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Beneficial effects of the Ca^{2+} sensitizer EMD 57033 in exercising pigs with infarction-induced chronic left ventricular dysfunction

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1 It is unknown how cardiac stimulation by Ca^{2+} sensitization modulates the cardiovascular response to exercise when left ventricular (LV) function is chronically depressed following a myocardial infarction. We therefore investigated the effects of EMD 57033 at rest and during exercise and compared these to those of the mixed Ca^{2+}-sensitizer/phosphodiesterase-III inhibitor pimobendan.

2 Pigs were chronically instrumented for measurement of cardiovascular performance. At the time of instrumentation, infarction was produced by coronary artery ligation (MI, $n=12$). Studies in MI were performed in the awake state, 2–3 weeks after infarction.

3 MI were characterized by a lower resting cardiac output (18%), stroke volume (30%) and LVdP/dt_{max} (18%), and a doubling of LV end-diastolic pressure, compared to normal pigs (N, $n=13$).

4 In 11 resting MI, intravenous EMD 57033 (0.2–0.8 mg kg^{-1} min^{-1}) increased LVdP/dt_{max} ($57\pm5\%$) and stroke volume ($26\pm6\%$) with no effect on heart rate, LV filling pressure, and myocardial O_2-consumption, similar to N.

5 In MI, the effects of EMD 57033 (0.4 mg kg^{-1} min^{-1}, IV) on stroke volume and LVdP/dt_{max} were maintained during treadmill exercise up to 85% of maximal heart rate, while heart rate was lower compared to control exercise (all $P<0.05$). In contrast, the effects of EMD57033 gradually waned in N at increasing intensity of exercise.

6 Compared to N, the cardiostimulatory effects of pimobendan (20 μg kg^{-1} min^{-1}, IV) were blunted in MI both at rest and during exercise compared to N.

7 In conclusion, the positive inotropic actions of the Ca^{2+} sensitizer EMD 57033 are unmitigated in resting and exercising MI compared to N, while those of the mixed Ca^{2+}-sensitizer/phosphodiesterase-III inhibitor pimobendan are blunted.

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Abbreviations: CHF, congestive heart failure; HR, heart rate; IV, intravenous; LV, left ventricular; LVdP/dt_{max}, maximal rate of rise of LV pressure; MAP, mean aortic pressure; MI, pigs with a myocardial infarction; MPAP, mean pulmonary artery pressure; N, normal pigs; SVR, systemic vascular resistance; PDE, phosphodiesterase

Introduction

Clinical trials exploring the usefulness of inotropic support in patients with chronic heart failure (CHF) have generally led to disappointing results, as in a large number of studies improvements in cardiovascular function and quality of life have been overshadowed by an excess of sudden death (Packer, 1993). Nevertheless, the depressed cardiac pump function remains a major concern in a significant proportion of patients, requiring treatment in order to prevent end organ failure because of lack of supply of nutrients. A major drawback of the traditional inotropic agents such as sympathomimetics and phosphodiesterase-III (PDE-III) inhibitors is that they exert their actions through a cyclic AMP-mediated enhancement of Ca^{2+}-transients which increases not only myocardial O_2-demand (Lee & Allen, 1997) but also the risk of ventricular arrhythmias. In contrast, Ca^{2+}-sensitizing agents augment myocardial contractility without increasing Ca^{2+}-transients and therefore with no or only minimal increments in myocardial O_2-consumption (Lee & Allen, 1997; Mori *et al*., 1997). In a recent study, Senzaki *et al*. (2000) showed that acute administration of the thiadiazinone derivative EMD 57033, a Ca^{2+}-sensitizing agent with negligible PDE-III inhibiting properties (Lues *et al*., 1993; White *et al*., 1993; Ventura *et al*., 1992), improved myocardial contractility and mechanoenergetics without compromising left ventricular diastolic function in conscious dogs with pacing-induced CHF. An adverse effect of Ca^{2+}-sensitizing agents on left ventricular diastolic function has been a major concern with this class of agents (Hajjar & Gwathmey, 1991; Soei *et al*., 1999), but is mainly based on *in vitro* studies (see Soei *et al*., 1999), as in several *in vivo* studies employing animals with normal, stunned or failing hearts no adverse effects on diastolic function were demonstrated (De Zeeuw *et al*., 2000b; Senzaki *et al*., 2000; Stubenitsky *et al*., 1997; Udvary *et al*., 1995). The observation that an improvement in systolic function can be obtained without increases in energy expenditure and without increasing the risk of arrhythmias may therefore open avenues for inotropic treatment of CHF.

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Evidence is emerging that physical activity may be beneficial in patients suffering from CHF (Belardinelli *et al*., 1999; Coats, 1999). If, and how, the responses to exercise are modulated when such patients are treated with Ca^{2+}-sensitizing agents is currently unknown. This appears to be relevant as we have earlier shown that in normal exercising pigs the EMD 57033-induced increase in myocardial contractility is partially offset by the increased β-adrenergic activity (Stubenitsky *et al*., 1997). This blunting effect of exercise on the inotropic actions of EMD 57033 was not present after β-adrenergic receptor blockade suggesting that EMD 57033 might be useful in patients with LV dysfunction who are still capable of performing moderate exercise despite a loss of cardiac β-adrenergic responsiveness (Bristow *et al*., 1990). We therefore investigated the cardiovascular effects of intravenous (IV) EMD 57033 in exercising pigs with chronic LV dysfunction caused by myocardial infarction. A suitable dose was chosen based on the haemodynamic response to three cumulative doses administered to these animals at rest. Furthermore, in order to establish whether a combination of Ca^{2+} sensitization and PDE-III inhibition is advantageous over Ca^{2+} sensitization alone, we compared the effects of EMD 57033 to those of pimobendan (Duncker *et al*., 1987; Kubo, 1994; Van der Giessen *et al*., 1989). Because in an earlier study (Stubenitsky *et al*., 1997) we have described the effects of EMD 57033 in normal exercising pigs, we have included in some Figures the responses of these normal pigs to establish whether responses of the drugs are different for pigs with infarction and normal pigs. Four new normal animals have been added to exclude confounding factors such as time.

Methods

Experiments were performed in pigs (Land race × Yorkshire) of either sex with approval of the Animal Care Committee of the Erasmus University Rotterdam and in accordance with the 'Guiding Principles in the Care and Use of Laboratory Animals' of the Council of the American Physiological Society. Adaptation of animals to the laboratory and experimental conditions started 1 week prior to instrumentation and was continued post-operatively.

Surgery

Full details of anaesthesia, surgical procedures and instrumentation have been described earlier (Duncker *et al*., 1998; Stubenitsky *et al*., 1997). After a thoracotomy under sterile conditions, midazolam/fentanyl anaesthetized pigs (23 ± 1 kg) were instrumented with fluid-filled polyvinylchloride 8Fr catheters for measurement of blood pressure in the aortic arch, pulmonary artery and left atrium and for blood sampling. An electromagnetic flow probe (Skalar) was positioned around the ascending aorta, and a Doppler flow probe (Triton) was placed around the proximal part of the left anterior descending coronary artery (LAD), while a microtipped pressure transducer (Konigsberg Instruments) was inserted into the left ventricle *via* the apex for recording of left ventricular (LV) pressure and LVdP/dt. Myocardial infarction was produced by permanent ligation of the proximal part of the left circumflex coronary artery, which causes infarction of approximately 20% of the total LV mass (Schaper *et al*., 1969, Brooks *et al*., 1977). In three of these animals a catheter was inserted into the anterior interventricular vein for coronary venous sampling. Three of the 17 animals that were subjected to infarction died within 1 h after occlusion due to recurrent ventricular fibrillation. After electrical wires and catheters had been tunnelled subcutaneously to the back, the chest was closed and the animals were allowed to recover. All electrical wires and catheters were protected with an elastic vest.

Post-surgical care

During the first 48 h after surgery, animals received daily intravenous injections of buprenorphine (0.3 mg) as treatment for pain, and during the first week also of amoxicillin (25 mg kg^{-1}) and gentamycin (5 mg kg^{-1}) to prevent infection. Catheters were flushed daily with physiological saline containing heparin (2,000 IU ml^{-1}). Two animals with a myocardial infarction died during the first night after surgery, and experimental protocols were therefore performed in 12 pigs.

Experimental protocols

The effects of consecutive 10-min IV infusions of EMD 57033 (0.2, 0.4 and 0.8 mg kg^{-1} min^{-1}, $n=12$) or vehicle (0.5, 1.0 and 2.0 ml min^{-1} propylene glycol, $n=10$) were studied approximately 3 weeks after instrumentation with the animals resting in a cage. Experiments were performed in random order and EMD 57033 and solvent infusions were separated by at least 24 h.

On different days, the effects of EMD 57033 ($n=6$) and pimobendan ($n=6$) on the responses to graded treadmill exercise were studied. After resting measurements (lying and standing on the treadmill) were made, the animals underwent a four-stage exercise protocol (1, 2, 3 and 4 km h^{-1}), with each level lasting 2–3 min (Stubenitsky *et al*., 1997). Measurements were made during the last 30 s of each level of exercise when haemodynamics had reached a stable level. Following completion of the exercise protocol, the animals were allowed to rest for 60 min, in which period all variables returned to baseline levels (Stubenitsky *et al*., 1997). The animals then received either EMD 57033 (0.4 mg kg^{-1} min^{-1}, IV) or pimobendan (20 μg^{-1} kg min^{-1}, IV) or no treatment. The EMD 57033 dose was chosen because it had no effect on heart rate in MI, and the effect on $LVdP/dt_{max}$ was unmitigated by propranolol in normal resting pigs (Stubenitsky *et al*., 1997), indicating negligible PDE-III inhibitory properties of this dose. In addition, it proved that at this dose, the vehicle propylene glycol did not contribute to the effects of the drug (see Table 1). The pimobendan dose (20 μg kg^{-1} min^{-1}, IV) was selected because it is equipotent to EMD 57033 (0.4 mg kg^{-1} h^{-1}, IV) with respect to their effects on $LVdP/dt_{max}$ in awake resting normal pigs (Duncker *et al*., 1987; Stubenitsky *et al*., 1997).

In order to evaluate whether the responses to EMD 57033 were altered in animals with myocardial infarction (MI) compared to normal pigs (N), we also present the responses to EMD 57033 in 13 N. The results of nine of these animals have been described before (Stubenitsky *et al*., 1997), but data of four additional animals were included in the present study to exclude time as a confounding factor.

Table 1 Systemic, pulmonary and coronary haemodynamic effects of propylene glycol in 10 resting swine with a myocardial infarction

	Baseline	*Propylene glycol* (ml min^{-1}) *0.5*	*1.0*	*2.0*
Systemic haemodynamics				
CO (l min^{-1})	2.8±0.2	2.9±0.3	2.9±0.3	3.0±0.3
HR (beats min^{-1})	134±5	134±6	140±7	139±7
SV (ml)	22±2	22±2	21±2	22±2
$LVdP/dt_{max}$ (mmHg s^{-1})	2630±220	2670±250	2650±230	2660±250
$LVdP/dt_{min}$ (mmHg s^{-1})	−2480±170	−2590±190	−2510±190	−2520±190
LVEDP (mmHg)	19±2	21±2	20±2	22±2*
Tau (ms)	37±4	38±3	39±3	39±4
LVSP (mmHg)	110±3	113±3	111±2	113±3
MAP (mmHg)	90±3	93±3	92±2	95±3
SVR (mmHg min l^{-1})	34.6±3.1	35.6±2.8	36.2±4.5	35.5±3.5
Pulmonary haemodynamics				
MPAP[a] (mmHg)	35±7	37±6	39±6	41±8*
MLAP[a] (mmHg)	17±3	18±3	19±3	20±3
PVR[b] (mmHg min l^{-1})	7.6±2.7	7.7±2.8	8.7±3.7	9.8±4.2
Coronary haemodynamics				
CBF (ml min^{-1})	63±12	68±12	70±12*	71±12*
CVR (mmHg min ml^{-1})	2.0±0.3	1.8±0.2	1.6±0.2	1.7±0.2
LVW (mmHg l min^{-1})	310±36	322±41	313±37	335±44*

CO, cardiac output; HR, heart rate; SV, stroke volume; $LVdP/dt_{max}$, maximum rate of rise of left ventricular pressure; $LVdP/dt_{min}$, maximum rate of fall of left ventricular pressure; LVEDP, left ventricular end-diastolic presssure; Tau, time constant of decay of LV pressure; LVSP, left ventricular peak systolic pressure; MAP, mean arterial pressure; SVR, systemic vascular resistance; MPAP, mean pulmonary artery pressure; MLAP, mean left atrial pressure; PVR, pulmonary vascular resistance; CBF, coronary blood flow; CVR, coronary vascular resistance; LVW, LV work (LVSP × CO). Data are mean ± s.e.mean; $n=10$; ([a]$n=7$, [b]$n=6$); *$P \leqslant 0.05$ *versus* baseline.

Data acquisition and analysis

Data were recorded and digitized on-line using an eight channel data-acquisition program CODAS (Dataq Instruments) and stored for analysis with a program written in MatLab (The Mathworks), as previously described (De Zeeuw *et al*., 2000b; Stubenitsky *et al*., 1997).

Statistical analysis

Analysis of the resting protocols was performed using three-way (group [MI *vs* N], treatment [EMD 57033 *versus* vehicle] and dose), two-way and one-way analysis of variance, followed by Dunnett's test, paired *t*-test or unpaired *t*-test, as appropriate. Statistical analysis of the exercise data was performed using two-way (treatment [control run *versus* intervention run], and exercise level) analysis of variance for repeated measures, followed by paired *t*-test (treatment) or one-way analysis of variance for repeated measures and Dunnett's test (exercise level). Significance (two-tailed) was accepted for $P \leqslant 0.05$. All data are mean ± standard error of the mean.

Results

Baseline characteristics of MI

MI were characterized by a lower cardiac output (18%), stroke volume (30%) and myocardial contractility ($LVdP/dt_{max}$, 18%), a higher heart rate (15%), a doubling of the LV end-diastolic pressure and an elevated systemic vascular resistance (SVR, 28%) compared to N (Figure 1). Mean arterial pressure (MAP) was normal in MI because the increases in SVR balanced the decrease in cardiac output. Mean pulmonary artery pressure (MPAP) had doubled, due to a quadrupling of mean left atrial pressure and a doubling of pulmonary vascular resistance (Figure 2).

EMD 57033 in resting pigs

In MI, EMD 57033 increased cardiac output dose-dependently (43±7%), primarily due to an increase in stroke volume (26±6%) as heart rate increased only at the highest dose (13±5%, Figure 1). The increase in stroke volume was caused by both an augmented $LVdP/dt_{max}$ (57±5%) and systemic vasodilation. MAP decreased only at the highest dose (18±4%) when the decrease in SVR exceeded the increase in cardiac output. $LVdP/dt_{min}$ (14±6%) and τ (63±21%) increased at the highest dose, but LV end-diastolic pressure remained unchanged. MPAP increased slightly, secondary to the increase in cardiac output as pulmonary vascular resistance was not affected (Figure 2).

Figures 1 and 2 show that the responses to EMD 57033 in MI were very similar to those in N, the only exception being the more pronounced systemic vasodilation at the highest infusion rate in MI. Except for the 10–15% increases in LVEDP, MPAP, CBF and LV work at the highest infusion rate, the vehicle did not contribute to the responses to EMD 57033 in MI (Table 1).

Myocardial blood flow and O_2-consumption in MI

Blood flow in the non-infarcted myocardium increased up to during EMD 57033 (24±5%, Figure 1), but approximately 50% of this effect must be ascribed to the solvent (Table 1). Myocardial O_2-consumption, although measured in only three pigs, increased less than 15% during EMD 57033 infusion despite a 59±15% increase in LV work (Figure 3), reflecting an increased LV mechanical efficiency (LV work/myocardial O_2-

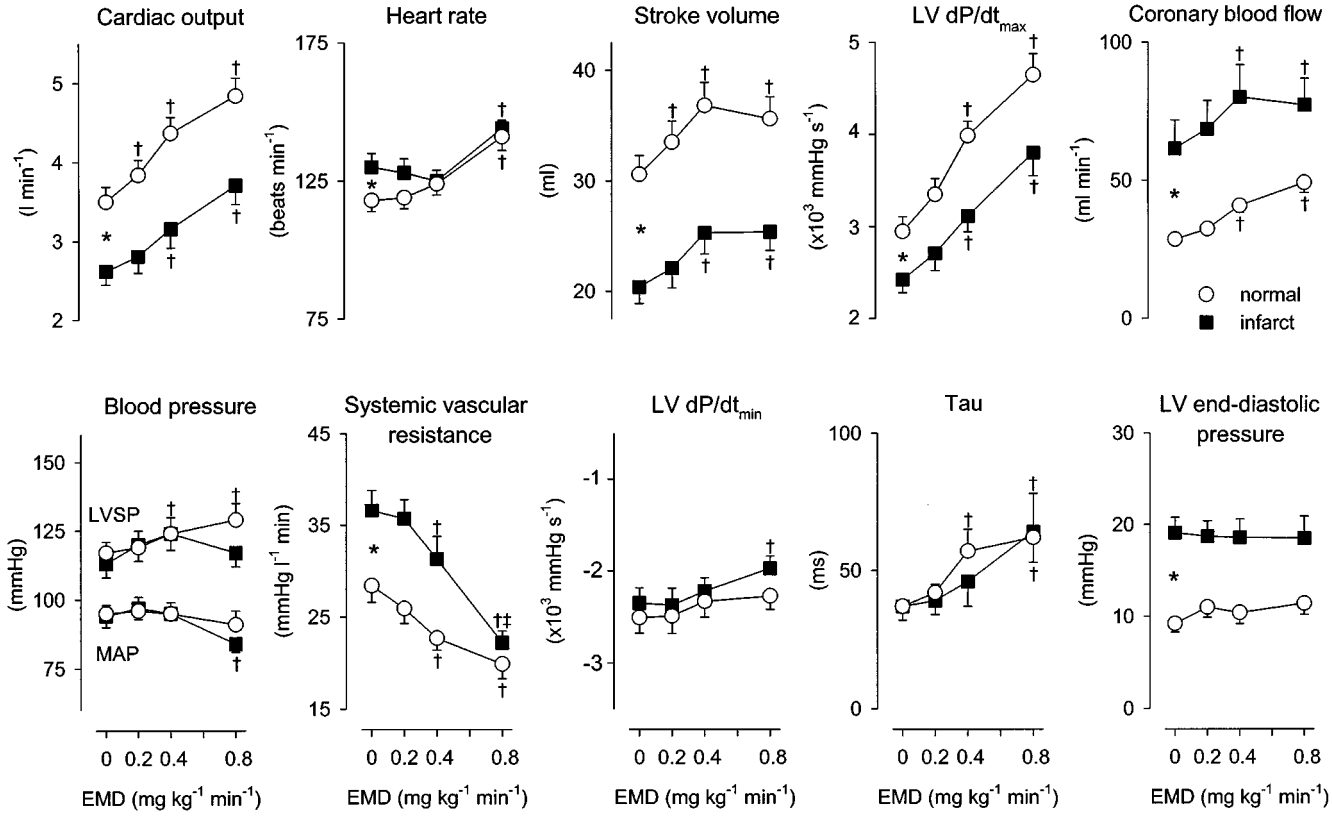

Figure 1 Systemic haemodynamic and cardiac effects of cumulative 10-min intravenous infusions of EMD 57033 in resting MI ($n=12$) and N ($n=13$); Tau = time constant of decay of left ventricular (LV) pressure. Data are mean ± s.e.mean; $*P \leqslant 0.05$ baseline MI *versus* baseline N, $\dagger P \leqslant 0.05$ *versus* baseline (0 mg kg^{-1} min^{-1}), ‡EMD 57033-induced change from baseline in MI is significantly different ($P \leqslant 0.05$) from EMD 57033-induced change from baseline in N.

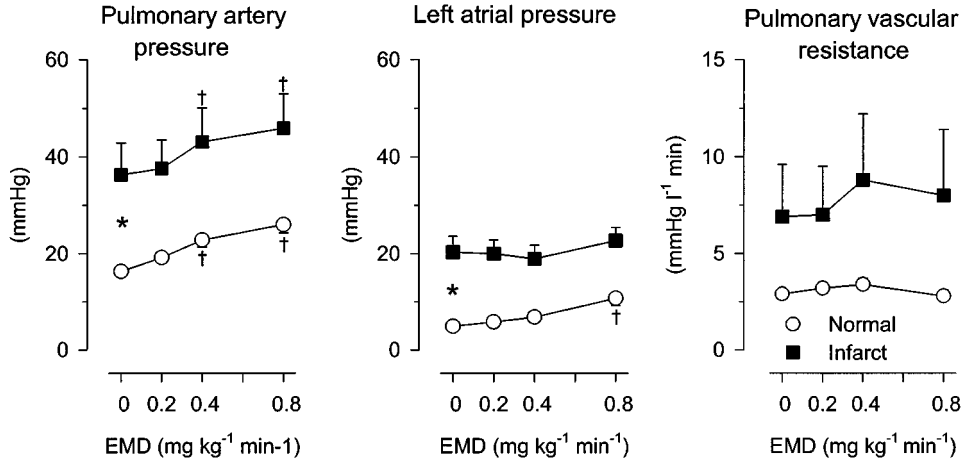

Figure 2 Pulmonary haemodynamic effects of cumulative 10-min intravenous infusions of EMD 57033 in resting MI ($n=5$) and N ($n=7$). For further details see Figure 1.

consumption). The contribution of the vehicle to the increase of LV work was of minor importance (Table 1).

Responses to exercise

During exercise at 4 km h^{-1}, heart rate of untreated MI increased to 235 ± 8 beats min^{-1} which was 66 ± 10% above its resting (standing) heart rate and approximately 85% of the maximum heart rate in the pig (Scheffer & Verdouw, 1983). Heart rate and cardiac output increased sharply at 1 km h^{-1} and more gradually thereafter (Table 2). Stroke volume remained unchanged, implying that tachycardia was responsible for the exercise-induced increase in cardiac output. MAP also remained unchanged as peripheral vasodilation balanced the increase in cardiac output. $LVdP/dt_{max}$ increased up to 50 ± 11% ($P<0.05$), while $LVdP/dt_{min}$

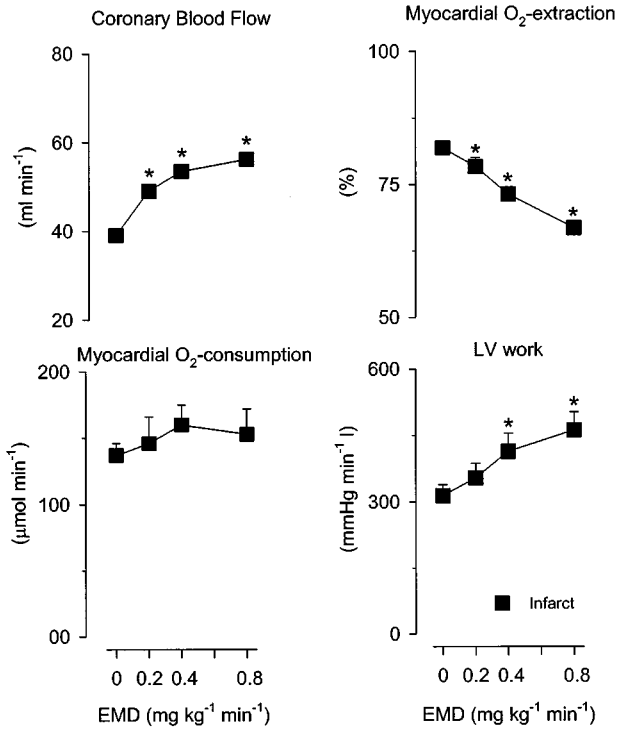

Figure 3 Effect of cumulative 10-min intravenous infusions of EMD 57033 on myocardial O_2-consumption and left ventricular (LV) work in resting MI ($n=3$). *$P \leqslant 0.05$ *versus* baseline (0 mg kg^{-1} min^{-1}).

became more negative ($14 \pm 5\%$, $P<0.05$) and τ did not change (44 ± 11 ms at rest and 39 ± 10 ms at 4 km h^{-1}) suggesting that exercise did not adversely affect LV relaxation. Figure 4 also shows that the slope of the relations between cardiac output and $LVdP/dt_{max}$ *versus* heart rate (i.e. exercise load) was less steep in MI than in N (both $P<0.05$). MPAP and left atrial pressure increased, with a trend towards vasodilation in the pulmonary vascular bed ($P=0.09$, Table 2).

Effects of EMD 57033 In MI, the responses of cardiac output and $LVdP/dt_{max}$ to exercise remained unaltered in the presence of EMD 57033, i.e. the EMD 57033-induced increases in the resting values of cardiac output, stroke volume and $LVdP/dt_{max}$ were maintained during exercise (Table 2 and Figure 4). The EMD 57033-induced increase in $LVdP/dt_{max}$ in MI at all levels of exercise differed from N in which $LVdP/dt_{max}$ values became similar at higher workloads, independent of the presence of EMD 57033 (Figure 4). $LVdP/dt_{min}$ (Table 2) and τ (42 ± 9 ms at 4 km h^{-1}) were not affected by EMD 57033 during exercise. Similar to the control run, MAP was not affected by exercise in the presence of EMD 57033. The response to exercise of the pulmonary circulation of MI was, similar to N (not shown), not modified by EMD 57033 (Table 2).

Effects of pimobendan The exercise-induced increases in cardiac output in MI during pimobendan were similar to those during EMD 57033 (Table 3). However, in contrast to EMD 57033, the cardiac output-heart rate relation was not affected by pimobendan (Figure 4), implying that the pimobendan-induced increase in cardiac output was solely caused by tachycardia. Similarly, the relation between $LVdP/dt_{max}$ and heart rate was virtually identical in untreated and pimobendan-treated MI, although at the highest exercise level, and at similar heart rates, $LVdP/dt_{max}$ tended to be higher in the pimobendan-treated than in the untreated animals (Figure 4). In contrast, for a given heart rate in N, $LVdP/dt_{max}$ was consistently higher during pimobendan. Similar to EMD 57033, the pimobendan-induced systemic vasodilatation balanced the increase in cardiac output, thereby leaving MAP unchanged. The response of the pulmonary circulation to exercise in MI was not modified by pimobendan in MI (Table 3) or N (*data* not shown).

Reproducibility of exercise responses When in MI the control run was repeated after the 60 min resting period all cardiovascular parameters (Figure 4), showed the same good reproducibility as N (Stubenitsky *et al*., 1997).

Discussion

The main findings of the present study in chronically instrumented pigs with a 3-week-old myocardial infarction are that (a) The positive inotropic action of EMD 57033 was virtually identical to that in N with minimal increments in myocardial O_2-consumption and only at the highest dose a minimal increase in heart rate and LV relaxation time; (b) The positive inotropic actions of EMD 57033 in exercising MI were similar to those in N. In contrast, the positive inotropic and chronotropic actions of pimobendan were blunted in exercising MI compared to N.

In vivo *models of heart failure*

Most large animal studies produce CHF by rapid ventricular pacing, although clinically the most common cause of CHF is myocardial infarction (Hasenfuss, 1998). We therefore elected to perform studies in a model in which LV dysfunction was produced by permanent coronary ligation. This model shows at 3–6 weeks a milder degree of LV dysfunction and neurohumoral activation compared to the pacing model. Thus, in pacing-induced CHF resting levels of catecholamines are markedly elevated within 6 weeks after onset of pacing (Spinale *et al*., 1997), whereas we found near normal resting catecholamine and angiotensin II levels up to 6 weeks after MI (Van Kats *et al*., 2000), although catecholamine levels are increased during treadmill exercise (Haitsma *et al*., 2001) and β-adrenergic receptor responsiveness is blunted (Van Woerkens *et al*., 1993). Moreover, the LV dilates and hypertrophies after infarction (Van Kats *et al*., 2000), whereas in pacing-induced CHF LV_{weight} fails to increase despite severe dilation resembling end-stage dilated cardiomyopathy (Hasenfuss, 1998).

Ca^{2+}-sensitizers in heart failure

Studies investigating the cardiovascular actions of Ca^{2+}-sensitizing agents have been hampered by the additional PDE-III inhibiting properties of most of these agents. In awake normal pigs and dogs (Asanoi *et al*., 1994; Duncker *et al*., 1987; Ohte *et al*., 1997), pimobendan increases cardiac output, $LVdP/dt_{max}$ and reduces LV filling pressure. The

Table 2 Haemodynamic responses to EMD 57033 in swine with a myocardial infarction during graded treadmill exercise

		Rest		*Exercise* (km h^{-1})			
	Treatment	*Lying*	*Standing*	*1*	*2*	*3*	*4*
Systemic haemodynamics							
CO (1 min^{-1})	Control	3.3±0.2	4.2±0.1*	5.2±0.1†	5.7±0.2†	5.8±0.2†	6.3±0.3†
	EMD 57033	3.9±0.1‡	4.4±0.2	5.3±0.2†	5.7±0.2†	6.0±0.2†	6.5±0.3†
HR (beats min^{-1})	Control	125±7	148±8*	176±7†	198±8†	221±8†	235±8†
	EMD 57033	125±6	140±6*	165±10†	178±8†‡	202±9†‡	223±11†‡
SV (ml)	Control	26±1	28±2*	30±2	29±2	27±2	27±1†
	EMD 57033	31±2‡	32±3‡	33±2‡	32±1‡	30±1‡	29±1‡
LV dP/dt_{max} (mmHg s^{-1})	Control	2310±150	2730±340	3340±440†	3720±500†	3740±460†	4260±470†
	EMD 57033	2800±230‡	3210±350‡	3670±480†	3930±470†	3970±490†‡	4490±580†
LV dP/dt_{min} (mmHg s^{-1})	Control	−2560±340	−2660±370	−2880±460	−2880±490	−2910±500	3300−±570
	EMD 57033	−2640±410	−2520±410	−2590±440	−2700±430	−2710±460	3180−±500
LVSP (mmHg)	Control	115±6	116±8	119±7	121±7	122±8	128±9
	EMD 57033	116±6	118±6	122±6	123±7	125±8†	131±10†
MAP (mmHg)	Control	101±4	96±7	94±6	95±6	95±6	97±6
	EMD 57033	97±5	91±5	88±6	88±5	90±6	92±6‡
SVR (mmHg min l^{-1})	Control	31.5±2.6	22.7±2.1*	18.1±1.0†	16.7±1.1†	16.4±1.0†	15.6±1.3†
	EMD 57033	24.6±1.7‡	21.7±2.7	16.1±1.2†‡	15.4±0.8†‡	14.9±0.8†‡	14.4±1.0†‡
Pulmonary haemodynamics							
MPAP (mmHg)	Control	27±5	28±4	32±4†	36±4†	37±4†	37±3†
	EMD 57033	27±3	28±4	34±5†	36±4†	39±4†	39±3†
MLAP (mmHg)	Control	14±3	12±2	14±2	15±2	17±3†	18±2†
	EMD 57033	13±1	11±2	14±3	16±2	19±2†	21±3†
PVR (mmHg min l^{-1})	Control	3.9±0.7	4.1±0.4	3.6±0.5	3.6±0.5	3.5±0.4	3.1±0.4
	EMD 57033	3.7±0.6	3.7±0.3	3.9±0.5	3.5±0.5	3.4±0.5	2.9±0.3

CO, cardiac output; HR, heart rate; SV, stroke volume; $LVdP/dt_{max}$, maximum rate of rise of left ventricular pressure; $LVdP/dt_{min}$, maximum rate of fall of left ventricular pressure; LVSP, left ventricular peak systolic pressure; MAP, mean arterial pressure; SVR, systemic vascular resistance; MPAP, mean pulmonary artery pressure; MLAP, mean left atrial pressure; PVR, pulmonary vascular resistance. Data are mean±s.e.mean; $n=6$; *$P\leqslant0.05$ standing *versus* lying; †$P\leqslant0.05$ exercise *versus* standing; ‡$P\leqslant0.05$ EMD 57033 *versus* corresponding control.

increase in cardiac output is predominantly heart rate-mediated (Asanoi *et al*., 1994; Duncker *et al*., 1987; Ohte *et al*., 1997), while the chronotropic and inotropic actions are markedly attenuated by beta-blockade (Duncker *et al*., 1987), indicating significant PDE-III inhibition. This explains why the chronotropic and inotropic actions of pimobendan are blunted in pigs and dogs with CHF (Asanoi *et al*., 1994; Van der Giessen *et al*., 1989), although they are better preserved than those of the pure PDE-III inhibitor amrinone (Ohte *et al*., 1997). In humans with LV dysfunction or overt CHF pimobendan results in short-term improvements of the haemodynamic status, but also increases exercise capacity and quality of life after long-term treatment (Hagemeijer *et al*., 1989; Kubo, 1997).

Levosimendan increases $LVdP/dt_{max}$ and cardiac output in awake dogs (Harkin *et al*., 1995), but the increases in heart rate and the amenability of the cardiostimulatory actions to autonomic blockade suggest a major action *via* PDE-III inhibition (Harkin *et al*., 1995). In patients with LV dysfunction, levosimendan caused a heart rate-mediated increase in cardiac output (Lilleberg *et al*., 1995), whereas in patients with severe CHF the dose-dependent increase in cardiac output was primarily due to an increase in stroke volume, with heart rate only contributing at higher doses (Slawsky *et al*., 2000). Similarly, in dogs with pacing-induced CHF, the chronotropic response was absent, while the inotropic response was blunted (Pagel *et al*., 1997; Udvary *et al*., 1995). Also (Todaka *et al*., 1996) reported in dogs a marked loss of the inotropic response to levosimendan in pacing-induced CHF, which paralleled the loss of response to isoproterenol. Because levosimendan also increases Ca^{2+}-transients dose-dependently in isolated rat hearts, these studies indicate that levosimendan exerts considerable PDE-III inhibition.

MCI-154, another mixed Ca^{2+}-sensitizer/PDE-III inhibitor, also improves LV contractility and cardiac output in dogs with pacing-induced severe CHF (Teramura *et al*., 1997) and in patients with LV dysfunction (Takaoka *et al*., 1997). Interestingly the inotropic effects of MCI-154 were very similar to those in normal dogs (Teramura *et al*., 1997), while in the patients with LV dysfunction in a dose that produced a similar increase in cardiac output as dobutamine, MCI-154 had no effect on heart rate and myocardial O_2-consumption (Takaoka *et al*., 1997; Teramura *et al*., 1997), whereas dobutamine increased O_2-consumption by 40% (Takaoka *et al*., 1997). Importantly, none of the aforementioned mixed agents affected diastolic function adversely and often improved diastolic performance (Asanoi *et al*., 1994; Hagemeijer *et al*., 1989; Lilleberg *et al*., 1995; Ohte *et al*., 1997; Pagel *et al*., 1997; Takaoka *et al*., 1997; Teramura *et al*., 1997; Udvary *et al*., 1995; Van der Giessen *et al*., 1989), which is likely related to PDE-III inhibition.

The principal mode of action EMD 57033 appears to be Ca^{2+} sensitization, both *in vitro* (Lues *et al*., 1993; White *et al*., 1993; Ventura *et al*., 1992) and *in vivo* (De Zeeuw *et al*., 2000a; Haeusler *et al*., 1997), although the drug possesses some minor PDE-III inhibitory effects *in vitro* (Ravens *et al*., 1996). Indeed, the inotropic responses to EMD 57033 were not affected by β-adrenoceptor blockade (De Zeeuw *et al*., 2000b; Stubenitsky *et al*., 1997), which explains why in the present study the effects of EMD 57033 in MI were virtually identical to those in N, despite a reduced β-adrenergic

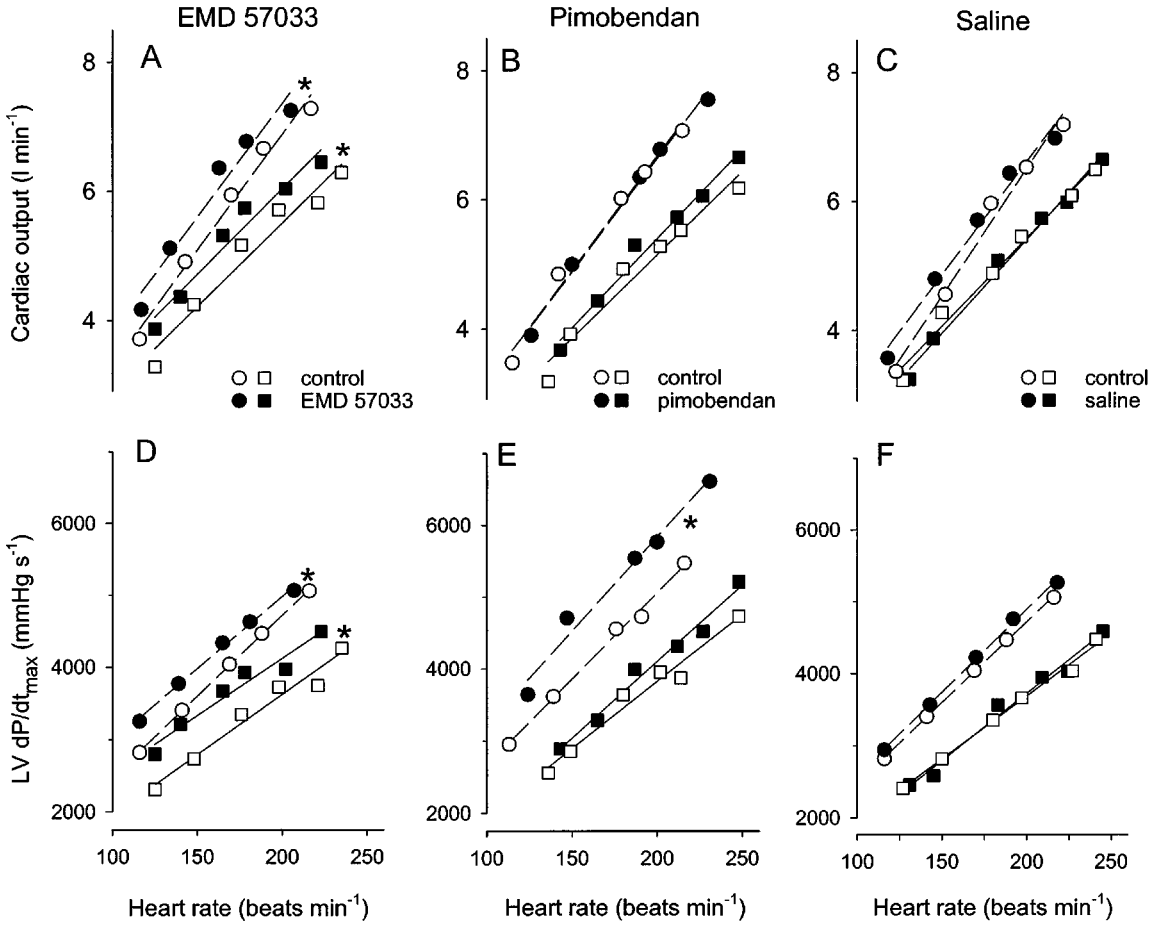

Figure 4 Responses of cardiac output and $LVdP/dt_{max}$ to exercise in the absence and presence of EMD 57033 (0.4 mg kg^{-1} min^{-1}) or pimobendan (20 μg kg^{-1} min^{-1}) in MI ($n=6$; squares) and N ($n=8$; circles). Data have been plotted as a function of heart rate. The reproducibility of two consecutive control exercise periods is presented in panels C and F. *$P \leqslant 0.05$ *versus* corresponding control conditions (first exercise period).

responsiveness (Van Woerkens *et al*., 1993). Drake-Holland *et al*. (1997) reported that EMD 57033 (0.6 mg kg^{-1} min^{-1}, IV) increased $LVdP/dt_{max}$ and cardiac output, which was accompanied by decreases in heart rate and LV end-diastolic pressure in dogs with pacing-induced CHF. Senzaki *et al*. (2000) also reported that EMD 57033 in a dose of 0.4 mg kg^{-1} min^{-1} IV produced similar inotropic effects in normal and failing dogs, whereas the increase in LV contractility by 0.8 mg kg^{-1} min^{-1} IV was blunted in failing compared to normal dogs, most likely due to modest PDE-III inhibition at this dose (Stubenitsky *et al*., 1997).

The negligible PDE-III inhibitory effects might raise concern that EMD 57033 exerts detrimental effects on diastolic function (Hajjar & Gwathmey, 1991; Hajjar *et al*., 1997). In pigs with a 3-week-old myocardial infarction, end-diastolic and end-systolic LV diameter and LV end-diastolic pressure are increased (Van Kats *et al*., 2000). However, early diastolic relaxation parameters ($LVdP/dt_{min}$ and τ) in MI were not different from N, possibly because the elevated heart rate masked subtle relaxation abnormalities in MI. EMD 57033 did not alter LV end-diastolic pressure of MI (whereas it increased during infusion of the vehicle), and only increased $LVdP/dt_{min}$ and τ at 0.8 mg kg^{-1} min^{-1} in resting swine. In addition, 0.4 mg kg^{-1} min^{-1} had no effect on τ either at rest or during exercise in MI, indicating that EMD 57033 at a dose that produces an increase in LV pump function had no adverse effect on this index of early relaxation or on LV filling. The contrasting observation of a lack of significant diastolic abnormalities in *in vivo* studies (Senzaki *et al*., 2000; the present study) and the impairment of diastolic function in isolated myocardial muscle strips (Hajjar *et al*., 1997) could be explained by increased elastic restoring forces in the *in vivo* heart, due to ejection to a lower end-systolic volume, which counteracts any potential untoward effects of EMD 57033 on diastolic function (Senzaki *et al*., 2000; Nikolic *et al*., 1988). This mechanism is only relevant in ejecting but not in isolated isometrically contracting tissues and may therefore explain why *in vitro* but not *in vivo* studies report an impairment of diastolic function with EMD 57033.

Ca^{2+}-sensitizers and exercise responses in LV dysfunction

In normal pigs (Stubenitsky *et al*., 1997) and dogs (Ohte *et al*., 1997), pimobendan enhances the exercise-induced increases in $LVdP/dt_{max}$. This facilitation of exercise-induced response of contractility is a typical feature of PDE-III inhibition and is most effective when β-adrenergic stimulation

Table 3 Haemodynamic responses to pimobendan in swine with a myocardial infarction during graded treadmill exercise

		Rest		*Exercise* (km h^{-1})			
	Treatment	*Lying*	*Standing*	*1*	*2*	*3*	*4*
Systemic haemodynamics							
CO (l min^{-1})	Control	3.2±0.3	4.0±0.2*	5.0±0.2†	5.3±0.3†	5.6±0.3†	6.2±0.3†
	Pimobendan	3.7±0.2‡	4.5±0.3*	5.4±0.3†‡	5.8±0.3†‡	6.1±0.3†‡	6.7±0.3†‡
HR (beats min^{-1})	Control	136±7	149±6	180±6†	202±9†	214±8†	248±5†
	Pimobendan	143±6‡	165±9‡	187±7†	212±10†‡	227±9†	248±4†
SV (ml)	Control	24±2	27±2*	28±2	26±1	26±1	25±1†
	Pimobendan	26±2‡	27±2	29±2	27±2	27±2	27±1
LV dP/dt_{max} (mmHg s^{-1})	Control	2560±210	2860±260	3640±310†	3960±430†	3870±410†	4730±590†
	Pimobendan	2890±240‡	3290±280	3990±400	4310±510†	4520±550†	5210±730†
LV dP/dt_{min} (mmHg s^{-1})	Control	−2540±350	−2520±360	−2680±330	−2690±350	−2710±360	−3160±540
	Pimobendan	−2580±400	−2450±320	−2700±420	−2730±420†	−2690±380	−3010±510†
LVSP (mmHg)	Control	113±5	109±6	118±4†	119±4†	117±4†	124±6†
	Pimobendan	112±5	111±4	120±6†	121±5†	120±4†	125±8†
MAP (mmHg)	Control	97±4	87±5*	88±3	88±4	88±4	93±5
	Pimobendan	93±5	86±4	89±6	88±5	86±4	88±5
SVR (mmHg min l^{-1})	Control	31.8±2.2	22.4±1.5*	18.0±0.8†	16.8±0.8†	16.1±0.7†	15.2±0.7†
	Pimobendan	26.0±1.8‡	20.1±1.8*	17.0±1.0†	15.5±0.8†	14.3±0.7†‡	13.3±0.8†‡
Pulmonary haemodynamics							
MPAP (mmHg)	Control	29±5	28±5	34±5†	38±5†	40±5†	39±3†
	Pimobendan	31±5	33±5	37±5	42±5†‡	42±5†	39±3†
MLAP (mmHg)	Control	15±3	10±3	12±3	14±2	17±2†	18±3†
	Pimobendan	14±3	13±3	15±3‡	17±3‡	17±3	18±4†
PVR (mmHg min l^{-1})	Control	4.9±0.9	4.0±0.4	4.5±0.6	4.7±0.6	4.4±0.6	3.4±0.3
	Pimobendan	4.6±0.8	4.6±0.6	4.4±0.7	4.4±0.7	4.3±0.8	3.2±0.3

CO, cardiac output; HR, heart rate; SV, stroke volume; $LVdP/dt_{max}$, maximum rate of rise of left ventricular pressure; $LVdP/dt_{min}$, maximum rate of fall of left ventricular pressure; LVSP, left ventricular peak systolic pressure; MAP, mean arterial pressure; SVR, systemic vascular resistance; MPAP, mean pulmonary artery pressure; MLAP, mean left atrial pressure; PVR, pulmonary vascular resistance. Data are mean±s.e.mean; $n=6$; *$P\leqslant0.05$ standing *versus* lying; †$P\leqslant0.05$ exercise *versus* standing; ‡$P\leqslant0.05$ Pimobendan *versus* corresponding control.

(and hence cyclic AMP production) is high, such as during exercise (Cheng *et al*., 1992; Ohte *et al*., 1997). Indeed the facilitation by pimobendan of exercise-induced inotropic stimulation was virtually abolished when normal pigs were pretreated with propranolol to minimize β-adrenoceptor-mediated cyclic AMP production (Stubenitsky *et al*., 1997).

The positive inotropic actions of EMD 57033 were blunted during exercise in N, which may be related to the exercise-induced increase in cyclic AMP which reduces myofilament Ca^{2+} sensitivity. Indeed, when cyclic AMP production was minimized by propranolol the positive inotropic effects of EMD57033 in N were maintained at all levels of exercise (Stubenitsky *et al*., 1997). These findings explain why in the present study the positive inotropic effects of EMD 57033 were maintained during exercise in MI, whereas those of pimobendan were blunted compared to N. Our findings are supported by the observations in dogs with pacing-induced CHF (Ohte *et al*., 1997), although in that study the 50% loss in inotropy with pimobendan was still less than the 80% loss observed with the pure PDE-III inhibitor amrinone. The findings are best explained by the loss of ß-adrenergic responsiveness that occurs not only in pacing-induced CHF (Vatner *et al*., 1985) but also in our MI model (Van Woerkens *et al*., 1993; Haitsma *et al*., in press). Interestingly, Hagemeijer *et al*. (1989) reported beneficial haemodynamic effects of pimobendan in patients with CHF, both at rest and during exercise. Thus, whereas in these patients pimobendan had no effect on heart rate, stroke volume was markedly increased and pulmonary capillary wedge pressure was decreased both at rest and during exercise. Moreover, the effects at rest were similar to the effects during exercise, much like the effect of EMD 57033 in the present study. These findings suggest that the contribution of PDE-III inhibition to the positive chronotropic and inotropic actions of mixed Ca^{2+}-sensitizers/PDE-III inhibitors is progressively lost with increasing severity of LV dysfunction and concomitant loss of ß-adrenergic responsiveness, whereas the Ca^{2+}-sensitizing effects are preserved. This also explains why in our MI the effects of EMD 57033 were fully maintained during exercise. Exercise in the presence of EMD 57033 resulted in slightly lower heart rates, whereas pimobendan resulted in higher heart rates compared to control exercise. This implies that exercise at a given workload will be accompanied by a lower myocardial O_2-demand during EMD 57033 compared to control exercise and particularly compared to pimobendan.

In conclusion, the positive inotropic actions of EMD 57033 were maintained in MI compared to N both at rest and during exercise, whereas the positive inotropic actions of pimobendan were blunted. Finally, for a drug to be attractive for daily use oral route of administration is desired. It is therefore of interest that oral administration of EMD 82571, a prodrug of EMD 57033, which is converted to EMD 57033 upon absorption increased myocardial contractility in 4 N (45% increase in $LVdP/dt_{max}$) and 6 MI (30% increase in $LVdP/dt_{max}$) up to 8 h after administration (unpublished data from our laboratory).

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