Endothelin Reverses the Effects of Acidosis on the Intracellular Ca$^{2+}$ Transient and Contractility in Ferret Myocardium

Jianxun Wang and James P. Morgan

Endothelin may play an important role in modulating myocardial contractility under certain pathophysiological conditions. To determine whether endothelin beneficially modulates myocardial contractility in the common clinical condition of acidosis, we compared the effects of endothelin-1 on intracellular Ca$^{2+}$ transients and isometric contractions under normal (extracellular pH [pH$_e$] 7.4) and acidic (pH$_e$, 6.4) conditions in ferret papillary muscles (n=33) loaded with the Ca$^{2+}$-regulated bioluminescent indicator aequorin. A pH$_e$ of 6.4 was induced by replacing 92% of HCO$_3^-$ with Cl$^-$ in the bathing medium. The effects of endothelin at pH$_e$ 6.4 differed from the effects at pH$_e$ 7.4 in that 1) the minimally effective concentration of endothelin was 30-fold lower (1x10$^{-10}$ M at pH$_e$ 6.4; 3x10$^{-7}$ M at pH$_e$ 7.4) and the concentration-response curve of endothelin was significantly shifted to the left with a decrease in log EC$_{50}$ from $-7.83\pm0.13$ to $-8.92\pm0.10$ ($p<0.001$), indicating an increased sensitivity of myocardium to endothelin; 2) endothelin produced an increase of $\approx$375% in tension development at pH$_e$ 6.4 ($\approx$62% at pH$_e$ 7.4) ($p<0.001$) without increasing peak [Ca$^{2+}$]$_i$ ($\approx$13% increase at pH$_e$ 7.4, $p<0.001$), indicating an increase in myofilament Ca$^{2+}$ responsiveness; and 3) endothelin significantly attenuated (=-19%, $p<0.001$) the prolonged intracellular Ca$^{2+}$ transient induced by acidosis (pH$_e$ 6.4). In addition, pretreatment with 10 μM of the Na$^+-$H$^+$ exchange inhibitor 5-(N-methyl-N-isobutyl)-amiloride significantly attenuated endothelin-induced effects on the intracellular Ca$^{2+}$ transient and contraction during acidosis. Results indicate that 1) acidosis decreased myocardial Ca$^{2+}$ responsiveness and prolonged the intracellular Ca$^{2+}$ transient, whereas endothelin enhanced myofilament Ca$^{2+}$ responsiveness and abbreviated the intracellular Ca$^{2+}$ transient by decreasing intracellular H$^+$ via Na$^+$-$H^+$ exchange during acidosis; and 2) endothelin exerts its cardiotonic effects at much lower concentrations during acidosis, presumably due to altered behavior of the receptor. Taken together, these findings suggest that endothelin could beneficially reverse acidosis-induced negative inotropic and lusitropic effects on the intracellular Ca$^{2+}$ transient and myocardial contractility. (Circulation Research 1992;71:631–639)

**KEY WORDS** • endothelin-1 • Ca$^{2+}$ transient • contraction • acidosis • Na$^+$-$H^+$ exchange • aequorin

Endothelin, a vasoactive peptide released by endothelial cells, circulates in the plasma of animals and humans, and has been found to potentiate both animal and human myocardial contractions. The density of myocardial endothelin receptors is high, and endothelin gene expression has been documented in atrium and ventricle. The release of endothelin is increased under certain conditions, such as hypoxia, stretch, and in the presence of angiotensin II and thrombin. Increases in circulating endothelin have been reported in animals and humans with heart failure and in cases of cardiogenic shock. These reports suggest that endothelin, released by the microvascular endothelium and/or endocardium, may directly modulate myocardial contractility in physiological and pathophysiological conditions. Whether endothelin is beneficial or detrimental to myocardium in a given pathophysiological state remains speculative.

Myocardial ischemia, reperfusion, and reoxygenation increase endothelin binding to cardiac membranes. Receptor binding of endothelin is increased under acidic conditions. Acidosis is a common clinical abnormality that has been shown to affect the concentration–response relations of many agents. We were therefore interested in studying the effects of endothelin on the intracellular Ca$^{2+}$ transient and myocardial contractility under normal (extracellular pH [pH$_e$] 7.4) and acidic (pH$_e$, 6.4) conditions to determine whether endothelin may beneficially modulate myocardial contractility under acidic conditions and to provide additional information about the cellular mechanisms responsible for endothelin’s cardiotonic actions.
Materials and Methods

Experiments (n=33) were performed on papillary muscles isolated from the right ventricles of young adult male ferrets (age, 14–16 weeks; body weight, 1.0–1.4 kg). Muscles were loaded with the Ca\(^{2+}\)-regulated bio-luminescent indicator aequorin by a modified chemical loading procedure.\(^{24}\) Briefly, muscles were first exposed to an oxygenated solution composed of (mM) NaCl 120, KCl 5.9, glucose 11.5, NaHCO\(_3\) 25, NaH\(_2\)PO\(_4\)·H\(_2\)O 1.2, MgCl\(_2\)·6H\(_2\)O 1.2, CaCl\(_2\) 0.1, and EDTA 0.1 for 2–4 minutes at 25°C. Instead of immersing the muscles into aequorin solution, 1–1.5 μl aequorin solution (1 mg/ml) was then pressure injected into muscles, just beneath the endocardium, through a glass micropipette. CaCl\(_2\) was gradually increased to 2.0 mM within 40 minutes and the temperature of the bath was slowly returned to 30°C. EDTA-containing solution in the muscle bath was then replaced by physiological salt solution containing (mM): NaCl 120, KCl 5.9, glucose 11.5, NaHCO\(_3\) 25, NaH\(_2\)PO\(_4\)·H\(_2\)O 1.2, MgCl\(_2\)·6H\(_2\)O 1.2, and CaCl\(_2\) 2.0. The solution was bubbled continuously with a mixture of 95% O\(_2\)–5% CO\(_2\) at 30°C that had a pH of 7.4. Muscles were vertically mounted between a miniature clamp and an isometric force transducer (MBl/5514-02, Sensotec) with a 5–0 Tevdek thread. A narrow chamber at the base of the glass muscle bath extended a short distance axially into an ellipsoidal reflector, allowing the muscle to be positioned near one focal point of the reflector; a photomultiplier (Thorn-EMI 9635 QA, Gencorn Inc., Fairfield, N.J.) was mounted so that its cathode was at the other focal point.\(^{25}\) This symmetrical reflecting system has high optical efficiency (the minimum current that can be reliably detected is 0.07 nA and the resting aequorin light from all preparations in this study was above 0.5 nA) and minimizes motion artifacts. Light and force signals were recorded on a VHS video cassette recorder (A.R. Vetter Co., Rebersburg, Pa.), and a multichannel analyzer (model 4094A, Nicolet Instrument Corp., Madison, Wis.) was used for averaging aequorin light signals (usually 20–40) to obtain a satisfactory signal-to-noise ratio. One hour after the loading of aequorin, the muscle at optimal length (the length at which the greatest peak twitch tension developed) was stimulated to contract at 4-second intervals with 5-msec square-wave pulses applied through a punctate platinum electrode located at the base of the muscle. Before starting the experimental protocols, the muscle was stimulated to contract for 2 hours to reach stable function (i.e., stable Ca\(^{2+}\)-transient, resting and developed tension), and to discharge all the aequorin remaining in the extracellular space (the aequorin was quickly discharged by 10\(^{-3}\) M [Ca\(^{2+}\), in the extracellular space). The full discharge of the aequorin in the extracellular space was assumed when no further decrease in resting aequorin light was detected during a period of 50–60 minutes.

In those experiments in which pH\(_i\) was varied, 23 mM HCO\(_3\)\(^-\) was omitted from the solution and replaced by equimolar Cl\(^-\) (pH, 6.4). A decrease in extracellular HCO\(_3\)\(^-\) or an increase in CO\(_2\) concentration (15% CO\(_2\), 85% O\(_2\)) produces changes in pH\(_i\), and intracellular pH (pHi),\(^{26,28}\) We chose HCO\(_3\)\(^-\) rather than CO\(_2\) to alter pH, and pHi, because we found that 1) an increase in external CO\(_2\) concentration (15% CO\(_2\), 85% O\(_2\)) significantly decreases the oxygen partial pressure from 515±14 mm Hg (n=10) to 401±9 mm Hg (n=10, p<0.001) in the bathing medium, thereby producing hypoxic effects on the preparations; and 2) tension development recovers gradually over a long period of time (usually for 2 hours) in the presence of 15% CO\(_2\), which was not suitable for this study. A pH\(_i\) of 6.4 was chosen for acidosis because it mimics the pH value in ischemia.\(^{29,30}\) For converting aequorin light signals into quantitative intracellular calcium concentrations ([Ca\(^{2+}\)], the following equation\(^{21}\) was used: [Ca\(^{2+}\)]\(_i\) = ([(L/L\(_{max}\)]\(^{1/3}\) [131.0] − 1)/(4.5×10\(^{-6}\) [1−(L/L\(_{max}\)])\(^{1/3}\)]). L is the particular light signal averaged during twitches and L\(_{max}\) is the maximal light emission that would be obtained if all the aequorin in the cells were exposed to a saturating concentration of Ca\(^{2+}\). To obtain this L\(_{max}\), 1) the volume of the organ bath was reduced from 55 ml to 5 ml (enough volume to just cover the muscle), and the temperature of the solution in the organ bath was maintained at 30°C; 2) warmed physiological salt solution containing Triton X-100 and high [Ca\(^{2+}\)] was rapidly flushed into the organ bath (2.5% Triton X-100, 20 mM [Ca\(^{2+}\)]) to lyse the preparation and discharge the remaining aequorin; 3) the emitted light during lysis was integrated until complete discharge of the aequorin; and 4) the time integral of this light signal was then corrected by the rate constant for aequorin consumption (2.11/sec) determined in vitro.\(^{31}\) Complete discharge of aequorin was assumed when no further light emission was detected during a 10-minute period, and the background had fallen to the level of the dark current.

It is conceivable that endothelin-1 or its metabolites might diffuse into myocardial cells and interact directly with the loaded aequorin to alter the luminescent reaction or the responsiveness of aequorin to Ca\(^{2+}\).\(^{32}\) Therefore, endothelin-1 was tested in vitro using the basic method and calibration device described by Blinks et al.\(^{33}\) These tests showed that neither the Ca\(^{2+}\)-dependent light emission nor the Ca\(^{2+}\)-independent light emission were affected by endothelin-1, indicating that endothelin-1 does not interact directly with aequorin in a way that would alter the experimental results. In vitro results show that a fall in pH causes a slight reduction in the light emission from aequorin.\(^{25,32,33}\) This indicates that the detected aequorin light at pH, 6.4 may provide a slight underestimate of quantitative [Ca\(^{2+}\)]. However, because this study is concerned principally with the effects of endothelin-1 on intracellular Ca\(^{2+}\) and contraction under steady-state acidosis (pH, 6.4), the slight effect of changing pH on aequorin light emission would not alter the experimental results; therefore, we have not made any corrections for this effect.

Data are expressed as mean±SEM. Student’s t test was used for paired data from the same muscle or unpaired data from different muscles. Number of experiments is indicated by n.

Chemicals

Pure synthetic bioactive endothelin-1 (Sigma Chemical Co., St. Louis, Mo.) was dissolved in deionized water to a concentration of 8 μM and stored below −20°C until used. All of the endothelin-1 used in this study was from the same batch (lot 21H02181). 5-(N-methyl-N-isobutyl)-amiloride (MIA) was purchased from Research Biochemicals Inc., Natick, Mass. Aequorin was
Effects of Reducing pH from 7.4 to 6.4

In contrast to the effects of reducing pH with 15% CO₂, in which developed tension declined rapidly at first but then recovered more slowly to an intermediate level, reducing pH by decreasing [HCO₃⁻] produced a monophasic decline of tension development in this study that is consistent with the results of others. 

Figure 1 shows the continuous recording of intracellular Ca²⁺ transients and isometric contractions at pH 6.4. There was usually a substantial increase in the amplitude of intracellular Ca²⁺ transient within the first 20 minutes at pH 6.4. Intracellular Ca²⁺ transient and tension development reached steady state approximately 50 minutes after reducing pH from 7.4 to 6.4 and remained stable for at least 100 minutes. The averaged results from five experiments are shown in Figure 2. In this study, endothelin-1 intervention was conducted when no further changes in intracellular Ca²⁺ transient and tension development were detected during a period of 30–40 minutes, therefore excluding the possibility of the time-dependent recovery of tension development at pH 6.4.

The effects of reducing pH from 7.4 to 6.4 on the intracellular Ca²⁺ transient and isometric contraction are shown in Figure 3. Acidosis alone produced two effects on myocardium: 1) it decreased peak tension by 73.7±2.88% (p<0.001, n=22) without significantly affecting the amplitude of the intracellular Ca²⁺ transient (0.74±0.01 μM at pH 7.4; 0.76±0.01 μM at pH 6.4, p>0.1, n=16); and 2) it prolonged the intracellular Ca²⁺ transient, as indicated by the 25.0±1.1% increase (p<0.001, n=16) in the time course (time course of the Ca²⁺ transient was measured from the stimulus artifact to 50% decline of peak Ca²⁺). The time course of tension development did not significantly change.

Myocardial Responsiveness to Endothelin

Endothelin concentration–response curves (cumulative administration) were obtained for two separate groups of muscles (n=6 for each group) because endothelin could not be washed out. Doses of endothelin were added to the bathing medium when peak tension reached stable plateau values. Endothelin produced concentration-dependent increases in the amplitude of isometric contraction both at pH 7.4 and pH 6.4 (Figure 4). The effects of endothelin at pH 6.4 differed from the effects at pH 7.4 in that 1) the minimally effective concentration of endothelin was 30-fold lower (1×10⁻¹⁰ M at pH 6.4; 3×10⁻⁹ M at pH 7.4) and the maximally effective concentration was 3.3-fold lower (3×10⁻⁹ M at pH 6.4; 1×10⁻⁷ M at pH 7.4); 2) the concentration–response curve was significantly shifted to the left with a decrease in log EC₅₀ from −7.83±0.13 to −8.92±0.10 (p<0.001) (Figure 4, lower panel); and 3) the maximum mean increment of peak tension produced by endothelin at pH 6.4 was 407±60% (p<0.01), which was significantly greater (p<0.01) than the...
Effects of Ca\(^{2+}\) abolishment was not significant (Table 1). The divisions along the y axis are not arithmetic due to the nonlinear relation between the aequorin signal and [Ca\(^{2+}\)].

Effects of Endothelin on Peak [Ca\(^{2+}\)], and Peak Tension

At pH\(_{6.4}\), endothelin produced a much greater increase in tension development (Figure 4) compared with pH\(_{7.4}\). To determine whether the greater increase in tension development was caused by a greater increase in [Ca\(^{2+}\)], we compared the effects of endothelin on peak [Ca\(^{2+}\)], and peak tension at pH\(_{6.4}\) with those at pH\(_{7.4}\) (Table 1). Maximal effective concentrations of endothelin (based on the data in Figure 4) were chosen for this comparison. At pH\(_{7.4}\) (n=10), endothelin produced a 61.9±8.4\% increase in tension development (p<0.001) and a 13.2±2.6\% increase in peak [Ca\(^{2+}\)] (p<0.001), whereas at pH\(_{6.4}\) (n=10), endothelin produced a 375.4±40.9\% increase in tension development (p<0.001) without increasing the peak [Ca\(^{2+}\)] (p>0.4). The results in Table 1 suggest that 1) the greater increases in tension development by endothelin at pH\(_{6.4}\) (Figure 4) were predominantly caused by enhanced myofilament Ca\(^{2+}\) responsiveness, and 2) the action of endothelin to increase peak [Ca\(^{2+}\)] at pH\(_{7.4}\) was abolished at pH\(_{6.4}\).

Effects of Endothelin on Time Courses of Ca\(^{2+}\) Transient and Contraction

Figure 5 shows typical tracings of the effect of 3×10^{-8} M endothelin at pH\(_{6.4}\), and demonstrates that maximum mean increment of 64±14\% (p<0.01) at pH\(_{7.4}\). In addition, all values (absolute and percent increments) along the concentration–response curve at pH\(_{6.4}\) were significantly greater (p<0.01) than the corresponding values at pH\(_{7.4}\). From the results in Figure 4, it is evident that both the efficacy (i.e., the maximal response shown in the upper and middle panels) and the potency (i.e., the relative effective doses shown in the lower panel) of endothelin were enhanced at pH\(_{6.4}\), suggesting an increased myocardial responsiveness to endothelin (presumably due to the altered behavior of the endothelin receptor).

Whereas the intracellular Ca\(^{2+}\) transient was abbreviated, the contraction was enhanced and prolonged. To illustrate these changes more clearly and to compare the changes at pH\(_{6.4}\) with those at pH\(_{7.4}\), we 1) summarized the averaged change of time courses in Table 2, and 2) superimposed responses with and without endothelin at the two different pH\(_{6.4}\) after

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**Figure 3.** Tracings showing effects of extracellular pH (pH\(_{6.4}\)) on the intracellular Ca\(^{2+}\) transient (upper panel) and contraction (lower panel). Stimulation interval was 4 seconds at 30°C. The resting [Ca\(^{2+}\)] is 0.22 μM. The divisions along the y axis are not arithmetic due to the nonlinear relation between the aequorin signal and [Ca\(^{2+}\)]. Tracings are from one of 16 similar experiments.

**Figure 4.** Graphs comparing endothelin-1 concentration–response curves at extracellular pH (pH\(_{6.4}\)) 6.4 and pH\(_{7.4}\). Separate groups (n=6 for each group) of muscles were used for comparison because endothelin could not be washed out. Upper panel: Absolute changes from control; middle panel: percent changes from control; lower panel: normalized changes (the maximum changes at pH\(_{6.4}\) 6.4 or pH\(_{7.4}\) were considered as 1.0 and other changes were referred to 1.0). The control values (before endothelin) at pH\(_{6.4}\) and pH\(_{7.4}\) were 20.1±2.1 mN/mm\(^2\) and 5.2±1.2 mN/mm\(^2\), respectively. *p<0.05 vs. control values (paired t test); #p<0.01 vs. corresponding values at pH\(_{6.4}\) 7.4 (unpaired t test).
Table 1. Effects of Endothelin-1 on Peak [Ca²⁺]i and Peak Tension at Extracellular pH 6.4 and 7.4

<table>
<thead>
<tr>
<th>pH  pH 6.4 (n=10)</th>
<th>Before</th>
<th>After</th>
<th>Change</th>
<th>[Ca²⁺]i (μM)</th>
<th>Before</th>
<th>After</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ca²⁺]i</td>
<td>5.3±0.9</td>
<td>23.6±2.7</td>
<td>37.5±40.9%*</td>
<td>0.76±0.02</td>
<td>0.75±0.02</td>
<td>-0.60±0.69%</td>
<td></td>
</tr>
<tr>
<td>[Ca²⁺]i</td>
<td>19.5±2.1</td>
<td>31.3±3.3</td>
<td>61.9±8.4%*</td>
<td>0.73±0.02</td>
<td>0.82±0.02</td>
<td>13.2±2.6%*</td>
<td></td>
</tr>
</tbody>
</table>

Before, before endothelin; After, after adding the maximally effective concentration of endothelin (3×10⁻⁸ M at extracellular pH 6.4 and 1×10⁻⁷ M at extracellular pH 7.4); Change, percent changes; pH, extracellular pH. Data are mean±SEM. Separate groups of muscles were compared because endothelin could not be washed out.

*p<0.001, vs. the values before endothelin (paired t test); †p<0.001 vs. the corresponding values at pH 7.4 or pH 6.4 (unpaired t test).

Adjusting the gains electronically to give tracings of the same amplitude (Figure 6). At pH 7.4 (n=10), endothelin did not abbreviate the Ca²⁺ transient (0.2±0.4%, p>0.4), although it produced a minor prolongation in the contraction (10.3±0.3%, p<0.001, Table 2); at pH 6.4 (n=10), endothelin clearly abbreviated the Ca²⁺ transient (−18.8±1.2%, p<0.001) while producing more pronounced prolongation of the contraction (13.4±0.6%, p<0.001, Table 2). Note that the longer intracellular Ca²⁺ transient in Figure 6B was caused by acidosis alone (see Figure 3). Moreover, at pH 6.4, there was a close relation between the degree of abbreviation of the Ca²⁺ transient and the degree of prolongation of the contraction in the same preparation after the administration of endothelin (Figure 7, upper panel). This close relation also existed in different preparations in the presence of 3×10⁻⁸ M endothelin (Figure 7, lower panel). The equation for the linear regression in the lower panel of Figure 7 is y=0.98+0.67x (r=0.92). The abbreviated intracellular Ca²⁺ transient associated with a prolongation of contraction (Figures 6B and 7, Table 2) suggests that endothelin potentiated the Ca²⁺ binding to troponin C during intracellular acidosis, which may account for the enhanced myofilament Ca²⁺ responsiveness at pH 6.4 (see “Discussion”).

Influence of MIA on the Effects of Endothelin at pH 6.4

To test the hypothesis that the reversal of intracellular acidosis by endothelin was related to alkalinization via the Na⁺-H⁺ exchanger of the sarcoplasm, comparisons were made of the effects of endothelin at pH 6.4 in the presence and absence of the Na⁺-H⁺ exchange inhibitor MIA, which is reported to be the most potent Na⁺-H⁺ exchange inhibitor of the amiloride derivatives.34-36 MIA (10 μM) was added to the bath medium when intracellular Ca²⁺ transient and tension development reached steady state at pH 6.4. MIA (10 μM) produced a slight decrease in tension development without decreasing the amplitude of intracellular Ca²⁺ transient. Pretreatment with MIA (10 μM) significantly attenuated endothelin-induced changes in the intracellular Ca²⁺ transient and isometric contractions (Figure 8), suggesting that intracellular alkalinization is the main mechanism underlying endothelin-induced recovery of myocardial contractility during acidosis.

Discussion

Previous studies have revealed that acidosis markedly decreases myofilament Ca²⁺ sensitivity in skinned fibers37,38 and aequorin-loaded intact fibers.27,28,39 Our result that a significant decrease in tension development was associated with a slight increase in peak [Ca²⁺], (Figure 3) is consistent with this conclusion. A likely explanation of the decrease of tension development during acidosis is that hydrogen ions (H⁺) could compete directly with Ca²⁺ for a site on troponin C, thereby decreasing the Ca²⁺ affinity of troponin C. Fuchs et al40 and Potter et al41 have found evidence for such direct competition. The prolonged intracellular Ca²⁺ transient (Figure 3) suggests that acidosis may also impair sarcoplasmic reticular Ca²⁺ uptake, which has been documented previously.28,37,42 Consistent with the results of others,27,28,37-39,42 acidosis produced two detrimental effects on myocardial contractility: 1) it decreased myofilament Ca²⁺ responsiveness, and 2) it prolonged the intracellular Ca²⁺ transient. The present study shows that endothelin can reverse acidosis-induced negative inotropic and lusitropic effects on myocardial contractility, as discussed below.

Sensitivity of Myocardium to Endothelin

One important finding of this study is that, compared with pH 7.4, the potentcy and efficacy of endothelin were significantly enhanced at pH 6.4, indicated by the leftward and upward shifts of the concentration-response curve (Figure 4). The leftward shift of the concentration-response curve (Figure 4, lower panel) suggests that binding of endothelin to its receptors was

![Figure 5. Tracings showing effects of 3x10^-8 M endothelin-1 (ET) on aequorin light signal (upper panel) and contraction (lower panel) of a ferret papillary muscle at extracellular pH 6.4 (from one of 10 similar experiments). Stimulation interval was 4 seconds at 30°C. The resting [Ca^2+], is 0.22 uM. The divisions along the y axis are not arithmetic due to the nonlinear relation between the aequorin signal and [Ca^2+].](image-url)
TABLE 2. Effects of Endothelin-1 on Time Courses of Ca\(^{2+}\) Transient and Contraction at Extracellular pH 6.4 and 7.4

<table>
<thead>
<tr>
<th>pH0</th>
<th>Time course of Ca(^{2+}) transient (msec)</th>
<th>Time course of contraction (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>pH6.4 (n=10)</td>
<td>120.0±2.3</td>
<td>97.5±1.9</td>
</tr>
<tr>
<td>pH7.4 (n=10)</td>
<td>97.0±2.0</td>
<td>97.2±1.8</td>
</tr>
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</table>

Before, before endothelin. After, after adding the maximally effective concentration of endothelin (3×10⁻⁸ M at extracellular pH 6.4 and 1×10⁻⁷ M at extracellular pH 7.4); Change, percent changes; pH0, extracellular pH. Data are mean±SEM. Separate groups of muscles were compared because endothelin could not be washed out. Time course of Ca\(^{2+}\) transient (or contraction) was measured from the stimulus artifact to 50% decline of peak Ca\(^{2+}\) (or peak tension).

*p<0.001 vs. the values before endothelin (paired t test); †p<0.001 vs. the corresponding values at pH7.4 (unpaired t test).

increased under acidotic conditions. This conclusion is supported by the results of Liu et al.,²² which indicated that the receptor binding of endothelin was increased as the pH was decreased from 10 to 4. Increased receptor binding of endothelin may be responsible for the enhanced efficacy of endothelin at pH 6.4 (Figure 4, upper and middle panels), but actions beyond the level of receptors, e.g., increased Ca\(^{2+}\) binding to troponin C via Na\(^+\)-H\(^+\) exchanger, may be also involved, as discussed below.

**Mechanism of the Reversal of Acidosis**

Another important finding of this study is that acidosis decreased myofilament Ca\(^{2+}\) responsiveness and prolonged the intracellular Ca\(^{2+}\) transient, whereas endothelin enhanced myofilament Ca\(^{2+}\) responsiveness and abbreviated the intracellular Ca\(^{2+}\) transient during acidosis. At pH 6.4, endothelin produced =375% mean increase in tension development without significantly increasing peak [Ca\(^{2+}\)] (Table 1), indicating an increased myofilament Ca\(^{2+}\) responsiveness. As reported previously,⁵ endothelin enhances myofilament Ca\(^{2+}\) responsiveness, but also increases peak [Ca\(^{2+}\)], at pH 7.4. At pH 6.4, the increments (=375%) of tension development produced by endothelin were much greater than those (=62%) at pH 7.4, whereas increases of peak [Ca\(^{2+}\)] did not occur (Table 1 and Figure 5).

The results shown in Figures 6B and 7 and Table 2 suggest that an increase in the Ca\(^{2+}\) affinity of troponin C might be involved in endothelin's action, which may account for the abbreviated Ca\(^{2+}\) transient and might have prolonged and enhanced contraction at pH 6.4. It has frequently been suggested that in-

![Figure 6](image_url)

**Figure 6.** Tracings comparing effects of endothelin-1 (ET) on the time courses of the Ca\(^{2+}\) transient and contraction of ferret papillary muscles at extracellular pH (pH0) 6.4 and pH7.4. Amplitudes of recordings have been electronically adjusted to facilitate comparison of their time courses. Panel A: at pH7.4, 1×10⁻⁷ M endothelin (maximum concentration), from one of 10 similar experiments; Panel B: at pH6.4, 3×10⁻⁸ M endothelin (maximum concentration), from one of 10 similar experiments. Stimulation interval was 4 seconds at 30°C.

![Figure 7](image_url)

**Figure 7.** Correlation between the degree of abbreviation of the intracellular Ca\(^{2+}\) transient and the degree of prolongation of the contraction induced by 3×10⁻⁸ M endothelin-1 at extracellular pH 6.4. Time course of Ca\(^{2+}\) transient (or contraction) was measured from the stimulus artifact to 50% decline of peak Ca\(^{2+}\) (or peak tension). Upper panel: Graph showing data from the same muscle taken 5, 10, 15, 25, 30, 35, 45, and 55 minutes after administration of endothelin. Lower panel: Scatterplot showing four or five data points from each of 10 muscles taken at different time intervals after administration of endothelin.
creased Ca\(^{2+}\) binding to troponin C will abbreviate the Ca\(^{2+}\) transient but prolong the contraction.\(^{45-47}\) Troponin C may be the most important Ca\(^{2+}\) sink in myocardial cytoplasm and binds Ca\(^{2+}\) almost as rapidly as it becomes available.\(^{48}\) Therefore, it would be expected that 1) an increase in the affinity of troponin C for Ca\(^{2+}\) would enhance binding and slow dissociation of Ca\(^{2+}\) from the regulatory sites, and 2) the sarcoplasmic reticulum should be able to lower the peak [Ca\(^{2+}\)] more rapidly because troponin C holds on to its Ca\(^{2+}\) more avidly. The combination of these two actions would abbreviate the Ca\(^{2+}\) transient and enhance and prolong the contraction, as observed in Figures 5, 6B, and 7 and Table 2. It is unlikely that changes of the function in sarcoplasmic reticulum Ca\(^{2+}\) release or uptake\(^{48,49,50}\) or Na\(^+-\)Ca\(^{2+}\) exchange\(^{49,50}\) were responsible for the changes of time course in Figures 5, 6B, and 7 because such changes would not produce the paradoxical effects on twitch duration, as opposed to the duration of the Ca\(^{2+}\) transient shown in Figures 5, 6B, and 7.

During acidosis, the increased intracellular H\(^+\) could compete directly with Ca\(^{2+}\) for a site on troponin C,\(^{40,41}\) decreasing Ca\(^{2+}\) binding to troponin C and, consequently, force generation. In contrast, endothelin could stimulate the sarcolemmal Na\(^+-\)H\(^+\) exchange in rat cardiac cells, resulting in a decrease in [H\(^+\)]\(_i\) (intracellular alkalization).\(^{51}\) Our results show that pretreatment with 10 \(\mu\)M MIA significantly attenuated endothelin-induced effects on the intracellular Ca\(^{2+}\) transient and contraction at pH\(_i\) 6.4 (Figure 8), indicating that the Na\(^+-\)H\(^+\) exchange was involved in endothelin's actions during acidosis. A decrease in [H\(^+\)]\(_i\) would decrease the competition of H\(^+\) with Ca\(^{2+}\), thereby increasing Ca\(^{2+}\) binding to troponin C and enhancing force generation. This may be the major mechanism by which endothelin reverses acidosis-induced effects on the intracellular Ca\(^{2+}\) transient and myocardial contractility. As indicated by the results in Figures 6 and 8, endothelin did not abbreviate the intracellular Ca\(^{2+}\) transient at pH\(_i\) 7.4, and its action was not completely eliminated by the Na\(^+-\)H\(^+\) exchange inhibitor MIA. This suggests that other mechanisms were involved in endothelin's myocardial actions, such as direct actions on myosin and actin, because endothelin could produce concentration-dependent increases in the myosin ATPase activity in cardiac cells.\(^{52}\)

**Pathophysiological Implications**

Our studies suggest that endothelin could modulate myocardial contractility not only in physiological but also in pathophysiological situations, such as acidosis. In favor of an important pathophysiological role of endothelin during acidosis are the observations that 1) the minimally effective concentration of endothelin was 30-fold lower and the EC\textsubscript{50} was 12.3-fold lower than at pH\(_i\) 7.4; 2) acidosis decreased myofilament Ca\(^{2+}\) responsiveness and prolonged the intracellular Ca\(^{2+}\) transient that could be detrimental to myocardial contractility, whereas endothelin enhanced myofilament Ca\(^{2+}\) responsiveness and abbreviated the intracellular Ca\(^{2+}\) transient during acidosis; and 3) endothelin did not increase either the resting or the peak [Ca\(^{2+}\)] during acidosis. Unlike many other Ca\(^{2+}\)-dependent inotropic agents that act predominantly to increase [Ca\(^{2+}\)], endothelin is unique in that it greatly potentiates myocardial contractility without increasing [Ca\(^{2+}\)], during acidosis. Therefore, during acidosis the locally produced endothelin may be beneficial to myocardium. Since the effective concentrations of endothelin in myocardium also produce vasoconstricting effects in smooth muscle,\(^{14,53,54}\) the use of endothelin as a therapeutic agent should be precluded for the time being. An especially exciting feature of endothelin is the possibility that one of its analogues may have more potent and selective effects on myocardium than on blood vessels; in this case a very low concentration of endothelin analogue may be of therapeutic benefit in conditions associated with acidosis, such as ischemia.

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