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Effects of Calcium and EMD-53998 on Oxygen Consumption in Isolated Canine Hearts

Pieter P. de Tombe, PhD; Daniel Burkhoff, MD, PhD; and William C. Hunter, PhD

Background. Most positive inotropic agents increase cardiac contractility by increasing the amount of Ca^{2+} cycled with each beat. The additional amount of oxygen that is consumed by the heart to cycle this additional Ca^{2+} is believed to reduce myocardial efficiency. On the other hand, it has been suggested that the agent EMD-53998 increases the Ca^{2+} sensitivity of the contractile proteins without affecting the intracellular Ca^{2+} transient in cardiac muscle. Therefore, application of this agent may increase cardiac contractility without decreasing myocardial efficiency. The purpose of the present study was to test this hypothesis.

Methods and Results. We measured myocardial oxygen consumption ($\dot{M}\dot{V}\text{O}_2$) in six isolated, isovolumically beating blood-perfused canine hearts. The hearts were paced at 120 beats per minute. Contractility was varied in each heart by infusion of either CaCl_2 or EMD-53998. With infusion of either agent, $\dot{M}\dot{V}\text{O}_2$ was a linearly proportional function of contractility. No significant difference between CaCl_2 and EMD-53998 could be detected in the interrelation between contractility and $\dot{M}\dot{V}\text{O}_2$.

Conclusions. We conclude that the "calcium-sensitizing agent" EMD-53998 is a potent positive inotropic agent in the isolated, blood-perfused canine heart. However, EMD-53998 does not provide an energetic advantage over currently used positive inotropic agents. (*Circulation* 1992;86:1945–1954)

KEY WORDS • inotropic agents • calcium • myocardium • myocardial oxygen consumption

Results of previous studies have shown that myocardial oxygen consumption ($\dot{M}\dot{V}\text{O}_2$) of the externally unloaded left ventricle (LV) ($\dot{M}\dot{V}\text{O}_{2\text{-unloaded}}$) varies directly with ventricular contractile state.^{1–4} This finding has been hypothesized to reflect altered energy demands for calcium cycling since changes in contractile state are usually brought about by changes in the amount of calcium cycled with each beat.^{5–7}

However, new classes of inotropic agents have emerged recently that reportedly affect contractile state by altering the sensitivity of the myofilaments to calcium instead of—or in addition to—altering the amount of calcium available for contraction.^{8–13} For example, it has been suggested that the compound EMD-53998 [5-(1-(3,4-dimethoxybenzoyl)-1,2,3,4-tetrahydro-6-quinolyl)-6-methyl-3,6-dihydro-2H-1,3,4-thiadiazin-2-one] (E. Merck Pharmaceuticals, Darmstadt, Germany) increases the calcium sensitivity of the myofilaments without affecting the intracellular calcium transient.^{12–14}

It has been suggested, therefore, that in the presence of EMD-53998, less calcium would have to be delivered to the myofilaments to achieve a certain contractile state.¹² The resultant lower amount of calcium cycled with each beat would be expected to be accompanied by a lower myocardial oxygen demand to achieve a contractile state similar to that induced by a conventional positive inotropic agent. Thus, one would predict that the relation between $\dot{M}\dot{V}\text{O}_{2\text{-unloaded}}$ and contractile state would be shifted downward by EMD-53998 compared with, for example, infusion of CaCl_2 . The purpose of the present study was to test this hypothesis in isolated, blood-perfused canine hearts.

Methods

Surgical Preparation

A total of six isolated canine ventricles were studied. The procedures used to isolate and support the canine heart have been previously described in detail.^{4,15} Briefly, two mongrel dogs (25–35 kg) anesthetized with sodium pentobarbital (30 mg/kg) were used for each experiment. The chest of the heart donor dog was opened under artificial ventilation. The subclavian artery and right atrium were cannulated and connected to a perfusion system that was used to supply oxygenated blood to the isolated heart. The perfusion system was connected to the femoral arteries and veins of a support dog. The support dog was premedicated with hydrocortisone (500 mg i.m.), diphenhydramine (50 mg i.v.), and indomethacin (25 mg p.r.). Heparin (5,000 units i.v.) was administered after the arteries and veins were cannulated and connected to the perfusion system. After ligation of the azygous vein, superior and inferior

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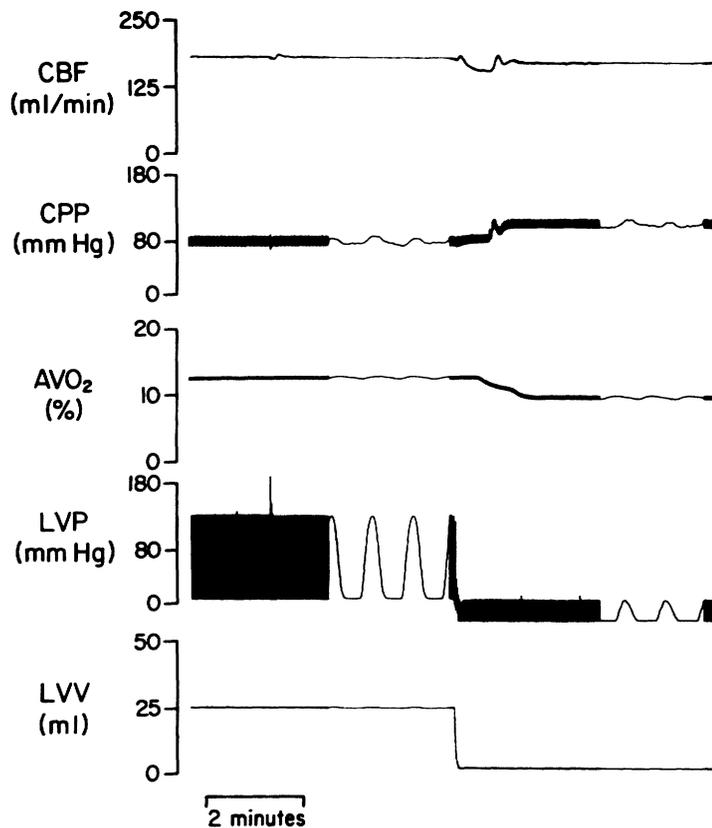


FIGURE 1. Representative original recording of a data run illustrating the protocol used to measure contractile state and myocardial oxygen consumption ($M\dot{V}O_2$) (measurement of P_{25} , $M\dot{V}O_{2\text{-loaded}}$, and $M\dot{V}O_{2\text{-unloaded}}$). Top to bottom panels: Coronary blood flow (CBF), coronary perfusion pressure (CPP), arterial-venous difference in oxygen content ($A\dot{V}O_2$), left ventricular pressure (LVP), and left ventricular volume (LVV). Several beats were also recorded at 100-fold increased chart speed for both the reference preload volume (LVV, 25 ml) and in the unloaded state (i.e., with the balloon collapsed; LVV, ≈ 2 ml). The large negative pressure present in the balloon in the unloaded state is not present in the left ventricle (see "Methods" in text). (Experiment no. 20/03/91 — EMD-53998 infusion was $1.3 \mu\text{M}$.)

venae cavae, brachiocephalic artery, descending aorta, and pulmonary hili, the heart was removed from the chest. From this time on, coronary perfusion was provided by arterial blood from the support dog, with temperature controlled at 37°C by a heat exchanger. The LV was vented, the left atrium was opened, and all chordae tendineae were cut. A ring adapter was sewn into the mitral annulus, and a thin latex balloon (filled with water) connected to a volume-control system was placed within the LV chamber. Left ventricular pressure (LVP) was measured inside the balloon with micromanometer (PC-380, Millar, Houston, Tex.). A bipolar surface ECG was obtained from two surface electrodes sutured to the LV.

Coronary perfusion pressure (CPP) was measured via a catheter placed through the brachiocephalic trunk into the aortic root. Two perfusion pumps (model 1215, Harvard Apparatus, South Natick, Mass.) maintained mean CPP between 80 and 120 mm Hg¹⁶ (see Figure 1 and below). The first pump withdrew arterial blood from the support dog at a constant rate, and the second pump diverted dog flow to bypass the isolated heart. With this arrangement, the hemodynamic demand on the support dog could be maintained constant, independent of coronary flow to the isolated heart. This helped minimize fluctuations in support dog catecholamine levels that might ordinarily result from varying flow demands of the isolated heart. This way, we avoided one source of instability of the contractile state of the isolated heart. In addition, we also induced complete β -blockade in the isolated heart to further limit the impact of circulating catecholamines on the results of the present study (see below and "Discussion"). The support dog was ventilated mechanically. Blood pH,

PO_2 , and PCO_2 were maintained in the normal range by adjusting the ventilation rate or by administering sodium bicarbonate or oxygen as dictated by results of periodic blood gas analyses (every 30 minutes).

The right ventricle (RV) and atrium of the isolated heart were drained via a wide-bore cannula with side holes. The right heart was made airtight by purse-string suturing around the cannula at the RV apex and ligation of superior and inferior venae cavae. Total coronary blood flow (CBF) (excluding the small amount of LV Thebesian flow¹⁷) was measured continuously by draining all blood from the right heart past an in-line ultrasonic flow probe (Transonics, Ithaca, N.Y.). The flow probe was calibrated by timed collection. The difference in oxygen content between arterial and coronary venous blood ($A\dot{V}O_2$) was measured continuously by absorption spectrophotometry (A-VOX Systems¹⁸) calibrated to an oxygen analyzer (Lex-O₂-Con). Total heart oxygen consumption ($M\dot{V}O_2$) was calculated as the product of CBF and $A\dot{V}O_2$.

Atrioventricular conduction block was induced by 0.1–0.3 ml of 10% formalin solution injection into the AV node. Hearts were then paced at the LV apex at a rate of 120 beats per minute. We have shown previously that LV contractile strength is greatest with apical pacing versus pacing from any other site on the ventricle.¹⁹

Experimental Protocol

After surgical preparation was complete, ventricular volume was increased by infusing 20 ml H_2O into the latex balloon. Because the balloon material occupied 5 ml, this resulted in an LV preload volume of 25 ml (reference preload volume). Next, the hearts were allowed to equilibrate for approximately 20 minutes, after

which β -blockade was induced by intracoronary infusion of propranolol (2 mg bolus) followed by a continuous intracoronary perfusion of propranolol (1 mg/hr). Preliminary studies showed that this procedure resulted in an apparent complete β -blockade of the isolated heart during the course of the entire experiment (i.e., absence of an inotropic response to a bolus of 5 μ g dobutamine injected into the coronary perfusion line). The rationale for performing the experiments during complete β -blockade was to minimize the impact of the phosphodiesterase inhibitory action of EMD-53998^{12,13} (see "Discussion") and to minimize the impact of varying levels of catecholamines in the blood of the support dog. β -Blockade reduced the contractile state of the hearts by approximately 50%; the post- β -blockade level of contractility will be referred to as the baseline contractile state. After β -blockade, hearts were again allowed to equilibrate for approximately 20 minutes.

In each heart, data were first collected in the baseline contractile state and at one or more levels of increased contractile state induced by CaCl₂ (0.5 M solution in saline) infusion at increasing rates. The maximum rate of CaCl₂ infusion was chosen such that LVP development was increased by approximately twofold to fourfold above the baseline level. The range of maximum CaCl₂ infusion rates was 0.08–0.4 ml/min, which corresponded to an increase of 0.27–1.29 mM (mean, 0.84 \pm 0.36 mM) total CaCl₂ added to the perfusing blood (note that the actual free concentration of Ca²⁺ was unknown). After discontinuation of the CaCl₂ infusion, contractile state was increased to one or more levels (twofold to fourfold baseline contractile state) by EMD-53998 infusion (10 mM solution in propylene glycol) at increasing rates. The maximum rate of EMD-53998 infusion ranged from 0.05 to 0.206 ml/min, which corresponded to 2.0–11.6 μ M (mean, 5.4 \pm 3.5 μ M) EMD-53998. Thus, a substantially wide and overlapping range of contractile states was examined in each heart, with both CaCl₂ infusion and EMD-53998 infusion. After each increase in the rate of EMD-53998 infusion, at least 10 minutes were allowed for equilibration (Figure 2). The final concentration of CaCl₂ and EMD-53998 in the blood perfusing the isolated heart was calculated from the infusion rate and the measured CBF. To allow for the direct comparison between the effects of EMD-53998 and CaCl₂ infusion, care was taken to ensure that at least one data run was performed in each group under conditions in which the end-systolic pressure was close to 120 mm Hg at a preload volume of 25 ml (Table 2), that is, conditions of matched contractile state. After the data run at the highest rate of EMD-53998 infusion, infusion of the drug was discontinued, and the heart was allowed to recover for approximately 20 minutes, after which one final data run was performed at that contractile state (washout). Finally, to examine the effect of propylene glycol itself (i.e., the solvent of EMD-53998; vehicle) on contractile state and M \dot{V} O₂, propylene glycol was infused at rates (range, 0.10–0.4 ml/min) that exceeded the range of infusion rates encountered during the infusion of EMD-53998; in four hearts, this measurement was made in between the CaCl₂ and EMD-53998 data runs, and in one heart, it was made after the washout of EMD-53998.

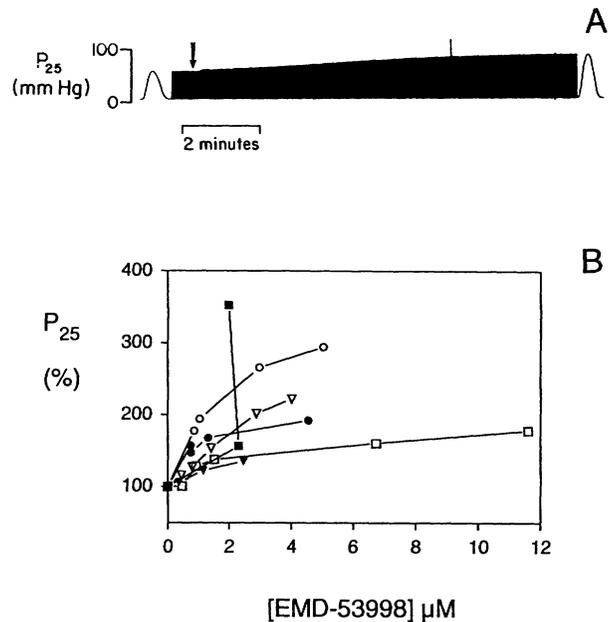


FIGURE 2. Plot of EMD-53998 dose–response curve. Panel A: Original recording in a representative heart illustrating the slow onset of the positive inotropic effect of EMD-53998. The drug was infused into the coronary perfusion line at the time indicated by the arrow (0.8 μ M final concentration). The heart contracted isovolumically at the reference preload volume (left ventricular volume, 25 ml). Experiment no. 05/03/91. Panel B: Increase in contractile state (as indexed by P₂₅) in all hearts as function of EMD-53998 concentration perfusing the heart. P₂₅ was normalized to the pre-drug infusion contractile state. From 1 to 7 μ M EMD-53998 was required to increase contractile strength of the hearts by 50%.

Data Collection

Figure 1 shows an original recording of a data run obtained in a representative heart. Hearts contracted isovolumically during the entire protocol. At each contractile state each experimental run consisted of making measurements at a preload ventricular volume of 25 ml, with the balloon almost totally collapsed (i.e., unloaded condition). Because the balloon material occupied approximately 4–5 ml, the latter preload volume amounted to <5 ml. Under these conditions, a large negative pressure is measured in the balloon. This pressure, however, is not present in the LV. That is, a similar pressure can be measured inside the balloon when the balloon is collapsed outside the LV. Furthermore, a significant negative pressure could not exist in the LV because leakage around the mitral annulus would quickly cancel any negative pressure. Thus, hearts do not beat isovolumically in the unloaded state,²⁰ but rather, LV volume decreases to approximately 4–5 ml during systole without significant pressure development. After the change in preload volume to the unloaded state, 2–3 minutes were required to ensure that data were obtained at steady state. Under normal circumstances, when perfusion pressure is fixed, unloading the heart would result in a decrease of CBF. Because CaCl₂ and EMD-53998 were infused at a constant rate into the coronary perfusion line, a decrease in CBF would lead to an increase of the concen-

tration of the infused agent and thus lead to an increase in contractile state. To eliminate this confounding factor, perfusion flow was set at each contractile state such that CPP was approximately 80 mm Hg when the heart contracted at the reference volume (LV volume [LVV], 25 ml); perfusion flow was then fixed at that level for the remainder of that data run. Thus, unloading of the heart resulted in an increase in CPP rather than a decrease in CBF (Figure 1), thereby ensuring a constant contractile state of the heart during the measurements in each data run.

Recorded signals in each data run in the steady state included LVP, LVV, CPP, CBF, $\dot{A}V_{O_2}$, and a surface ECG. Signals were digitized (12-bit resolution) at a sampling rate of 200 Hz, stored on diskettes, and analyzed off-line. Several beats of data were recorded at each of the two preload volumes. At the end of the experiment, the weights of the RV wall and the LV (LV free wall plus septum) were measured. RV free wall mass averaged 53.2 ± 9.0 g, and LV mass averaged 129.9 ± 7.0 g in the six hearts studied.

Data Analysis

Contractile state of the heart, which traditionally is indexed by the slope of the end-systolic pressure-volume relation (ESPVR) in our preparation,^{4,15} was indexed in this study by LV developed pressure at the reference preload volume (LVV, 25 ml; P_{25}). This procedure was adopted to account for the nonlinear ESPVR that is observed at low and high levels of contractile state in the isolated canine heart.²¹ In the hearts used in the present study, the reference preload volume (25 ml) corresponded to a volume on the ESPVR at which developed pressure is most sensitive to changes in contractile state.^{20,21} Adoption of this protocol as opposed to the full evaluation of the ESPVR allowed for a greater number of data runs (i.e., different contractile strength with different inotropic agents) to be evaluated in each heart because less time was required to acquire the data at each contractile state.²

The oxygen consumption of the entire heart ($\dot{M}V_{O_2}$) represents the sum of the oxygen consumed by the work-performing LV plus the unloaded, but still oxygen-consuming, RV. To correct for this, we assumed that the RV consumed an amount of the total unloaded oxygen consumption—measured when the LV was also unloaded—that was proportional to its weight, and we subtracted this amount of consumed oxygen from all the data at each contractile state.³ Note that the amount of this correction was assessed at each contractile state. LV $\dot{M}V_{O_2}$ was normalized to the weight of the LV and expressed in milliliters of O_2 per minute per gram. $\dot{M}V_{O_2}$ measured in the unloaded state is called $\dot{M}V_{O_2\text{-unloaded}}$; $\dot{M}V_{O_2}$ measured at the reference preload volume (LVV, 25 ml) will be called $\dot{M}V_{O_2\text{-loaded}}$.

In addition to increasing the sensitivity of the myofilaments to the binding of calcium ions, EMD-53998 has been shown to inhibit the activity of intracellular phosphodiesterases.^{12,14} This action of the drug could lead to an increase in intracellular levels of cyclic-AMP (cAMP). Increased levels of cAMP would then lead to increased levels of intracellular calcium, which would cause an increase in both contractile state and $\dot{M}V_{O_2}$.⁷ For this reason, the present study was performed during complete β -blockade. Nevertheless, if this effect of

EMD-53998 were to occur, this would confound the interpretation of the present study. Therefore, we sought to measure the impact of this factor. Increased levels of cAMP cause the rate of pressure development and relaxation to increase.^{6,7} We therefore measured the effects of $CaCl_2$ and EMD-53998 infusions on parameters that index the time course of the pressure waveform at matched contractile states (i.e., with end-systolic pressure close to 120 mm Hg; see above). For this purpose, in each drug infusion group at the reference preload volume, we determined peak developed pressure (PP), time required for pressure to develop from 10% PP to PP (TTP), and time required for developed pressure to decay from PP to 10% PP (Relax).²⁰

The effect of EMD-53998 on smooth muscle tone (i.e., contractile state) of the coronary arteries was assessed by determining the mean resistance to coronary flow (R_{coronary}), defined simply as mean CPP divided by mean CBF. These measurements were made at matched contractile states induced by either EMD-53998 or $CaCl_2$ infusion with the LV contracting in the unloaded state.

Statistical Analysis

The oxygen consumption at the two loading conditions (i.e., $\dot{M}V_{O_2\text{-unloaded}}$ and $\dot{M}V_{O_2\text{-loaded}}$) was plotted as function of contractile state (P_{25}) in each heart. To test whether EMD-53998 infusion affected the relation between P_{25} and $\dot{M}V_{O_2}$ compared with $CaCl_2$ infusion, we used multiple linear regression analysis to data obtained in each individual heart as well as to pooled data from all six hearts.²² In the regression model for the individual hearts, one dummy variable coded for the presence or absence of EMD-53998. In the regression model for pooled data, an additional dummy variable coded for the experiment number. The effect of washout of EMD-53998 and infusion of propylene glycol (vehicle) was tested separately against $CaCl_2$ infusion in a similar manner. All other parameters (e.g., those measured at matched contractile states) were tested by paired Student's *t* test. Commercially available software was used (SYSTAT, Evanston, Ill.). Data are presented as mean \pm SD. A value of $p < 0.05$ was considered significant.

In one heart (experiment no. 17/04/91), infusion of EMD-53998 at the highest rate caused an increase in CBF slightly above that observed in the other five hearts. The calculated EMD-53998 concentration during that data run was lower than might be expected from the increase in contractile state that was observed (see dose-response curve indicated by the filled squares in Figure 2B). Because no criterion other than the above average CBF could be identified for this data run, we included it in the present study. However, neither the conclusions nor the level of statistical significance of either the mechanical or energetic aspects of the current study was affected by inclusion of this data run.

Results

Contractile State

A total of 30 and 28 contractile states were analyzed with $CaCl_2$ and EMD-53998 infusion, respectively, in six hearts. On average, LV pressure development in the baseline contractile state at the reference preload vol-

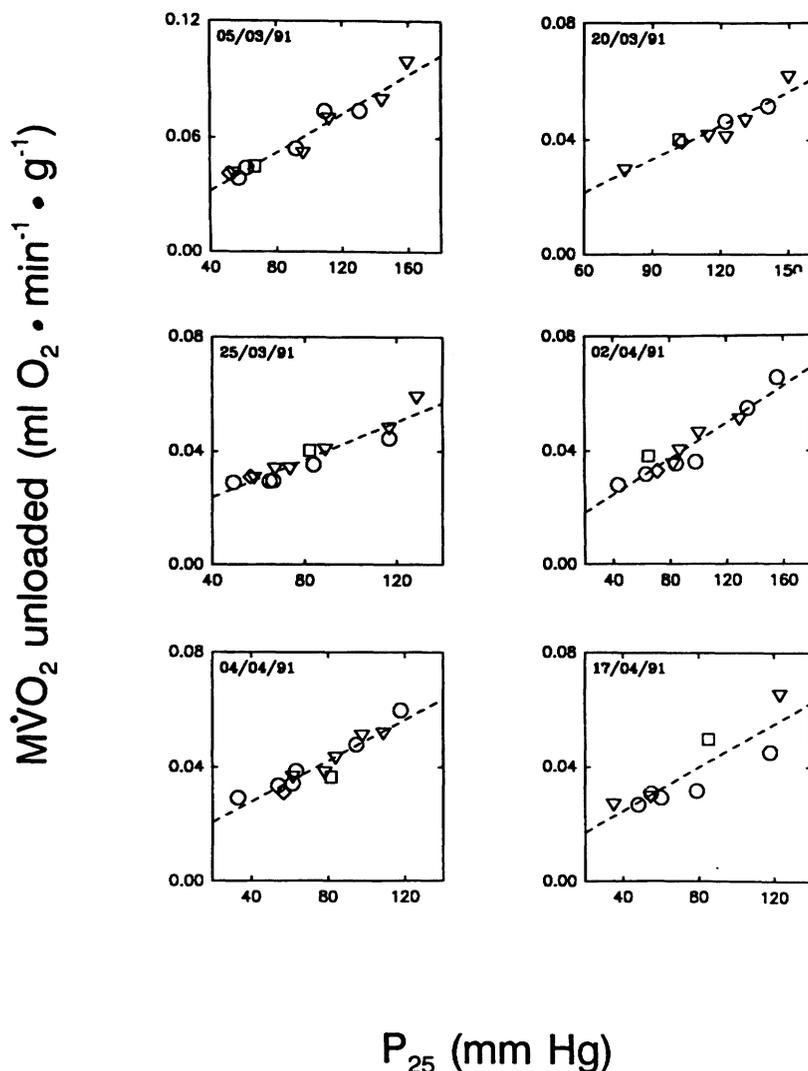


FIGURE 3. Scatterplots of unloaded left ventricular myocardial oxygen consumption ($M\dot{V}O_{2\text{-unloaded}}$) measured as function of contractile state (as indexed by P_{25}) in six isolated canine hearts. Contractile state was increased either by CaCl_2 infusion (\circ) or by EMD-53998 infusion (\triangle). Twenty minutes after washout of EMD-53998, an additional datum point was acquired (\square). Infusion of propylene glycol alone (vehicle for EMD-53998) is indicated by \diamond . Multiple linear regression analysis (see Table 1) revealed no significant effect of EMD-53998 on the relation between $M\dot{V}O_{2\text{-unloaded}}$ and P_{25} . ----, Linear regression analysis of the combined CaCl_2 and EMD-53998 data.

ume (LVV, 25 ml; P_{25}) was 51.5 ± 15.2 mm Hg. At the highest rate of CaCl_2 infusion, P_{25} was increased to 130.0 ± 15.8 mm Hg ($p < 0.001$). After the infusion of CaCl_2 , P_{25} returned to 61.4 ± 17.1 mm Hg ($p = 0.27$ versus baseline P_{25}).

The impact of EMD-53998 on contractile state is summarized in Figure 2. A consistent finding in all hearts was the slow onset of action of the drug as is illustrated in Figure 2A. Therefore, at least 10 minutes were required after each increase in the EMD-53998 infusion rate to ensure steady state. Figure 2B shows the dose-response curves for EMD-53998 obtained in all six hearts. Infusion of EMD-53998 at concentrations between 1 and 7 μM caused a 50% increase of P_{25} , which is consistent with recent results in isolated papillary muscles¹² and isolated myocytes.^{13,14} However, even though some indication of saturation of the response to the drug could be observed in five of the six hearts, a clear maximum saturated response was not observed in any of the hearts. Therefore, an EC_{50} value for the response to EMD-53998 infusion could not be obtained in the present study. At the highest rate of EMD-53998 infusion (5.4 ± 3.5 μM), P_{25} was increased from 61.4 ± 17.1 mm Hg to 130.2 ± 20.6 mm Hg ($p = 0.002$ versus the baseline contractile state obtained

after CaCl_2 washout); end-diastolic pressure increased, on average, from 4.9 ± 2.3 to 5.6 ± 2.5 mm Hg ($p = 0.07$). Therefore, a potentially undesirable effect of calcium-sensitizing drugs to increase diastolic stiffness¹³ appeared not to play a significant role in the isolated, blood-perfused canine heart.

Myocardial Oxygen Consumption

We next examined whether the positive inotropic action of EMD-53998 would be energetically favorable compared with that of CaCl_2 infusion. Figure 3 shows unloaded oxygen consumption of the left ventricle ($M\dot{V}O_{2\text{-unloaded}}$) as a function of contractile state (as indexed by P_{25}). In all six hearts, infusion of CaCl_2 caused an increase in both contractile state and P_{25} (open circles), which is consistent with previous findings in the isolated canine heart.^{1-4,20} Similarly, infusion of EMD-53998 caused an increase in P_{25} and $M\dot{V}O_{2\text{-unloaded}}$ in each heart (open triangles). The coefficients of the multiple linear regression analysis that compare the $M\dot{V}O_{2\text{-unloaded}}-P_{25}$ relations between CaCl_2 and EMD-53998 infusion are presented in the top panel of Table 1. Each of the columns in Table 1 indicates the magnitude and level of significance of the effects of contractility per se on $M\dot{V}O_2$ no matter which agent was used to

TABLE 1. Coefficients of Multiple Linear Regression Analyses of $\dot{M}\dot{V}O_{2\text{-unloaded}}$ or $\dot{M}\dot{V}O_{2\text{-loaded}}$ as Function of P_{25}

Experiment date	CaCl ₂ /EMD infusions (No.)	Constant ($\cdot 10^{-3}$)	α ($\cdot 10^{-4}$)	β ($\cdot 10^{-3}$)	γ ($\cdot 10^{-5}$)
$\dot{M}\dot{V}O_{2\text{-unloaded}}$					
05/03/91	5/5	11.2	5.1*	-3.3	2.7
20/03/91	3/5	1.0	3.7*	-7.3	5.7
25/03/91	5/6	14.6*	2.5*	-6.7	2.2
02/04/91	6/4	9.7	3.3*	2.9	2.9
04/04/91	6/5	14.5*	3.7*	-2.3	0.2
17/04/91	5/3	15.1*	2.4*	-7.1	21.2
Overall analysis	30/28	9.1	3.7*	-1.2	2.6
$\dot{M}\dot{V}O_{2\text{-loaded}}$					
05/03/91	5/5	11.3	7.7*	-4.2	0.6
20/03/91	3/5	2.0	5.8*	0.3	0.4
25/03/91	5/6	20.4*	4.7*	-15.2*	21.0*
02/04/91	6/4	19.1*	4.4*	-20.7*	25.4*
04/04/91	6/5	19.4*	4.7*	-12.9	14.1
17/04/91	5/3	11.6	4.2*	-4.9	16.3
Overall analysis	30/28	13.6	5.3*	-6.6	0.4*

P_{25} , developed pressure; $\dot{M}\dot{V}O_2$, myocardial oxygen consumption.

*A coefficient is significantly ($p < 0.05$) different from zero by multiple linear regression.

Multiple linear regression²² analysis was performed in each individual heart on the $\dot{M}\dot{V}O_{2-x}-P_{25}$ relation ($x = \text{unloaded}$ or loaded state; see Figures 3 and 4) using the following regression equation:

$$\dot{M}\dot{V}O_{2-x} = \text{constant} + \alpha \cdot P_{25} + \beta \cdot \text{EMD} + \gamma \cdot \text{EMD} \cdot P_{25}$$

EMD was a dummy variable that indicated either CaCl₂ or EMD-53998 infusion (respectively, EMD=0, 1). In the overall analysis that incorporated the data obtained in all six hearts, an additional set of five dummy variables was included in the regression model to code for the differences in slope and intercept of the $\dot{M}\dot{V}O_{2-x}-P_{25}$ relation in each individual heart during CaCl₂ infusion.

change contractility (column α), a uniform increase or decrease of $\dot{M}\dot{V}O_2$ at all levels of contractility induced by EMD-53998 compared with CaCl₂ (column β), and a change in the impact of contractility on $\dot{M}\dot{V}O_2$ induced by EMD-53998 compared with CaCl₂ (column γ). The data in Table 1 indicate that EMD-53998 did not affect either the slope (column β) or the intercept (column γ) of this relation. Furthermore, this was true for the analysis applied to data from each individual heart and for the analysis of data pooled from all hearts. Thus, in terms of the relation between contractile state and oxygen consumption of the unloaded LV, EMD-53998 infusion was indistinguishable from CaCl₂ infusion.

Because EMD-53998 may affect the amount of oxygen consumed to fuel the process of pressure development, we also examined the oxygen consumption of the LV contracting isovolumically at the reference preload volume (LVV, 25 ml; $\dot{M}\dot{V}O_{2\text{-loaded}}$). The results for the six hearts are shown in Figure 4 (similar format as Figure 3). Multiple linear regression (see bottom panel of Table 1) revealed a significant effect of EMD-53998 infusion on the intercept (column β) of the $\dot{M}\dot{V}O_{2\text{-loaded}}-P_{25}$ relation in two of the six hearts. However, in analysis of data pooled from all six hearts, this effect was not significant. In the same two hearts, the slope of the $\dot{M}\dot{V}O_{2\text{-loaded}}-P_{25}$ relation (column γ) was significantly affected by EMD-53998. This effect on slope achieved statistical significance ($p = 0.01$) in the overall analysis using all six hearts. However, although this effect was statistically significant, it also was much smaller (approximately 50-fold) than the effect of contractile state per se (see column α) on $\dot{M}\dot{V}O_{2\text{-loaded}}$. Furthermore, the influence of EMD-53998 on the slope was countered by an opposite effect on the intercept, so the

data from both inotropic agents still clustered close to a common regression line. Consistent with this notion is the observation that neither $\dot{M}\dot{V}O_{2\text{-unloaded}}$ nor $\dot{M}\dot{V}O_{2\text{-loaded}}$ at matched contractile state (see Table 2 and below) was significantly different for CaCl₂ and EMD-53998 infusions.

Comparison at Matched Contractile State

Table 2 summarizes the results of the comparison between the effects of CaCl₂ and of EMD-53998 infusion at matched contractile state. We matched contractile state by regulating the infusion rate of either drug such that end-systolic pressure at the reference preload volume (LVV, 25 ml) was close to 120 mm Hg (P_{25} averaged 113.7 ± 8.6 and 112.7 ± 9.4 mm Hg in the CaCl₂ and EMD-53998 groups; $p = 0.7$). Neither the time to peak ventricular pressure (TTP; $p = 0.1$) nor the time of pressure relaxation to 10% peak pressure (Relax; $p = 0.2$) was significantly affected by EMD-53998, suggesting that inhibition of intracellular phosphodiesterases did not play a significant role in the positive inotropic action of EMD-53998. Resistance to coronary flow (R_{coronary}) in the unloaded state at matched contractile states, on the other hand, was 32% lower during EMD-53998 infusion ($p = 0.04$). Therefore, EMD-53998 appears to possess potent coronary vasodilatory properties.

Effects of Propylene Glycol and Washout of EMD-53998

Infusion of propylene glycol, the vehicle of EMD-53998, did not affect the contractile state of the heart: P_{25} was 69.6 ± 18.1 mm Hg before infusion of the vehicle

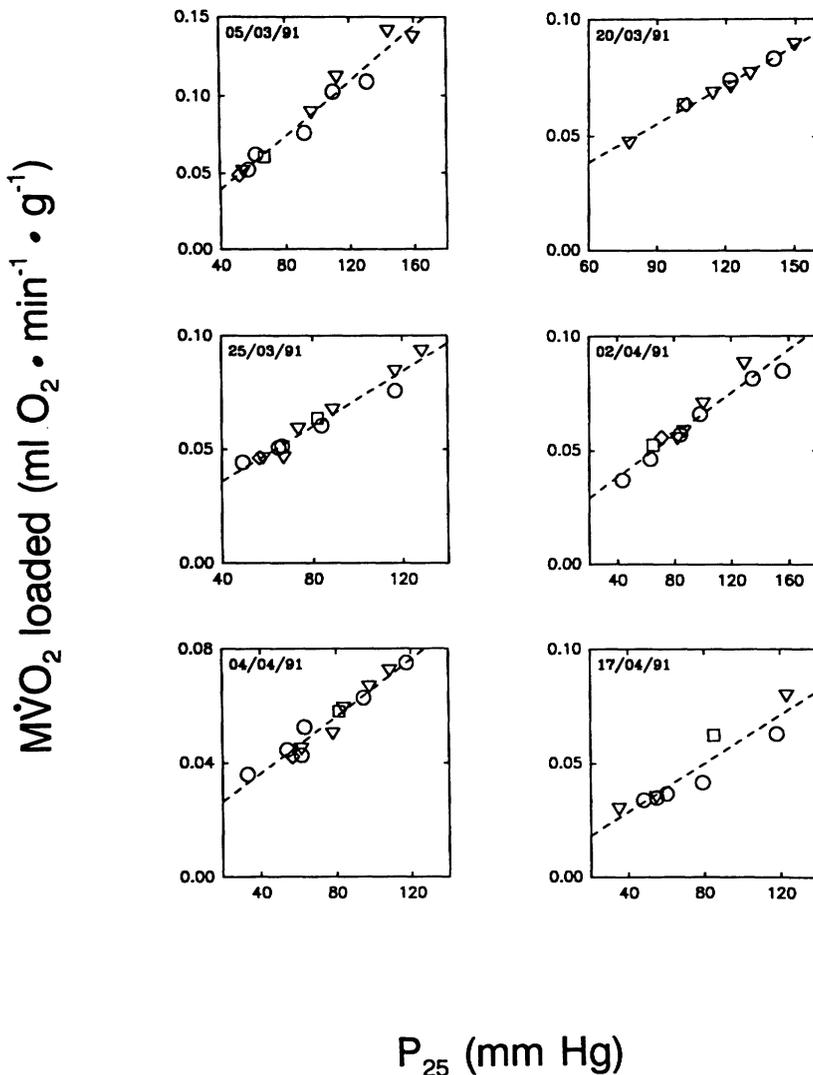


FIGURE 4. Scatterplots of left ventricular myocardial oxygen consumption at the reference preload volume (left ventricular volume, 25 ml; $M\dot{V}O_{2\text{-loaded}}$) measured as function of contractile state. \circ , CaCl₂ infusion; Δ , EMD-53998 infusion; \square , 20 minutes after EMD-53998 washout; \diamond , infusion of propylene glycol alone. Data from both CaCl₂ or EMD-53998 infusions clustered around a single regression line (----). Nevertheless, multiple linear regression analysis (see Table 1) revealed a small but significant effect of EMD-53998 on the slope of the $M\dot{V}O_{2\text{-loaded}}-P_{25}$ relation. (For details, see legend to Figure 3.)

and 67.7 ± 20.9 mm Hg ($p=0.6$) during infusion of approximately twice the maximum rate of infusion used during the experiment (0.1–0.4 ml/min). A similar result was obtained for resistance to coronary flow ($p=0.2$). Furthermore, as shown in Figures 3 and 4 (open diamonds), infusion of propylene glycol did not affect the $M\dot{V}O_{2\text{-unloaded}}-P_{25}$ ($p>0.9$) or $M\dot{V}O_{2\text{-loaded}}-P_{25}$ relation ($p>0.3$).

Twenty minutes after the infusion of EMD-53998, contractile state remained 28% higher than pre-drug infusion contractile state ($P_{25}=78.4 \pm 16.3$ versus 61.4 ± 17.1 mm Hg). Although this phenomenon was observed in five of the six hearts, this difference did not reach statistical significance ($p=0.13$). In addition, resistance to coronary flow in the unloaded state remained lower after washout of the drug (0.86 ± 0.21 versus 1.36 ± 0.41 mm Hg \cdot ml⁻¹ \cdot min⁻¹ for EMD-53998 washout and before drug infusion, respectively; $p=0.02$).

Discussion

In the present study, we investigated the effects of the "calcium-sensitizing agent" EMD-53998 on the relation between contractile state and oxygen consumption in cross-circulated canine hearts. EMD-53998 caused a

dose-dependent increase in contractile state as judged by P_{25} . Contrary to our prediction, however, we found that the relation between contractile state and $M\dot{V}O_{2\text{-unloaded}}$ was not influenced by EMD-53998. A similar result was obtained for $M\dot{V}O_2$ measured with the ventricle loaded at a common preload volume ($M\dot{V}O_{2\text{-loaded}}$).

$M\dot{V}O_2$ measured with the heart externally unloaded ($M\dot{V}O_{2\text{-unloaded}}$) has been hypothesized to reflect the amount of energy expended by the heart to sustain basal metabolism and excitation–contraction coupling.³ In addition, there may be energy expended to mechanically deform the unloaded heart, although it has been argued that this component is small.²³ It is generally assumed that any change in $M\dot{V}O_{2\text{-unloaded}}$ with altered contractility is due to a change in the energy required for excitation–contraction coupling (i.e., the amount of calcium cycled during each beat³).

To our knowledge, the effect of EMD-53998 on $M\dot{V}O_2$ has not been studied previously. Recently, Suga and colleagues^{24–26} studied the effects of several of the newer cardiotoxic agents (e.g., OPC-8212, sulmazole, milrinone, and DPI 201-106) in the isolated canine heart. Similar to the results of the present study, these investigators observed that those compounds caused an increase in $M\dot{V}O_{2\text{-unloaded}}$ in proportion to the increase in

TABLE 2. Comparison Between EMD-53998 and CaCl₂ at Matched Contractile States

Parameter	CaCl ₂	EMD-53998
P ₂₅ (mm Hg)	113.7±8.6	112.7±9.4
TTP (msec)	125.2±9.3	133.0±10.2
Relax (msec)	149.8±13.9	142.5±9.5
R _{coronary} (mm Hg · ml ⁻¹ · min ⁻¹)	0.91±0.23	0.62±0.17*
CBF (ml · min ⁻¹ · g ⁻¹)	0.57±0.12	0.86±0.16*
M \dot{V} O _{2-unloaded} (ml O ₂ · min ⁻¹ · g ⁻¹)	0.051±0.014	0.054±0.011
M \dot{V} O _{2-loaded} (ml O ₂ · min ⁻¹ · g ⁻¹)	0.076±0.014	0.082±0.011

M \dot{V} O₂, myocardial oxygen consumption.

**p*<0.05 for difference between CaCl₂ and EMD-53998 by paired Student's *t* test.

Values are expressed as mean±SD. CaCl₂ or EMD-53998 was infused such that end-systolic pressure at the reference preload volume (left ventricular volume, 25 ml) was close to 120 mm Hg. Developed pressure (P₂₅) was used as index of contractile state. Duration of pressure development (TTP) and pressure relaxation (Relax) and resistance to mean coronary blood flow (R_{coronary}), were calculated as described in "Methods." Coronary blood flow (CBF) is normalized to total heart weight (i.e., right ventricle plus left ventricle); M \dot{V} O₂ is normalized to left ventricular weight.

contractile state. Furthermore, for these agents, the magnitude of the increase in M \dot{V} O_{2-unloaded} as a function of contractile state was similar to previous results in that preparation, using either CaCl₂ or epinephrine as positive inotropic agents. In our study, the sensitivity to detect small changes in cardiac energetics was greatly enhanced by directly comparing the effects of EMD-53998 and CaCl₂ infusion in the same heart. Contrary to the proposed mechanisms of action of EMD-53998,²⁷ the principal mode of action of the compounds tested by Suga and colleagues^{24–26} was inhibition of phosphodiesterase activity rather than a calcium-sensitizing action. However, similar to the present study, in one of those studies,²⁴ complete β -blockade was used, which would be expected to greatly diminish the impact of any phosphodiesterase inhibitory action by an inotropic agent. However, because we did not measure cAMP levels in our hearts after exposure to EMD-53998, we cannot fully exclude this mode of action of this agent.

The failure of EMD-53998 to change the M \dot{V} O_{2-unloaded}-P₂₅ relation (see Figure 3 and Table 1) could be the result of several factors. First, EMD-53998 could alter the amount of calcium cycled during each beat. The effect of EMD-53998 on the intracellular calcium transient has not been studied extensively. Recent reports, however, using different preparations and animal species have shown that EMD-53998 ($\approx 5 \mu\text{M}$) causes a substantial increase in contractile state while also minimally affecting peak intracellular calcium concentration reached during the cardiac calcium transient.^{12–14} On the other hand, the recorded calcium transient does not measure the total amount of calcium cycled in the myocyte during each beat.^{28,29} It should be recognized that if EMD-53998 were to increase the myofibrillar affinity to calcium ions, one would expect a decrease in unbound cytoplasmic calcium for a constant amount of calcium cycled each beat.²⁸ Therefore, the reported actions of EMD-53998 on the calcium transient in myocardium^{12–14} do not exclude an effect of EMD-53998 on the total amount of calcium cycled each beat. It is generally assumed that the oxygen consumed by the unloaded heart (i.e., M \dot{V} O_{2-unloaded}) is proportional to the

amount of calcium cycled for excitation-contraction coupling each beat.³ Therefore, the effect of EMD-53998 on M \dot{V} O₂ that we observed in the present study could be consistent with the notion that EMD-53998 causes an increase in the total amount of calcium cycled each beat. Such an effect might be expected if the effect of EMD-53998 were to enhance the sensitivity of calcium binding to troponin-C, an "upstream" effect in the terminology of Blinks and Endoh.⁸ That is, increased affinity for calcium binding by troponin-C is not expected to alter the relation between bound calcium and force development. If troponin-C were the dominant calcium buffer in the myocyte, then a similar amount of calcium cycling probably would be required each beat to achieve a particular contractile state, despite changes in the sensitivity of troponin-C for calcium. Unfortunately, the effect of calcium-sensitizing agents on calcium binding by troponin-C has not been studied extensively. In one report, Solaro and Ruegg³⁰ reported increased calcium binding induced by sulmazole. Lee and Allen,¹² on the basis of indirect evidence, recently suggested a similar mode of action for EMD-53998. No direct measurement of the effect of EMD-53998 on calcium-binding affinity of troponin-C has been reported. Preliminary results, however, indicate that EMD-53998 does not affect troponin-C calcium affinity (R.J. Solaro, personal communication), which would tend to suggest that EMD-53998 acts "downstream" of the process of calcium binding. A recent preliminary report by Strauss et al³¹ also suggests that EMD-53998 may directly affect the cardiac cross-bridge such that less ATP is consumed to sustain force development. The energetic consequence of such an effect of EMD-53998 in the intact blood-perfused canine heart, however, appeared to be small, since only a minor effect, if any, of EMD-53998 was detected on the M \dot{V} O_{2-loaded}-P₂₅ relation (see Figure 4). Further studies are required to resolve the molecular mechanism that underlies the mode of action of EMD-53998.

A second possible explanation of our finding could be related to the fact that even though the heart is externally unloaded at the volume at which the pressure-volume area equals zero, some energy is required to deform the heart. The amount of deformation, and thus the energy required to accomplish this deformation, probably would increase in proportion to contractile state. It is difficult to estimate the magnitude of the energy that is required for this deformational work, although indirect evidence has been put forward suggesting that this component of M \dot{V} O₂ in the intact heart is small.^{3,20} Because pressure development is minimal in the unloaded state, any energy consumed by the unloaded heart for mechanical work is used mostly to support shortening, which translates to geometric deformation of the ventricle. Energy consumption for shortening per se has been shown to be minimal in both the intact heart²³ and isolated cardiac muscle.^{32–34}

Recently, Goto et al³⁵ showed in the isolated, blood-perfused rabbit heart that an increase in CBF induced by adenosine infusion causes both an increase in contractile state of the heart and an increase in M \dot{V} O₂. In that study, however, the increase in CBF was much larger (100% versus 50% in the present study; see Table 2) than that observed in the present study relative to the positive inotropic action of EMD-53998. Therefore, it is unlikely that the inotropic and energetic properties of

EMD-53998 that we observed in the present study were entirely due to the effect of EMD-53998 on CBF.

It has been shown previously in the isolated canine heart that the magnitude of the increase in oxygen consumption associated with an increase in contractile state (i.e., the energy cost of contractility) is affected by the manner in which the positive inotropy is brought about.³ That is, the energy cost of contractility is higher for epinephrine or CaCl₂ infusion, less for digitalis, and almost absent for cooling.³ Similarly, it has been shown that the unloaded oxygen consumption (M \dot{V} O_{2-unloaded}) in stunned myocardium is higher than that expected from the contractile state.³⁶ Thus, it is unlikely that the failure to detect a shift in the P₂₅-M \dot{V} O₂ relation on EMD-53998 infusion compared with CaCl₂ infusion was due to an inherent lack of sensitivity of the energetic methods that we have used in the present study. However, small changes in energy consumption by the heart induced by EMD-53998 in each beat could have been below the detection limit of our method.

Recently, Holubarsch and colleagues³⁷ reported the effect of several inotropic agents, including a calcium-sensitizing agent (UDCG-115), on the amount of heat produced by isolated papillary muscles. In that study, tension-independent heat was the myothermic variable most similar to M \dot{V} O_{2-unloaded} that we measured in the present study. Both variables are believed to reflect the amount of calcium cycled during each beat. In the myothermic study,³⁷ both tension-independent heat and peak isometric force after application of UDCG-115 fell midway between the values for low and high calcium concentrations in the bathing solution. Because there were no savings in tension-independent heat relative to force development with UDCG-115, these myothermic results in isolated papillary muscles are consistent with those of the present study, despite the differences in preparation and calcium-sensitizing agent.

In conclusion, the results of our study indicate that EMD-53998 increases both unloaded (non-work related) and loaded M \dot{V} O₂ of the heart. However, the relations between contractile state and M \dot{V} O₂ were not influenced by EMD-53998, which indicates that in the intact heart EMD-53998 does not act energetically, as expected for a pure myofibrillar calcium-sensitizing agent. Thus, EMD-53998 does not provide an energetic advantage over currently used positive inotropic agents. The positive inotropic property of this agent per se, however, may make this agent useful under some circumstances. For example, increased calcium sensitivity of the myofilaments may be beneficial in the setting of congestive heart failure where reduced responsiveness of the heart to β -adrenergic stimulation or phosphodiesterase inhibition has been observed.³⁸⁻⁴⁰

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