximum velocity of shortening was 81% of its peak value, whereas extrapolated isometric force was 72±13%, and maximum power was 59±6% of the final values. At this time in the contraction, hypoxia decreased maximum velocity of shortening by 23±13%, extrapolated isometric force by 59±15%, and maximum power by 57±6%. Hypercapnia decreased maximum velocity of shortening by 14±4%, extrapolated isometric force by 52±8%, and maximum power by 54±12%. These percent reductions are similar to the effect of hypoxia and hypercapnia at 90% of the time to peak isometric force, suggesting that the relative time course of activation was not greatly altered by hypoxia or hypercapnia. Postextrasystolic potentiation had a similar effect at the time to 50% peak isometric force.

As described previously, there is a small but definite dependence of maximum velocity of shortening on twitch force. Therefore, it is necessary to account for this dependence when assessing changes in maximum velocity of shortening. We do this in three ways. First, we directly compare hypoxia and hypercapnia, which produced approximately the same degree of peak isometric force reduction in nonpotentiated contractions. As shown in Figure 5 and Table 3, maximum velocity of shortening was slightly (9%) but significantly (p<0.01) lower during hypoxia than during hypercapnia. Second, postextrasystolic potentiated contractions during hypoxia or hypercapnia were compared with nonpotentiated contractions under control conditions, because postextrasystolic potentiation increased peak isometric force toward control levels (Table 2). Maximum velocity of shortening during hypercapnia was fully restored to control levels, whereas that during hypoxia was significantly (p<0.05) below control levels (Table 3). Thus, hypoxia reduced maximum velocity of shortening slightly but definitely, whereas hypercapnia did not decrease maximum velocity of shortening when peak isometric force was restored to control levels by postextrasystolic potentiation. Third, we suggest that the changes in maximum velocity of shortening with twitch force can be accounted for by a small internal load equivalent to 6% of the peak isometric force developed in control, nonpotentiated contractions as previously reported. The apparent maximum velocity of shortening

### Table 3. Force–Velocity Relations in Rabbit Myocardium

<table>
<thead>
<tr>
<th>Contractions</th>
<th>Control</th>
<th>Hypoxia</th>
<th>Hypercapnia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpotentiated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extrapolated isometric force (mN/mm²)</td>
<td>35.2±16.7</td>
<td>16.6±11.6*</td>
<td>18.1±13.8*</td>
</tr>
<tr>
<td>Extrapolated isometric force divided by peak isometric force</td>
<td>1.09±0.09</td>
<td>1.09±0.05</td>
<td>1.13±0.00</td>
</tr>
<tr>
<td>Maximum velocity of shortening (Lmax/sec)</td>
<td>1.97±0.46</td>
<td>1.55±0.40*</td>
<td>1.70±0.44*</td>
</tr>
<tr>
<td>Peak power (Lmax/sec/m²)</td>
<td>6.4±2.4</td>
<td>2.9±1.8*</td>
<td>3.3±2.4*</td>
</tr>
<tr>
<td>Peak power divided by peak isometric force (Lmax/sec)</td>
<td>0.204±0.044</td>
<td>0.198±0.043</td>
<td>0.209±0.046</td>
</tr>
<tr>
<td>Potentiated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extrapolated isometric force (mN/mm²)</td>
<td>46.6±17.1</td>
<td>28.3±18.4*</td>
<td>34.3±14.1*</td>
</tr>
<tr>
<td>Extrapolated isometric force divided by peak isometric force</td>
<td>1.06±0.09</td>
<td>1.10±0.09</td>
<td>1.15±0.22</td>
</tr>
<tr>
<td>Maximum velocity of shortening (Lmax/sec)</td>
<td>2.05±0.48</td>
<td>1.80±0.43†</td>
<td>2.02±0.57</td>
</tr>
<tr>
<td>Peak power (Lmax/sec/m²)</td>
<td>8.9±3.1</td>
<td>5.2±3.1†</td>
<td>6.4±2.5†</td>
</tr>
<tr>
<td>Peak power divided by peak isometric force (Lmax/sec)</td>
<td>0.203±0.045</td>
<td>0.203±0.039</td>
<td>0.212±0.042</td>
</tr>
</tbody>
</table>

Values are mean±SD; n=10 muscles. Lmax, length at which maximum active isometric force was developed.

*p<0.01 and †p<0.05 vs. control.
is reduced, because the contractile elements cannot be fully unloaded. As illustrated in Figure 5, the apparent reduction of maximum velocity of shortening may be explained by internal load during hypercapnia but not during hypoxia.

This internal load appears as an apparent dependence of the asymptote a in the Hill equation on extrapolated isometric force, without any change in the asymptote b. We examined the data in the present experiments for a dependence of a on extrapolated isometric force and a lack of such dependence for b. As shown in Figure 6, there is no such dependence for b and a strong dependence for a. The value of the internal load calculated from the relation between a and extrapolated isometric force (Figure 6) was ~6% of peak isometric force in the control condition and was similar for all three conditions. This value is comparable to the value of 1.5% reported by de Tombe and ter Keurs, 4% reported by Fabiato and Fabiato, and 6% reported by Chiu et al.

**Discussion**

The principle new finding in this study is that hypoxia and hypercapnia have very little influence on the shortening kinetics of the muscles, at least when their effects are not pushed to irreversible levels. The small changes in maximum velocity of shortening that occur can be largely explained quantitatively by a small internal load, suggesting that these interventions do not have a substantial direct effect on power generation by the activated cross-bridges. This is a very different conclusion than has been reached in earlier studies using afterloaded contractions. This degree of hypoxia appears to have a small additional effect on maximum velocity of shortening, which at most may represent a small decrease in the maximum rate of actomyosin ATPase cycling. Furthermore, it is important to note that maximum power is reduced in proportion only to the reduction in twitch force and is not doubly reduced because of reductions in both the force- and velocity-generating capacities of myocardium.

**Effects of Hypercapnia**

We have regularly used postextrasystolic potentiation as a positive inotropic intervention in the belief that it changes only the level of calcium activation. Its effects are so rapid that it seems unlikely to cause other chemical alterations in the contractile apparatus. When it was used here to reverse the depressant effects of hypercapnia, it also fully reversed the
changes in maximum velocity of shortening. Since the pH decrease produced by hypercapnia was still present during postextrasystolic potentiation, it is concluded that this level of acidosis did not affect maximum velocity of shortening or the kinetics of the activated cross-bridges.

This level of hypercapnia was associated with a reduction in the bathing solution pH of −0.5. Other studies suggest that this would be associated with a reduction in intracellular pH of −0.2.24,25 This level of intracellular acidosis may impair force generation in a number of ways. Orchard and Kentish26 summarize mechanisms impairing calcium release including inhibition of the calcium current, reduced release of calcium from the sarcoplasmic reticulum, and shortening of the action potential. Alternatively, calcium release may be enhanced by prolongation of the action potential that is sometimes observed. Bioluminescence studies show that hypercapnia can decrease twitch force without changing the calcium transient.17,27 This is consistent with the observation that the main effect of intracellular acidosis accompanying hypercapnia is decreased sensitivity of the contractile proteins to calcium.28,29 Decreased sensitivity of the contractile proteins may be due to inhibition of calcium binding to troponin by acidic pH.30 This may also account for our observation that there is proportionately less hypercapnic depression of potentiated peak isometric force than nonpotentiated peak isometric force (Table 2 and Figure 3). That is, the increased intracellular Ca2+ associated with postextrasystolic potentiated contractions may to some extent overcome the competitive inhibition of calcium binding to troponin by intracellular H+.30 That potentiated peak isometric force is minimally depressed.

When the decrease in pH is approximately five times that in our study, there is evidence that maximum velocity of shortening does decrease in skinned skeletal muscle fibers.31 so that, at this level of acidosis, intracellular hydrogen ion may also have a direct action on the contractile proteins, reducing velocity and potential force-generating capability.31 It is less likely that this second mechanical consequence of the intracellular acidosis associated with hypercapnia can be overcome by postextrasystolic potentiation, which increases intracellular calcium.

Effects of Hypoxia

This degree of hypoxia slightly but significantly decreased maximum velocity of shortening compared with control and hypercapnia. We observed this slight decrease by comparing hypoxia with a level of hypercapnia that reduced peak isometric force to the same extent. To the extent that this experimental design identified a true decrease, it follows that hypoxia limits the maximum rate of actomyosin ATPase cycling because maximum velocity of shortening reflects the maximum rate of actomyosin ATPase cycling.32,33 A likely possibility is that either the substrate for contraction begins to become depleted or that one of the reaction products begins to accumulate at this level of hypoxia. Lowered pH,31 ATP depletion,34 and ADP accumulation,35 but not inorganic phosphate accumulation,31 have been shown to be associated with a decrease in maximum velocity in skinned skeletal muscle fibers. The finding that irreversible changes occur when hypoxia is further increased suggests that cell damage is associated with some metabolic limitation. There are several reasons for believing that acidosis was not responsible for the irreversible effects on twitch force or the reduction of maximum velocity of shortening during hypoxia. First, the depression of twitch force seen with hypercapnia was approximately equal to that seen with hypoxia. Since hypoxia results in several additional depressant effects in addition to intracellular acidosis, it seems likely that the intracellular pH was at least as high and probably higher during hypoxia than during hypercapnia. Second, during the onset of hypercapnia, twitch force fell substantially below the final steady level, probably because of the rapid diffusion of CO2 into cells, with only later equilibration of buffer ions. Since these effects were reversible, it seems unlikely that the effects of hypoxia are due to low intracellular pH, because we found hypoxic reduction in force to that level was always associated with irreversibility. Finally, Allen and Orchard36 have shown that, in whole hearts, hypoxia does not lead to much lowering of intracellular pH, as measured by nuclear magnetic resonance spectroscopy. They suggest that the accumulation of reaction products, notably inorganic phosphate, might inhibit contraction and thereby inhibit further production of hydrogen ion. In their experiments, large decreases in pH do not occur until the ATP concentration begins to fall. At this point, irreversible contracture develops.36 These considerations suggest that the metabolic change associated with the small decrease in maximum velocity of shortening during hypoxia is either ATP depletion, ADP accumulation, or both.

Technical Considerations

The major technical difference between these experiments and previous work is the use of a servo system to produce critically damped, rapid steps to isotonic loads. This improvement enabled us to measure all the force–velocity points for a given curve at the same time in different contractions. Since the twitches were all identical up to the time of release, the level of activation and the contractile element lengths are presumed to be the same at the time of release. After this time, the conditions diverge because the rate and extent of shortening vary at different loads. Therefore, it is imperative that the measurements be made as soon after the release as possible. Ideally, the measurements might be made immediately after the release. Several practical limitations prevent this type of ideal experiment: the steps are not instantaneous, the recoil of the series elastic elements is not instantaneous,15 and the con-
tractile elements do not reach their steady-state value immediately.

We have studied these problems in detail previously\(^5.37\) and have concluded that the measurement interval used here (6–10 msec after the release) gives the best compromise among the several competing influences. At this time, the damped recoil of the series elastic elements, which increases the apparent muscle velocity, has died away, as have most of the velocity transients. At the same time, the contractile elements have shortened very little. To estimate the amount of shortening that could have occurred, we note that, at the lowest loads studied, velocity of shortening was \(\sim 2\) muscle lengths/sec. The time from the midpoint of the step, 1–2 msec after the onset of the step, to the midpoint of the measurement interval, 8 msec after the onset of the step, was 6–7 msec. During this time, at 2 muscle lengths/sec, the contractile elements would shorten by \(\sim 1\%\). Our previous studies have shown that this degree of shortening is likely to have only a small influence on the measured velocity of shortening.\(^37\)

An additional problem created by the series elastic compliance is that there is likely to be a small difference in the contractile element lengths during interventions that produce a change in developed force, making it more difficult to correlate muscle length with sarcomere length.\(^21.38\) To estimate the magnitude of this problem, we note that the contractile elements are 5–6\% shorter at the peak of a control twitch than they are during rest.\(^15.39\) This degree of extension cannot, however, be linearly interpolated to determine the difference in contractile element lengths at forces other than control isometric force. The force-extension curve of the series elastic elements is nonlinear, becoming stiffer at higher forces. Thus, the contractile element length at the peak of the control twitch is likely to be only \(\sim 1\%\) shorter than that at the peak of a twitch producing 50\% of control force. Since twitch force declines from its maximum to zero as the muscle is shortened from its optimum length to 75\% of this optimum, a 1\% difference in length is likely to produce a 4\% difference in developed force. These considerations suggest that, because of the measured force depression caused by hypoxia and hypercapnia, the effects of these interventions on force have been slightly underestimated (\(\sim 2\%\)). The small differences in length would have even less effect on velocity, since maximum velocity of shortening changes little in the range of lengths studied here.\(^10.40\) Furthermore, since we compare hypoxia to hypercapnia and control at approximately equal forces, the confounding effects of series elastic extension are avoided to a large extent.

Relation to Whole Heart Pressure–Volume Relations

Progressive hypoxia in whole hearts increases end-systolic volume at a constant afterload; that is, hypoxia decreases contractility.\(^2\) End-systolic volume increases more by an increase of the volume axis intercept of the end-systolic pressure–volume relation than by a decrease of the slope of the end-systolic pressure–volume relation. This contrasts propranolol\(^41\) and other inotropic interventions, which do not change the volume axis intercept but decrease the slope of the end-systolic pressure–volume relation. We previously speculated that the volume axis intercept could be interpreted as the end point of a theoretically unloaded contraction.\(^2\) Given the same starting volume and contraction time, it follows that a decrease in maximum velocity of shortening could account for a smaller ejected volume and an increase in the volume axis intercept. The present results are consistent with this hypothesis, but the observed hypoxic reduction of the maximum velocity of shortening is small (\(\sim 9\%\) less than control or hypercapnic maximum velocity of shortening compared at similar peak isometric force). However, the whole ventricle is a three-dimensional structure, and if one considers a 9\% decrease in wall shortening of a sphere, there has been a 9\% decrease in shortening velocity, then a 25\% increase in final volume is produced. This increase in end-systolic volume of the theoretically unloaded contraction is less than the \(\sim 35\%\) increase in the volume axis intercept of the end-systolic pressure–volume relation that we previously observed.\(^2\) Nevertheless, the present data are consistent with the hypothesis that hypoxia may mediate part of its global myocardial depressant effects by a decrease in maximum velocity of shortening.

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