Disparate effects of three types of extracellular acidosis on left ventricular function

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Berger, David S., Susan K. Fellner, Kimberly A. Robinson, Katherine Vlasica, Ivan E. Godoy, and Sanjeev G. Shroff. Disparate effects of three types of extracellular acidosis on left ventricular function. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H582–H594, 1999.—Effects of acidosis on muscle contractile function have been studied extensively. However, the relative effects of different types of extracellular acidosis on left ventricular (LV) contractile function, especially the temporal features of contraction, have not been investigated in a single model. We constituted perfusion buffers of identical ionic composition, including Ca$^{2+}$ concentration ([Ca$^{2+}$]), to mimic physiological control condition (pH 7.40) and three types of acidosis with pH of 7.03: inorganic (IA), respiratory (RA), and lactic (LA). Isolated rabbit hearts (n = 9) were perfused with acidotic buffers chosen at random, each preceded by the control buffer. Under steady-state conditions, instantaneous LV pressure ($P_v$) and volume ($V_v$) were recorded for a range of $V_v$. The results were as follows. 1) LV passive (end-diastolic) elastance increased with IA and RA. However, this increase may not be a direct effect of acidosis; it can be explained on the basis of myocardial turgor. 2) Although LV inotropic state (peak active $P_v$ and elastance) was depressed by all three acidotic buffers, the magnitude of inotropic depression was significantly less for LA. 3) Temporal features of $P_v$ were altered differently. Whereas IA and RA reduced time to peak $P_v$ ($t_{max}$) and hastened isovolumic relaxation at a common level of LV wall stress, LA significantly increased $t_{max}$ and retarded relaxation. These results and a model-based interpretation suggest that cooperative feedback (i.e., force-activation interaction) plays an important role in acidosis-induced changes in LV contractile function. Furthermore, it is proposed that LA-induced responses comprise two components, one due to intracellular acidosis and the other due to pH-independent effects of lactate ions.

pH; left ventricular inotropic state; left ventricular relaxation; cooperative feedback; activation-cross bridge dynamics; lactate ion

Most biochemical processes are sensitive to the pH of the environment in which the processes occur. Such dependence on pH makes alteration of H$^+$ concentration ([H$^+$]) a useful tool for examining muscle physiology. Experimental models of acidosis usually employ inorganic acidosis (IA; decreasing the amount of bicarbonate buffer in the perfusate or superfusate) or respiratory acidosis (RA; increasing the percentage of CO$_2$ in the perfusion gas mixture). Clinically, RA can occur when blood pCO$_2$ rises in the setting of pulmonary dysfunction, whereas bicarbonate wasting from the kidneys or small intestine can cause IA. A form of metabolic organic acidosis, lactic acidosis (LA), occurs as a result of anaerobic metabolism, often because of cardiovascular dysfunction.

Various forms of acidosis have been studied extensively in skeletal and cardiac muscle preparations, all sharing a common observation: acidosis reduces inotropic state as measured by the ability of the muscle to generate active force (17, 34, 45). Reduced myofilament Ca$^{2+}$ sensitivity (rightward shift of steady-state force-pCa relationship) is thought to be the primary cause of negative inotropy; however, changes in cross-bridge dynamics (reduced cycling rate and force per unit attached cross bridge) may also play a role. Most of these studies have used RA or IA and have focused on the magnitude of active force generation. Temporal features of force generation (e.g., rates of rise and relaxation) can provide additional insights into muscle contractile function. These aspects, when studied, have been shown to be influenced variably by pH (34, 49, 51). However, recent studies have demonstrated that the proper analysis of certain temporal features (e.g., those describing relaxation) should incorporate changes in the magnitude of active pressure (force) (4, 23, 50).

In the present study, we examined the effects of all three types of acidosis on left ventricular contractile function in isolated rabbit hearts. Identical levels of extracellular acidosis were produced, with perfusion buffers varying only in bicarbonate, CO$_2$, or lactate concentration; Na$^+$, K$^+$, Mg$^{2+}$, P$i$, and Ca$^{2+}$ concentrations ([Na$^+$], [K$^+$], [Mg$^{2+}$], [P$i$], and [Ca$^{2+}$], respectively) were identical. Our results and model-based interpretations suggest that cooperative feedback (i.e., force-activation interaction) plays an important role in acidosis-induced changes in left ventricular contractile function. In addition, we found that effects of LA were markedly different from those of RA or IA, especially regarding the temporal aspects of contraction. We hypothesize that these disparities are caused by pH-independent effects of lactate ions.

METHODS

All protocols were reviewed and approved by The University of Chicago Institutional Animal Care and Use Committee and conform with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health [DHHS Publication No. (NIH) 85-23, Revised 1985, Office of Science and Health Reports, Bethesda, MD 20892].
Experimental Preparation and Isolated Heart Setup

Experiments were performed on hearts isolated from adult male rabbits (New Zealand White) weighing 2.0–3.0 kg. Rabbits were preanesthetized with 5.0 mg/kg xylazine (Ben Venue Laboratory, Bedford, OH) and 0.01 mg/kg glycopyrrolate (Robinson-V; Elkins-Sinn, Cherry Hill, NJ). After 10 min, rabbits were anesthetized with 30–50 mg/kg ketamine (Kedalar; Parke-Davis, Morris Plains, NJ) and 1.0 mg/kg acepromazine (Fermenta Animal Health, Kansas City, MO). Thoracotomy was performed after anesthesia, and rabbits were artificially ventilated (Harvard Ventilator, model 663; Harvard Apparatus, South Natick, MA) with a 95% O2-5% CO2 gas mixture at a respiratory rate of 43 breaths/min and a tidal volume of 25–30 ml. After median sternotomy and ligation of great vessels, a metal cannula connected to the perfusion system was inserted into the brachiocephalic artery and immediately flushed with heparinized saline (3.0 ml, 1,000 U/ml). Retrograde perfusion of the coronary arteries was then begun at a constant perfusion pressure of 80 mmHg. The heart was perfused at 37°C with oxygenated modified Krebs-Henseleit solution (see Perfusate Formulary), which was not recirculated. Connective tissue was cut away and the heart removed from the chest while being constantly perfused. Thus at no time was coronary circulation interrupted. Nine animals were studied.

A thin latex balloon, secured at the end of a piston-cylinder device attached to a linear motor, was positioned in the left ventricle via the mitral orifice. A thread tied to the end of the balloon was passed through the apex of the left ventricle to secure the balloon in the chamber. A purse string was tied around the mitral orifice to secure the heart to the piston-cylinder device. The piston position was sensed by a linear voltage-displacement transformer. All hearts were paced using unipolar electrodes attached to the apex of the left ventricle. More comprehensive details of the isolated heart preparation have been described previously (3, 44).

Perfusate Formulary

The control perfusate (CT) was made by adding the ingredients listed in Table 1 to double-distilled water at a temperature of 30°C. When the perfusate was warmed to 37°C and bubbled through the appropriate gas mixture (Table 1), free ionic concentrations of H+, Ca2+, and other electrolytes reached their desired control values. The three acidotic perfusates were modified from the control solution as follows (Table 1).

Respiratory acidosis. RA in this perfusate was achieved by increasing the %CO2-to-%O2 ratio from its control value.

Inorganic acidosis. IA perfusate was formulated by reducing the concentration of bicarbonate (NaHCO3) in solution.

Lactic acidosis. LA perfusate was created by adding racemic lactic acid to the solution. The dissociation of this compound results in increased [H+].

All three perfusion buffers were designed to achieve identical [Na+], [K+], [Mg2+], [P-], and [Ca2+]. Only sodium bicarbonate- and sodium chloride-containing compounds were varied to achieve this (Table 1). It should be noted that tide-fial pH and free ionic concentrations are very sensitive to temperature and actual gas mixture. For this reason, high-tolerance gas mixtures were used (±5% instead of the typical ±10%) and pH, electrolytes, and free Ca2+ were monitored throughout the experiment. In addition, perfusion buffers were prepared in 4-liter batches so that an entire experiment could be performed using the same buffers.

Experimental Protocol and Data Collection

In this study, the single-beat Frank-Starling (SBFS) protocol (10) was used from which active and passive functional states of the heart were evaluated for each experimental condition (control and acidosis). In the SBFS protocol, the heart was allowed to beat isovolumetrically at a constant reference volume (Vref) until it reached steady state, at which time the left ventricular chamber volume (Vv) was changed over a short period of time in late diastole. After several cardiac cycles occurred at this perturbed volume, Vv was changed back to Vref again in late diastole. The left ventricular pressure (Pv) and Vv from two cardiac cycles were sampled: one steady state at Vref and the other as the first beat after the volume change (the beat that will be analyzed). A full SBFS protocol consisted of 10– 12 equispaced volume changes centered around Vref. If an arrhythmia occurred during the data collection, for example, a mechanically induced premature contraction, that Pv-Vv pair was omitted from the analysis. This was not common with the control perfusate but occurred more frequently with acidosis, not a surprising outcome given that acidosis is known to be arrhythmogenic.

The following data-collection protocol was used for all hearts. After the heart was isolated and instrumented, an SBFS protocol was performed and analyzed (see Data Analysis) to identify Vmax, the volume that resulted in maximum active Pp. The volume was adjusted to be 80% of Vmax and served as Vref for the rest of the experiment. The heart was then subjected to six SBFS protocols, with the perfusate being changed after each one. The order of the type of acidosis was chosen at random from each acidotic perfusate used once and preceded by control perfusate, for example, CT-IA-CT-LA-CT-RA. Ample time, typically 10–12 min, was provided after a perfusate switch so that the heart reached steady state as identified by the stability of the Pp time course. Thus, after surgery, each experiment lasted ~75 min. Also, pH of the perfusate was monitored continuously (pH Meter 59003 20; Cole-Parmer, Vernon Hills, IL). A perfusate sample (14 ml) was taken just before each SBFS protocol, from which relevant ionic concentrations were measured (NOVA 2 Ionized Calcium Analyzer; NOVA Biomedical, Waltham, MA, and Clinical System Synchron CXSCE; Beckman, Schaumburg, IL).

Data Analysis

All analyses were performed using left ventricular pressure and volume data obtained from the first perturbed beat of the SBFS protocol. The effect of acidosis on the left ventricular end-diastolic (passive) pressure-volume relationship was evaluated quantitatively by comparing end-diastolic Pp (Pp ed) and passive elastance (Ep) at Vref. Ep was determined by first fitting the Pp-Vv data to the following exponential relationship:

\[ Pp = Pp_0 - Ep \times Vv \]

Table 1. Perfusate formulary

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Respiratory Acidosis</th>
<th>Inorganic Acidosis</th>
<th>Lactic Acidosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl, g/l</td>
<td>7.240</td>
<td>7.417</td>
<td>8.176</td>
<td>7.240</td>
</tr>
<tr>
<td>KCl, g/l</td>
<td>0.37</td>
<td>0.37</td>
<td>0.37</td>
<td>0.37</td>
</tr>
<tr>
<td>CaCl2, g/l</td>
<td>0.198</td>
<td>0.187</td>
<td>0.176</td>
<td>0.198</td>
</tr>
<tr>
<td>NaHCO3, g/l</td>
<td>2.016</td>
<td>1.764</td>
<td>0.672</td>
<td>2.016</td>
</tr>
<tr>
<td>NaH2PO4, g/l</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>MgCl2 · 6H2O, g/l</td>
<td>0.22</td>
<td>0.22</td>
<td>0.22</td>
<td>0.22</td>
</tr>
<tr>
<td>Glucose, g/l</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Insulin, U/l</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Lactic acid, ml/l</td>
<td>0.0</td>
<td>0.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Gas, %O2/%CO2</td>
<td>95/5</td>
<td>89/11</td>
<td>95/5</td>
<td>95/5</td>
</tr>
</tbody>
</table>
ACIDOSIS AND LEFT VENTRICULAR FUNCTION

Fig. 1: A: left ventricular (LV) active (●) and passive (○) pressure-volume data derived from single-beat Frank-Starling (SBFS) protocol. Passive (end-diastolic) and active pressure-volume relationships were fit to an exponential function (Eq. 1) and a second-degree polynomial, respectively (solid lines). Passive and active elastance were calculated from these fits according to Eqs. 2 and 3. B: quantitation of temporal features of LV active stress waveform. Except for an amplitude scale factor, stress and pressure waveforms are identical for an isovolumic contraction. Maximum active stress value (σmax) was attained at t = tin. Relaxation time (Tr) was calculated as difference between times at which active stress (σact) falls to 75 and 25% of σmax. Rise time (Trise) was defined similarly as time difference between rise to 75% and rise to 25% of σmax.

Additional temporal features of the left ventricular pressure waveform were determined. These were the time at which developed pressure (stress) reached its maximum value (τmax) and the rise time (Trise). Tτmax was defined similarly to Tr except that the rising portion of the Px time course is used (Fig. 1B).

Statistical Analysis

One-way analysis of variance (ANOVA) was performed to compare compositions of the different perfusate solutions. If data did not satisfy the normality condition, Kruskal-Wallis one-way ANOVA on ranks was performed (noted in Table 2). If differences among the groups were statistically significant (P < 0.05), all pairwise multiple comparisons were made using either the Student-Newman-Keuls or Dunn’s (non-normal data) method.

To determine whether acidosis affects physiological function, values of physiological variables for control and acidosis, as well as their percent changes from control, were analyzed using one-way repeated-measures ANOVA (RM-ANOVA). If the normality condition was not satisfied, Friedman RM-ANOVA on ranks was performed. If differences among the groups were statistically significant (P < 0.05), all pairwise multiple comparisons were made using either the Student-Newman-Keuls or Dunn’s (non-normal data) method.

To further investigate the Tτσmax relationship over the entire σmax range, repeated-measures analysis of covariance (RM-ANCOVA) was performed. RM-ANCOVA was implemented using multiple linear regression with dummy variables (19). With this implementation, effects of individual experimental conditions on the Tτσmax relationship (slope and intercept) could be identified readily. Effects coding was used to construct the dummy variables to identify intercondition (Cj) and interrabbit (Ri) differences

\[
C_j = \begin{cases} +1 & \text{condition } j \\ -1 & \text{condition } c \end{cases}, \quad R_i = \begin{cases} +1 & \text{rabbit } i \\ -1 & \text{rabbit } n \end{cases}
\]

where j = 1, ..., nc − 1, with nc as the number of experimental

Table 2. pH and ionic concentrations in different perfusates

<table>
<thead>
<tr>
<th>Perfusate</th>
<th>[Ca²⁺], mM</th>
<th>pH</th>
<th>[Na⁺], mM</th>
<th>[K⁺], mM</th>
<th>[P_i], mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.31 ± 0.04</td>
<td>7.40 ± 0.02</td>
<td>151 ± 1</td>
<td>5.2 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Lactic acidosis</td>
<td>1.29 ± 0.04</td>
<td>7.03 ± 0.03*</td>
<td>152 ± 3</td>
<td>5.3 ± 0.3</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Inorganic acidosis</td>
<td>1.31 ± 0.03</td>
<td>7.03 ± 0.03*</td>
<td>153 ± 2*</td>
<td>5.3 ± 0.1</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Respiratory acidosis</td>
<td>1.31 ± 0.02</td>
<td>7.03 ± 0.03*</td>
<td>155 ± 2*</td>
<td>5.3 ± 0.1*</td>
<td>1.0 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SD. [Ca²⁺], [Na⁺], [K⁺], and [P_i] are concentrations of Ca²⁺, Na⁺, K⁺, and P_i, respectively. ANOVA on ranks was performed on [Ca²⁺] and [K⁺]. *P < 0.05 vs. control.
conditions \( (n_c = 6; \text{three acidotic and three control}) \) and \( i = 1, \ldots, n_r - 1, \) with \( n_r \) as the number of rabbits \( (n_r = 9). \) The experimental conditions were coded for \( j \) as 1, control IA; 2, IA; 3, control RA; 4, RA; 5, control LA; and 6, LA. The multiple regression model reads

\[
T_r = b_0 + b_2 \sigma_{\text{max}} + b_2 \sigma_{\text{max}}^2 \\
+ \sum_{j=1}^{n_c} [c_{0,j}C_j + c_{1,j}(C_j \sigma_{\text{max}}) + c_{2,j}(C_j \sigma_{\text{max}}^2)] \\
+ \sum_{i=1}^{n_r} [r_{0,i}R_i + r_{1,i}(R_i \sigma_{\text{max}}) + r_{2,i}(R_i \sigma_{\text{max}}^2)]
\]  

(7)

The overall \( T_r-\sigma_{\text{max}} \) relationship (quadratic function) is quantified by coefficients \( b_0, b_1, \) and \( b_2, \) which correspond to the mean values for the entire data set (i.e., all conditions, all animals). Coefficients \( c_{0,j}, c_{1,j}, \) and \( c_{2,j} \) quantify the deviations of the \( j \)th experimental condition from the overall \( T_r-\sigma_{\text{max}} \) relationship. Similarly, coefficients \( r_{0,i}, r_{1,i}, \) and \( r_{2,i} \) represent deviations of the \( i \)th rabbit from the overall \( T_r-\sigma_{\text{max}} \) relationship. Thus the effect of a particular type of acidosis can be identified readily by examining the statistical significance of coefficients \( c_{0,j}, c_{1,j}, \) and \( c_{2,j}. \) This analysis was carried out using the stepwise regression algorithm (Minitab, version 10.2).

RESULTS

Table 2 contains values (means ± SD) for pH and \([\text{Ca}^{2+}], [\text{Na}^{+}], [\text{K}^-], \) and \([\text{Pi}] \) measured for control and each acidotic perfusate. The control values are those at the start of the experiment just before the first SBFS. As shown, pH values for the three acidotic perfusates were the same and significantly lower than control value. Equally important, concentrations of relevant electrolytes, particularly \([\text{Ca}^{2+}] \), were the same in each acidotic perfusate. Although \([\text{Na}^{+}] \) or \([\text{K}^-] \) in certain acidotic perfusates were different statistically from control values (Table 2), these differences were very small and, therefore, not likely to be functionally important.

Figure 2 contains raw \( P_v \) and \( V_v \) data (perturbed beat from the SBFS protocol) from all six conditions from one representative experiment. These data were used to analyze left ventricular passive and active pressure-volume relationships and relaxation behavior.

**Left Ventricular End-Diastolic (Passive) Pressure-Volume Relationships**

Passive pressure-volume relationships from a single experiment (same as in Fig. 2) are shown in Fig. 3A. Only IA and RA showed a small but consistent increase in \( P_{ed} \) for all \( V_{ed}. \) When data from all hearts are considered (Table 3), the control values of \( P_{ed} \) and \( E_p \) at \( V_v = V_{ref} \) were not different. Relative to their control conditions, only IA and RA increased \( P_{ed} \) (at \( V_{ref} \)) and \( E_p \) (at \( V_{ed} \)) significantly (Fig. 3, B and C); the absolute values of these quantities are given in Table 3.

**Left Ventricular Peak Isovolumic (Active) Pressure-Volume Relationships**

The active pressure-volume curve was shifted down compared with control in all types of acidosis (Fig. 4A, derived from the data in Fig. 2). Reduction of \( P_{\text{max}} \) for a given \( V_{ed} \) was greater for IA and RA compared with LA. Once again, analysis of data from all animals revealed that control values of \( P_{\text{max}} \) and \( E_p \) at \( V_v = V_{ref} \) were not different (Table 3). This group analysis also showed
that both $P_{\text{max}}$ and $E_a$ at $V_v = V_{\text{ref}}$ were reduced significantly by IA and RA, whereas only $P_{\text{max}}$ at $V_{\text{ref}}$ was diminished significantly by LA. Furthermore, whereas the negative inotropic effects of IA and RA were not different from each other, both had a significantly larger negative inotropic effect than LA. The same statistical results were obtained from the analysis of percent changes from control (Fig. 4, B and C).

**Left Ventricular Relaxation**

Figure 5A shows the $T_r-\sigma_{\text{max}}$ relationships from the same experimental data as in Fig. 2. Again, effects of IA and RA were similar to each other; the $T_r-\sigma_{\text{max}}$ points were shifted downward such that relaxation was hastened at all $\sigma_{\text{max}}$. In contrast, the $T_r-\sigma_{\text{max}}$ points were shifted upward in LA, indicating that LA slowed left ventricular relaxation.

Analysis of $T_r-\sigma_{\text{max}}$ relationships revealed that the model given by Eq. 7 fit the entire data set well (all $\sigma_{\text{max}}$ points, all conditions, all rabbits; $n = 429$; $R^2 = 0.95$). Except for the intercept for RA control, none of the control condition coefficients was significantly different from the overall mean values. The difference in the intercept for RA control was very small ($1.1\text{ ms}$). In Table 3.

**Summary indexes of left ventricular function**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Inorganic Acidosis</th>
<th>Respiratory Acidosis</th>
<th>Lactic Acidosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{\text{ed}}$, mmHg</td>
<td>8.9 ± 3.2</td>
<td>10.8 ± 3.4*†</td>
<td>8.7 ± 2.9</td>
</tr>
<tr>
<td>$E_p$, mmHg/ml</td>
<td>26.0 ± 13.0</td>
<td>31.2 ± 14.9</td>
<td>26.0 ± 10.7</td>
</tr>
<tr>
<td>$P_{\text{max}}$, mmHg</td>
<td>102.9 ± 15.9</td>
<td>77.9 ± 14.0*†</td>
<td>104.2 ± 14.4</td>
</tr>
<tr>
<td>$E_a$, mmHg/ml</td>
<td>39.8 ± 10.9</td>
<td>34.6 ± 12.9*†</td>
<td>41.5 ± 10.5</td>
</tr>
<tr>
<td>$\sigma_{\text{max}}$, mmHg</td>
<td>51.4 ± 11.0</td>
<td>39.0 ± 9.7*†</td>
<td>52.2 ± 10.6</td>
</tr>
<tr>
<td>$T_r$, ms</td>
<td>82.3 ± 10.8</td>
<td>75.9 ± 11.9*†</td>
<td>84.3 ± 9.1*</td>
</tr>
<tr>
<td>$T_{\text{rise}}$, ms</td>
<td>55.9 ± 5.1</td>
<td>53.9 ± 6.6†</td>
<td>56.2 ± 5.3</td>
</tr>
<tr>
<td>$\sigma_{\text{max}}$, ms</td>
<td>213 ± 23</td>
<td>197 ± 25*†</td>
<td>213 ± 23</td>
</tr>
</tbody>
</table>

Values are means ± SD and were all measured at reference volume. $P_{\text{ed}}$, end-diastolic pressure; $E_p$, passive elastance; $P_{\text{max}}$, maximum active pressure; $E_a$, peak active elastance; $\sigma_{\text{max}}$, maximum active stress; $T_r$, relaxation time; $T_{\text{rise}}$, rise time; $\tau_{\text{max}}$, time to peak active stress. *$P < 0.05$, acidosis vs. control. †$P < 0.05$, IA or RA vs. LA. ANOVA on ranks was performed on $E_p$. 

Fig. 3. A: effects of IA, RA, and LA on LV passive pressure ($P_{ed}$)-volume ($V_v$) relationships in 1 heart. Raw data for these plots are from Fig. 2. B: percent change in $P_{ed}$ at $V_v = V_{\text{ref}}$ for all hearts. C: percent change in passive elastance ($E_p$) at $V_v = V_{\text{ref}}$ for all hearts. All significant acidosis-induced changes in $P_{ed}$, $E_p$, and $P_{ed}-V_v$ relationships could be explained by coronary turgor (see DISCUSSION). Data are means ± SE. *$P < 0.05$ vs. control. †$P < 0.05$ for RA or IA vs. LA. ANOVA on ranks was performed on $E_p$. 

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contrast, all intercepts for the acidotic conditions were significantly different from the overall mean values (P < 0.05), as indicated by the significant coefficients \( c_{0.2}, c_{0.4}, \) and \( c_{0.6}. \) The stress-associated coefficients for the acidotic groups were not different from the mean values. Figure 6 shows the regression results for all six conditions plotted together. Note that the three control conditions are together, whereas LA lies above these values (slower relaxation) and both RA and IA lie below them (faster relaxation). From this analysis, it is clear that the entire \( T_r-\sigma_{\text{max}} \) relationships are similarly affected by IA and RA and that LA affects this relationship differently over the entire range of \( \sigma_{\text{max}}. \) Furthermore, from continuity, we can conclude that the effects of LA on the \( T_r-\sigma_{\text{max}} \) relationship are significantly different from those of IA or RA.

We want to provide a quantitative feel for \( \sigma_{\text{max}} \)-dependent and -independent components responsible for the acidosis-induced net changes in \( T_r \) at \( V_{\text{ref}}. \) Because the effects of IA and RA on \( \sigma_{\text{max}} \) and \( T_r \) were similar (Table 3), data from these two conditions were combined. With respect to the control condition, acidosis (IA and RA combined) reduced \( \sigma_{\text{max}} \) and \( T_r \) at \( V_{\text{ref}} \) by 24% (from 52 to 40 mmHg) and 11% (from 83 to 74 ms), respectively (measured data, Table 3). According to the data presented in Fig. 6, a portion (5.6%) of this fall in

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**Fig. 4.** A: effects of IA, RA, and LA on LV maximum active pressure (\( P_{\text{max}} \))-volume (\( V_v \)) relationships in 1 heart, showing reduced \( P_{\text{max}} \) at all \( V_v. \) B: percent change in \( P_{\text{max}} \) at \( V_v = V_{\text{ref}} \) for all hearts. C: percent change in active elastance (\( E_a \)) at \( V_v = V_{\text{ref}} \) for all hearts. Data are means ± SE. *P < 0.05 vs. control. †P < 0.05 for RA or IA vs. LA.

**Fig. 5.** Effects of IA, RA, and LA on \( T_r-\sigma_{\text{max}} \) relationships in 1 heart. Both IA and RA hastened relaxation, whereas LA slowed it.
### DISCUSSION

IA, RA, and LA were studied together in the whole heart while concentrations of Ca$^{2+}$ and other relevant electrolytes in the perfusates were tightly controlled (Table 2). Two primary observations will be discussed in detail. The first is that the effects of RA and IA on all aspects of left ventricular function were broadly similar. We will discuss these findings in light of previous studies that, in general, showed similar results. The second observation, unique to our study, is that LA has distinctly different, and in some cases opposite, effects on LV function compared with those of IA and LA.

**Additional Temporal Features of Left Ventricular Pressure Waveform**

Normalized (i.e., magnitude ranging from 0 to 1) $P_{act}$ time-course curves for each type of acidosis and its control (at $V_v = V_{ref}$) are shown in Fig. 7A. These plots show the effects of acidosis on important temporal landmarks. Specifically, RA and IA caused maximum pressure to occur sooner. Once again, LA had different effects; pressure rise was slower, and the occurrence of peak pressure was delayed. As shown in Table 3 (absolute values) and Fig. 7, B and C (percent changes), these observations are borne out quantitatively for all hearts. $T_{rise}$ and $t_{max}$ were not different among the three controls and were increased significantly by LA. In contrast, IA and RA caused a small but significant reduction in $t_{max}$.

![Graph showing $T_r$ vs $\sigma_{max}$](image.png)

$T_r$ was due to the reduction in $\sigma_{max}$; the rest (5.4%) was due to the IA/RA-induced hastening of relaxation. In contrast, LA reduced $\sigma_{max}$ at $V_{ref}$ by 10%, whereas $T_r$ increased by 5% (measured data, Table 3). Thus the expected fall in $T_r$ due to reductions in both $\sigma_{max}$ and pH with LA is more than offset by some factor that slows relaxation.

![Graphs showing temporal features of LV pressure waveform](image.png)

**Fig. 7.** Acidosis-induced changes in temporal features of LV pressure waveform. A: LV normalized active pressure (i.e., peak value of 1) at $V_v = V_{ref}$ in 1 heart. Both IA and RA hastened pressure rise and fall, whereas LA retarded rise and fall. B and C: percent change in $T_{rise}$ and $t_{max}$, respectively, at $V_v = V_{ref}$ for all hearts. Data are means ± SE.

* $P < 0.05$ vs. control. † $P < 0.05$ for RA or IA vs. LA.
Acidosis: Effect on Passive Pressure-Volume Relationships

Both IA and RA rotated left ventricular end-diastolic (passive) pressure-volume relationships leftward (Table 3 and Fig. 3), indicating an increase in passive chamber stiffness. Because coronary perfusion pressure \( (P_{cor}) \) was constant, the decrease in \( P_v \) with acidosis has a potential to increase myocardial volume (intravascular, extravascular, or both), which can augment both systolic and diastolic \( P_v \) at a fixed \( V_t \) (22). In a previous study, we showed that, at a given \( V_t \), \( P_{max} \) reductions of 35–40%, induced with hypocalcemia \([Ca^{2+}] = 0.63 \text{ mM}\), lead to 18–25% increases in \( P_{ed} \) and 25–30% increases in \( E_p \) (43). Similar increases in \( P_{ed} \) and \( E_p \) were found when \( P_{cor} \) was increased (4). In the present study, RA caused a 23% reduction in \( P_{max} \) coincident with a 40% increase in \( P_{ed} \) and a 28% increase in \( E_p \). Similarly, IA caused \( P_{max} \) to fall 26% while \( P_{ed} \) and \( E_p \) increased 25 and 28%, respectively. Thus changing the pressure gradient between \( P_{cor} \) and \( P_v \) in this study was accompanied by changes in diastolic properties similar to those seen previously (43).1 Furthermore, acidosis does not appear to alter passive force at a fixed muscle length in isolated cardiac muscle studies 

1 The \( V_{sat} \) at which changes in \( P_{ed} \) and \( E_p \) were compared in the hypocalcemia study was the volume at which control \( P_{ed} \) was equal to 5 mmHg. \( V_{sat} \) in the present study was larger, typically resulting in control \( P_{ed} \) equal to 8–10 mmHg. Absolute \( P_{ed} \) values and percent changes in \( P_{ed} \) and \( E_p \) are augmented at higher volumes.

have slowed, not hastened as observed here. Furthermore, caffeine or ryanodine pretreatment greatly affected the transient responses (both free \( Ca^{2+} \) and mechanical), yet they resulted in similar steady-state tension reductions with acidosis. Therefore, it is unlikely that the steady-state responses in ventricular function observed in the present study are due to SR-induced changes in activator \( Ca^{2+} \). The reduced active pressure seen with IA and RA is consistent with a general negative inotropy observed in all previous studies of acidosis in skeletal muscle (12–14, 17, 30), isolated cardiac muscle (17, 25, 39, 45), and the whole heart (15, 24, 42, 47, 48). Furthermore, this reduced inotropy is consistent with the well-known acidosis-induced reduction of maximally activated force \( (F_{max}) \) and a rightward shift of the force-pCa curve (i.e., decreased pCa at half-maximal activation \( (pCa_{50}) \)). To facilitate understanding of acidosis-induced negative inotropy, it is appropriate to consider the following four aspects of contractile and activation processes: 1) reduced force per attached cross bridge; 2) altered kinetic properties of cross-bridge cycling such that cross-bridge detachment is favored relative to its attachment; 3) reduced \( Ca^{2+} \)-induced myofilamental activation; and 4) reduced cooperative feedback. Although all of these factors may contribute to acidosis-induced changes, we propose that reduced cooperative feedback, induced by acidosis, can reconcile observed changes in the temporal features of the pressure waveform and also be consistent with other known effects of acidosis. This proposal is based in part on a simple model of activation/cross-bridge dynamics described in the Appendix (Fig. 8). Briefly, the cross-bridge cycle consists of three states, two attached (force producing and non-force producing) and one unattached. The \( Ca^{2+} \)-induced myofilamental activation is governed by the effective rate constants \( K_{on} \) and \( K_{off} \). This reversible reaction includes both \( Ca^{2+} \)-binding to troponin C and switching of thin filament regulatory units. Cooperative feedback is represented by the following: 

K_{off} = K_{off,0} \left(1 + fX_{cf} \right)

This reversible reaction includes both \( Ca^{2+} \)-binding to troponin C and switching of thin filament regulatory units. Cooperative feedback is represented by the following: 

\[ K_{off} = K_{off,0} \left(1 + fX_{cf} \right) \]

where \( K_{off} \) is the rate constant for detachment of the \( Ca^{2+} \)-binding site, \( K_{off,0} \) is the rate constant for detachment of the free site, \( f \) is the fraction of sites that are attached, \( X_{cf} \) is the concentration of free \( Ca^{2+} \), and \( g \) is the cooperativity parameter (i.e., \( X_{cf} \) and a cooperativity parameter (\( g \)).
by modulating $K_{off}$ (parameter $\beta$ in Fig. 8); $K_{off}$ decreases in the presence of attached, force-producing cross bridges, thus facilitating myofilamental activation. Other forms of cooperative feedback, such as nearest-neighbor thin filament and/or cross-bridge interaction and active force cross-bridge kinetic rate constant interaction, are known to exist (46); our nonspecific and simple formulation probably does not represent these processes well. In the following discussion, force and pressure are used interchangeably.

Reduced force per attached cross bridge can occur either by a diminution of force per attached, force-producing cross bridge (17) or by a redistribution of attached cross bridges that favors the non-force-producing population (41). Because the first possibility simply scales all force values, it cannot alter any temporal features of the pulse. The redistribution of attached cross bridges can only be achieved by changing cross-bridge kinetic properties, specifically reduced $h$, increased $g$, or both (see APPENDIX, Eq. A7).

The model-based analysis is now used to evaluate the remaining candidates (i.e., cross-bridge kinetic- and activation-related parameters). For this purpose, we attempt to reproduce the following experimental observations with acidosis (respiratory or inorganic). In terms of the left ventricular pressure pulse (Table 3 and RESULTS), acidosis reduces $F_{max}$, $t_{max}$, and $T_r$, at a fixed volume (i.e., $V_{ref}$) by 24, 7, and 11%, respectively. It is generally thought to be diffusion limited, it is unlikely that $K_{on}$ is affected by acidosis. In addition, a reduced $K_{on}$ would increase $t_{max}$ (model-based analysis), which is contrary to our observations. An increase in $K_{on}$, implemented via a decrease in cooperative feedback (decreased $\beta$), reproduces all of the acidosis-induced changes except for $F_{max}$ and $S_{rel}$ (Table 4). Thus it is reasonable to propose that acidosis affects both $h$ and $\beta$; a concomitant decrease in $h$ and $\beta$ reproduces all of the observed changes well (Table 4 and Fig. 9). In summary, the modeling efforts, together with the experimental observations, lead us to conclude that 1) the presence of cooperative feedback is necessary, and 2) inhibition of the transition to the force-producing state and reduced cooperative feedback are likely mechanisms for the acidosis-induced changes.

In the presence of cooperative feedback, increasing $g$ alone could reproduce the acidosis-induced effects reasonably well (Table 4). However, the experimental evidence in support of such an increase in $g$ is inconclusive. Most experimental results, based on unloaded velocity of shortening or relaxation of tetanic contractions, suggest that $g$ is either decreased or unchanged with acidosis (35, 41, 51). The only evidence for increased $g$ is based on the observation that acidosis reduces both isometric force and ATPase activity, with the reduction in ATPase activity less than that in isometric force (13, 38). According to the Brenner scheme (7), this would suggest an increase in $g$ with acidosis (38).

Evidence for acidosis-induced decrease in rate of force-producing cross-bridge formation exists (31, 41). This could be achieved by a decrease in $f$, $h$, or both. However, because acidosis increases relative stiffness in both skeletal and cardiac muscle (29, 32, 41), a decrease in $h$ is most likely. Our model-based analysis indicated that a decrease in $h$ could reproduce acidosis-induced changes reasonably well, except perhaps for the too large change in $t_{max}$ (Table 4).

Finally, a reduction in Ca$^{2+}$-induced myofilamental activation with acidosis has been observed in a variety of preparations (5, 16, 26). A reduced $K_{on}$-to-$K_{off}$ ratio would be necessary to yield observed changes in force pCa relationship and inotropic state. Because $K_{on}$ is generally thought to be diffusion limited, it is unlikely that $K_{on}$ is affected by acidosis. In addition, a reduced $K_{on}$ would increase $t_{max}$ (model-based analysis), which is contrary to our observations. An increase in $K_{on}$, implemented via a decrease in cooperative feedback (decreased $\beta$), reproduces all of the acidosis-induced changes except for $F_{max}$ and $S_{rel}$ (Table 4). Thus it is reasonable to propose that acidosis affects both $h$ and $\beta$; a concomitant decrease in $h$ and $\beta$ reproduces all of the observed changes well (Table 4 and Fig. 9). In summary, the modeling efforts, together with the experimental observations, lead us to conclude that 1) the presence of cooperative feedback is necessary, and 2) inhibition of the transition to the force-producing state and reduced cooperative feedback are likely mechanisms for the acidosis-induced changes.

### Table 4. Results of model-based analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Change, %</th>
<th>$F_{peak}$, %</th>
<th>$t_{max}$, %</th>
<th>$T_r$, %</th>
<th>$F_{max}$, %</th>
<th>$p_{Ca_{50}}$, pCa unit</th>
<th>$S_{rel}$, %</th>
<th>Parameter</th>
<th>Change, %</th>
<th>$F_{peak}$, %</th>
<th>$t_{max}$, %</th>
<th>$T_r$, %</th>
<th>$F_{max}$, %</th>
<th>$p_{Ca_{50}}$, pCa unit</th>
<th>$S_{rel}$, %</th>
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<tbody>
<tr>
<td>$g$</td>
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<td>-3.3</td>
<td>-15.5</td>
<td>-17.2</td>
<td>-0.14</td>
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<td>-2.3</td>
<td>-1.1</td>
<td>-21.6</td>
<td>-0.03</td>
<td>21.6</td>
</tr>
<tr>
<td>$f$</td>
<td>-29</td>
<td>-24.0</td>
<td>-1.0</td>
<td>-17.8</td>
<td>-13.9</td>
<td>-0.17</td>
<td>0.0</td>
<td>$f$</td>
<td>-31</td>
<td>-24.0</td>
<td>-0.6</td>
<td>-2.2</td>
<td>-15.0</td>
<td>-0.09</td>
<td>0.0</td>
</tr>
<tr>
<td>$h$</td>
<td>-22</td>
<td>-24.0</td>
<td>-1.4</td>
<td>-13.9</td>
<td>-16.9</td>
<td>-0.13</td>
<td>17.2</td>
<td>$h$</td>
<td>-28</td>
<td>-24.0</td>
<td>0.0</td>
<td>0.0</td>
<td>-21.9</td>
<td>-0.03</td>
<td>23.6</td>
</tr>
<tr>
<td>$\beta$</td>
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<td>-24.0</td>
<td>-7.1</td>
<td>-10.7</td>
<td>0.0</td>
<td>-0.29</td>
<td>0.0</td>
<td>$\beta$</td>
<td>-46</td>
<td>-24.0</td>
<td>-7.1</td>
<td>-11.1</td>
<td>-15.0</td>
<td>-0.08</td>
<td>23.6</td>
</tr>
<tr>
<td>$\beta, h$</td>
<td>-30, -16</td>
<td>-24.0</td>
<td>-2.9</td>
<td>-14.2</td>
<td>-12.0</td>
<td>-0.20</td>
<td>11.2</td>
<td>$\beta, h$</td>
<td>-30, -16</td>
<td>-24.0</td>
<td>-2.9</td>
<td>-14.2</td>
<td>-12.0</td>
<td>-0.20</td>
<td>11.2</td>
</tr>
</tbody>
</table>

$g$, $f$, and $h$, Rate constants for cycling-cross bridge model (Fig. 8); $\beta$, cooperative feedback parameter (Fig. 8); $F_{peak}$, $t_{max}$, and $T_r$, peak force, time to peak force, and relaxation time, respectively, for a twitch contraction; $F_{max}$, $p_{Ca_{50}}$, $S_{rel}$, maximally activated force, pCa, at half-maximal activation, and relative stiffness, respectively, in constantly activated state. Control values of $g$, $f$, and $h$ were 75 s$^{-1}$, 400 s$^{-1}$, 50 s$^{-1}$, and 0.035 µM$^{-1}$, respectively. Model simulations were expected to reproduce the following changes with acidosis (see text for details): $F_{peak}$, -24%; $t_{max}$, -7%; $T_r$, -11%; $F_{max}$, -15% pCa$_{50}$, -0.15 pCa units; and $S_{rel}$, 10%. Indicated parameter was adjusted to yield a 24% reduction in $F_{peak}$, remaining variables emerged from simulation.
Lactic Acidosis: Disparate Results

Effects on ventricular function due to LA are very different from those due to IA and RA (Table 3, Figs. 3–7). The first observation is that the negative inotropic effect (reduced $P_{\text{max}}$ and $E_a$) is mitigated in LA compared with IA and RA. Because intracellular pH is a more important determinant of muscle function than extracellular pH (37), one possible explanation for the differences in inotropic responses is that intracellular pH is not reduced as much in LA as in IA and RA. Although we did not measure intracellular pH, such differential changes in intracellular pH have been reported (42, 47, 53). Because the negative inotropic effect of reduced intracellular pH follows a dose response (34), the above explanation would be warranted, especially if one focuses on developed pressure data alone. However, the effects of LA on $T_{\text{rise}}$, $t_{\text{max}}$, and the $T_{\text{rise}}$-$t_{\text{max}}$ relationship require further explanation. Less intracellular pH reduction would, at most, move $T_{\text{rise}}$, $t_{\text{max}}$, and $T$, toward their control values, not beyond. Directionally opposite changes in these variables suggest that some factor in addition to an increase in $[H^+]$ is involved with the disparate LA response.

One obvious candidate for this additional factor is the lactate ion. The cell membrane is permeable to lactate ions; thus addition of lactate to the perfusion buffer will increase intracellular lactate ([lactate]). It has been shown that increased [lactate], even without any change in pH, reduces active force development in skeletal muscle (2, 21). However, its effects on twitch characteristics of cardiac muscle under normoxic conditions are not known. Xu et al. (52) have shown that SR Ca$^{2+}$ transport is critically dependent on the ATP generated endogenously by SR-associated glycolytic enzymes. Specifically, glycolytic ATP supported a 15-fold greater Ca$^{2+}$ transport than the exogenously supplied ATP at the same concentration. Under normoxic conditions, increased [lactate] is known to suppress the glycolytic pathway, which could reduce glycolytic ATP generation and, consequently, impair SR Ca$^{2+}$ uptake. Another possibility is based on the observation that there is a positive correlation between ATP phosphorylation potential (i.e., ATP concentration divided by the product of ADP and P$e$, concentrations [ATP]/[ADP]($P_e$)) and SR Ca$^{2+}$ uptake and release (28). Increased [lactate], via its effects on the cytosolic redox state, can reduce ATP phosphorylation potential, resulting in impaired SR Ca$^{2+}$ uptake. Thus it is likely that [lactate]-induced changes in SR Ca$^{2+}$ handling, specifically reduced SR uptake, is the mechanism underlying the slowing of relaxation and delayed time to peak pressure (force).

As a preliminary assessment of the independent effects of lactate ion, two additional experiments were performed. Hearts were perfused first with the control perfusate and then with a perfusate containing sodium lactate (racemic mixture) such that the concentration of lactate ions was the same as in LA (~11 mM). Both perfusates had the same pH (7.40). Although $P_{\text{max}}$ at $V_{\text{ref}}$ was not affected by lactate (control, 137 mmHg; lactate, 132 mmHg), pressure wave morphology was altered significantly. As shown in Fig. 10, lactate alone increases $t_{\text{max}}$ and greatly slows relaxation. Although a
more thorough evaluation of the independent effects of lactate is necessary, we propose the following hypothesis to reconcile the disparities observed between LA and both RA and IA. LA-induced responses can be thought of as the sum of two components, one due to intracellular acidosis and the other due to a direct effect of lactate ions. The change in intracellular acidosis with LA is likely to be not as great as that with RA or IA. Nonetheless, the acidosis component of LA response will reduce isotropic state (lower P_max and E_t) and hasten pressure rise and fall (lower T_1/2, t_max, and T_r) compared with control. Therefore, the direct component of the LA response (Fig. 10) must retard rate processes to such an extent that the net effect is significant slowing of pressure rise and relaxation with respect to the control state. Because we used a racemic mixture (i.e., both L- and D-lactate ions were present), we cannot comment at this time on the mechanism for the direct effects of lactate ions; both metabolic and nonmetabolic causes are possible.

**APPENDIX**

To gain some insight into the mechanisms for acidosis-induced changes in left ventricular contractile function, a model-based interpretation was pursued. A simple cross-bridge cycling-based model of cardiac muscle force generation was used for this purpose (Fig. 8).

**Model Description**

The salient features of the model are as follows. 1) All states in which troponin-tropomyosin regulatory units are off (Ca^{2+} not bound to low-affinity site on thin filament) are given by a single noncycling state (X_{nc}). 2) All cycling, unattached states are given by X_{co}, K_{co}, and K_{off} are the apparent rate constants governing the transition between noncycling and cycling states. It should be noted that this reversible process includes both Ca^{2+} binding to troponin C and the switching on and off of thin filament regulatory units. 3) Cycling, attached cross bridges exist in either non-force- or force-producing states, given by X_{d} and X_{ac}. Only the force-producing state gives rise to developed force (F). Thus, at any time (t), F = X_{d}. Rate constants f and d govern the cycling between unattached and attached non-force-producing states, whereas the unidirectional transition between non-force-producing and force-producing attached bridges, the power stroke, is governed by h. Finally, the detachment of force-producing cross bridges is governed by the rate constant g. 4) The total number of attached cross bridges in the force-producing conformation governs the cooperative feedback (6, 20). This phenomenon was implemented by modulating the off rate constant according to the function K_{off} = K_{off,0}(1 + \beta X_d^2), where K_{off,0} is the initial off rate (i.e., zero attached cross bridges) and \beta is a fixed parameter. Investigators have previously implemented this feedback as a linear function of X_d (8, 27). We chose the quadratic function instead because it yields a parabolic relationship between the apparent Ca^{2+} binding coefficient (K_{ass}/K_{off}) and normalized force, which is consistent with experimental findings (20). Thus increasing \beta will promote the transition from noncycling to cycling states.

X_T, the total concentration of available cross bridges (X_T = X_{nc} + X_{co} + X_{ac} + X_{d}), is proportional to the concentration of low-affinity Ca^{2+} binding sites on troponin C and is fixed in this simulation of isometric (isovolumic) contraction. With [Ca] representing intracellular free Ca^{2+} concentration, this model can be described completely by the following system of three coupled differential equations:

\[
\begin{align*}
\frac{dX_{co}}{dt} &= -(f + K_{off} + K_{ass}[Ca])X_{co} + (d - K_{ass}[Ca])X_{cn} \\
&\quad - K_{ass}[Ca]X_{d} + K_{ass}[Ca]X_{T} \\
\frac{dX_{cn}}{dt} &= fX_{co} - (d + h)X_{cn} \\
\frac{dX_{d}}{dt} &= hX_{cn} - gX_{d}
\end{align*}
\]

Activation for this system is provided through [Ca], which can be either constant or time varying, to simulate excitation-contraction.

**Constant Activation: Force and Stiffness**

For the case of steady-state constant activation, [Ca] and X_{d} are constant and all derivatives equal zero. Solving the resultant algebraic system for X_{d} yields steady-state force for a given [Ca], F([Ca]), which reads

\[
F([Ca]) = F_{max} \frac{16\left[Ca\right]^{2}(K_{2}^{2} - 3\beta K_{3}^{2})}{6[Ca]^{2}K_{2}K_{3}^{2}} + 2[Ca]K_{3} - 16\beta K_{1}K_{3} + (2K_{3})^{2}
\]

\[
A4
\]

**Fig. 10.** A: normalized LV active pressure under control conditions and during perfusion with a solution containing sodium lactate in 1 heart. Addition of sodium lactate to control perfusate resulted in the same free ionic concentration of lactate as in LA, without fall in pH. B: T_{1/2}, t_{max} relationship for control and perfusion with sodium lactate in same heart as in A. These data demonstrate that lactate ion has a direct effect that significantly slows pressure rise and fall. Similar results were obtained from a 2nd heart.
where $K_1$, $K_2$, $K_3$, $K_r$, and $K_c$ are constants given by

$$K_1 = K_{oi}(d + f)g + f + gh$$

$$K_2 = K_{af} f h \beta X_c$$

$$K_3 = K_{df} g(d + h)$$

$$K_r = 4[\beta K_3 [Ca]_i + [Ca] K_2 - [Ca] K_3]^2$$

$$+ [Ca] K_2 [9 \beta K_3 K_r - 2(9 \beta K_2^2 + K_3)^2]$$

$$K_c = 2 [Ca]^2 (K_2 + 9 \beta K_3 K_r - 9 \beta [Ca] K_c K_r + \sqrt{[Ca] K_r}$$

Two additional solutions for $F([Ca])$ are discarded because one yields $F < 0$ for all $[Ca] > 0$ and one is complex. $F_{\text{max}}$ in Eq. A4 is the maximum possible force under constant activation (limit as $[Ca] \to \infty$) and is given by

$$F_{\text{max}} = \frac{f h}{(d + f) g + (f + g)h} X_T$$

(A5)

The factor multiplying $F_{\text{max}}$ in Eq. A4 is the normalized force-pCa relationship (bounded by 0 and 1). Thus, whereas the shape and position of the force-pCa curve depend on all aspects of the model (cross-bridge kinetics, activation, and $X_T$), $F_{\text{max}}$ is independent of activation. Equations A4 and A5 were used to construct the force-pCa plots in Fig. 9 and to determine the pCa that yields half-maximal activation ($pCa_{50}$).

Whereas the $X_d$ state provides force, both $X_d$ and $X_c$ contribute to stiffness ($S$), and they do so equally such that $S = (X_d + X_c)$. For any level of constant $[Ca]$ and steady-state conditions, $X_d$ and $X_c$ are related (Eq. A3 with $dX_{df}/dt = 0$) as

$$X_{\text{st}} = \frac{g}{h} X_d$$

(A6)

Thus steady-state relative stiffness of the model under constant activation ($S_{\text{rel}}$) is

$$S_{\text{rel}} = \frac{S}{F} = 1 + \frac{g}{h}$$

(A7)

Therefore, a change in $S_{\text{rel}}$ can only be achieved by altering $h$ or $g$.

Simulation With Time-Varying [Ca]

To simulate contraction, the following time-varying $[Ca]$ forcing function was applied

$$[Ca](t) = \frac{t}{t_{Ca_{\text{max}}} + e^{-t/t_{Ca_{\text{max}}}}}$$

(A8)

where $[Ca]_{\text{max}}$, $t_{Ca_{\text{max}}}$, and $\tau$ are maximum $[Ca]$, time to $[Ca]_{\text{max}}$, and the parameter controlling the shape of the $[Ca]$ waveform, respectively. For all simulations (both control and acidic conditions), $[Ca]_{\text{max}} = 1.2 \mu M$, $t_{Ca_{\text{max}}} = 0.15 s$, and $\tau = 2.0$, which yielded a $[Ca]$ pulse with a physiologically realistic time course and amplitude (1, 36). With $[Ca]$, thus described, free Ca$^{2+}$ diminishes to zero well before the end of the cycle. With this assumption, the following initial conditions emerge

$$X_{\text{co}}(0) = X_{\text{co}}(0) = X_{\text{co}}(0) = 0$$

(A9)

For control conditions, values for model parameters were $X_t = 70 \mu M$ (40), $K_{an} = 40 s^{-1} \mu M^{-1}$ (40), $K_{df} = 135 s^{-1}$, $\beta = 0.035 \mu M^{-2}$, $g = 75 s^{-1}$, $h = 50 s^{-1}$, $f = 400 s^{-1}$, and $d = 400$

s$^{-1}$. $K_{or} = 0$ and $\beta$ values were chosen so that the experimentally observed pressure wave morphology was reproduced for the control state. The value of $g$ was based on the short-time scale left ventricular dynamics data (9, 44). The h-to-g ratio is $\frac{1}{2}$ (11, 54); this defined the value of $h$. The reversible transition between $X_{co}$ and $X_{an}$ is considered to be rapid, i.e., values of $f$ and $d$ should be large compared with $g$ or $h$. As a first approximation, we made $f$ and $d$ equal. The nonlinear system in Eqs. A1–A3 and A9 was integrated numerically (Runge-Kutta $\frac{1}{2}$ rule) over a 1.0-s interval using a 0.005-s step size.

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