MCI-154, a Ca\(^{2+}\) sensitizer, decreases the oxygen cost of contractility in isolated canine hearts

Onishi, Katsuya, Kiyotsugu Sekioka, Ryoichi Ishisu, Yuji Abe, Hideyuki Tanaka, Mashiho Nakamura, Yuji Ueda, and Takeshi Nakano. MCI-154, a Ca\(^{2+}\) sensitizer, decreases the oxygen cost of contractility in isolated canine hearts. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H1688–H1695, 1997.—An increase in the responsiveness of the contractile machinery to Ca\(^{2+}\) could theoretically enhance the mechanoenergetics of the heart. To clarify this unresolved issue, we studied the effects of MCI-154, a Ca\(^{2+}\) sensitizer, on the mechanoenergetics in terms of the left ventricular contractility index [slope of end-systolic pressure-volume relationship (E\(_{\text{max}}\))] and the relationship between myocardial oxygen consumption (V\(_{\text{O}}2\)) and left ventricular pressure-volume area in excised cross-circulated canine hearts. MCI-154 increased E\(_{\text{max}}\) by 42 ± 31% (SD), although the slope of the V\(_{\text{O}}2\)-PVA relationship (an indicator of contractile efficiency) was unchanged by MCI-154. Despite equal increases in E\(_{\text{max}}\), the relative increase in unloaded V\(_{\text{O}}2\) (\(\Delta V_{\text{O}}2/\Delta E_{\text{max}}\)) during infusion of MCI-154 was, however, significantly less than that during CaCl\(_2\) infusion (0.0016 ± 0.0018 vs. 0.0059 ± 0.0054; P < 0.05). By contrast, \(\Delta V_{\text{O}}2/\Delta E_{\text{max}}\) for milrinone was the same as that for CaCl\(_2\) (0.0043 ± 0.0041 vs. 0.0039 ± 0.0045; P > 0.05). Basal metabolism in KCl-affected hearts was unchanged by MCI-154, indicating that MCI-154 consumes less energy than CaCl\(_2\) for excitation-contraction coupling. These findings suggest that MCI-154 acts energetically as a Ca\(^{2+}\) sensitizer in beating canine whole hearts.

Materials and Methods

Positive Inotropic Agents have a Prominent Role in the Management of Chronic Heart Failure (5, 6, 28). With respect to myocardial energetics, however, positive inotropic agents such as catecholamines (6, 29, 35) are disadvantageous because, to relax, the Ca\(^{2+}\) pump of the sarcoplasmic reticulum of myocardial cells requires additional ATP to reduce the increased intracellular levels of Ca\(^{2+}\) (8, 9, 28, 31, 37). Recognition of the problems caused by compounds that achieve their inotropic effects through increased Ca\(^{2+}\) levels has led to an interest in drugs that enhance tension by a Ca\(^{2+}\) sensitization of myofilibrillar proteins (7, 10, 26). As measured by the heat liberated from guinea pig papillary muscles, pimobendan, which has a Ca\(^{2+}\)-sensitizing effect, has been shown to improve contractile economy (12). The myocardial energetics of whole hearts have been assessed in terms of the left ventricular (LV) contractility index [slope of end-systolic pressure-volume relationship (E\(_{\text{max}}\))] and the relationship between myocardial oxygen consumption (V\(_{\text{O}}2\)) and the LV pressure-volume (P–V) area (PVA). Pimobendan and EMD-53998, however, have failed to improve myocardial energetics in isolated canine hearts (7, 10), probably due to a combination of adenosine 3',5'-cyclic monophosphate (cAMP)-mediated and the Ca\(^{2+}\)-sensitizing effects, which would mask the pure effect of Ca\(^{2+}\) sensitization on myocardial energetics.

MCI-154 [6-[4-(4-pyridylaminophenyl)-4,5-dihydro-3(2H)-pyridadinone hydrochloride trihydrate; synthesized by Mitsubishi Chemical, Yokohama, Japan] is a potent novel cardiotonic agent (15, 16). Although MCI-154 slightly inhibits cAMP phosphodiesterase (PDE), especially at high concentrations, its positive inotropic action at low concentrations is primarily due to an increase in the Ca\(^{2+}\) sensitivity of the contractile apparatus (1, 4, 18). Several investigators (1, 2, 16) have indicated that MCI-154 increases both myofilibrillar Ca\(^{2+}\) sensitivity and maximum Ca\(^{2+}\)-activated force in skinned fibers from guinea pig, dog, and human hearts. Furthermore, Abe et al. (1) previously showed that an effect on Ca\(^{2+}\) sensitivity on intact beating whole hearts in guinea pigs by MCI-154 is stronger than that by pimobendan. In the present study, we investigated the effects of MCI-154 on LV contractility and energetics in terms of E\(_{\text{max}}\) and the V\(_{\text{O}}2\)-PVA relationship (31, 33, 34) in excised, blood-perfused canine hearts.

Materials and Methods

Surgical Preparation

We used an excised, cross-circulated dog heart preparation (7, 10, 26, 30). Forty-six pairs of mongrel dogs were anesthetized with pentobarbital sodium (30 mg/kg iv) and heparinized (500 U/kg). Support dogs weighed 18–30 kg, and donor dogs weighed 14–17 kg. The left subclavian artery and right ventricle of the donor dog were cannulated and connected to the femoral arteries and external jugular veins, respectively, of the support dog. The brachiocephalic artery was cannulated to monitor coronary perfusion pressure. The azygos vein, superior and inferior venae cavae, brachiocephalic artery, descending aorta, and pulmonary hili were ligated. The heart was removed from the chest, and a drainage tube was inserted into the apex of the LV. The chordae tendineae of the mitral valve were cut, and a ring adapter was sewn onto the mitral annulus. A thin latex balloon (unstressed volume ~50 ml) was placed in the LV, and the space between the balloon and the ventricular wall was minimized by a vent in the apex. The balloon adapter was connected to a servo-pump system (see Servo-Pump Hardware).

The bundle of His was ablated electrically to obtain a complete arterioventricular block. The heart was paced at a constant rate (100–110 beats/min, a normal rate for conscious dogs) with a pair of electrodes that was sutured onto the apical epicardium of the LV. A pair of electrodes was sutured onto the right atrial and right ventricular (RV) epicardia to record the electrocardiogram.

To maintain coronary arterial pressure at 90 mmHg throughout the experiment, we used three pumps (26). The first pump drew the blood flow from the support dog at a constant rate to minimize the neurohumoral factor from the
support dog and to provide coronary flow to the donor heart. The second (bypass) pump was placed between the first pump and the donor heart, and its speed was controlled through a feedback control system to maintain coronary arterial pressure of the donor heart at 90 mmHg. The bypass pump was connected to a third pump that returned venous blood from the RV draining tube to the jugular veins of the support dog.

The temperature of the heart was maintained at 36–37°C. Arterial pH, Po2, and Pco2 of the supporting dog were measured intermittently with a blood gas analyzer (model Lex O2 Con, Lexington Instruments, Waltham, MA). These parameters were maintained within their physiological ranges by artificial ventilation, with oxygen supplementation and administration of bicarbonate solution as needed.

Servo-Pump Hardware

Details of the volume servo-pump design and its performance have been described elsewhere (30). A high-performance linear motor (VTS 100, Vibration Test System, Aurora, OH) controlled the piston position of a rolling-diaphragm cylinder (Bellofram, Fujikura Gomu, Omiya, J apan). A latex balloon was secured to a tube connected to the fluid port of the cylinder. The cylinder, connecting tube, and balloon were all filled with water. A position-sensitive detector (linearily arrayed photo diode; S1352, Hamamatsu Photonics, Hamamatsu, J apan) sensed the position of the piston, producing a signal proportional to the balloon volume. The signal was used in a negative feedback loop for comparison with a volume-command signal that represented the desired instantaneous volume. The error signal resulting from this comparison was supplied to a power amplifier, which, in turn, drove the linear motor. The phase delay between the volume command and measured volume was compensated for by adjusting the gains of the feedforward and feedback signals.

Impedance Loading System

LV pressure (LVP) was measured with a micromanometer (P-7, Konigsburg, Pasadena, CA) placed inside the balloon. The pressure was digitized at 1 kHz with a 12-bit, analog-to-digital (A/D) converter and a personal computer (PC98RA, NEC, Tokyo, J apan; central processing unit, Intel 80386 with numeric data processor 80387, Santa Clara, CA). The instantaneous LV volume (LVV) command was calculated in real time based on a simulated LV preload and afterload model. It was fed to the volume-control servo system via a 12-bit, digital-to-analog converter (volume resolution 0.02 ml).

Myocardial VO2

Total coronary blood flow (CBF) was measured with an electromagnetic flowmeter (FF040T, Nihon Kohden, Tokyo, J apan) in the middle of the tube that hydrostatically drained the coronary venous blood that returned to the RV. The RV was collapsed by this drainage, and, therefore, it was assumed that it consumed oxygen at a negligibly low, constant rate. We ignored thebesian coronary venous return to the LV because it represented only a small percentage of the total coronary flow.

The difference in oxygen content between coronary arterial and venous blood (a-vO2D) was continuously measured with an oximeter (A-Vox system, San Antonio, TX), which was calibrated against a galvanometric oxygen-content analyzer (model Lex O2 Con, Lexington Instruments, Waltham, MA) at both the beginning and the end of the experiment.

CBF and a-vO2D were sampled on-line with the personal computer through a 12-bit A/D converter. Myocardial VO2 per beat was calculated by integrating the products of the 2 variables during 10 beats.

At the end of each experiment, the LV (LV free wall plus septum) and the RV free walls were weighed. VO2 was normalized with respect to LV weight to give VO2 in milliliters of oxygen per beat per 100 g LV.

Real-Time Calculation of PVA

The LV systolic PVA is that area in the P–V diagram that is circumscribed by the end-systolic (ESPVR) and end-diastolic P–V relationships and the systolic segment of the P–V trajectory. LVP and LVV were sampled on-line at 1 kHz with the personal computer through a 12-bit A/D converter and were displayed in real time on the computer monitor, and the PVA was calculated (26, 30).

Experimental Protocol

After surgery, β-blockade was induced by an intracoronary infusion of propranolol (1 mg bolus) followed by a continuous intracoronary perfusion of propranolol (0.6 mg/h) to minimize the impact of varying levels of catecholamines in the blood of the support dog (7). The infusion pump was placed between the first and the bypass pumps to keep the concentration of propranolol constant despite changes in CBF. Propranolol, being negatively inotropic, induces a parallel downward shift of the VO2–PVA relationship, opposite to the effect of catecholamines. β-blockade reduced Emax by 45 ± 7% (SD) during propranolol infusion; the post-β-blockade level of contractility was referred to as the baseline contractile state. After β-blockade, the hearts were allowed to equilibrate for 20 min.

VO2–PVA relationship studies. For control runs, we obtained Emax and the VO2–PVA relationship of steady-state contractions at five to six different LV end-diastolic pressures (2–14 mmHg) in 16 hearts, waiting 2–3 min between interventions to get steady states.

MCI-154 runs. MCI-154 (5 nM solution) was administered into the coronary perfusion tube of eight hearts at a constant rate (0.055–0.165 ml/min) with a constant-flow infusion pump that was placed between the first and the bypass pumps to keep the concentration of propranolol constant despite changes in CBF. The hearts were allowed to equilibrate for 20 min until LVP, LVV, CBF, and a–vDO2 had all stabilized; Emax and VO2–PVA relationship were then obtained in a manner similar to the control runs.

MILRINONE RUNS. Milrinone (0.1 µM solution; Sigma Chemical) was infused in eight hearts, and Emax and VO2–PVA relationships were obtained in a manner similar to the MCI-154 runs.

Chloride runs. After the second control run, MCI-154 was infused in eight hearts at the same rate as in the VO2–PVA MCI-154 runs. MCI-154 (5 nM solution) was administered into the coronary perfusion tube of eight hearts at a constant rate (0.055–0.165 ml/min) with a constant-flow infusion pump that was placed between the first and the bypass pumps to keep the concentration of propranolol constant despite changes in CBF. The hearts were allowed to equilibrate for 20 min until LVP, LVV, CBF, and a–vDO2 had all stabilized; Emax and VO2–PVA relationship were then obtained in a manner similar to the control runs.

Oxygen cost of contractility studies. Emax and unloaded VO2 were measured under the control contractile state in another 22 hearts. Then, data were obtained under a heightened contractile state induced by infusing a calcium solution (1% CaCl2) at a constant rate (0.6 meq/min). When a steady state was reached after 4–5 min, Emax and unloaded VO2 were again measured. After the CaCl2 infusion was stopped, the heart was allowed to recover for 10.2 ± 0.3 min. Then, Emax and unloaded VO2 were measured as the second control runs.

REPEATED CACL2 RUNS. After the second control run, the contractile state was again increased by CaCl2 infusion in six hearts. Four to five minutes were allowed for equilibration, and Emax and unloaded VO2 were obtained. There were no significant differences in the ratio of the increase in unloaded VO2 to the increase in Emax (AVO2/ΔEmax) between the first and the second CaCl2 infusions (0.0052 ± 0.0020 vs. 0.0054 ± 0.021; P > 0.05).

MCI-154 runs. After the second control run, MCI-154 was infused in eight hearts at the same rate as in the VO2–PVA
relationship studies. The hearts were allowed to equilibrate for 20 min until LVP, LVV, CBF, and a-vDO₂ had stabilized, and unloaded VO₂ and Eₘₐₓ were obtained for each heart.

**MILRINONE RUNS.** After the second control run, milrinone (0.1 µM solution) was infused in eight hearts. The hearts were allowed to equilibrate for 20 min, and unloaded VO₂ and Eₘₐₓ were obtained for each heart.

Basal metabolism studies. Twenty-four hearts (eight hearts in the control state, eight hearts infused with MCI-154 in the VO₂-PVA study, and eight hearts infused with milrinone) were arrested at the volume zero (V₀) by injecting KCl (5- to 6-ml bolus dose of 0.75 eq/l followed by a continuous infusion of 1–3 meq/min) into the coronary artery tube (23). When both CBF and a-vDO₂ had stabilized (15 min of arrest), VO₂ was determined as the basal metabolic VO₂ under KCl arrest.

**Data Analysis**

Contractility. The LV contractile state was assessed in terms of Eₘₐₓ, which sensitively reflects ventricular contractility and is largely independent of ventricular loading conditions (31, 33). Eₘₐₓ was computed as the maximal value of the ratio of P(t) to [V(t) – V₀], where P(t) and V(t) are the instantaneous values of LVP and LVV, respectively, during each contraction. Eₘₐₓ was normalized for LV weight and is presented as millimeters of mercury per milliliter per 100 g LV.

VO₂-PVA relationship. The data for VO₂ and PVA were subjected to linear regression analysis to obtain a regression equation: VO₂ = aPVA + b, where a is the slope of the regression line, b is the VO₂ intercept, and the reciprocal of a (1/a) represents the contractile efficiency (31, 33, 34). aPVA represents the PVA-dependent VO₂, and b represents the PVA-independent VO₂, which reflect the VO₂ component of both excitation-contraction (E-C) coupling and basal metabolism.

Oxygen cost of enhanced contractility. To compare the oxygen cost of contractility, we calculated \( \frac{\Delta VO₂}{\Delta Eₘₐₓ} \) in the contractile states enhanced by CaCl₂, MCI-154, and milrinone (9, 24, 25).

**Statistical Analysis**

Correlation analysis and linear regression analysis were applied to a set of VO₂-PVA data from each of the control and MCI-154 runs (3). Correlation coefficients (r) were calculated, and the linear regression lines for VO₂ vs. PVA were then determined.

Comparison of the VO₂-PVA regression lines from the control and MCI-154 runs in each heart was performed by analysis of covariance (3). Statistical significance was tested by the F-test. Comparisons of the mean values of the slope and VO₂ intercept of the VO₂-PVA regression lines from the MCI-154 or milrinone runs with the control runs were performed with the unpaired t-test. A value of P < 0.05 was assumed to be statistically significant. Data are presented as means ± SD.

Analysis of variance (ANOVA) was applied to compare \( \frac{\Delta VO₂}{\Delta Eₘₐₓ} \) from the CaCl₂ and the MCI-154 or milrinone runs (3). When ANOVA showed statistical significance by the F-test, the mean values were compared by the least significant difference method.

The difference in KCl-arrested VO₂ among the control, MCI-154, and milrinone runs was also tested by ANOVA (3). A value of P < 0.05 was judged to be statistically significant.

**RESULTS**

Effects of MCI-154 on Cardiac Contractility

Figure 1, A and B, shows the effect of MCI-154 on the LV P-V loops of a representative heart. MCI-154 increased Eₘₐₓ. As shown in Fig. 1C, MCI-154 significantly increased Eₘₐₓ by 42 ± 31% (P < 0.01) in eight hearts.

**VO₂-PVA Relationships**

Figure 2A shows the VO₂-PVA data points from the control and MCI-154 runs in a representative heart. VO₂ correlated linearly and closely with PVA in each run. Correlation coefficients between VO₂ and PVA were 0.99 and 0.99 for the control runs and MCI-154 runs, respectively. In the MCI-154 run, the VO₂-PVA regression line shifted upward in parallel from the control run without an apparent change in its slope. There was no significant difference in the slope between the two regression lines (by analysis of covariance).

Figure 2B and C, shows the mean values of the slope and intercept of the VO₂-PVA relationship from the control and MCI-154 runs in eight hearts. MCI-154
heart, and the VO2 intercept (unloaded VO2) reflects the sum of the energy utilization for E-C coupling and basal metabolism. This result indicates that MCI-154 does not affect contractile efficiency but increases energy utilization for basal metabolism and/or E-C coupling.

Oxygen Cost of Contractility

We next examined whether the positive inotropic action of MCI-154 would be energetically favorable compared with that of CaCl2 or milrinone. Because of the long-lasting action of MCI-154 and milrinone, the subsequent comparisons of the two drugs were performed in two ways with CaCl2: the data were compared between CaCl2 and MCI-154 and between CaCl2 and milrinone. Table 1 summarizes the mean values of E\(_{\text{max}}\), CBF, and unloaded VO2 in the CaCl2, MCI-154, and milrinone runs. The positive inotropic effects of CaCl2 and MCI-154 are compared in Fig. 3, A and B, which shows the relative magnitudes of E\(_{\text{max}}\) and PVA-independent VO2 for CaCl2 and MCI-154 relative to the control state in eight hearts. The concentration of MCI-154 in the CBF was 1.4–11.0 nM (mean 4.6 nM) in eight hearts (calculated from the infusion rate and the measured CBF). MCI-154 increased E\(_{\text{max}}\) by 45 ± 38% (P < 0.05) and unloaded VO2 by 16 ± 33% (P > 0.05); CaCl2 increased E\(_{\text{max}}\) by 34 ± 30% (P < 0.05) and unloaded VO2 by 23 ± 8% (P < 0.01). Note that the inotropic effect of MCI-154 was stronger than that of CaCl2 but that unloaded VO2 with MCI-154 was smaller than that with CaCl2. The augmentation of contractility by CaCl2 and MCI-154 are compared in Fig. 3C, which shows the relative change ΔVO2/ΔE\(_{\text{max}}\) for CaCl2 and MCI-154 in the same heart. The mean value of ΔVO2/ΔE\(_{\text{max}}\) (the oxygen cost of contractility) for MCI-154 was significantly lower than that for CaCl2 (0.0016 ± 0.0018 vs. 0.0059 ± 0.0054; P < 0.05). Thus the oxygen cost of contractility for MCI-154 was 32 ± 28% of that for CaCl2.

The positive inotropic effects of CaCl2 and milrinone are compared in Fig. 4, A and B, which shows the relative magnitudes of E\(_{\text{max}}\) and PVA-independent VO2 for CaCl2 and milrinone relative to the control state. The concentration of milrinone in the CBF was 0.02–0.19 µM (mean 0.09 µM) in eight hearts. Milrinone increased E\(_{\text{max}}\) by 43 ± 20% (P < 0.01) and VO2 by 28 ± 15% (P < 0.01); CaCl2 increased E\(_{\text{max}}\) by 46 ± 21% (P < 0.01) and VO2 by 25 ± 14% (P < 0.01). By contrast, with MCI-154, ΔVO2/ΔE\(_{\text{max}}\) for milrinone was the same as

Table 1. E\(_{\text{max}}\), CBF, and unloaded VO2 in CaCl2, MCI-154, and milrinone runs

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<th>MCI-154 (Unloaded)</th>
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<td>Control CaCl2</td>
<td>Control MCI-154</td>
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<td>E(_{\text{max}})</td>
<td>4.6 ± 1.3</td>
<td>5.7 ± 2.0*</td>
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<td>CBF</td>
<td>26 ± 12</td>
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<td>VO2</td>
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Values are means ± SD. E\(_{\text{max}}\), left ventricular contractility index (slope of end-systolic pressure-volume relationship) in mmHg·ml\(^{-1}\)·100 g left ventricle; CBF, coronary blood flow in ml/min; VO2, myocardial oxygen consumption in ml O2·beat\(^{-1}\)·100 g left ventricle. Significant difference from control run: *P < 0.01; †P < 0.05.
that for CaCl$_2$ (0.0043 ± 0.0004 vs. 0.0054 ± 0.0045; \(P > 0.05;\) Fig. 4C). The oxygen cost of contractility for milrinone was 122 ± 77% of that for CaCl$_2$. These results showed that the oxygen cost of contractility for MCI-154 was lower than that for milrinone.

KCl Arrest

Basal metabolic VO$_2$ during KCl arrest was $1.09 \pm 0.50$ ml O$_2$·min$^{-1}$·100 g LV$^{-1}$ for the control hearts (eight hearts), $1.31 \pm 0.35$ ml O$_2$·min$^{-1}$·100 g LV$^{-1}$ for the MCI-154 hearts (eight hearts), and $1.25 \pm 0.27$ ml O$_2$·min$^{-1}$·100 g LV$^{-1}$ for the milrinone hearts (eight hearts). Thus MCI-154 did not influence the VO$_2$ for KCl-arrested basal metabolism. This result indicates that MCI-154 decreased the oxygen cost for E-C coupling.

DISCUSSION

We investigated the effects of MCI-154, which is known to increase the response of the myofilament to...
Ca\textsuperscript{2+}, on cardiac mechanoenergetics in terms of E\textsubscript{max} and the VO\textsubscript{2}-PVA relationship in blood-perfused canine whole hearts. MCI-154 increases E\textsubscript{max} and the VO\textsubscript{2} intercept (unloaded VO\textsubscript{2}) of the VO\textsubscript{2}-PVA relationship but does not affect the slope of the relationship and basal metabolism. The increase in unloaded VO\textsubscript{2} with MCI-154, however, is less than that with CaCl\textsubscript{2} or milrinone despite an equal increase in E\textsubscript{max}. MCI-154, therefore, significantly reduces the oxygen cost of contractility. These findings suggest that MCI-154 acts energetically as a Ca\textsuperscript{2+} sensitizer in beating canine whole hearts.

Effects of MCI-154 on Cardiac Energetics

We assessed the effects of MCI-154 on cardiac energetics using E\textsubscript{max} and the VO\textsubscript{2}-PVA relationship (31, 33, 34). The present study showed that MCI-154 did not affect contractile efficiency but increased energy utilization for basal metabolism and/or E-C coupling. In KCl-arrested hearts, the basal metabolism was not affected by MCI-154. These findings suggest that the increase in unloaded VO\textsubscript{2} by MCI-154 is primarily due to an increase in energy utilization for E-C coupling, which may be associated with the inhibition of PDE by this compound. Complete β-blockade was used, which would be expected to greatly diminish the impact of any PDE inhibitory action by an inotropic agent (7, 10). However, because we did not measure cAMP levels in our hearts after exposure to MCI-154, we cannot fully exclude this mode of action of this agent. In fact, in these experiments, milrinone also increased the intercept of the VO\textsubscript{2}-PVA relationship.

MCI-154 has been shown to possess a prominent Ca\textsuperscript{2+}-sensitizing action in skinned cardiac muscles (16, 17, 27). If MCI-154 could act as a Ca\textsuperscript{2+} sensitizer in intact cardiac muscles, the relative increase in energy utilization for E-C coupling by MCI-154 should be less than that of a conventional inotropic agent that increases Ca\textsuperscript{2+} transients (7, 11, 28, 31, 37). To test this hypothesis, we compared the mechanoenergetic effects of MCI-154 with those of CaCl\textsubscript{2}. Our results indicate that, compared with CaCl\textsubscript{2}, MCI-154 consumes less energy for E-C coupling. The oxygen cost of contractility with MCI-154 was 32 ± 28% of that with CaCl\textsubscript{2}, so the contribution of its Ca\textsuperscript{2+}-sensitizing action to its inotropic action can be calculated as 68%. This clearly suggests that MCI-154 exerts a positive inotropic effect, mainly through Ca\textsuperscript{2+} sensitization. Moreover, the oxygen cost of contractility with a PDE inhibitor, milrinone, was 122 ± 77% of that with CaCl\textsubscript{2}. Thus the oxygen cost of contractility with MCI-154 is remarkably lower than that with milrinone. Taken together, these results indicate that MCI-154 acts energetically, mainly by increasing Ca\textsuperscript{2+} sensitivity and not by inhibiting PDE in beating whole hearts.

Comparison With Prior Studies

Ca\textsuperscript{2+}-sensitizing action of MCI-154. MCI-154 has been shown to increase the myofibrillar Ca\textsuperscript{2+} sensitivity and the maximum Ca\textsuperscript{2+}-activated force in skinned cardiac fibers obtained from guinea pig, dog, and human hearts (16, 17, 27). Moreover, biochemical studies (15, 20) have revealed that the Ca\textsuperscript{2+}-sensitizing action of MCI-154 may be responsible for the stimulation of Ca\textsuperscript{2+} binding to troponin C. However, it was unknown whether MCI-154 also acted as a Ca\textsuperscript{2+}-sensitizing agent in intact beating hearts. In the present study, we have demonstrated that in blood-perfused canine hearts MCI-154 does act energetically as expected for a Ca\textsuperscript{2+}-sensitizing agent. It should be noted that the concentrations of MCI-154 producing a Ca\textsuperscript{2+}-sensitizing action in the previous studies using skinned fibers are 10\textsuperscript{-6} to 10\textsuperscript{-4} mol/l (16, 17, 27), far higher than that in the present study (4.6 ± 2.9 nmol/l). To learn whether MCI-154 acts as a Ca\textsuperscript{2+} sensitizer at such low concentrations in intact whole hearts, Abe et al. (1) recently exposed indocyanine loaded Langendorff guinea pig hearts to MCI-154 at 10\textsuperscript{-10} to 10\textsuperscript{-6} mol/l. In these experiments they found that MCI-154 exerts a positive inotropic effect, mainly through its Ca\textsuperscript{2+}-sensitizing action. In that study, the effects of MCI-154 were compared with CaCl\textsubscript{2}. Interestingly, the contribution of its Ca\textsuperscript{2+}-sensitizing action to its inotropic effect was calculated to be ~60%, which is close to the value (68%) in the present experiments. Therefore, it can be concluded that MCI-154 does act as a Ca\textsuperscript{2+}-sensitizing agent in the beating whole heart.

Effect of MCI-154 on VO\textsubscript{2}. It is often suggested that the use of Ca\textsuperscript{2+}-sensitizing agents is an energetically favorable way of producing an inotropic effect (12, 26, 31). In fact, Onishi et al. (26) demonstrated that respiratory alkalosis, which increases the sensitivity of the contractile protein to Ca\textsuperscript{2+}, simultaneously causes a decrease in the oxygen cost of contractility. This may lead to the conclusion that Ca\textsuperscript{2+} sensitization of the contractile protein would provide an energetic benefit for the hearts (26). In anesthetized open-chest canine hearts, Abe et al. (2) found that MCI-154 exerts a positive inotropic effect and hence corrects the contractile abnormalities without increasing VO\textsubscript{2} during coronary artery stenosis. Moreover, Mori et al. (22) demonstrated that MCI-154 provides an advantageous energetic profile for patients with coronary artery disease. However, in those studies, the systemic administration of MCI-154 produced a decrease in systemic blood pressure, offsetting the increase in VO\textsubscript{2} by decreasing wall stress during the augmentation of the contractile state. Thus the indirect effect of MCI-154 on cardiac energetics through systemic vasodilation could not be ruled out. By using the isolated canine whole heart, this study has provided more direct evidence showing that MCI-154 possesses an advantageous energetic profile, as expected for a Ca\textsuperscript{2+}-sensitizing agent.

Ca\textsuperscript{2+} sensitization and mechanoenergetics. The energy utilization of the heart increases in proportion to augmentation of myocardial contractility (8, 31, 35). In each cardiac cycle, a certain amount of ATP is utilized by Ca\textsuperscript{2+}-adenosinetriphosphatase on the sarcoplasmic reticulum and sarcolemma to return the high systolic Ca\textsuperscript{2+} concentrations to their low resting levels during diastole (8, 9, 28, 31, 37). Theoretically, sensitization of
the contractile machinery to Ca\(^{2+}\) could improve the mechanoenergetics of the heart. Thus evaluation of the effects of Ca\(^{2+}\)-sensitizing agents on myocardial energetics is of great importance not only to assess the clinical values of these compounds but also to improve our understanding of the energetic aspects of the regulation of myocardial contractile function. Our results showed that the hearts treated with MCI-154 consume less energy for E-C coupling than hearts treated with CaCl\(_2\) when these drugs increase the myocardial contractile state. In contrast, Hata et al. (10) showed that pimobendan does not provide an energetic advantage over dobutamine within the framework of E\(_{\text{max}}\) and the VO\(_2\)-PVA relationship in blood-perfused canine whole hearts. Although pimobendan increases myofibrillar Ca\(^{2+}\) sensitivity of skinned cardiac fibers and stimulates Ca\(^{2+}\) binding to troponin C, it failed to produce a net Ca\(^{2+}\) sensitization in intact ventricular muscles, probably due to the decrease in the myofibrillar Ca\(^{2+}\) sensitivity by the phosphorylation of troponin I (19). Furthermore, the Ca\(^{2+}\) sensitization effect induced by MCI-154 on the intact beating heart was much stronger than that induced by pimobendan (1). Recently, de Tombe et al. (7) reported that a more selective Ca\(^{2+}\) sensitizer, EMD-53998, also fails to provide an advantageous energetic profile compared with CaCl\(_2\). Ishisu et al. (13) also found that EMD-53998 fails to produce a net Ca\(^{2+}\) sensitization in beating intact hearts of guinea pigs. These results are possibly due to PDE inhibition caused by the (-)-enantiomer EMD-57439, which is a potent PDE inhibitor with almost no Ca\(^{2+}\)-sensitizing action (21, 36). Therefore, the present study has shown, for the first time, that cardiac energetics can be improved by pharmacological intervention by sensitizing the contractile machinery to Ca\(^{2+}\).

Limitations of the Study

MCI-154 has been shown to increase the maximal Ca\(^{2+}\)-activated force and tension development induced by Mg\(^{2+}\)-ATP in the absence of free Ca\(^{2+}\) in skinned cardiac muscles (15), suggesting that the compound may affect the interaction between actin and myosin. We hypothesized that MCI-154 may affect not only energy utilization for E-C coupling (assessed by unloaded VO\(_2\)) but also contractile efficiency (assessed by the slope of the VO\(_2\)-PVA relationship) in the framework of E\(_{\text{max}}\) and the VO\(_2\)-PVA relationship in canine whole hearts. However, we found that MCI-154 decreased the oxygen cost of contractility but did not affect the slope of the VO\(_2\)-PVA relationship. A previous study (32) also revealed that the slope of the VO\(_2\)-PVA relationship did not change during cardiac cooling, which is known to decrease the rate of cross-bridge cycling. Recently, Onishi et al. (26) also found that the positive inotropic produced by respiratory alkalosis, which would change the cycling rate of the cross bridge, decreases the oxygen cost of contractility but does not affect the slope of the VO\(_2\)-PVA relationship. Taken together, there may be a potential limitation to studies using the VO\(_2\)-PVA relationship to assess contractile efficiency.

The limitations of the use of E\(_{\text{max}}\) to assess cardiac contractility should be pointed out. E\(_{\text{max}}\) provides a measure of the end-systolic chamber stiffness over a given loading range (14). Ca\(^{2+}\) sensitizers may change the end-systolic chamber stiffness of the LV, and whether the stiffness is what we mean by contractile state is controversial.

Summary

In the present study, we assessed the effects of MCI-154 on cardiac energetics using E\(_{\text{max}}\) and the VO\(_2\)-PVA relationship in blood-perfused canine whole hearts. MCI-154 exerted a positive inotropic effect at therapeutically relevant concentrations. MCI-154 did not affect the slope of the VO\(_2\)-PVA relationship (contractile efficiency) and basal metabolism of the heart, but it did increase the VO\(_2\) intercept of the relationship. The increase in unloaded VO\(_2\) with MCI-154, however, is less than that with CaCl\(_2\) or milrinone despite an equal increase in E\(_{\text{max}}\), resulting in a significant decrease in the oxygen cost of contractility. These findings suggest that MCI-154 acts energetically as a Ca\(^{2+}\) sensitizer in canine whole hearts.

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