Mechanism of preserved positive lusitropy by cAMP-dependent drugs in heart failure

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Tanigawa, Takeo, Masafumi Yano, Michihiro Kohno, Takeshi Yamamoto, Takayuki Hisaoka, Kaoru Ono, Takeshi Ueyama, Shigeki Kobayashi, Yuhji Hisamatsu, Tomoko Ohkusa, and Masunori Matsuzaki. Mechanism of preserved positive lusitropy by cAMP-dependent drugs in heart failure. Am. J. Physiol. Heart Circ. Physiol. 278: H313–H320, 2000.—In tachycardia-induced heart failure (HF), positive lusitropic effects of milrinone or dobutamine were assessed by evaluating the time constant of left ventricular (LV) pressure decay (τ) and Ca²⁺-ATPase activity of the sarcoplasmic reticulum (SR). The peak value of the positive first derivative of LV pressure (±dP/dt) was less increased, either by dobutamine (2–10 µg·kg⁻¹·min⁻¹) or by milrinone (4–20 µg·kg⁻¹), in HF than in control (P < 0.05), whereas τ was shortened to an extent similar to that in control with dobutamine (P = not significant [NS]) and to an even greater extent with milrinone (P < 0.05). Ca²⁺-ATPase activity increased similarly in HF and control with dobutamine (1 µM; +11% in HF vs. +12% in control, P = NS), whereas it increased more with milrinone (1 µM; +19% in HF vs. +11% in control, P < 0.05). Ca²⁺-ATPase activity-cAMP relationships were shifted to the left by milrinone or dobutamine in HF compared with control. Thus, in HF, the sensitivity of Ca²⁺-ATPase activity to cAMP was increased on addition of cAMP-dependent inotropic agents, contributing to the preservation of positive lusitropy.

IN HEART FAILURE, the contractile response to β-adrenergic stimulation is attenuated through the mechanism by which the basal intracellular cAMP level is decreased, i.e., downregulation of myocardial β-adrenergoreceptor and increase in inhibitory guanine-nucleotide binding proteins (Gᵢ) (4, 5, 29). Both β-adrenergic agonists and phosphodiesterase (PDE) inhibitors have the capability to increase cAMP levels, leading to an enhancement of cardiac contractility (22). Although the clinical benefits of positive inotropism by these drugs have been well established in heart failure, the beneficial role of these cAMP-dependent inotropic agents on left ventricular (LV) relaxation in heart failure remained to be elucidated. In this regard, diastolic dysfunction is a major clinical problem in cardiac hypertrophy and/or failure as well as systolic dysfunction, and sometimes cardiac failure can be induced only by diastolic dysfunction even though systolic function is well preserved (3, 9, 10). Therefore, for the clinical use of these cAMP-dependent drugs, it is important to clarify the differences in positive lusitropic effects between the drugs.

Abnormal regulation of intracellular Ca²⁺ by the sarcoplasmic reticulum (SR) has been shown to be involved in the mechanism of contractile and relaxation dysfunction in heart failure (30, 32). Several investigators demonstrated that Ca²⁺ uptake by SR is decreased in association with the decreased density of Ca²⁺-ATPase in cardiac hypertrophy and/or failure (8, 11, 18, 21, 23, 25, 28). In a previous report (42), we demonstrated that a low dose of milrinone substantially improved LV relaxation in normal dogs and that this positive lusitropic effect of milrinone was coupled with a direct acceleration of Ca²⁺ uptake by SR, probably caused by an inhibition of membrane-bound PDE III in SR and hence local elevation of cAMP.

The goal of this study was to evaluate the effects of two different cAMP-dependent drugs, milrinone and dobutamine, on LV relaxation in parallel with the assessment of SR Ca²⁺-ATPase activity in tachycardia-induced heart failure. Tachycardia induced by chronic pacing causes well-defined, predictable, and progressive LV dilatation, contractile dysfunction, and neurohormonal activation (2, 7, 27, 33, 41), and hence this model may more clearly resemble cardiac failure in humans than do previous studies of small-animal models of cardiac hypertrophy and/or failure.

MATERIALS AND METHODS

Heart failure was induced in beagle dogs of either sex by 3 wk of rapid ventricular pacing at a rate of 250 beats/min using an externally programmable miniature pacemaker (Medtronic, Minneapolis, MN). The specific details of the chronic instrumentation were as follows. Beagle dogs (n = 7 for control; n = 7 for rapid ventricular pacing) were sedated with morphine sulfate (15 mg sc) and thiopental sodium (150 mg iv). They were then anesthetized with isoflurane (2%, 1.5 l/min) and a mixture of nitrous oxide and oxygen (2:1), intubated with a cuffed endotracheal tube, and ventilated at a tidal volume of 22 ml/kg and a respiratory rate of 15 breaths/min. A bipolar pacing lead was fixed to the endocardial rapid

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ventricular pacing surface and the distal lead was tunneled to
a subcutaneous pocket constructed on the animal's back, and
they were connected to a pacemaker (Medtronic). Cefazolin (1 g iv)
was administered before and after surgery. The control
dogs underwent only a sham operation without pacing.

After 1 wk was allowed for animal recovery, the pacemaker
was programmed to 250 beats/min. Dogs were monitored
daily for clinical signs and symptoms of heart failure. With
the pacing off after 3 wk of rapid ventricular pacing, the dogs
were anesthetized after sedation as described above. LV
ventricular pacing was programmed to 250 beats/min. Dogs were monitored
daily for clinical signs and symptoms of heart failure. With
the pacing off after 3 wk of rapid ventricular pacing, the dogs
were anesthetized after sedation as described above. LV

Hemodynamics before and after infusion of milrinone or dobutamine are summarized in Table 1. After chronic rapid ventricular pacing, heart rate, LV end-diastolic pres-
sure, and LV internal diameters were all increased, compared with control. As for the parameters of LV systolic function, the peak +dP/dt of LV pressure, cardiac output, and fractional shortening were signifi-
cantly decreased. As for the parameters of LV diastolic function, the time constant of LV pressure decay during isovolumic relaxation period (τ) was prolonged after rapid right ventricular pacing.

Hemodynamic changes after administration of milrinone or dobutamine. Hemodynamics before and after infusion of milrinone or dobutamine are summarized in Table 2. After the administration of dobutamine (2–10 µg·kg⁻¹·min⁻¹), LV peak pressure was slightly in-
creased in control conditions and unchanged in heart
failure. LV end-diastolic pressure tended to increase in
control and was unchanged in heart failure. Peak +dP/dt increased in control and was unchanged in heart failure. Peak +dP/dt increased in control and was unchanged in heart failure. As shown in Fig. 1, peak +dP/dt increased to a lesser extent in heart failure than in control, whereas τ was
shortened to a similar extent as in control.

Table 1. Hemodynamics and echocardiographic data

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 7)</th>
<th>Heart Failure (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>87 ± 2</td>
<td>107 ± 4*</td>
</tr>
<tr>
<td>LVPSP, mmHg</td>
<td>101 ± 6</td>
<td>105 ± 6</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>4.4 ± 0.7</td>
<td>20.0 ± 1.3*</td>
</tr>
<tr>
<td>Peak +dP/dt, mmHg/s</td>
<td>2.12 ± 189</td>
<td>1.398 ± 0.8*</td>
</tr>
<tr>
<td>τ, ms</td>
<td>29.3 ± 1.2</td>
<td>42.1 ± 1.5*</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>1.7 ± 0.1</td>
<td>1.1 ± 0.2*</td>
</tr>
<tr>
<td>SV, ml</td>
<td>19.8 ± 1.0</td>
<td>10.1 ± 1.0*</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>27.8 ± 1.4</td>
<td>31.8 ± 1.2*</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>18.8 ± 0.9</td>
<td>29.0 ± 1.0*</td>
</tr>
<tr>
<td>%FS</td>
<td>32.4 ± 2.0</td>
<td>9.7 ± 1.4*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of dogs. HR, heart rate; LVPSP, left ventricular (LV) peak systolic pressure; LVEDP, LV end-diastolic pressure; peak +dP/dt, peak value of first derivative of LV pressure; τ, time constant of LV pressure decay during isovolumic relaxation period; CO, cardiac output; SV, stroke volume; LVEDD, LV end-diastolic diameter; LVESD, LV end-systolic diameter; %FS, % fractional shortening [LVEDD – LVESD]/LVEDD×100. *P < 0.05 vs. control.
Table 2. Effect of dobutamine or milrinone on hemodynamics in control and heart failure

<table>
<thead>
<tr>
<th></th>
<th>Dobutamine, µg·kg⁻¹·min⁻¹</th>
<th>Milrinone, µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>87</td>
<td>2</td>
</tr>
<tr>
<td>Heart failure</td>
<td>107</td>
<td>4†</td>
</tr>
<tr>
<td>LVPSP, mmHg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>101</td>
<td>6</td>
</tr>
<tr>
<td>Heart failure</td>
<td>105</td>
<td>6</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Heart failure</td>
<td>20.0</td>
<td>1.3‡</td>
</tr>
<tr>
<td>Peak +dP/dt, mmHg/s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.124</td>
<td>189</td>
</tr>
<tr>
<td>Heart failure</td>
<td>1.398</td>
<td>88‡</td>
</tr>
<tr>
<td>τ, ms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>29.3</td>
<td>1.2</td>
</tr>
<tr>
<td>Heart failure</td>
<td>42.1</td>
<td>1.5‡</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 dogs for each group. *P < 0.05 vs. baseline; †P < 0.05 vs. control.

After the administration of milrinone, heart rate was slightly increased in control and unchanged in heart failure. LV peak pressure was unchanged in either control or heart failure. LV end-diastolic pressure decreased in both groups. Peak +dP/dt increased and τ was shortened in both groups. As shown in Fig. 2, peak +dP/dt increased to a lesser extent in heart failure than in control, whereas τ was shortened much more than in control by low doses of milrinone (4–12 µg/kg).

Ca²⁺-ATPase activity and cAMP levels in heart failure were significantly decreased in heart failure compared with control. After the addition of dobutamine, the cAMP increased in a dose-dependent manner to a lesser extent in heart failure than in control (Fig. 3A). Ca²⁺-ATPase activity increased in a dose-dependent manner in both groups (Fig. 3B). There was no significant difference in the percent increase of Ca²⁺-ATPase activity from baseline between control and heart failure. On the other hand, after milrinone was added, cAMP increased in both groups (Fig. 4A), whereas Ca²⁺-ATPase activity increased to a greater extent in heart failure than in control at low doses of milrinone (Fig. 4B).

Figure 5 shows the relationship between the percent change of Ca²⁺-ATPase activity and cAMP in the presence of dobutamine (Fig. 5A) or milrinone (Fig. 5B). In the presence of either dobutamine or milrinone, Ca²⁺-ATPase activity (%) and cAMP (%) relationship curves were shifted to the left in heart failure compared with control, indicating higher sensitivity of Ca²⁺-ATPase activity to cAMP in heart failure. Compared with dobutamine, low doses of milrinone exerted a substantial increase in Ca²⁺-ATPase activity in heart failure, at a given increase in cAMP.

In the presence of 1 µM thapsigargin (SR Ca²⁺-ATPase inhibitor), Ca²⁺-ATPase activity was decreased by 17.7 ± 3.1% in normal homogenate and by 19.6 ± 1.8% in heart failure homogenate. There was no significant difference in the percentage of the thapsigargin-sensitive portion of Ca²⁺-ATPase activity between normal and heart failure.

Figure 6 shows the effect of dobutamine or milrinone on the thapsigargin-insensitive portion of Ca²⁺-ATPase activity at various doses of dobutamine or milrinone.
DISCUSSION

The major findings of this study are as follows. First, in heart failure, the positive lusitropic effect of either milrinone or dobutamine was well preserved in association with the increased sensitivity of SR Ca$^{2+}$-ATPase activity to cAMP. Second, in particular, the positive lusitropic effect of low doses of milrinone was more prominent in heart failure than in normal conditions, associated with a marked stimulation of SR Ca$^{2+}$-ATPase activity.

Preservation of positive lusitropy in heart failure. Much evidence has accumulated that in heart failure, the positive inotropic response to catecholamine is significantly decreased, whereas positive lusitropy is well preserved (26, 31). The reduction of the positive inotropic action of dobutamine after heart failure might be caused by high production of NO (17). Keaney et al. (16) found that intracoronary infusion of the nitric oxide synthase inhibitor N$^G$-nitro-L-arginine methyl ester increased peak $\pm$ dP/dt in response to intracoronary infusions of either dobutamine or isoproterenol in the in situ canine heart. Consistent with these previous reports, we found that both dobutamine and milrinone caused less increase in LV contractility in heart failure than under normal conditions, whereas lusitropic responses to both drugs were well preserved. Although the underlying mechanism is still unclear, in heart failure the lusitropic response might be coupled more efficiently to cAMP than the inotropic responses on stimulation of either dobutamine or milrinone. Sensitivity of the lusitropic cascade (i.e., phosphorylation of phospholamban, interaction of phospholamban to Ca$^{2+}$-ATPase, etc.) to cAMP may be increased in heart failure. As a matter of fact, we found that Ca$^{2+}$-ATPase

Table 3. Ca$^{2+}$-ATPase activity and cAMP level

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Heart Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca$^{2+}$-ATPase activity, µM</td>
<td>$0.54 \pm 0.04$</td>
<td>$0.31 \pm 0.02^*$</td>
</tr>
<tr>
<td>cAMP, pmol/mg protein</td>
<td>$24.3 \pm 1.5$</td>
<td>$13.6 \pm 1.5^*$</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 dogs. *P < 0.05 vs control.
Recently, a particulate, high-affinity type IV cAMP-PDE activity was shown to exist in cardiac SR (14). In this regard, much evidence has accumulated that certain cardiotonic agents (milrinone, imazodan, and amrinone) inhibit this SR membrane-bound “low K_m” or “cGMP-inhibited” PDE type IV isozyme (35, 38, 39) and exert their contractile effects through subtle alterations in the metabolism of cAMP (15, 19). With regard to this, functional compartmentation of cAMP and protein kinases was previously proposed for cardiac muscle (1, 6), and intracellular Ca^{2+} mobilization might be affected by cAMP located in the particulate compartment of canine cardiac myocytes (6). Because milrinone, at submicromolar concentrations, inhibits specifically SR membrane-bound PDE III activity (19), the acceleration of SR Ca^{2+}-ATPase activity by low doses of milrinone might be caused by an inhibition of membrane-bound PDE III in SR, followed by a local elevation of cAMP, not the global cytosolic elevation of cAMP. Indeed, we previously demonstrated (42) that a low dose of milrinone significantly enhanced LV relaxation activity in heart failure was more enhanced at a given increase in cAMP either by dobutamine or by milrinone than under normal conditions (Fig. 5). As another possibility, the recently proposed functional compartmentation of cAMP and protein kinases was previously proposed for cardiac muscle (1, 6), and intracellular Ca^{2+} mobilization might be affected by cAMP located in the particulate compartment of canine cardiac myocytes (6). Because milrinone, at submicromolar concentrations, inhibits specifically SR membrane-bound PDE III activity (19), the acceleration of SR Ca^{2+}-ATPase activity by low doses of milrinone might be caused by an inhibition of membrane-bound PDE III in SR, followed by a local elevation of cAMP, not the global cytosolic elevation of cAMP. Indeed, we previously demonstrated (42) that a low dose of milrinone significantly enhanced LV relaxation...
in association with the substantial increase in the rate of Ca\(^{2+}\) uptake by cardiac SR. This effect of milrinone might also explain why, in this study using LV homogenate, milrinone exerted more increase in the Ca\(^{2+}\)-ATPase activity in heart failure than dobutamine at a given increase in cAMP (Fig. 5).

Limitations. Milrinone exerts a vasodilating effect as well as positive inotropic and lusitropic effects. Therefore, afterload reduction by this drug may possibly induce acceleration of LV relaxation. With regard to this, when LV pressure was increased by 25% (mean 30 mmHg) by addition of phenylephrine together with milrinone, \(\tau\) was not significantly influenced in normal dogs (unpublished data). Furthermore, in the present study, the low dose of milrinone (4 \(\mu\)g/ml) did not change peak LV pressure and LV end-diastolic pressure, whereas \(\tau\) was shortened by 16% in heart failure. Therefore, the PDE-inhibitory effect of milrinone might be predominantly involved in the positive lusitropic effect, particularly at a low dose. At higher doses of milrinone, the mixed effects of PDE III inhibition and vasodilatation may play important roles in the improvement of LV relaxation.

In the present study, we measured whole Ca\(^{2+}\)-ATPase activities in myocardium. However, the SR Ca\(^{2+}\)-ATPase activity alone comprises \(\sim 25\%\) of the total muscle homogenate activity, and \(\sim 75\%\) of total Ca\(^{2+}\)-ATPase activity in muscle homogenate is provided by intracellular organs other than SR, i.e., Ca\(^{2+}\)-Mg\(^{2+}\)-ATPase of the plasmalemma and myofibrils (36). Therefore, we should address the reaction of these other Ca\(^{2+}\)-ATPase activities to milrinone or dobutamine in this study. As shown in Fig. 6, thapsigargin-insensitive Ca\(^{2+}\)-ATPase activity, which comprises \(\sim 80\%\) of total Ca\(^{2+}\)-ATPase activity, was influenced by neither dobutamine nor milrinone. Only the thapsigargin-sensitive portion of Ca\(^{2+}\)-ATPase activity was changed by these drugs, indicating that these positive inotropic agents indeed affect SR Ca\(^{2+}\)-ATPase activity.

In the present study, the degree of heart failure appears moderate (heart rate \(\sim 100\) beats/min, no change in LV systolic pressure, LV end-diastolic pressure \(< 25\) mmHg) compared with the hemodynamic values reported in the literature (20, 26, 37). In severe heart failure, the cytosolic level of cAMP and the protein expression of Ca\(^{2+}\)-ATPase might substantially...
decrease, and hence the positive lusitropic responses to cAMP-dependent drugs may deteriorate no matter how SR Ca^{2+}-ATPase activity is hypersensitized to cAMP. Also, it is likely that the sensitivity of SR Ca^{2+}-ATPase activity to cAMP may change depending on the severity of heart failure. Clearly, more work is needed.

In conclusion, 1) positive lusitropic effects by cAMP-dependent drugs were well preserved, probably because of the higher sensitivity of SR Ca^{2+}-ATPase activity to cAMP in heart failure, and 2) a low dose of milrinone substantially improved LV relaxation in association with stimulation of SR Ca^{2+}-ATPase activity in heart failure much more than under normal conditions.

The authors thank Dr. Kenichi Yoshida, Department of Legal Medicine, Yamaguchi University School of Medicine, for helpful technical advice.

This work was supported by a grant-in-aid for scientific research in Japan (Grants C 09670722 and C 11670684) and by a Health Sciences Research Grant for Comprehensive Research on Aging and Health from the Ministry of Health and Welfare, Japan.

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Received 3 May 1999; accepted in final form 18 August 1999.

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