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Cardiovascular effects of the novel Ca^{2+}-sensitiser EMD 57033 in pigs at rest and during treadmill exercise

René Stubenitsky, Rick W.P. van der Weerd, David B. Haitsma, Pieter D. Verdouw & Dirk J. Duncker[1]

Experimental Cardiology, Thoraxcenter, Cardiovascular Research Institute COEUR, Erasmus University Rotterdam, Rotterdam, The Netherlands

1 To date no study has described the cardiovascular effects of increased myofilament Ca^{2+} responsiveness in awake animals both under resting conditions and during treadmill exercise. In the present study we therefore investigated the systemic, pulmonary and coronary haemodynamic actions of the Ca^{2+} sensitizer EMD 57033 in 16 chronically instrumented awake pigs at rest and during treadmill exercise, and compared these to the haemodynamic actions of the Ca^{2+} sensitizer/phosphodiesterase inhibitor pimobendan.

2 Under resting conditions EMD 57033 (0.2, 0.4 and 0.8 mg kg^{-1} min^{-1}, i.v.) produced dose-dependent increases in $LVdP/dt_{max}$ (up to $65 \pm 17\%$ (mean $\pm$s.e.mean), $P \leqslant 0.05$) and stroke volume (up to $20 \pm 3\%$, $P \leqslant 0.05$), with an increase in heart rate only after the highest dose ($22 \pm 5\%$, $P \leqslant 0.05$), while mean aortic blood pressure and $LVdP/dt_{min}$ were not altered. EMD 57033 had also no effect on pulmonary vascular resistance, but produced dose-dependent decreases in systemic vascular resistance ($32 \pm 4\%$, $P \leqslant 0.05$), and coronary vascular resistance ($44 \pm 2\%$, $P \leqslant 0.05$). These effects were essentially unchanged when animals were pretreated with non-selective β-adrenoceptor blockade, indicating that phosphodiesterase inhibition did not contribute to the positive inotropic actions of EMD 57033.

3 During exercise at 2, 3, and 4 km h^{-1}, the positive inotropic actions of EMD 57033 gradually waned at higher levels of exercise. This may have been caused by the exercise-induced increase in β-adrenergic activity, because after pretreatment with propranolol the positive inotropic actions of EMD 57033 were preserved at all levels of exercise. In contrast, the positive inotropic and chronotropic effects of pimobendan were amplified during exercise, but were abolished (at rest) or markedly attenuated (during exercise) after pretreatment with propranolol.

4 The responses to EMD 57033 during exercise after combined α- and β-adrenergic receptor blockade were not different from those after β-adrenergic receptor blockade alone, indicating that the positive inotropic actions of EMD 57033 were not mediated via or did not depend on intact α-adrenergic receptor activity.

5 In conclusion, EMD 57033 increases left ventricular myocardial contractility in awake pigs. During exercise this effect is partially offset by the increased β-adrenergic activity, with no effect of α-adrenergic activity, suggesting that EMD 57033 may be most effective in patients with severe loss of β-adrenergic responsiveness.

Keywords: Adrenergic receptor blockade; awake swine; Ca^{2+}-sensitization; EMD 57033; coronary circulation; exercise; haemodynamics; phosphodiesterase inhibition; pulmonary circulation; systemic circulation

Introduction

Inotropic pharmacotherapy of heart failure has traditionally included glycosides and sympathomimetic drugs. These compounds act by increasing intracellular Ca^{2+} levels via inhibition of the Na^+/K^+ ATPase or increased cAMP levels (phosphodiesterase inhibitors, β-adrenoceptor agonists and adenylyl cyclase activators), respectively. Untoward side-effects of increased cytosolic Ca^{2+} levels include increased oxygen consumption and increased risk of arrhythmias (Katz, 1986). Moreover, in patients with impaired left ventricular pump function β-adrenergic receptor responsiveness is often decreased (Bristow *et al*., 1982; 1986), thereby attenuating the efficacy of particularly the sympathomimetic agents (Colucci *et al*., 1988; 1989; van der Giessen *et al*., 1989). Over the past 15 years a class of drugs has emerged that stimulates cardiac contractile force at least in part by increasing the Ca^{2+} sensitivity and, to a lesser extent, maximum Ca^{2+}-induced contractile force of myocardial contractile proteins. The potential advantage of these 'Ca^{2+} sensitisers' could lie in their ability to increase contractile force without increasing energy consuming Ca^{2+} transients (Holubarsch *et al*. 1989; Gross *et al*., 1993). Moreover it is possible that these drugs are less susceptible to impairment of the β-adrenoceptor-adenylyl cyclase system than the phosphodiesterase inhibitors.

Putative Ca^{2+} sensitisers include sulmazole, pimobendan, MCI-154, levosimendan and EMD 53998, all of which exert significant additional PDE-III inhibitory actions (Ruegg & Solaro, 1993; Kubo, 1994; Haikala and Lindén, 1995), and EMD 60263 (Ravens *et al*., 1996). EMD 53998 is a racemate consisting of (−)EMD 53998 (i.e. EMD 57439, a pure PDE III inhibitor) and (+) EMD 53998 (i.e. EMD 57033), a Ca^{2+} sensitiser with minimal PDE III inhibitory actions *in vitro* (Ventura *et al*., 1992; Lues *et al*., 1993). Data on the cardiovascular actions of EMD 57033 *in vivo* are lacking. Consequently, the present study was performed to investigate the systemic, pulmonary and coronary haemodynamic actions of EMD 57033 in chronically instrumented awake pigs and to compare these to the previously reported actions of pimobendan in the same model (Duncker *et al*., 1987a). To determine the contribution of PDE III inhibition to the cardiostimulatory actions of EMD 57033, we also administered the same dose regimen in animals pretreated with the non-selective β-adrenergic receptor antagonist propranolol.

[1] Author for correspondence: Experimental Cardiology, Thoraxcenter, Erasmus University Rotterdam, PO Box 1738, 3000 DR Rotterdam, The Netherlands.

Abnormalities in left ventricular pump function of patients are often exacerbated or may become apparent only during increased physical activity. Therefore, it is important to study novel inotropic therapies not only under resting conditions but also during exercise. Consequently, we performed an additional set of experiments to study the cardiovascular effects of EMD 57033 during graded treadmill exercise and compared its actions to those of pimobendan. The contribution of PDE III inhibition to the actions of these compounds during treadmill exercise was evaluated by studying the effect of these agents after pretreatment with β-adrenergic receptor blockade. Finally, to exclude the possibility that the EMD 57033-induced increase in Ca^{2+} responsiveness was mediated by α-adrenoceptors (Endoh & Blinks, 1988; Terzic *et al*., 1992), we studied the effects of EMD 57033 on left ventricular performance at rest and during treadmill exercise in the presence of combined α- and β-adrenergic blockade.

Methods

A total number of 16 crossbred Landrace × Yorkshire pigs (24±1 kg) of either sex were used in the present study. All experiments were performed in accordance with the 'Guiding Principles in the Care and Use of Laboratory Animals' as approved by the Council of the American Physiological Society and with the prior approval of the Animal Care Committee of the Erasmus University Rotterdam. Adaptation of animals to the laboratory conditions started 1 week prior to the day of surgery and continued until 1 week after surgery.

Surgical procedures

After an overnight fast, pigs were sedated with ketamine (30 mg kg^{-1} i.m.; Ketalin®, Apharmo BV, Arnhem, The Netherlands), anaesthetised with thiopental (15 mg kg^{-1}, i.v.; Nesdonal®, Rhone-Poulenc Rorer BV, Amstelveen, The Netherlands), intubated and mechanically ventilated with a mixture of oxygen and nitrous oxide (1 : 2) to which 0.2–1% isoflurane (Forene®, Abbott BV, Amstelveen, The Netherlands) was added. Anaesthesia was maintained with midazolam (2 mg kg^{-1} + 0.5 mg kg^{-1} h^{-1}, i.v.; Dormicum®, Roche BV, Mijdrecht, The Netherlands) and fentanyl (10 μg kg^{-1} + 10 μg kg^{-1} h^{-1}, i.v.; Fentanyl-Janssen®, Janssen-Cilag BV, Tilburg, The Netherlands). Under sterile conditions, the chest was opened via the fourth left intercostal space and an 8 French (F) fluid-filled polyvinylchloride (PVC) catheter was inserted into the aortic arch, for the measurement of central aortic blood pressure, and secured with a purse string suture. After the pericardium was opened, an electromagnetic flow probe (Skalar, Delft, The Netherlands) was positioned around the ascending aorta for measurement of aortic blood flow. A high fidelity pressure transducer (Konigsberg Instruments Inc., Pasadena, CA, U.S.A.) was inserted into the left ventricle (LV) via the apical dimple for recording of LV pressure and its first derivative (LVdP/dt; obtained via electrical differentiation). An 8 F PVC catheter was also inserted into the LV for calibration of the Konigsberg transducer signal. Similar catheters were introduced into the left atrium and into the pulmonary artery for the measurement of blood pressure and administration of drugs. A Doppler flow probe (2.0 or 2.5 mm in diameter, emitting frequency (f_0) = 20 MHz; Crystal Biotech, Northboro, MA, U.S.A.) was placed around the proximal part of the left anterior descending coronary artery to measure the coronary blood flow. Electrical wires and catheters were tunnelled subcutaneously to the back, the chest was closed and the animals allowed to recover. All electrical wires and catheters were protected with a vest (Tubigrip®, Seton Healthcare Group, Oldham, U.K.).

Post-surgical period

During the first 48 hours after surgery animals received pain treatment consisting of buprenorphine (0.3 mg, i.v., once daily; Temgesic®, Reckitt and Colman Products, Kingston-upon-Hull, U.K.). During the first week after surgery animals received intravenous injections of 25 mg kg^{-1} amoxicillin (Clamoxil®, Beecham Farma B.V., Amstelveen, The Netherlands) and 5 mg kg^{-1} gentamycin (Gentamycine 5%®, A.U.V., Cuijk, The Netherlands) on a daily basis to prevent infection. Catheters were flushed daily with physiologic saline containing 2000 IU ml^{-1} heparin.

Table 1 Systemic, pulmonary and coronary haemodynamic effects of propylene glycol in awake resting swine

		Propylene glycol (ml min^{-1})		
	Baseline	*0.5*	*1.0*	*2.0*
Systemic haemodynamics				
CO (l min^{-1})	3.6±0.2	3.8±0.2	3.8±0.2	4.1±0.2*
HR (beats min^{-1})	118±4	124±5	121±5	124±4
SV (ml)	31±2	31±2	32±2	34±2*
$LVdP/dt_{max}$ (mmHg s^{-1})	3290±160	3260±150	3200±150	3220±160
$LVdP/dt_{min}$ (mmHgs^{-1})	−2750±210	−2620±250	−2700±200	−2650±230
LVEDP (mmHg)	8±1	9±1	9±1	12±1*
LVSP (mmHg)	119±4	119±4	121±4	121±3
MAP (mmHg)	97±3	97±4	99±4	98±4
DAP (mmHg)	72±3	73±4	75±5	74±4
SVR (mmHg min l^{-1})	27.3±1.4	26.4±1.4	26.5±1.6	24.2±1.3*
Pulmonary haemodynamics				
MPAP (mmHg)	17±2	19±1*	19±2*	21±1*
MLAP (mmHg)[a]	5±2	6±1	6±1	7±1*
PVR (mmHg min l^{-1})[a]	3.1±0.2	3.7±0.4*	3.5±0.3	3.3±0.3
Coronary haemodynamics				
CBF (ml min^{-1})	28±1	29±1	30±2	32±2*
CVR (mmHg min ml^{-1})	3.5±0.2	3.4±0.3	3.4±0.3	3.3±0.5
MW (mmHg l min^{-1})	440±40	450±30	470±30	500±30*

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 pressure; LVSP = left ventricular peak systolic pressure; MAP = mean arterial pressure; DAP = diastolic 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; MW = myocardial work (LVSP × CO). Data are mean±s.e.mean; $n = 10$, ([a]$n = 8$), *$P \leq 0.05$ vs baseline.

Experimental protocols

Haemodynamic responses to EMD 57033 in awake resting swine Resting studies were performed in a total of 11 pigs at 1–2 weeks after surgery with animals lying unrestrained in a cage. All resting studies were performed in random order at a minimum interval of 24 h.

1. *Solvent*. Systemic, pulmonary and coronary haemodynamic responses to propylene glycol (the solvent of EMD 57033) were studied in 10 resting pigs. After baseline haemodynamic measurements had been recorded, animals received three consecutive 10 min i.v. infusions of solvent administered at rates of 0.5, 1.0 and 2.0 ml min^{-1}, respectively. Haemodynamic measurements were made at the end of each infusion period and during the subsequent two hour washout period.
2. *EMD 57033*. The haemodynamic effects of three consecutive 10 min intravenous infusions of EMD 57033 in doses of 0.2, 0.4 and 0.8 mg kg^{-1} were studied in 10 pigs. Data were collected at the end of each infusion period and during the subsequent 2 h washout period.
3. *EMD 57033 in the presence of β-adrenergic receptor blockade*. After baseline haemodynamic measurements had been made, 10 pigs received a slow intravenous injection of propranolol (0.5 mg kg^{-1}) followed by an infusion (0.5 mg kg^{-1} h^{-1}, i.v.). This dose regimen results in >95% inhibition of isoproterenol-induced increases in heart rate and $LVdP/dt_{max}$ in awake swine (Duncker *et al*., 1987b). Ten minutes later, the post-propranolol data were recorded and the EMD 57033 infusion protocol (0.2, 0.4 and 0.8 mg kg^{-1} min^{-1}, i.v.) performed.

Effects of EMD 57033 and pimobendan on the haemodynamic responses to graded treadmill exercise Studies were performed in 14 pigs at 2–4 weeks after surgery with animals exercising on a motor driven treadmill. All exercise protocols were performed in random order at a minimum interval of 24 h.

1. *EMD 57033 and pimobendan*. With swine resting on the treadmill, measurements were made both lying and standing. Then, animals underwent a 3-stage exercise protocol (2, 3 and 4 km h^{-1}), with each level lasting 2–3 min. 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, animals were allowed to rest. Sixty minutes later, when all variables had returned to baseline levels, animals received either no treatment (control, $n=14$) to determine the reproducibility of the haemodynamic responses to exercise, EMD 57033 (0.4 mg kg^{-1} min^{-1}, i.v., $n=12$) or pimobendan (20 μg kg^{-1} min^{-1} i.v., $n=9$). The dose of EMD 57033 was chosen as in the resting study protocols (see above) it had no effect on heart rate and its effects on $LVdP/dt_{max}$ were unmitigated by propranolol, suggesting that this dose of EMD 57033 exhibited negligible phosphodiesterase inhibiting effects. The dose of pimobendan (20 μg kg^{-1} min^{-1}, i.v.) was chosen to be approximately equipotent (Duncker *et al*., 1987a) to EMD 57033 (0.4 mg kg^{-1} min^{-1}, i.v.) with respect to their effects on $LVd/P/dt_{max}$ during resting conditions.
2. *EMD 57033 and pimobendan in the presence of β-adrenergic receptor blockade*. Ten minutes after administration of propranolol (0.5 mg kg^{-1}, i.v.), resting measurements were made with animals both lying and standing. Then animals underwent the 3-stage exercise protocol as described above. Following completion of the exercise protocol, animals were allowed to rest. Sixty minutes later, when all variables had returned to baseline, animals received propranolol (0.2 mg kg^{-1}, i.v.) followed by either no additional treatment (control, $n=9$), EMD 57033 (0.4 mg kg^{-1} min^{-1}, i.v., $n=9$) or pimobendan (20 μg kg^{-1} min^{-1}, i.v., $n=8$). Five minutes later resting measurments were obtained and the exercise protocol was repeated.
3. *EMD 57033 in the presence of combined α- and β-adrenergic receptor blockade*. In seven swine we studied the effects of combined α- and β-adrenergic receptor blockade on haemo-

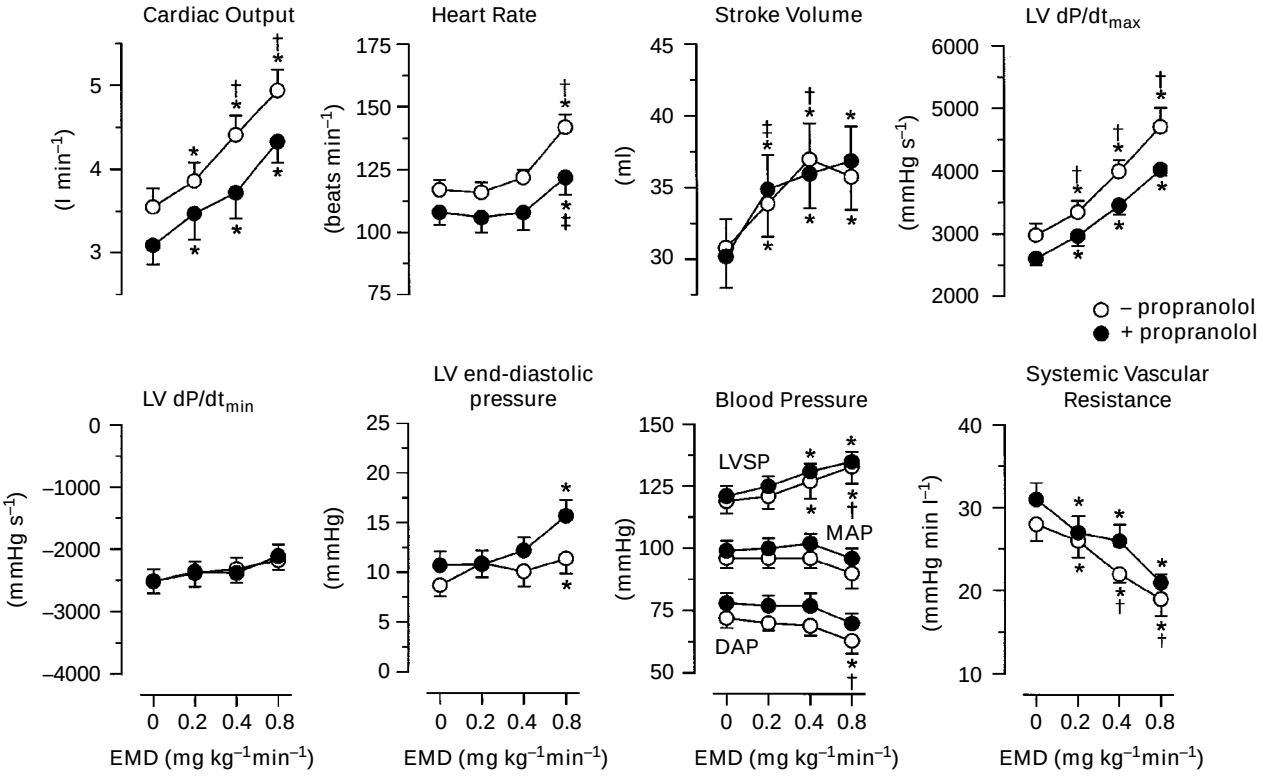

Figure 1 Systemic haemodynamic actions of cumulative intravenous 10 min infusions of EMD 57033, in the absence (−propranolol) and in the presence of β-adrenoceptor blockade (+propranolol) in awake resting (lying) swine. $LVdP/dt_{max}$ = maximum rate of rise of LV pressure; $LVdP/dt_{min}$ = maximum rate of fall of LV pressure; LVSP = LV peak systolic pressure; MAP = mean aortic pressure; DAP = diastolic aortic pressure. Data are mean ± s.e.mean, $n=10$. *$P \leq 0.05$ vs baseline (0 mg kg^{-1} min^{-1}); †$P \leq 0.05$ vs solvent-induced change from baseline; ‡$P \leq 0.05$ vs EMD 57033 induced change in the absence of β-adrenoceptor blockade.

dynamic responses to EMD 57033 during treadmill exercise. For this purpose, animals received propranolol (0.5 mg kg^{-1}, i.v.) and phentolamine (1.0 mg kg^{-1}, i.v.); the dose of phentolamine results in >95% inhibition of the noradrenaline (0.3 μg kg^{-1}, intracarotid) induced increase in carotid vascular resistance in anaesthetized swine (Verdouw *et al.*, 1984). Ten minutes later, resting measurements were made with animals both lying and standing followed by the 3-stage exercise protocol as described above. After completion of all measurements, animals were allowed to rest for 60 min. Then, animals received propranolol (0.2 mg kg^{-1}, i.v.) and phentolamine (0.4 mg kg^{-1}, i.v.) followed by either no treatment (control, $n=7$) or EMD 57033 (0.4 mg kg^{-1} min^{-1}, i.v., $n=7$). Five minutes later resting measurements were obtained and the exercise protocol was repeated.

Data acquisition and analysis

Data were recorded and digitized on-line using an eight channel data-acquisition program ATCODAS (Dataq Instruments, Inc., Akron Ohio, U.S.A.) and stored on a computer for later post-acquisition off-line analysis with a program written in MatLab (The Mathworks Inc., Mass, U.S.A.). A minimum of 15 consecutive beats were selected for analysis of the digitised haemodynamic signals. From these the heart rate, LV peak systolic, LV end-diastolic, mean left atrial, mean aortic, and mean pulmonary artery pressures, $LVdP/dt_{max}$, $LVdP/dt_{min}$, aortic blood flow and the mean coronary Doppler shift were determined.

Mean coronary blood flow was computed from the mean Doppler shift using the equation $Q=1.25\bullet\Delta f\bullet d^2$, where Q is the coronary blood flow (ml min^{-1}), Δf is the Doppler shift (KHz), d is the internal diameter of the coronary artery (mm) within the flow probe (Ishida *et al.*, 1983). The factor 1.25 is a constant derived from the speed of sound in tissue ($C=1.5\ 10^5$ cm s^{-1}), the frequency of the emitted sound beam ($f_0=20$ MHz), the cosine of the angle at which the sound beam is emitted (45°C), and unit conversion factors: $(C\bullet 0.75\ \pi)$ / $(2f_0\bullet\cos\ 45°C)$. As in chronically instrumented animals the flow probe is tightly adherent to the coronary artery, the internal diameter of the flow probe is equal to the external diameter of the artery. To obtain the inner diameter of the coronary artery we subtracted 10% of the external diameter of the coronary artery, which is approximately the arterial wall thickness. In this way any error in computation of the coronary internal diameter would affect control and intervention conditions equally.

Cardiac output was computed as ascending aorta flow (measured with the electromagnetic flow probe) + 2.5•coronary blood flow (as the left anterior descending coronary artery supplies approximately 40% of the LV). Systemic vascular resistance was computed as the ratio of mean aortic pressure and cardiac output; pulmonary vascular resistance was calculated as the ratio of the mean pulmonary artery pressure − mean left atrial pressure difference and cardiac output; coronary vascular resistance was computed as the ratio of mean aortic pressure and coronary blood flow. Stroke volume was calculated as the ratio of cardiac output and heart rate, while myocardial work was defined as the product of cardiac output and LV peak systolic blood pressure.

Statistical analysis of the resting protocols was performed using one-way analysis of variance for repeated measures. When a significant effect was observed post-hoc testing was done using Dunnett's test. To compare the changes between the three resting protocols two-way (dose and treatment) analysis of variance for repeated measures was performed. Statistical analysis of the exercise protocols was performed

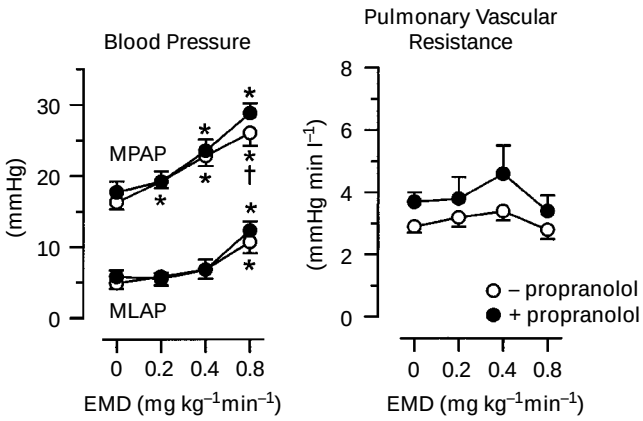

Figure 2 Pulmonary haemodynamic actions of cumulative intravenous 10 min infusions of EMD 57033, in the absence (−propranolol) and in the presence of β-adrenoceptor blockade (+propranolol) in awake resting swine. Data are mean ± s.e.mean; $n=10$, 8, and 8 for mean pulmonary artery pressure (MPAP), mean left atrial pressure (MLAP), and pulmonary vascular resistance, respectively. *$P\leqslant 0.05$ vs baseline (0 mg kg^{-1} min^{-1}); †$P\leqslant 0.05$ vs solvent-induced change from baseline; ‡$P\leqslant 0.05$ vs EMD 57033-induced change in the absence of β-adrenoceptor blockade.

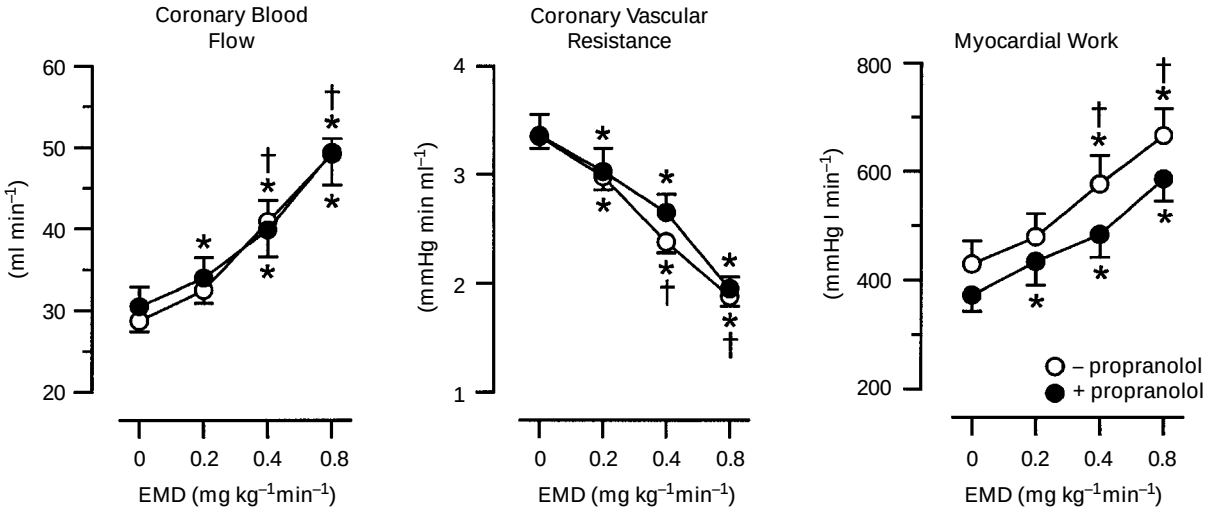

Figure 3 Coronary haemodynamic actions of cumulative intravenous 10 min infusions of EMD 57033, in the absence (−propranolol) and in the presence of β-adrenoceptor blockade (+propranolol) in awake resting swine. Myocardial work is the product of LV peak systolic pressure and cardiac output. Data are mean ± s.e.mean; $n=10$.*$P\leqslant 0.05$ vs baseline (0 mg kg^{-1} min^{-1}); †$P\leqslant 0.05$ vs solvent-induced change from baseline; ‡$P\leqslant 0.05$ vs EMD 57033-induced change in the absence of β-adrenoceptor blockade.

using two-way (exercise and treatment) analysis of variance for repeated measures. When a significant effect of exercise was observed, one-way analysis of variance for repeated measures was performed followed by post-hoc testing with a paired *t* test. When a significant effect of treatment was observed differences were tested using a paired *t* test. A *P* value of less than or equal to 0.05 was considered statistically significant (two-tailed). All data are presented as mean ± s.e.mean.

Drugs

EMD 57033 (the (+) enantiomer of 5-[1-(3,4-dimethoxybenzoyl)-1,2,3,4-tetrahydro-6-quinolyl]-6-methyl-3,6-dihydro-2H-1,3,4-thiadiazin-2-one; courtesy of Dr P Schelling, E. Merck KGaA, Darmstadt, Germany) and pimobendan (courtesy of Dr J. Dämmgen, Thomae, Biberach, Germany) were dissolved in 40°C propylene glycol (Sigma Chemical Co, St Louis, MO, U.S.A.). Phentolamine (10 mg ml^{-1}, Regitine®, Ciba-Geigy BV, Arnhem, The Netherlands) was dissolved in water containing glucose (35 mg ml^{-1}) and further diluted in saline to produce a final concentration of 0.1 mg kg^{-1} ml^{-1}. Propranolol (Sigma Chemical Co, St Louis, MO, U.S.A.) was dissolved in 30°C saline to produce a concentration of 0.05 mg kg^{-1} ml^{-1}. Fresh drug solutions were prepared on the day of each experiment.

Results

Haemodynamic responses to EMD 57033 in awake resting swine

Solvent (Table 1) Intravenous infusion of the lower two doses of solvent had no systemic or coronary haemodynamic effects, but produced slight increases in pulmonary artery pressure (2 ± 1 mmHg). The highest dose of solvent (2 ml min^{-1}) also produced slight increases in LV filling pressure (4 ± 1 mmHg) resulting in an increase in stroke volume (10 ± 3%) and hence cardiac output (15 ± 4%), and a small decrease in systemic vascular resistance (11 ± 4%), but had no effect on $LVdP/dt_{max}$. Upon termination of infusion of the highest rate of solvent, all variables returned to baseline values within 15 min, with the exception of cardiac output and pulmonary artery pressure which had returned to baseline at 30 min (data not shown).

EMD 57033 in the absence and presence of β-adrenergic receptor blockade

1. *Systemic haemodynamics (Figure 1).* EMD 57033 produced a dose-dependent increase in cardiac output (up to 41 ± 6%; $P \leqslant 0.01$ vs solvent-induced increase), which was principally due to an increase in stroke volume at lower doses while a modest increase in heart rate (22 ± 5%, $P \leqslant 0.01$) contributed during the highest dose. The increase in stroke volume resulted most likely from an increase in contractility (reflected by a 65 ± 17% increase in $LVdP/dt_{max}$ during infusion of the highest dose; $P \leqslant 0.01$ vs solvent) as LV end-diastolic pressure was minimally elevated (3 ± 1 mmHg, P = NS vs 4 ± 1 mmHg increase by solvent), while LV systolic pressure increased slightly ($P \leqslant 0.05$ vs solvent.). Despite the increase in cardiac output, EMD 57033 had no effect on mean aortic blood pressure, implying that systemic vascular resistance had decreased (32 ± 4%, $P \leqslant 0.01$ vs solvent). EMD 57033 had no significant effect on $LVdP/dt_{min}$ although the trend towards a decrease may not have reached statistical significance at the highest dose ($P = 0.12$) due to the EMD 57033-induced increase in LV systolic pressure. Fifteen minutes after the infusion was terminated, all haemodynamic variables had returned

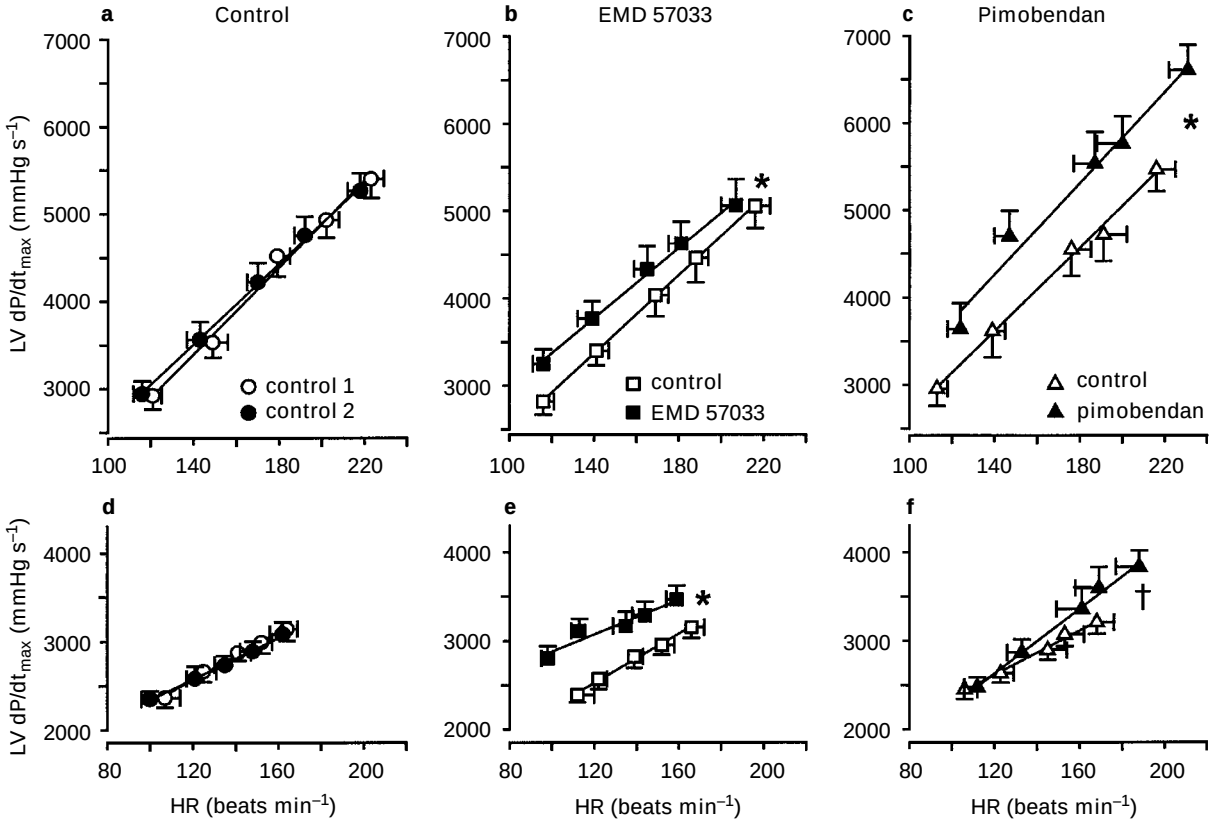

Figure 4 Reproducibility of inotropic responses to two consecutive control exercise periods (a, d), and the inotropic actions of EMD 57033 (0.4 mg kg^{-1} min^{-1}, b, e) and pimobendan (20 μg kg^{-1} min^{-1}, c, f) in swine during graded treadmill exercise in the absence (a–c) and in the presence of β-adrenoceptor blockade (d–f). The maximum rate of rise of LV pressure ($LVdP/dt_{max}$) data have been plotted as a function of heart rate (HR). Data are mean ± s.e.mean; n = 14, 12, 9, 9, 9, 8 for a–f, respectively. *$P \leqslant 0.05$ vs corresponding control conditions (first exercise period), †$P \leqslant 0.05$ vs corresponding change in the absence of β-adrenoceptor blockade.

to baseline values, with the exception of $LVdP/_{max}$ which was still $26\pm7\%$ higher than at baseline and $LVdP/dt_{min}$ which had decreased to $17\pm4\%$ below baseline (both $P\leqslant0.05$ vs solvent). At 60 min after the infusion had been stopped, $LVdP/dt_{min}$ had returned to baseline, but $LVdP/dt_{max}$ remained elevated above baseline ($30\pm7\%$ and $32\pm5\%$ at 1 and 2 h, respectively, both $P\leqslant0.05$ vs solvent) during the entire 2 h washout period (data not shown).

β-adrenergic receptor blockade with propranolol resulted in significant reductions in heart rate ($7\pm2\%$), $LVdP/dt_{max}$ ($21\pm5\%$) and cardiac output ($9\pm3\%$) and increases in LV end-diastolic pressure ($22\pm6\%$) (all $P\leqslant0.01$), but did not alter the EMD 57033-induced haemodynamic responses, except for a slight attenuation of the increases in heart rate ($P\leqslant0.05$ vs EMD 57033 without propranolol) and $LVdP/dt_{max}$ ($P=0.08$ vs EMD 57033 without propranolol) produced by the highest dose. In addition, the highest dose of EMD 57033 produced a 5 ± 2 mmHg increase in LV end-diastolic pressure ($P\leqslant0.05$ vs baseline), but this increase was not different from 4 ± 1 mmHg increase produced by the equivalent infusion rate of solvent. During washout, haemodynamic variables behaved similarly as in animals with intact β-adrenergic receptors, so that at 15 min washout $LVdP/dt_{max}$ was still $20\pm3\%$ above and $LVdP/dt_{min}$ had decreased to $16\pm5\%$ below pre-EMD 57033 baseline (both $P\leqslant0.05$). Thirty minutes after the infusion was stopped, $LVdP/dt_{min}$ had returned to baseline, while $LVdP/dt_{max}$ again remained elevated above baseline during the entire 2 h washout period, although this was slightly less pronounced ($18\pm4\%$ and $14\pm4\%$ at 1 and 2 h, respectively, both $P\leqslant0.05$) compared to animals with intact β-adrenoceptors.

2. *Pulmonary haemodynamics (Figure 2).* EMD 57033 produced a dose-dependent increase in mean pulmonary artery pressure, which was the result of an increased mean left atrial pressure as well as an increase in cardiac output. Consequently, pulmonary vascular resistance did not change ($P=$NS vs baseline or solvent). Propranolol had no significant effect on pulmonary vascular resistance and did also not alter the pulmonary haemodynamic responses to EMD 57033. Fifteen minutes after the infusion was stopped, all pulmonary haemodynamic variables had returned to baseline.

3. *Coronary haemodynamics (Figure 3).* EMD 57033 produced a dose-dependent increase in coronary blood flow (up to $74\pm7\%$ of baseline, $P\leqslant0.05$ vs solvent), which could in part be explained by the $59\pm9\%$ increase in the myocardial work (triple product of heart rate•stroke volume•LV systolic pressure). Propranolol caused a $9\pm2\%$ ($P\leqslant0.05$) decrease in

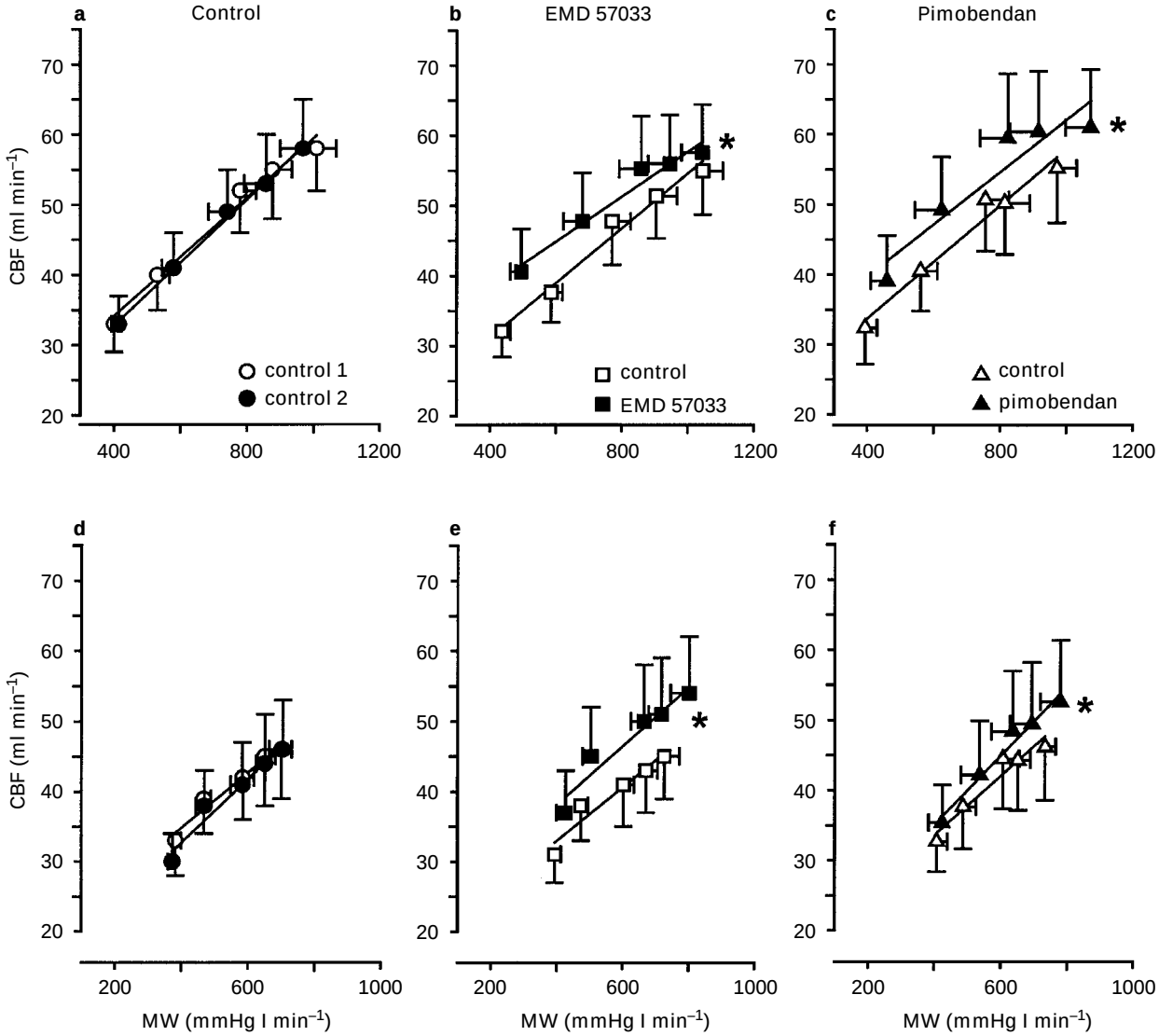

Figure 5 Reproducibility of coronary blood flow responses to two consecutive control exercise periods (a, d), and the coronary blood flow responses to EMD 57033 (0.4 mg kg^{-1} min^{-1}, b, e) and pimobendan (20 μg kg^{-1} min^{-1}, c, f) in swine during graded treadmill exercise in the absence (a–c) and in the presence of β-adrenoceptor blockade (d–f). Coronary blood flow (CBF) data have been plotted as a function of myocardial work (MW, cardiac output•LV peak systolic pressure). Data are mean$\pm$s.e.mean; $n=14$, 12, 9, 9, 9 and 8, for a–f, respectively. *$P\leqslant0.05$ vs corresponding control conditions (first exercise period), †$P\leqslant0.05$ vs corresponding change in the absence of β-adrenoceptor blockade.

Table 2 Systemic, pulmonary and coronary haemodynamic responses to two consecutive trials of graded treadmill exercise in swine

		Rest		*Exercise* (km h^{-1})		
	Treatment	*Lying*	*Standing*	*2*	*3*	*4*
Systemic haemodynamics						
CO	Control 1	3.4±0.1	4.6±0.2*	6.1±0.3*	6.7±0.4*	7.3±0.4*
(l min^{-1})	Control 2	3.6±0.2	4.8±0.2*	5.8±0.3*	6.6±0.4*	7.1±0.4*
HR	Control 1	121±4	149±7*	179±6*	202±6*	223±6*
(beats min^{-1})	Control 2	116±4	143±6*	170±5*	192±5*	218±6*
SV	Control 1	29±2	32±2*	35±2*	34±2*	33±2*
(ml)	Control 2	32±2	35±2*	35±2*	35±2*	33±2
$LVdP/dt_{max}$	Control 1	2920±160	3530±170*	4520±240*	4930±200*	5400±220*
(mmHg s^{-1})	Control 2	2940±140	3560±200*	4220±220*	4760±210*	5260±200*
$LVdP/dt_{min}$	Control 1	−2530±120	−2480±110	−2690±140	−2800±140*	−3190±160*
(mmHg s^{-1})	Control 2	−2530±130	−2370±130	−2570±120	−2710±160	−3020±210*
LVSP	Control 1	119±3	117±3	129±2*	133±3*	140±3*
(mmHg)	Control 2	118±3	122±3	129±4*	131±4*	138±4*
MAP	Control 1	102±2	92±3*	95±3*	97±2*	99±3
(mmHg)	Control 2	100±3	93±3*	95±3	95±3	97±2
SVR	Control 1	30.2±1.5	20.3±1.3*	16.0±0.9*	15.0±1.0*	13.8±0.8*
(mmHg min l^{-1})	Control 2	28.4±1.7	19.5±0.8*	16.7±0.9*	14.7±0.8*	13.8±0.7*
Pulmonary haemodynamics						
MPAP	Control 1	15±1	15±2	22±2*	27±2*	29±2*
(mmHg)	Control 2	15±1	18±2	23±1*	26±1*	30±1*
MLAP[a]	Control 1	7±1	2±2*	6±1	9±1	11±1*
(mmHg)	Control 2	7±1	4±2*	7±1	8±1	10±1*
PVR[a]	Control 1	2.4±0.3	2.7±0.3	2.4±0.3	2.6±0.3	2.4±0.3
(mmHg min l^{-1})	Control 2	2.2±0.2	2.7±0.3*	2.8±0.3*	2.6±0.2	2.7±0.3
Coronary haemodynamics						
CBF	Control 1	33±4	40±4*	51±6*	54±6*	57±6*
(ml min^{-1})	Control 2	33±4	41±4*	49±5*	53±6*	57±6*
CVR	Control 1	3.6±0.4	2.6±0.3*	2.2±0.2*	2.1±0.2*	2.0±0.2*
(mmHg min ml^{-1})	Control 2	3.4±0.3	2.6±0.2*	2.3±0.3*	2.1±0.2*	1.9±0.2*
MW	Control 1	410±20	540±40*	800±50*	890±60*	1030±60*
(mmHg l min^{-1})	Control 2	430±20	590±30*	760±60*	870±70*	990±70*

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; CBF = coronary blood flow, CVR = coronary vascular resistance, MW = myocardial work (LVSP × CO). Data are mean±s.e.mean; $n = 14$ ($^{a}n = 11$). *$P \leqslant 0.05$ vs Rest; †$P \leqslant 0.05$ control 2 vs control 1.

coronary blood flow but did not alter the responses of coronary blood flow and vascular resistance to EMD 57033. Fifteen minutes after the infusion was stopped, all coronary heamodynamic variables had returned to baseline.

Effects of EMD 57033 and pimobendan on the haemodynamic responses to graded treadmill exercise

Control (Table 2, Figures 4 and 5) The first period of exercise produced increases in heart rate from 121±4 beats min^{-1} at rest (lying) to 223±6 beats min^{-1} at 4 km h^{-1}, in LV systolic pressure from 119±3 mmHg to 140±3 mmHg, in $LVdP/dt_{max}$ from 2920±160 mmHg s^{-1} to 5400±220 mmHg s^{-1} and in cardiac output from 3.4±0.11 min^{-1} to 7.3±0.4 l min^{-1} (all $P \leqslant 0.01$). While mean aortic pressure did not change from rest lying, mean pulmonary artery pressure almost doubled from 15±1 to 29±2 mmHg ($P \leqslant 0.01$). These different pressure responses of the systemic and pulmonary vascular beds reflect the intense systemic vasodilation (54±1% decrease in systemic vascular resistance, $P \leqslant 0.01$) vs the unaltered pulmonary vascular resistance. Coronary blood flow increased from 33±4 ml min^{-1} at rest to 57±6 ml min^{-1} during the highest level of exercise. After 60 min of rest, at a time when all variables had returned to baseline levels, the second period of exercise resulted in highly reproducible systemic, pulmonary and coronary haemodynamic responses.

EMD 57033 (Table 3, Figures 4 and 5) EMD 57033 in a dose of 0.4 mg kg^{-1} min^{-1}, i.v. produced an increase in $LVdP/dt_{max}$ from 2820±150 to 3250±170 mmHg s^{-1} in animals lying on the treadmill ($P \leqslant 0.01$) (Table 3, Figure 4) and an increase in cardiac output from 3.9±0.2 to 4.3±0.2 l min^{-1} ($P \leqslant 0.01$) (Table 3). In the presence of EMD 57033, exercise at 2 km h^{-1} resulted in a 9±4% higher $LVdP/dt_{max}$ ($P = 0.07$) than during exercise under control conditions. However, at 3 and 4 km h^{-1} $LVdP/dt_{max}$ and cardiac output were no longer elevated compared to control exercise. The responses of LV systolic and mean aortic blood pressure to exercise in the presence of EMD 57033 were not different from their respective responses to control exercise. EMD 57033 decreased systemic vascular resistance at rest and during exercise at 2 km h^{-1}, but at 3 and 4 km h^{-1} systemic vascular resistance was no longer different from control exercise. In the presence of EMD 57033 exercise resulted in elevated pulmonary artery pressure and left atrial pressure compared to control exercise, but pulmonary vascular resistance was not different from control exercise. EMD 57033 produced coronary vasodilation resulting in higher coronary blood flows during exercise in the presence of EMD 57033 compared to control exercise, which could only in part be explained by the higher myocardial work loads.

Pimobendan (Table 4, Figures 4 and 5) Pimobendan (20 μg kg^{-1} min^{-1}, i.v.) produced increases in $LVdP/dt_{max}$ from 2960±190 to 3640±290 mmHg s^{-1} ($P \leqslant 0.01$) and in cardiac output from 3.5±0.3 to 3.9±0.3 l min^{-1} ($P \leqslant 0.01$), during lying resting conditions. Exercise in the presence of pimobendan resulted in significantly higher levels of $LVdP/dt_{max}$ compared to control exercise, so that at 4 km h^{-1} of exercise $LVdP/dt_{max}$ was 5470±250 mmHg s^{-1} during control conditions and was 6610±290 mmHg s^{-1} during exercise in the presence of pimobendan. Pimobendan produced systemic vasodilation at rest and at each level of exercise resulting in slightly lower aortic pressures at 2 and 4 km h^{-1}, compared to

Table 3 Systemic, pulmonary and coronary haemodynamic responses to EMD 57033 during graded treadmill exercise in swine

		Rest		*Exercise* (km h^{-1})		
	Treatment	*Lying*	*Standing*	*2*	*3*	*4*
Systemic haemodynamics						
CO	Control	3.9±0.2	5.1±0.2*	6.2±0.3*	6.9±0.4*	7.6±0.3*
(l min^{-1})	EMD	4.3±0.2†	5.3±0.3*	6.6±0.3*†	7.1±0.4*	7.5±0.3*
HR	Control	116±5	141±6*	169±6*	188±6*	216±7*
(beats min^{-1})	EMD	116±5	139±7*	165±6*	181±6*	207±7*
SV	Control	34±2	37±2*	37±2*	37±2*	36±2
(ml)	EMD	38±2†	39±3*	41±3*†	40±2*†	37±2
$LVdP/dt_{max}$	Control	2820±150	3400±160*	4040±240*	4460±280*	5060±250*
(mmHg s^{-1})	EMD	3250±170†	3770±200*†	4330±260*	4630±250*	5060±300*
$LVdP/dt_{min}$	Control	−2600±170	−2500±150	−2650±150	−2820±120	−2920±130
(mmHg s^{-1})	EMD	−2280±200†	−2380±140	−2630±170*	−2690±150*	−2990±250*
LVSP	Control	114±3	117±3	126±4*	132±3*	140±3*
(mmHg)	EMD	116±3	125±4*	131±4*	135±4*	142±4*
MAP	Control	98±2	90±3*	88±3*	92±3*	94±3*
(mmHg)	EMD	94±3	88±3*	86±3	90±3	92±3
SVR	Control	26.2±1.8	18.0±1.1*	14.6±0.9*	13.8±1.0*	12.6±0.7*
(mmHg min l^{-1})	EMD	22.4±1.3†	17.1±1.1*	13.4±0.9*†	13.0±0.8*	12.3±0.7*
Pulmonary haemodynamics						
MPAP	Control	14±1	16±1	19±2*	24±2*	29±2*
(mmHg)	EMD	19±1†	21±2†	27±2*†	30±2*†	34±2*†
MLAP[a]	Control	4±1	2±1*	2±1*	4±1	7±1
(mmHg)	EMD	5±1	4±1	5±1†	9±1*†	12±1*†
PVR[a]	Control	2.4±0.3	2.8±0.3	2.8±0.3	2.9±0.3	2.9±0.3
(mmHg min l^{-1})	EMD	3.2±0.4	3.2±0.5	3.2±0.4	3.2±0.4	3.0±0.4
Coronary haemodynamics						
CBF	Control	32±3	38±4*	48±6*	51±5*	55±6*
(ml min^{-1})	EMD	40±6†	47±6*†	55±7*†	55±6*†	57±6*
CVR	Control	3.5±0.4	2.7±0.3*	2.1±0.3*	2.1±0.3*	2.0±0.3*
(mmHg min ml^{-1})	EMD	2.8±0.4†	2.2±0.3*†	1.9±0.2*†	1.9±0.2*†	1.9±0.3*
MW	Control	450±30	600±40*	790±60*	920±60*	1070±60*
(mmHg l min^{-1})	EMD	510±30†	670±50*†	880±70*†	970±70*	1060±70*

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; CBF = coronary blood flow, CVR = coronary vascular resistance, MW = myocardial work (LVSP × CO), EMD = EMD 57033 (0.4 mg kg^{-1} min^{-1}, i.v.). Data are mean±s.e.mean; $n = 12$ ([a]$n = 9$). *$P \leqslant 0.05$ vs Rest; †$P \leqslant 0.05$ EMD 57033 vs control.

control exercise. Pimobendan had no significant effect on pulmonary haemodynamics. Similar to EMD 57033, pimobendan produced higher coronary blood flows which in part paralleled the higher myocardial work loads.

Effects of EMD 57033 and pimobendan on the haemodynamic responses to graded treadmill exercise in the presence of β-adrenergic receptor blockade

Control (Table 5, Figures 4 and 5) β-adrenergic receptor blockade markedly blunted the exercise-induced increases in heart rate, $LVdP/dt_{max}$, LV systolic pressure, cardiac output, and coronary blood flow compared to exercise with normal β-adrenergic activity. After 60 min of rest, when all variables had returned to baseline values, the second exercise period in the presence of β-adrenergic receptor blockade, resulted in highly reproducible systemic, pulmonary and coronary haemodynamic responses.

EMD 57033 (Table 6, Figures 4 and 5) After pretreatment with β-adrenergic receptor blockade, exercise in the presence of EMD 57033 (0.4 mg kg^{-1} min^{-1}) resulted in higher levels of $LVdP/dt_{max}$ and stroke volume compared to exercise in the presence of propranolol alone. After propranolol, EMD 57033 produced increases in pulmonary artery pressure at rest and during exercise which appeared to be the result of pulmonary vasoconstriction, but these effects were not significantly different from the effects of solvent on pulmonary haemodynamics. During exercise in the presence of propranolol, the effects of EMD 57033 on coronary blood flow and vascular resistance were maintained.

Pimobendan (Table 7, Figures 4 and 5) In the presence of β-adrenergic receptor blockade the increases in $LVdP/dt_{max}$ and stroke volume were abolished at rest and markedly attenuated during exercise, compared to control conditions, indicating that the cardiostimulatory actions of pimobendan depended on the degree of β-adrenoceptor activity. In contrast, the systemic vasodilator effects were unmitigated in the presence of propranolol at rest and during exercise. During β-adrenoceptor blockade pimobendan produced less coronary vasodilation at rest (in parallel with an unchanged myocardial work) but its vasodilatory actions were maintained during exercise.

Effects of EMD 57033 on the haemodynamic responses to graded treadmill exercise in the presence of combined α- and β-adrenergic receptor blockade

Control (Figure 6) In the presence of combined α- and β-adrenergic receptor blockade exercise produced similar haemodynamic responses compared to exercise during single β-adrenergic receptor blockade, with the exception of a lower systemic vascular resistance and mean aortic pressure (data not shown). Two consecutive exercise periods in the presence of combined α- and β-adrenergic receptor blockade separated by 60 min of rest, resulted in highly reproducible systemic, pulmonary and coronary haemodynamic responses.

EMD 57033 (Figure 6) Combined α- and β-adrenergic receptor blockade did not alter the positive inotropic responses to EMD 57033 (0.4 mg kg^{-1} min^{-1}, i.v.) either at rest or during exercise indicating that the inotropic actions of EMD 57033 were not mediated via α-adrenoceptors. The coronary

Table 4 Systemic, pulmonary and coronary haemodynamic responses of pimobendan during graded treadmill exercise in swine

		Rest		*Exercise* (km h^{-1})		
	Treatment	*Lying*	*Standing*	*2*	*3*	*4*
Systemic haemodynamics						
CO	Control	3.5±0.3	4.8±0.3*	6.1±0.4*	6.5±0.4*	7.2±0.4*
(l min^{-1})	PIM	3.9±0.3†	5.0±0.4*	6.3±0.5*	6.9±0.5*	7.6±0.4*†
HR	Control	113±5	139±6*	176±9*	191±11*	216±9*
(beats min^{-1})	PIM	124±6†	147±7*†	187±10*	200±12*	231±9*†
SV	Control	30±2	35±2*	34±2*	34±2*	33±2
(ml)	PIM	31±1	34±2	34±1*	34±1*	33±1
$LVdP/dt_{max}$	Control	2960±190	3620±300*	4550±300*	4730±300*	5470±250*
(mmHg s^{-1})	PIM	3640±290†	4640±320*	5540±360*†	5770±310*†	6610±290*†
$LVdP/dt_{min}$	Control	−2550±90	−2530±110	−2640±150	−2830±230	−3090±200*
(mmHg s^{-1})	PIM	−2470±140	−2580±180	−2970±250†	−3210±360†	−3340±310*
LVSP	Control	116±4	119±5	126±4*	128±4*	138±4*
(mmHg)	PIM	119±5	126±6	131±5*	135±5*†	143±4*
MAP	Control	99±3	92±3*	89±2*	88±3*	95±3
(mmHg)	PIM	95±3	91±3	85±3*†	88±4*	89±4†
SVR	Control	29.9±2.7	20.0±1.9*	15.4±1.4*	14.2±1.2*	13.6±1.1*
(mmHg min l^{-1})	PIM	25.2±1.4†	19.2±1.5*	14.1±1.3*†	13.3±1.2*†	12.1±1.2*†
Pulmonary haemodynamics						
MPAP	Control	15±2	17±2	22±2*	24±2*	28±2*
(mmHg)	PIM	17±1	18±2	22±2	26±3*	29±3*
MLAP[a]	Control	4±1	2±1*	4±2	6±2	9±2*
(mmHg)	PIM	6±3	1±1	3±1	6±3	8±1
PVR[a]	Control	3.0±0.6	2.8±0.4	2.6±0.5	2.6±0.4	2.5±0.5
(mmHg min l^{-1})	PIM	3.0±0.5	3.3±0.4†	2.8±0.5	2.7±0.5	2.5±0.5
Coronary haemodynamics						
CBF	Control	32±5	40±6*	51±7*	50±7*	55±8*
(ml min^{-1})	PIM	39±6†	49±8*†	59±9*†	60±9*†	61±8*†
CVR	Control	3.9±0.8	2.9±0.6*	2.2±0.5*	2.2±0.5*	2.1±0.4*
(mmHg min ml^{-1})	PIM	3.2±0.6†	2.2±0.3*†	1.9±0.4*†	1.9±0.4*†	1.9±0.5*†
MW	Control	400±40	570±50*	770±70*	830±80*	990±60*
(mmHg l min^{-1})	PIM	470±50†	640±80*	840±90*	940±90*†	1090±80*†

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; CBF = coronary blood flow, CVR = coronary vascular resistance, MW = myocardial work (LVSP × CO); PIM = Pimobendan (20 μg kg^{-1} min^{-1}, i.v.). Data are mean±s.e.mean; $n=9$ ([a]$n=6$). *$P\leqslant0.05$ vs Rest; †$P\leqslant0.05$ pimobendan vs control.

vasodilator response to EMD 57033 was also not further modified by additional α-adrenergic receptor blockade, but the systemic vasodilator response was attenuated (data not shown).

Discussion

The present study describes the cardiovascular effects of the Ca^{2+} sensitizer EMD 57033 in awake pigs, free from the effects of anaesthesia and acute surgical trauma, in which we previously studied the cardiovascular profile of the mixed Ca^{2+} sensitizer/PDE-III inhibitor pimobendan (Duncker *et al*., 1987a). The main findings under resting conditions were: (1) EMD 57033 produced dose-dependent increases in global LV contractility and stroke volume, with a minimal effect on heart rate and blood pressure, and produced a decrease in systemic vascular resistance, (2) these effects were essentially unchanged when animals were pretreated with β-adrenoceptor blockade, indicating that phosphodiesterase inhibition does not contribute significantly to the positive inotropic actions of EMD 57033. Furthermore, the present study demonstrated that during exercise (3) the positive inotropic actions of EMD 57033 persisted at low levels of exercise but were no longer observed at higher levels of exercise, which may have been caused by the exercise-induced increases in β-adrenergic activity and cAMP levels, because pretreatment with propranolol resulted in preservation of the positive inotropic actions at all levels of exercise; (4) in contrast, the positive inotropic and chronotropic effects of pimobendan were amplified during exercise with intact β-adrenoceptors, but were abolished at rest and markedly blunted during exercise in the presence of β-adrenoceptor blockade, and (5) finally, combined α- and β-adrenergic receptor blockade did not further alter the responses to EMD 57033 during exercise, compared to the responses to EMD 57033 during β-adrenergic receptor blockade alone, indicating that the positive inotropic actions of EMD 57033 were not mediated via or did not depend on intact α-adrenergic receptor activity.

Effects of EMD 57033 on systemic, pulmonary and coronary haemodynamics in awake resting pigs

During resting conditions EMD 57033 produced dose-dependent increases in $LVdP/dt_{max}$, stroke volume and cardiac output. These effects were only minimally affected by pretreatment with propranolol, suggesting that PDE inhibition contributed only minimally to the cardiostimulatory actions. Only during the highest dose did we observe an increase in heart rate which was blunted by pretreatment with propranolol suggesting that EMD 57033 produced slight phosphodiesterase inhibition at this dose (Nankervis *et al*., 1994). In contrast, we previously observed that the pimobendan-induced increases in cardiac output, heart rate and $LVdP/dt_{max}$ were markedly attenuated by pretreatment with propranolol (Figure 7; Duncker *et al*., 1987a), indicating that this purported Ca^{2+} sensitiser exerts a major part of its actions via phosphodiesterase inhibition in awake pigs. In awake dogs, another Ca^{2+} sensitiser levosimendan also produced dose-dependent increases in $LVdP/dt_{max}$ and cardiac output. Autonomic blockade with propranolol, atropine and hexamethonium, markedly attenuated (>50%) the chronotropic and inotropic responses of even the

Table 5 Systemic, pulmonary and coronary haemodynamic responses to two consecutive trials of graded treadmill exercise in swine in the presence of non-selective β-adrenoceptor blockade

		Rest		*Exercise* (km h^{-1})		
	Treatment	*Lying*	*Standing*	*2*	*3*	*4*
Systemic haemodynamics						
CO	$\ominus\beta_{1,2}$ Control 1	3.4±0.2	4.2±0.2*	5.1±0.3*	5.6±0.3*	5.9±0.4*
(l min^{-1})	$\ominus\beta_{1,2}$ Control 2	3.3±0.2	4.2±0.3*	5.1±0.3*	5.5±0.3*	5.8±0.4*
HR	$\ominus\beta_{1,2}$ Control 1	107±7	125±6*	141±6*	152±5*	164±5*
(beats min^{-1})	$\ominus\beta_{1,2}$ Control 2	100±4	121±4*	135±5*†	148±6*	162±6*
SV	$\ominus\beta_{1,2}$ Control 1	32±1	34±2	36±2*	7±1*	36±1*
(ml)	$\ominus\beta_{1,2}$ Control 2	33±1	35±2	37±2*	37±2*	36±1*
$LVdP/dt_{max}$	$\ominus\beta_{1,2}$ Control 1	2370±110	2670±120*	2880±90*	2990±120*	3140±130*
(mmHg s^{-1})	$\ominus\beta_{1,2}$ Control 2	2350±90	2580±140*	2740±110*†	2890±110*	3090±130*
$LVdP/dt_{min}$	$\ominus\beta_{1,2}$ Control 1	−2490±130	−2400±170	−2440±160	−2420±160	−2410±160
(mmHg s^{-1})	$\ominus\beta_{1,2}$ Control 2	−2500±150	−2420±150	−2400±170	−2400±150	−2400±140
LVSP	$\ominus\beta_{1,2}$ Control 1	117±3	116±3	117±3	120±3	123±3*
(mmHg)	$\ominus\beta_{1,2}$ Control 2	117±3	114±3	118±2	123±3*	124±2*
MAP	$\ominus\beta_{1,2}$ Control 1	100±2	95±3	93±4	94±3*	93±3
(mmHg)	$\ominus\beta_{1,2}$ Control 2	99±3	93±3*	90±3*	94±3	95±3
SVR	$\ominus\beta_{1,2}$ Control 1	30.7±2.3	23.4±1.7*	19.0±1.7*	17.5±1.5*	16.6±1.6*
(mmHg min l^{-1})	$\ominus\beta_{1,2}$ Control 2	31.0±2.1	22.9±1.9*	18.5±1.7*	17.7±1.4*	16.9±1.4*
Pulmonary haemodynamics						
MPAP	$\ominus\beta_{1,2}$ Control 1	16±1	20±2	21±2*	24±2*	27±2*
(mmHg)	$\ominus\beta_{1,2}$ Control 2	15±2	17±2	20±2*	23±2*	28±2*
MLAP[a]	$\ominus\beta_{1,2}$ Control 1	7±1	6±4	6±2	7±2	10±2
(mmHg)	$\ominus\beta_{1,2}$ Control 2	5±2	2±2	5±2	7±1	10±1*
PVR[a]	$\ominus\beta_{1,2}$ Control 1	2.7±0.4	3.4±0.4	3.0±0.5	2.9±0.4	2.9±0.4
(mmHg min l^{-1})	$\ominus\beta_{1,2}$ Control 2	3.0±0.6	3.3±0.5	3.0±0.6	2.9±0.5	3.1±0.6
Coronary haemodynamics						
CBF	$\ominus\beta_{1,2}$ Control 1	33±5	39±5*	42±6*	45±7*	46±7*
(ml min^{-1})	$\ominus\beta_{1,2}$ Control 2	30±4	38±5*	41±6*	44±7*	46±7*
CVR	$\ominus\beta_{1,2}$ Control 1	3.5±0.5	2.8±0.4*	2.6±0.4*	2.5±0.4*	2.5±0.4*
(mmHg min ml^{-1})	$\ominus\beta_{1,2}$ Control 2	3.8±0.6	2.9±0.5*	2.6±0.4*	2.6±0.4*	2.5±0.4*
MW	$\ominus\beta_{1,2}$ Control 1	400±20	480±20*	600±30*	670±30*	720±30*
(mmHg l min^{-1})	$\ominus\beta_{1,2}$ Control 2	380±10	480±30*	600±40*	670±30*	720±40*

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; CBF = coronary blood flow, CVR = coronary vascular resistance, MW = myocardial work (LVSP × CO). Data are mean±s.e.mean; $n=9$ ([a]$n=7$). *$P\leqslant0.05$ vs Rest; †$P\leqslant0.05$ control 2 vs control 1.

lowest doses of levosimendan, suggesting that the cardiostimulatory actions were most likely the result of both PDE-III inhibition and Ca^{2+} sensitization (Harkin *et al*., 1995). Interestingly, the increase in cardiac output was entirely due to an increase in heart rate, with no change in stroke volume which may have resulted from the decrease in LV preload. These findings with levosimendan in awake dogs are very similar to our data with pimobendan in awake normal pigs (Duncker *et al*., 1987a). In healthy resting humans, levosimendan also increased cardiac output, which was due to an increase in stroke volume at a lower dose, while at a higher dose it resulted entirely from an increase in heart rate (Lilleberg *et al*., 1994; Sundberg *et al*., 1995).

Although we failed to find evidence to support a significant contribution of phosphodiesterase inhibition to the actions by EMD 57033, direct evidence that the positive inotropic actions of EMD 57033 are mediated by an increase in responsiveness of the myofilaments to Ca^{2+} has so far been obtained exclusively in *in vitro* studies. Thus, in isolated ferret hearts EMD 57033 at concentrations of 0.3–10 μM increased the force of contraction despite a slight decrease in Ca^{2+} transient, whereas its (−) enantiomer the phosphodiesterase inhibitor EMD 57439 increased the Ca^{2+} transient together with an increase in contractile force (White *et al*., 1993). In skinned ventricular fibers isolated from guinea pig hearts EMD 57033 in concentrations of up to 3–10 μM increased the Ca^{2+} sensitivity of the myofilaments reflected by a decrease in the Ca^{2+} concentration that results in 50% of the maximum response of the Ca^{2+}-force relation (0.2–0.5 log units increase in pCa_{50}) and increased the maximum contractile force by 10–15% (Lues *et al*., 1993). Importantly, in concentrations lower than 10 μM EMD 57033 had minimal effects on isoprenaline-mediated production of cAMP, suggesting negligible effects on phosphodiesterase inhibiting activity at these concentrations. In support of the latter finding, the increase in contractile force in intact guinea pig papillary muscle produced by EMD 57033 was not affected by the presence of a low dose of the β-adrenoceptor agonist isoprenaline (which by itself increased basal contractile force by 15%). In contrast, the increase in force produced by EMD 57439 was markedly potentiated by the dose of isoprenaline (Lues *et al*., 1993). These findings support our *in vivo* observations and provide, albeit indirect evidence that the positive inotropic actions observed in the present *in vivo* study are most likely mediated via an increase in Ca^{2+} responsiveness (either an increase in sensitivity or maximum force) of the myofilaments.

To demonstrate that EMD 57033 also increases the myofilament responsiveness to Ca^{2+} *in vivo*, we studied the effects of EMD 57033 on the regional myocardial contractile responses to intracoronary infusions of Ca^{2+} in two pentobarbital anaesthetized open-chest pigs, in which β-adrenoceptor activity was blocked to exclude phosphodiesterase activity by EMD 57033. The animals were instrumented with an LV microtipped pressure transducer, a coronary Doppler flow probe around the left anterior descending coronary artery, and distal to the probe an intracoronary catheter for infusion of Ca^{2+}. In the myocardium perfused by the left anterior descending coronary artery ultrasonic crystals were implanted for the measurement of End-Systolic Elastance (E_{ES}), the directional coefficient of the LV end-systolic pressure segment relation, as a measure of regional myocardial contractile function (Krams *et al*., 1993); LV preload was varied by inflation of a balloon positioned in

Table 6 Systemic, pulmonary and coronary haemodynamic responses of EMD 57033 during graded treadmill exercise in swine in the presence of non-selective β-adrenoceptor blockade

		Rest		*Exercise* (km h^{-1})		
	Treatment	*Lying*	*Standing*	*2*	*3*	*4*
Systemic haemodynamics						
CO	$\ominus\beta_{1,2}$	3.5±0.2	4.2±0.2*	5.2±0.3*	5.6±0.3*	6.0±0.3*
(l min^{-1})	$\ominus\beta_{1,2}$+EMD	3.6±0.2	4.3±0.2*	5.4±0.3*	5.7±0.3*†	6.2±0.4*
HR	$\ominus\beta_{1,2}$	112±8	122±4	139±4*	152±6*	166±6*
(beats min^{-1})	$\ominus\beta_{1,2}$+EMD	98±3	113±4*†	135±6*	144±6*†	159±5*†
SV	$\ominus\beta_{1,2}$	32±2	34±1*	37±1*	37±1*	36±1*
(ml)	$\ominus\beta_{1,2}$+EMD	36±1†	38±2*†	40±2*†	40±1*†	39±2†
$LVdP/dt_{max}$	$\ominus\beta_{1,2}$	2390±80	2570±120*	2820±130*	2960±110*	3150±120*
(mmHg s^{-1})	$\ominus\beta_{1,2}$+EMD	2810±140†	3110±130*†	3170±160*†	3290±150*†	3470±160*†
$LVdP/dt_{min}$	$\ominus\beta_{1,2}$	−2430±160	−2430±140	−2380±140	−2420±130	−2370±110
(mmHg s^{-1})	$\ominus\beta_{1,2}$+EMD	−2390±160	−2400±160	−2270±120	−2300±120	−2360±110
LVSP	$\ominus\beta_{1,2}$	117±3	115±3	119±2	123±3*	124±2*
(mmHg)	$\ominus\beta_{1,2}$+EMD	123±3†	122±2†	125±3*†	129±3*†	133±3*†
MAP	$\ominus\beta_{1,2}$	100±3	93±3	91±3*	94±2	93±3
(mmHg)	$\ominus\beta_{1,2}$+EMD	102±3	97±4	92±4*	96±4*	97±4
SVR	$\ominus\beta_{1,2}$	29.6±2.2	22.6±1.5*	17.9±1.1*	17.3±1.2*	16.2±1.3*
(mmHg min l^{-1})	$\ominus\beta_{1,2}$+EMD	29.4±2.3	23.4±1.7*	17.4±1.3*	17.3±1.3*	16.3±1.3*
Pulmonary haemodynamics						
MPAP	$\ominus\beta_{1,2}$	15±1	17±1	22±1*	25±1*	29±2*
(mmHg)	$\ominus\beta_{1,2}$+EMD	20±2†	21±2	26±1*†	29±2*†	34±2*†
MLAP[a]	$\ominus\beta_{1,2}$	6±2	3±2	6±2	7±1	10±1*
(mmHg)	$\ominus\beta_{1,2}$+EMD	4±1	3±2	6±1	8±1	12±1*
PVR[a]	$\ominus\beta_{1,2}$	2.9±0.5	3.2±0.4	3.1±0.4	3.0±0.3	3.1±0.4
(mmHg min l^{-1})	$\ominus\beta_{1,2}$+EMD	4.4±0.6†	3.5±0.7	3.8±0.5†	3.7±0.5†	3.4±0.4
Coronary haemodynamics						
CBF	$\ominus\beta_{1,2}$	31±4	38±5*	41±6*	43±6*	45±6*
(ml min^{-1})	$\ominus\beta_{1,2}$+EMD	37±6†	45±7*†	50±8*†	51±8*†	54±8*†
CVR	$\ominus\beta_{1,2}$	3.8±0.6	2.9±0.5*	2.6±0.4*	2.6±0.4*	2.5±0.4*
(mmHg min ml^{-1})	$\ominus\beta_{1,2}$+EMD	3.5±0.7†	2.7±0.5*†	2.3±0.4*†	2.3±0.4*†	2.2±0.4*†
MW	$\ominus\beta_{1,2}$	400±20	480±20*	620±30*	680±40*	740±50*
(mmHg l min^{-1})	$\ominus\beta_{1,2}$+EMD	440±30	520±30*	680±40*†	740±40*†	820±60*†

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; CBF = coronary blood flow, CVR = coronary vascular resistance, MW = myocardial work (LVSP × CO), EMD = EMD 57033 (0.4 mg kg^{-1} min^{-1}, i.v.). Data are mean±s.e.mean; $n=9$ ([a]$n=7$). *$P\leqslant0.05$ vs Rest; †$P\leqslant0.05$ EMD 57033 vs control.

the vena cava. Intracoronary infusion of Ca^{2+} in a dose of 2.2 mg min^{-1} (at a rate 0.75 ml min^{-1}), resulting in computed intracoronary concentrations of 38 (pig 1) and 63 (pig 2) μg per ml of whole blood, increased E_{ES} from 51 to 75 mmHg mm^{-1} (47%) in pig 1 and from 68 to 88 mmHg mm^{-1} (29%) in pig 2. Infusion of EMD 57033 (0.2 mg kg^{-1} min^{-1}, i.v.) increased E_{ES} to 75 and 135 mmHg mm^{-1} in pig 1 and 2, respectively. Intracoronary infusion of Ca^{2+} (2.2 mg min^{-1}), resulting in intracoronary blood concentrations of 29 (pig 1) and 31 (pig 2) μg ml^{-1}, now increased E_{ES} to 150 mmHg mm^{-1} (100%) in pig 1 and to 220 mmHg mm^{-1} (63%) in pig 2. These findings indicate that also *in vivo* the myofilament responsiveness to Ca^{2+} is enhanced by EMD 57033. The mechanism by which EMD 57033 induces these alterations in the interaction between Ca^{2+} and the myofilaments is still incompletely understood. A favoured hypothesis is that EMD 57033 increases the sensitivity of troponin C to Ca^{2+} (Pan & Johnson, 1996), although modulation of the cooperative interaction between adjacent troponin-tropomyosin units along the thin filaments or an effect on the cross-bridge kinetics may also contribute (Solaro *et al*., 1993; Lues *et al*., 1993).

In view of its potential use in patients with LV dysfunction, the effects of EMD 57033 on LV filling pressure deserve comment. In resting pigs with intact β-adrenoceptor activity, EMD 57033 in a dose of 0.8 mg kg^{-1} min^{-1} increased LV end-diastolic pressure by 3±1 mmHg, while in the presence of β-adrenoceptor blockade, LV end-diastolic pressure was increased by 5±2 mmHg. Although this would appear to be an unfavourable effect, it is important to note that the equivalent rate of solvent infusion (2 ml min^{-1}) produced a 4±1 mmHg increase in LV end-diastolic pressure. These findings are best interpreted as a lack of an effect of EMD 57033 per se on LV filling pressure. However, EMD 57033 does not decrease LV filling pressure,which contrasts with the decrease in LV filling pressure observed in pigs during infusion of pimobendan (Duncker *et al*., 1987a) or the pure phosphodiesterase inhibitor amrinone (Hartog *et al*., 1986).

The increase in Ca^{2+} responsiveness produced by EMD 57033 did not result in constriction of either the pulmonary or systemic vascular bed. The decrease in systemic vascular resistance could have resulted in part from withdrawal of α-adrenergic tone to prevent an increase in aortic blood pressure secondary to the EMD 57033-induced increase in cardiac output. This is suggested by the observation that the decrease in resistance was attenuated in the presence of α-adrenergic blockade. It is also possible that systemic vasodilation resulted in part from PDE-III inhibition, although this would seem unlikely as pretreatment with β-adrenoceptor blockade did not alter the degree of systemic vasodilation. However, in vascular smooth muscle β-adrenoceptors are, unlike in the myocardium, not an exclusive source for cAMP production as adenosine (Olsson *et al*., 1991) and prostaglandins (Bassenge, 1995) also increase cAMP production, so that PDE-III inhibition could still have resulted in peripheral vasodilation even when β-adrenoceptors were blocked. Finally, there is some evidence that EMD 57033 can produce endothelium-dependent vasodilation, which may also have contributed to its vasodilator action in the present study (Nankervis *et al*., 1994).

EMD 57033 increased coronary blood flow in excess of the increase in myocardial work. This action of EMD 57033 was

Table 7 Systemic, pulmonary and coronary haemodynamic responses of pimobendan during graded treadmill exercise in swine in the presence of non-selective β-adrenoceptor blockade

		Rest		*Exercise* (km h^{-1})		
	Treatment	*Lying*	*Standing*	*2*	*3*	*4*
Systemic haemodynamics						
CO	$\ominus\beta_{1,2}$	3.6±0.3	4.4±0.4*	5.2±0.4*	5.5±0.4*	6.0±0.3*
(l min^{-1})	$\ominus\beta_{1,2}$+PIM	3.8±0.3	4.9±0.5*	5.7±0.5*†	6.1±0.5*†	6.5±0.4*†
HR	$\ominus\beta_{1,2}$	106±6	123±6*	145±9*	153±9*	168±8*
(beats min^{-1})	$\ominus\beta_{1,2}$+PIM	112±6†	133±7*	161±12*†	169±11*†	188±11*†
SV	$\ominus\beta_{1,2}$	33±2	36±3	36±2*	36±2*	36±2*
(ml)	$\ominus\beta_{1,2}$+PIM	34±2	37±2*	36±2	36±2	35±2
$LVdP/dt_{max}$	$\ominus\beta_{1,2}$	2450±110	2630±110*	2900±110*	3070±130*	3210±130*
(mmHg s^{-1})	$\ominus\beta_{1,2}$+PIM	2480±110	2870±150*	3360±250*†	3600±240*†	3840±180*†
$LVdP/dt_{min}$	$\ominus\beta_{1,2}$	−2460±120	−2320±150	−2370±120	−2350±130*	−2450±120
(mmHg s^{-1})	$\ominus\beta_{1,2}$+PIM	−2370±90	−2290±80*	−2520±150	−2630±170	−2710±150*
LVSP	$\ominus\beta_{1,2}$	119±4	115±4	119±3	122±4	127±5
(mmHg)	$\ominus\beta_{1,2}$+PIM	115±4	112±4	113±4†	116±4	122±5*
MAP	$\ominus\beta_{1,2}$	103±3	94±5*	95±3*	96±3*	97±4
(mmHg)	$\ominus\beta_{1,2}$+PIM	95±3†	86±3*	85±4*†	87±3*†	89±3*†
SVR	$\ominus\beta_{1,2}$	30.5±3.1	23.8±3.9*	19.1±1.9*	18.1±1.8*	16.9±1.6*
(mmHg min l^{-1})	$\ominus\beta_{1,2}$+PIM	26.7±3.0†	19.2±2.9*†	15.8±1.8*†	15.0±1.5*†	14.2±1.4*†
Pulmonary haemodynamics						
MPAP	$\ominus\beta_{1,2}$	18±1	19±2	28±2*	29±2*	33±2*
(mmHg)	$\ominus\beta_{1,2}$+PIM	18±1	21±2	26±2*	28±2*	33±2*
MLAP[a]	$\ominus\beta_{1,2}$	7±1	4±2	9±1	8±1	11±1*
(mmHg)	$\ominus\beta_{1,2}$+PIM	6±1	5±1	5±2	6±2	10±2
PVR[a]	$\ominus\beta_{1,2}$	3.3±0.3	3.2±0.4	3.8±0.4	3.7±0.4	3.7±0.4
(mmHg min l^{-1})	$\ominus\beta_{1,2}$+PIM	3.2±0.2	3.5±0.3	3.6±0.3	3.6±0.3	3.6±0.4
Coronary haemodynamics						
CBF	$\ominus\beta_{1,2}$	33±4	38±6*	44±7*	44±7*	46±8*
(ml min^{-1})	$\ominus\beta_{1,2}$+PIM	35±5	42±8	48±9*	50±9*	53±9*†
CVR	$\ominus\beta_{1,2}$	3.6±0.5	3.0±0.6*	2.5±0.4*	2.6±0.4*	2.6±0.5*
(mmHg min ml^{-1})	$\ominus\beta_{1,2}$+PIM	3.3±0.6	2.5±0.4*	2.2±0.4*†	2.2±0.4*†	2.1±0.4*†
MW	$\ominus\beta_{1,2}$	420±30	500±40*	620±50*	670±40*	750±30*
(mmHg l min^{-1})	$\ominus\beta_{1,2}$+PIM	440±40	550±60*	650±70*	710±70*	800±60*

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; CBF = coronary blood flow, CVR = coronary vascular resistance, MW = myocardial work (LVSP × CO); PIM = Pimobendan (20 μg kg^{-1} min^{-1}, i.v.). Data are mean±s.e.mean; $n=8$ ([a]$n=6$). *$P\leqslant0.05$ vs Rest; †$P\leqslant0.05$ pimobendan vs control.

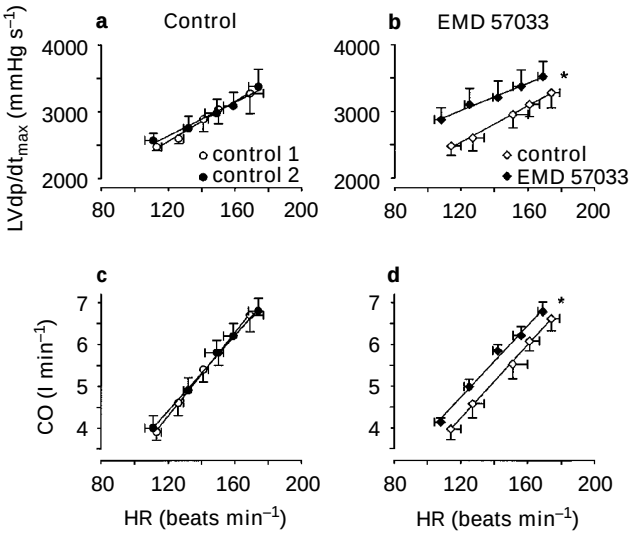

Figure 6 Reproducibility of $LVdP/dt_{max}$ and cardiac output responses to two consecutive control exercise periods (a, c) and the responses to EMD 57033 (0.4 mg kg^{-1} min^{-1}, b, d) in swine during graded treadmill exercise in the presence of combined α- and β-adrenoceptor blockade. $LVdP/dt_{max}$ and cardiac output (CO) data have been plotted as a function of heart rate (HR). Data are mean±s.e.mean, $n=7$. *$P\leqslant0.05$ vs corresponding control conditions (first exercise period).

not affected by pretreatment with either β-adrenoceptor blockade or combined α- and β-adrenoceptor blockade, suggesting that EMD 57033 produced at least in part direct coronary vasodilation possibly via release of endothelium derived substances (Nankervis *et al*., 1994). In contrast, the coronary vasodilation produced by pimobendan under resting conditions was abolished after pretreatment with propranolol suggesting that the vasodilation resulted principally from an increase in myocardial work but possibly also from PDE-III inhibition.

Haemodynamic actions of EMD 57033 during treadmill exercise

An interesting finding in the present study was that the cardiostimulatory actions of EMD 57033 gradually waned when the work load was increased. Thus, in the presence of EMD 57033 exercise resulted in $LVdP/dt_{max}$ levels that were not different compared to control exercise. Similar findings have been reported for levosimendan in healthy humans (Sundberg *et al*., 1995). It is possible that exercise-induced increments in β-adrenergic activity, via cAMP-mediated phosphorylation of troponin-I, neutralizes the levosimendan or EMD 57033-mediated increases in Ca^{2+} responsiveness of the myofilaments (Herzig *et al*., 1981). In the present study, EMD 57033 produced an increase in contractility at all levels of exercise in the presence of β-adrenoceptor blockade, which is consonant with the hypothesis that during exercise

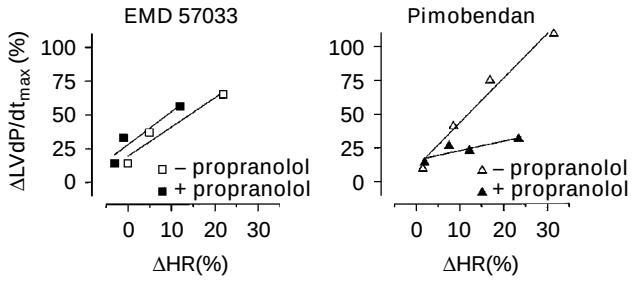

Figure 7 Positive inotropic actions of EMD 57033 (0.2, 0.4 and 0.8 mg kg^{-1} min^{-1}, $n=10$) and pimobendan (10, 25, 50 and 100 μg kg^{-1} min^{-1}, $n=6$) in the absence (−propranolol) and in the presence of β-adrenoceptor blockade (+propranolol) in awake resting swine. Data have been plotted as a function of the increase in heart rate (HR). Pimobendan data have been reported previously (Duncker *et al*., 1987a).

in the normal heart a pharmacologically-induced increase in myofilament sensitivity to Ca^{2+} can be offset by very high levels of β-adrenergic activity. In contrast, the positive inotropic actions of pimobendan were enhanced at higher levels of exercise, but were markedly blunted during exercise in the presence of β-adrenoceptor blockade, findings that are consistent with a predominantly PDE-III inhibitory action of pimobendan.

An alternative explanation could be that EMD 57033 increased Ca^{2+} responsiveness via an α-adrenergic receptor dependent mechanism (Endoh & Blinks, 1988; Terzic *et al*., 1992), so that with increasing exercise levels the progressively greater α-adrenergic receptor stimulation resulted in near maximal increments of myofilament Ca^{2+} responsiveness. As a result EMD 57033 could then have been unable to produce a further increase in Ca^{2+} responsiveness via this mechanism. In the present study, EMD 57033 produced an increase in contractility at all levels of exercise in the presence of β-adrenoceptor blockade, while the addition of α-adrenoceptor blockade to the β-adrenoceptor blockade did not further alter this response. Thus, the positive inotropic responses to EMD 57033 were not mediated via α-adrenoceptor stimulation and did not depend on intact α-adrenoceptor activity.

The reduced effectiveness of EMD 57033 during higher levels of physical activity could limit the drug's usefulness in humans where abnormalities in cardiac pump function often become apparent only during exercise. It is important to note, however, that in patients with heart failure loss of β-adrenergic receptor responsiveness and density is correlated with the loss of LV pump function. The results from the present study suggest that those patients with the greatest impairment in function could benefit the most from an increase in Ca^{2+} responsiveness.

During exercise, EMD 57033 produced coronary vasodilation which resulted in an upward shift of the relation between myocardial work and coronary blood flow. This action was again not affected by pretreatment with either β-adrenoceptor blockade or combined α- and β-adrenoceptor blockade, suggesting that EMD 57033 produced at least in part direct coronary vasodilation. In contrast, the coronary vasodilation produced by pimobendan during exercise was attenuated after pretreatment with propranolol suggesting that it resulted from PDE-III inhibition. Importantly, the present study suggests that even when diastolic duration is reduced, such as during exercise-induced tachycardia, the positive inotropic actions of either EMD 57033 or pimobendan do not appear to compromise myocardial perfusion.

Conclusions

EMD 57033 results in dose-dependent increases of left ventricular contractility in awake pigs. This positive inotropic action is partially offset during exercise by the increased β-adrenergic activity, but occurs independently of α-adrenergic activity. These finding suggest that EMD 57033 will be most effective in patients with severe loss of β-adrenergic responsiveness.

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