Influence of Acidosis on Cardiotonic Effects of Milrinone

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Background: The present study was designed to determine whether augmentation of cardiac performance by milrinone is affected by acidosis in in vitro canine and in vitro guinea pig preparations, and to elucidate a mechanism in relation to the cyclic adenosine monophosphate (cAMP) formation.

Methods: Halothane-anesthetized, ventilated dogs were randomly assigned to a control group (arterial pH [pHa] = 7.4, base excess [BE] > -2 mm; n = 7), mild acidosis group (pHa = 7.2, BE < -9 mm; n = 7); or severe acidosis group (pHa < 7, BE < -20 mm; n = 6). Arterial blood pressure, left ventricular pressure (including maximum rate of increase, 1V dp/dtmax), and pulmonary blood flow (PBF) were measured. Acidosis was induced by transient hypoxia and maintained with hydrogen chloride infusion. Hemodynamic responses to milrinone infusions at 2 and 5 μg·kg⁻¹·min⁻¹ were then studied. In addition, left atria and right ventricular strips were dissected from guinea pig hearts and suspended in HEPES-Tyrode solution, with pH values adjusted to 7.4, 7, or 6.6. The concentration–response relation of isometric contractions for milrinone (10⁻⁷ to 10⁻³ M) and 8-bromo-cAMP (10⁻⁴ to 10⁻³ M) were determined.

Results: In the control group of dogs, significant increases in 1V dp/dtmax (2.67 ± 0.82 to 3.999 ± 1.016 mmHg/s [means ± SD]) and PBF (2.04 ± 0.98 to 2.44 ± 0.96 l/min [means ± SD]) were seen with a milrinone infusion of 5 μg·kg⁻¹·min⁻¹. In the mild acidosis group, 5 μg·kg⁻¹·min⁻¹ milrinone also increased 1V dp/dtmax and PBF. However, neither 1V dp/dtmax nor PBF changed in the severe acidosis group. In in vitro experiments, milrinone exerted a positive inotropic effect in a concentration-dependent manner on the right ventricular preparations at pH 7.4, but not at pH 7 and 6.6, whereas no significant difference was observed in isometric responses to 8-bromo-cAMP at pH values of 6.6, 7, and 7.4 on the right ventricular strips. In the right ventricular in vitro preparation, 10⁻⁷ M milrinone was accompanied by a significant increase in intracellular cAMP content at a pH of 7.4 but not 7.

Conclusions: These results indicate that the inotropic effect of milrinone is attenuated by acidosis due, at least in part, to decreased cAMP formation in acidic muscle. (Key words: Cyclic adenosine monophosphate; phosphodiesterase III inhibitor; positive inotropy.)

MILRINONE, a bipyridine derivative, is a new agent with both inotropic and vasodilatory effects.1 It is structurally unrelated to the catecholamines or the digitalis glycosides. Its inotropic effect results mainly from increased concentrations of intracellular cyclic adenosine monophosphate (cAMP) by specifically inhibiting phosphodiesterase isozyme III (PDE III), thereby enhancing protein phosphorylation and ultimately augmenting intracellular calcium availability at the myofilaments.1 In contrast to catecholamines, the PDE III inhibitor milrinone has the unique ability to improve hemodynamic states by virtue of inotropic and vasodilatory effects.1 In addition, milrinone has been shown to improve hemodynamic states associated with altered β-adrenergic responsiveness, such as in patients with acute heart failure due to myocardial infarction2 and with low output syndrome after cardiac surgery.3,4 These clinical situations are frequently accompanied by metabolic acidosis of varying degree, and the correction of severe acid-base disturbances may first be considered to improve basal hemodynamic status and to restore cardiovascular responsiveness to inotropic agents, as seen with various catecholamines.5–9 Although the cause of the diminished myocardial response to catecholamines during acidosis is not completely understood, several findings suggest that it is due to decreased β-adrenoceptor numbers10 or affinity for agonists,11 decreased cAMP production as the result of depressed adenylyl cyclase activ-

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Received from School of Medicine, Akita University, Akita; the Institutes of Basic Medical Science and Clinical Medicine, University of Tsukuba, Tsukuba, Japan. Submitted for publication April 18, 1997. Accepted for publication October 23, 1997. Presented in part at the annual meeting of the American Society of Anesthesiologists, October 21–25, 1995, Atlanta, Georgia.

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Anesthesiology, V 88, No 3, Mar 1998
ity, inhibition of calcium ion exchange, decreased affinity of calcium ion to myofilaments, or all of these.

Because the final common pathway for improved inotropy is similar for catecholamines and PDE III inhibitors, we speculated that severe acidosis may also attenuate the improvement of cardiac performance in response to milrinone by a mechanism similar to that seen with catecholamines. Accordingly, the present investigation was designed to test this hypothesis in an in vitro canine preparation. In addition, to examine myocardial contractility independent of the influence of preload, afterload, and heart rate (HR) and to elucidate the mechanism of pH-induced modulation of contractile function and the possible contribution of cAMP production, positive inotropic responses to milrinone at several pH values were examined in an isolated guinea pig atrial and right ventricular preparation.

Methods

Preparations for In Vivo Experiments

The study protocol was approved by our institutional animal care committee. Animal preparations were made as described previously. Briefly, adult mongrel dogs of either sex (weighing 7.5–17.5 kg) were anesthetized with 15 mg/kg intravenous thiamylal and the trachea was intubated. The lungs were mechanically ventilated with room air supplemented with oxygen through a cuffed tracheal tube using a volume-cycled ventilator (model R-60; Aika Co., Ltd, Tokyo). Anesthesia was maintained with halothane (end-tidal concentrations, 0.65–0.75%) in oxygen and air. End-tidal carbon dioxide and halothane concentrations were measured continuously (Capnomac Ultima, DATEX; Helsinki, Finland). Succinylcholine chloride (20 mg) was injected intravenously at the time of tracheal intubation followed by continuous infusion of 10 mg/h thereafter. During all measurements, pulmonary arterial blood temperature was maintained between 35.8 and 38°C. Lactated Ringer’s solution was administered constantly at a rate of 10 ml·kg⁻¹·h⁻¹ during the surgical preparation, followed by 5 ml·kg⁻¹·h⁻¹ during the equilibration period and hemodynamic measurements.

An intravenous cannula was inserted into a forelimb vein to administer lactated Ringer’s solution. Lead II electrocardiograms were taken to continuously monitor heart activity (model 2236A; NEC San-ei Instruments Co., Ltd, Tokyo) using subcutaneous electrodes. Heart rate was measured by a cardiotachometer (model 1321; NEC San-ei Instruments Co.) triggered by lead II of the electrocardiogram. Arterial pressure measurement and blood sampling for blood gas analysis, and measurements of electrolytes and hemoglobin concentration (288 Blood Gas System; Ciba-Corning, Medfield, MA) were performed using a right femoral arterial catheter. A flow-directed, balloon-tipped pulmonary artery catheter (5 French Thermodilution Catheter; Arrow, Reading, PA) was inserted into the right external jugular vein to permit continuous monitoring of pulmonary artery pressure. Another catheter was positioned in the right atrium from the right femoral vein to monitor right atrial pressure. A 5-French-gauge catheter-tipped transducer (model MPC-500; Miller Instruments, Houston, TX) was inserted through the left femoral artery and positioned in the left ventricle to obtain left ventricular end-diastolic pressure (LVEDP) and maximum rate of increase in the left ventricular pressure (LV dP/dtmax) using a differential amplifier (model 1323; NEC San-ei Instruments Co.).

After the pulmonary trunk was dissected out through a left-sided thoracotomy in the fourth intercostal space, an electromagnetic flow probe of appropriate size (model FR 12–16 mm; Nihon-Koden Co., Ltd, Tokyo, Japan) was placed around the pulmonary trunk for continuous measurement of pulmonary blood flow (PBF) measured with a square-wave electromagnetic blood flowmeter (model MFV-5200, Nihon-Koden Co., Ltd.). All recordings were made on the eight-channel recorder (model RECTHORIZ8K, NEC San-ei Instruments Co.).

Establishment and Maintenance of Acidosis

The dogs were randomized to one of three groups: the control group, the mild acidosis group, or the severe acidosis group. After a stabilization period of at least 1 h after surgical procedures, dogs in the mild and severe acidosis groups were ventilated with room air and nitrogen so that the fractional inspired oxygen concentrations were 0.08–0.12. They were kept on the hypoxic gas mixture until the target acidosis (base excess less than −9 mm [range, −9 to −11.4]; pH ≈ 7.2 in the mild acidosis group; base excess less than −20 mm [range, −21.3 to −23.7], pH < 7 in the severe acidosis group) were verified by frequent arterial blood gas determinations. Typically, 2 and 3 h were needed to induce mild and severe metabolic acidosis, respectively. After the target acidosis was established, dogs were ventilated with air and oxygen so that arterial oxygen saturation was >97%. Hydrochloric acid (2 N) was ad-
ministered continuously at a rate of 5–15 ml/h for the mild acidosis group and 6–20 ml/h for the severe acidosis group, to maintain acidosis for 1 h as an equilibration period. The partial pressure of carbon dioxide in arterial blood was maintained between 37–43 mmHg throughout the experiment.

**Measurement of Hemodynamic Variables**

When stable arterial blood gas data were verified by serial analyses and stable hemodynamic variables were obtained, continuous infusion of milrinone was started through the right atrial catheter at a rate of 2 or 5 \( \mu g \cdot kg^{-1} \cdot min^{-1} \) for 1 h. In the control group, a 3-h equilibration period was ensured before starting the milrinone infusion. Milrinone was prepared in a concentration of 1 mg/ml. Hemodynamic variables including HR, mean arterial pressure, mean pulmonary artery pressure, right atrial pressure, LVEDP, LV dp/dtmax, and PBF were measured immediately before starting the milrinone infusion (baseline values), and at the completion of each dose. In addition, systemic and pulmonary vascular resistance were calculated using standard formulas. Ventilator settings were kept constant during these measurements.

**Measurement of Cardiac Contraction In Vitro**

Male albino guinea pigs (400–580 g) were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally), and the hearts were quickly removed and immersed in ice-cold HEPES-Tyrode solution adjusted for pH 7.4. The Tyrode solution was composed of 137 mm Na\(^+\), 4 mm K\(^+\), 1.8 mm Ca\(^2+\), 1 mm Mg\(^2+\), 148.6 mm Cl\(^-\), 10 mm HEPES (N-2-hydroxyethylpiperazine-N-2-ethanesulphonic acid), and 5.5 mm glucose; and NaOH was added to reach the appropriate pH while the total concentration of Na\(^+\) was maintained at a constant level.

The isometric contractions of the left atrium and a piece (1 × 15 mm) of the right ventricular wall were measured with a force displacement transducer (model TB-612T; Nihon Kohden) connected to an amplifier (model AP-601G; Nihon Kohden), as described previously. Briefly, the isolated preparations were suspended by threads in organ baths containing HEPES-Tyrode solution (30 ml) maintained at 37°C and aerated with 100% oxygen, placed between a pair of platinum electrodes, and stimulated electrically at a rate of 2 Hz by suprathreshold (1.5 × threshold) square-wave pulses lasting 1 ms generated through a Nihon Kohden SEN-7103 stimulator. The resting tensions were applied at L\(_{max}\) (approximately 1 g for left atria and 0.5 g for ventricular strips). Tensions were recorded using a thermal pen recorder (model WT687G; Nihon Kohden). All preparations were allowed to equilibrate in HEPES-Tyrode solution adjusted for pH 7.4, 7, or 6.6 for at least 1 h before drug applications and allowed to respond to each concentration of milrinone for 3 min. This time interval represented a peak contractile effect for most concentrations of milrinone in our experiment. The concentration–response relation of isometric contractions for milrinone were studied between 10\(^{-7}\) and 10\(^{-4}\) M based on the information from previous similar studies. Higher concentrations of milrinone were not studied because the vehicle associated with higher concentrations contained sufficient lactate to decrease the pH of HEPES-Tyrode solution.

**Determination of Cyclic Adenosine Monophosphate Content**

The cAMP content in the right ventricle was measured as described previously. Briefly, the isolated right ventricular strips were mounted in an organ bath and electrically driven as described before. When the maximum response to 10\(^{-4}\) M milrinone was obtained, the preparation was taken out of the bath and frozen by immersion into liquid nitrogen within 4 s. For the control, vehicle-treated tissues were allowed to respond for 4 min before immersion into liquid nitrogen. The frozen samples were homogenized in 1 ml of 6% trichloroacetic acid and centrifuged at 2,000 g for 15 min at 4°C. The supernatants were extracted four times with 5 ml water-saturated diethyl ether. The upper ether layer was discarded after each wash. The remaining aqueous extract was dried under a stream of nitrogen at 60°C. The dried extracts were reconstituted in a suitable volume of assay buffer consisting of 0.05 M acetate buffer with 0.02% bovine serum albumin and 0.005% thimerosal (pH 5.8). The amount of cAMP was quantitated in duplicate by enzyme immunoassay with commercially available kits (cAMP enzyme immunosassay system; Amersham Life Science, Buckinghamshire, UK), and the mean of two determinations was used for data analyses and statistical comparison. The results were expressed as picomoles of cAMP per wet weight of the tissues.

**Materials and Statistics**

Milrinone was provided by Yamanouchi Pharmaceutical Co. (Tsukuba, Japan). The vehicle contained 70 mg lactic acid and 380 mg glucose in 10 ml distilled water with NaOH adjusted for pH 3.5. 8-bromo-cAMP was purchased from Sigma Chemical Company (St. Louis,
Table 1. Arterial Blood Gas Values, Electrolytes, Hemoglobin Values, and Fractional Inspired Oxygen and End-Tidal Halothane Concentrations

<table>
<thead>
<tr>
<th></th>
<th>Control Group (n = 7)</th>
<th>Mild Acidosis Group (n = 7)</th>
<th>Severe Acidosis Group (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.42 ± 0.03</td>
<td>7.23 ± 0.02</td>
<td>6.98 ± 0.02</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td>38 ± 4</td>
<td>40 ± 2</td>
<td>41 ± 2</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>126 ± 40</td>
<td>120 ± 34</td>
<td>111 ± 10</td>
</tr>
<tr>
<td>Base excess (mm)</td>
<td>0.3 ± 1.1</td>
<td>-10.3 ± 1.1</td>
<td>-22.5 ± 0.9</td>
</tr>
<tr>
<td>Na (mEq/L)</td>
<td>145 ± 6</td>
<td>143 ± 4</td>
<td>142 ± 3</td>
</tr>
<tr>
<td>K (mEq/L)</td>
<td>3.2 ± 0.5</td>
<td>3.5 ± 0.5</td>
<td>3.5 ± 0.5</td>
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<tr>
<td>Ca²⁺ (mm)</td>
<td>1.0 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>1.1 ± 0.1</td>
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<tr>
<td>Hemoglobin (g/dl)</td>
<td>15.7 ± 2.1</td>
<td>14.5 ± 1.8</td>
<td>15.2 ± 1.5</td>
</tr>
<tr>
<td>FiO₂</td>
<td>0.42 ± 0.05</td>
<td>0.45 ± 0.11</td>
<td>0.50 ± 0.10</td>
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<tr>
<td>End-tidal halothane (%)</td>
<td>0.69 ± 0.06</td>
<td>0.69 ± 0.02</td>
<td>0.69 ± 0.05</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

*P < 0.01 versus the other two groups.

MO). All values are expressed as means ± SD. Analysis of changes from baseline values and comparisons of the data between groups were performed using one-way and two-way analysis of variance, respectively, followed by Bonferroni’s multiple comparison. P < 0.05 was considered the minimum level of statistical significance.

Results

Arterial Blood Gas Data

Arterial blood gas analysis before milrinone infusion revealed significant differences in pH, and base excess among the three groups (P < 0.01), whereas there were no significant differences in carbon dioxide tension, oxygen tension, electrolyte values, inspired oxygen and end-tidal halothane concentrations, and hemoglobin concentration (table 1). No significant intragroup changes were found in pH, oxygen tension, carbon dioxide tension, base excess, electrolyte values, inspired oxygen and end-tidal halothane concentrations, and hemoglobin concentration before milrinone infusion and at the completion of two milrinone infusion rates (data not shown).

Hemodynamic Changes Due to Milrinone Infusion

Table 2 shows the hemodynamic responses to milrinone infusion in the control, the mild acidosis, and the severe acidosis groups. There was no significant difference in baseline hemodynamic variables except for mean arterial pressure between the control and severe acidosis groups. Intravenous infusion of milrinone produced significant increases in LV dp/dtmax, PBF, and HR, and significant decreases in LVEDP and systemic vascular resistance in the control and mild acidosis groups, whereas these variables were unchanged in the severe acidosis group. Dose-dependent increases in HR and PBF and decreases in LVEDP and systemic vascular resistance were seen in the control group, whereas in the mild acidosis group only LVEDP and systemic vascular resistance showed dose-dependent decreases. Mean arterial pressure in the severe acidosis group was decreased by milrinone, although milrinone even at the higher dose did not cause any significant changes in mean arterial pressure in the control and mild acidosis groups. Increases in LV dp/dtmax from baseline values at both milrinone infusion rates, 2 and 5 μg·kg⁻¹·min⁻¹, in the severe acidosis group were significantly less than those of the control and mild acidosis groups (P < 0.05; fig. 1).

Positive Inotropic Responses to Milrinone and 8-bromo-cyclic Adenosine Monophosphate

As shown in figure 2, milrinone produced a positive inotropic response in both isolated right ventricular and left atrial preparations in a concentration-dependent manner at pH 7.4. At pH 7.7, the effect of milrinone was also produced in the left atrial preparation, whereas milrinone produced no significant increase in isometric contractions in the right ventricular preparation. At pH 6.6, no significant changes in isometric contractions were elicited by milrinone at concentrations up to 10⁻⁴ M in both preparations. The increase in isometric contractions at pH 7.4 in the right ventricular strips was significantly greater than those at pH 7.6 and 6.6 at concentrations of 10⁻⁶ M and higher. In the left atrial preparation, the response to milrinone at pH 7.4 was significantly greater than that at pH 6.6 at concentrations of 3 × 10⁻⁶ M and higher, but no significant difference was seen in the concentration–response relations between pH 7.4 and 7. In either preparation, the maximal responses to milrinone were not measured in the present study, because the vehicle itself caused a significant reduction in pH of the HEPES-Tyrode solution and produced a negative inotropic response if milrinone at concentrations >10⁻⁴ M was administered.

8-bromo-cAMP produced positive inotropic responses.
in the right ventricular preparation and the left atrial preparation in a concentration-dependent manner (fig. 3). In contrast to milrinone, no significant difference was observed in the concentration–response relation for the inotropic effects of 8-bromo-cAMP among three different pH values studied in either preparation.

**Cyclic Adenosine Monophosphate Formation and Positive Inotropy**

Figure 4 shows the effects of milrinone (10^{-4} M) on the cAMP content in the guinea pig right ventricular preparation at pH values of 7.4 and 7. At pH 7.4, the cAMP content after the application of milrinone was significantly greater than that after vehicle, whereas no such difference was seen at pH 7. This result corresponded well with the reduced positive inotropic response to milrinone at pH 7 in the right ventricular preparation.

**Discussion**

Depressed inotropic response to catecholamines during acidosis is an important clinical issue for anesthesiol-
ogists. Ample evidence suggests that severe acidosis, from either respiratory or metabolic origin, depresses cardiac performance in *in vivo* and *in vitro* experiments.³⁹ Ford *et al.*³ demonstrated in an *in vivo* canine lactic acidosis model that the inotropic and the maximum chronotropic responses to norepinephrine were depressed when arterial pH was < 7. Wildenthal *et al.*⁶ also reported in dogs that the inotropic effect of norepinephrine was attenuated at pH 7.1 and 6.9 values under the conditions of constant HR and aortic pressure and flow. Although the cause of the diminished myocardial responses to catecholamines has not been completely elucidated, decreased β-adrenoceptor numbers⁰ or affinity for agonists,¹¹ decreased cAMP production,⁹ inhibition of calcium ion exchange,¹² decreased affinity of calcium ion to myofilaments,¹³,¹⁴ or all of these appear to play a role. Among those proposed mechanisms, decreased cAMP formation as a result of depressed adenylate cyclase activity,⁷ which has been shown to be highly pH sensitive,⁰ is important to the pharmacologic action of milrinone, because augmentation of cAMP production is considered to be the primary mechanism of cardiotoxic effect through its inhibition of PDE III.

To the best of our knowledge, this is the first investigation of dose-dependent changes in hemodynamic variables by a PDE III inhibitor at several degrees of metabolic acidosis *in vivo*. Changes in LV dP/dtmax and PBF by milrinone were significantly less at pH < 7 than at pH = 7.4 and 7.2, whereas no significant differences in hemodynamic changes produced by milrinone infusions at 2 and 5 μg·kg⁻¹·min⁻¹ were seen between pH = 7.4 and pH = 7.2. The *in vitro* experiments also showed that concentration-dependent inotropic effects of milrinone were significantly depressed at low pH.

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Fig. 1. (A) Changes in the maximum rate of increase in left ventricular pressure (LV dP/dtmax) and (B) changes in pulmonary blood flow (PBF) from baseline values at 2 and 5 μg·kg⁻¹·min⁻¹ of milrinone infusions in the control (arterial pH [pHa] = 7.4; n = 7), mild acidosis (pHa = 7.2; n = 7), and severe acidosis (pHa < 7; n = 6) groups. Data are shown as means ± SD. *P < 0.05 versus the control group. †P < 0.05 versus the mild acidosis group.
Fig. 2. Concentration–response relations for positive inotropic responses to milrinone in isolated (A) right ventricular preparation (RV), and (B) left atrial preparation (LA) of the guinea pig at pH values of 7.4, 7, or 6.6. Muscles were electrically driven at 2 Hz. Data are shown as means ± SD of seven to nine experiments for RV and seven experiments for LA. Baseline isometric contractions of RV at pH 7.4, 7, and 6.6 were 0.037 ± 0.018 g, 0.032 ± 0.027 g, and 0.024 ± 0.009 g, respectively. Baseline isometric contractions of LA at pH 7.4, 7, and 6.6 were 0.280 ± 0.178 g, 0.152 ± 0.105 g, and 0.081 ± 0.039 g, respectively. *P < 0.05 versus pH 7.4. †P < 0.05 versus pH 7.

Fig. 3. Concentration–response relations for positive inotropic responses to 8-bromoadenosine-3,5-cyclic monophosphate (8-bromo-cAMP) in isolated (A) right ventricular preparation (RV) and (B) left atrial preparation (LA) of the guinea pig at pH 7.4, 7, or 6.6. Muscles were electrically driven at 2 Hz. Data are shown as means ± SD of six or seven experiments for RV and six experiments for LA. Baseline isometric contractions of RV at pH 7.4, 7, and 6.6 were 0.033 ± 0.015 g, 0.028 ± 0.017 g, and 0.024 ± 0.013 g, respectively. Baseline isometric contractions of LA at pH 7.4, 7, and 6.6 were 0.210 ± 0.135 g, 0.173 ± 0.064 g, and 0.093 ± 0.039 g, respectively.

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values in the isolated atrial and ventricular muscles. These results indicate that cardiotonic effects of milrinone are decreased in severely acidic hearts.

In the *in vitro* experiments, PBF was unaltered by milrinone at \( \text{pH} \leq 7 \), whereas the drug significantly increased PBF at physiologic \( \text{pH} \) and \( \text{pHa} \) values \( \approx 7.2 \). Similarly, milrinone augmented LV dP/dtmax at physiologic \( \text{pH} \) and at \( \text{pHa} \) values \( \approx 7.2 \) in a dose-dependent manner, whereas the response was minimal at \( \text{pHa} \) values \( < 7 \). These results may be interpreted with some constraints; although we measured dP/dtmax as an index of cardiac performance, this parameter is largely affected by preload, afterload, and HR. Therefore, we performed further experiments using an isolated preparation *in vitro* to evaluate the direct effects of pH on the inotropy by milrinone. The results showed that positive inotropic responses to milrinone in isolated left atrial and right ventricular preparations were largely depressed by lowered pH, indicating that the acidosis directly suppresses the inotropic effects of milrinone on cardiac muscles. However, we must emphasize that our *in vivo* and *in vitro* experiments were performed using two different species. The results of the two sets of studies, therefore, should be compared with caution.

It has been established that milrinone inhibits PDE III, which in turn increases intracellular cAMP content. By activating cAMP-dependent protein kinase, cAMP enhances \( \text{Ca}^{2+} \) release from the sarcoplasmic reticulum, resulting in positive inotropic responses. The reduced inotropy of milrinone during acidosis may, therefore, be due to decreased cAMP production, decreased responses to cAMP, or both. However, it is unlikely that the responses to cAMP are reduced by acidosis because the inotropic effect of 8-bromo-cAMP was not altered during acidosis. 8-bromo-cAMP is thought to pass through the cell membrane and to activate protein kinase directly. This suggests that the processes downstream to cAMP production are not depressed in the acidic muscles. We also found that the increase in isometric contractions of the isolated right ventricular strips by milrinone was accompanied by an increase in intracellular cAMP content at \( \text{pH} \approx 7.4 \), but not at \( \text{pH} \approx 7 \). The effects of milrinone on cAMP contents at \( \text{pH} \approx 7.4 \) in the present study (68% increase) is in accordance with a previous report by Silver *et al.*\(^{19}\), in which cAMP content increased by approximately 60% by \( 10^{-4} \text{M} \) milrinone in guinea pig isolated right ventricular muscle. These results suggest that the depressed inotropic response to milrinone by decreased \( \text{pH} \) is at least in part, due to the decreased cAMP production in the acidic cardiac muscles, where the inhibition of PDE III could only elicit a limited effect with the diminished amount of cAMP available. We cannot, however, exclude other possibilities, such as changes in the binding affinity of milrinone for PDE III in the acidic hearts.

Nakanishi *et al.*\(^{19}\) found that in isolated rabbit heart, inotropic effects of isoproterenol are suppressed at \( \text{pH} \approx 6.8 \) with an intact number of \( \beta \)-adrenoceptors and responsiveness to exogenously administered CAMP. Interestingly, the study also showed that basal contractile function and tissue cAMP content at \( \text{pH} \approx 6.8 \) were similar to those at \( \text{pH} \approx 7.4 \), although the basal activity of adenylate cyclase was largely depressed at \( \text{pH} \approx 6.8 \). In the present *in vitro* study, the basal contractile function and the basal cAMP content in the isolated right ventricular preparation at \( \text{pH} \approx 7 \) were similar to those at \( \text{pH} \approx 7.4 \). Nevertheless, at \( \text{pH} \approx 7 \), increases in isometric contractions and cAMP content induced by the inhibition of PDE III were nearly abolished. It is likely that acidosis suppresses not only adenylate cyclase but also PDE III, thus preventing profound decreases in the basal level of cAMP and thus basal contractile function in the acidic hearts.

The doses of milrinone used in the *in vivo* experiments were remarkably large compared with those for humans. The apparent species differences in the inotropic responses to milrinone are known. A dose-dependent positive inotropic effect has been demonstrated in dogs, guinea pigs, cats, and rabbits, whereas the sensitivity of ventricular muscle to the inotropic effects of
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milrinone have been shown to be poor for hamsters and rats.\textsuperscript{21,22} In rabbit cardiac muscle, PDE III localized at the sarcoplasmic reticulum has been identified as the primary modulator of cAMP levels and cAMP-dependent positive inotropic responses. In contrast, PDE IV rather than PDE III appears to be an important regulator of rat cardiac muscle, in which a selective PDE IV inhibitor has been shown to increase cardiac inotropy in the presence of isoproterenol.\textsuperscript{23} Further, two subclasses of PDE III, membrane-bound and soluble fraction, are distributed in the ventricular muscle.\textsuperscript{24} It is conceivable that different roles of PDE isozymes as regulators of cardiac muscle contractility and variations in intracellular localizations of PDE III subclasses may explain species differences in responsiveness to milrinone.

Administration of milrinone \textit{in vivo} caused the significant, dose-dependent increase in HR and the decrease in systemic vascular resistance at normal pH\textsubscript{a} values and mild acidosis, whereas milrinone did not cause any changes in those parameters in severe acidosis. Electrophysiologic studies have shown that milrinone enhances conduction of canine Purkinje fibers\textsuperscript{25} and human atrioventricular conduction.\textsuperscript{26} Whether chronotropic and vasodilatory effects of milrinone are also affected by acidosis remain to be determined.

Our study may have several shortcomings. First, using two different kinds of animals in our study limits comparisons of our results between \textit{in vivo} and \textit{in vitro} experiments and extrapolations of our results to humans. Although the concentration–response relations are similar for dogs and guinea pigs,\textsuperscript{17,19,21,27–29} pH\textsubscript{a}-induced modulation of positive inotropic effects of milrinone may not be observed to a similar extent in both species. Second, considerably larger doses of milrinone without a loading dose have been infused in our \textit{in vivo} study than the recommended dosing regimen in humans. Because plasma milrinone concentrations have not been determined in our study, it was not clear from the results of our study whether steady-state concentrations of milrinone were reached and whether dose-related hemodynamic changes could be obtained in our \textit{in vivo} study. However, previous open-chest canine preparations showed that continuous infusion of 3 \textmu g·kg\textsuperscript{-1}·min\textsuperscript{-1} milrinone produced maximal blood pressure and contractile responses within 60 min of administration.\textsuperscript{30} In addition, bolus injection of milrinone in dogs produces a dose-related inotrophic effect over a wide range of doses (10 \textmu g/kg to 10 mg/kg).\textsuperscript{17,30}

Based on the fact that the plasma half-life of milrinone is <60 min, it is likely that hemodynamic changes seen in our results over a dose range of 120–300 \textmu g·kg\textsuperscript{-1}·h\textsuperscript{-1} in dogs were associated with dose-related positive inotropic effects.

Third, we did not enzymatically confirm the absence of severe myocardial damage or perform autopsies after the \textit{in vivo} experiments. Therefore, the possibility that severe hypoxic injury to the myocardium at the time of induction of metabolic acidosis contributed to the altered hemodynamic responses to milrinone in the mild or severe acidosis groups cannot be ruled out. However, no episodes of bradycardia consistent with a terminal rhythm or changes in electrocardiographic structure, including S-T changes or premature ventricular contractions, were evident during hypoxic challenge or maintenance of acidosis. In addition, pre-milrinone hemodynamics (LV dP/dt\textsubscript{max}, PBF, LVEDP, and right atrial pressure) were similar for the groups and therefore do not support the presence of severe myocardial damage in the canine model with mild or severe acidosis.

Fourth, infusion of hydrochloric acid to maintain acidosis in our \textit{in vivo} experiment may not have produced hemodynamic alterations similar to those seen in more clinically relevant lactic acidosis. To the best of our knowledge, no previous study has shown different hemodynamic responses to inotropic agents when organic and inorganic acidosis were compared. Poole-Wilson and Cameron\textsuperscript{31} demonstrated in rabbits that intravenous infusion of hydrochloric acid produced continuous linear decreases in intracellular pH for as long as 60 min after decreasing the dose of hydrochloric acid infusion to 1/6, indicating that an equilibration period of at least 60 min was needed before hydrochloric acid-induced changes in extracellular pH were reflected in the intracellular space. Therefore, we used a 1-h equilibration period during the maintenance of acidosis before milrinone infusions.

Finally, halothane, a potent inhibitor of cardiovascular system,\textsuperscript{32} including arterial baroreflex function,\textsuperscript{33} was used in our \textit{in vivo} study. Our results may have been different with other anesthetic agents that possessed less cardiovascular depressive actions.

In conclusion, our investigation showed that severe metabolic acidosis (pH\textsubscript{a} <7), but not mild metabolic acidosis (pH\textsubscript{a} ≈ 7.2), attenuates cardiac performance mediated by milrinone in halothane-anesthetized, mechanically ventilated dogs. In \textit{in vitro} experiments using guinea pig isolated hearts, concentration-dependent inotropic effects of milrinone were found at pH 7.4, but inotropic efficacy was significantly depressed in the

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right ventricular tissues at pH values of 7 and 6.6, and in the left atrial preparation at pH 6.6. In contrast, isotropic responses to 8-bromo-cAMP were not altered by pH in either heart muscle preparation. The increase in isometric force of the right ventricular preparation at pH 7.4, but not at pH 7, was accompanied by the increase in intracellular cAMP content. These results suggest that the depressed isotropic response to milrinone by decreased pH is, at least in part, due to decreased cAMP production.

References