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Cardiovascular profile of the calcium sensitizer EMD 57033 in open-chest anaesthetized pigs with regionally stunned myocardium

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1 Ca^{2+} sensitizers enhance systolic function, but may impair relaxation *in vitro*; these effects may differ in stunned and normal myocardium. We therefore studied the effect of EMD 57033 on systolic and diastolic function of normal and stunned porcine myocardium *in vivo*.

2 Myocardial stunning by 15 min coronary occlusion and 30 min reperfusion abolished systolic shortening (SS) (baseline $13\pm1\%$) and decreased end-systolic elastance (E_{es}) from 67 ± 7 to 47 ± 5 mmHg mm^{-1} (both $P<0.05$). Maximum rate of fall of myocardial elastance (dE/dt_{min}) decreased from -850 ± 100 to -320 ± 30 mmHg mm^{-1} s^{-1}, while the time constant τ_e of the decay of elastance increased from 58 ± 3 to 68 ± 6 ms (both $P<0.05$). End-diastolic elastance (E_{ed}) was unchanged although the zero pressure intercept ($L_{0,ed}$) had increased.

3 In the stunned region, EMD 57033 (0.2 mg kg^{-1} min^{-1} for 60 min, i.v., $n=7$) increased SS to $19\pm2\%$, E_{es} to 287 ± 40 mmHg mm^{-1}, dE/dt_{min} to -3630 ± 640 mmHg mm^{-1} s^{-1} and decreased τ_e to 50 ± 3 ms, while E_{ed} remained unchanged. In the normal region, EMD 57033 increased SS from 14 ± 2 to $18\pm3\%$, E_{es} from 59 ± 4 to 263 ± 23 mmHg mm^{-1}, dE/dt_{min} from -480 ± 70 to -2280 ± 700 mmHg mm^{-1} s^{-1} and decreased τ_e from 91 ± 12 to 61 ± 3 ms (all $P<0.05$), while E_{ed} remained unchanged. These responses were minimally affected by adrenoceptor blockade ($n=7$). Vehicle ($n=7$) had no effect on either region.

4 EMD 57033 increased cardiac output (up to $27\pm8\%$) and $LVdP/dt_{max}$ ($86\pm19\%$). Mean aortic pressure decreased ($19\pm7\%$) due to systemic vasodilation that was not amenable to blockade of adrenoceptors or NO synthesis.

5 In conclusion, EMD 57033 restored systolic and diastolic function of stunned myocardium, and produced similar improvements in systolic and diastolic function in normal myocardium.
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Abbreviations: dE/dt_{min}, maximum rate of fall of regional myocardial elastance; EDL, end-diastolic length; E_{ed}, end-diastolic elastance; E_{es}, end-systolic elastance; ESL, end-systolic length; EW_{beat}, external work per beat; LADCA, left anterior descending coronary artery; LCXCA, left circumflex coronary artery; $L_{0,ed}$, zero pressure length intercept of the left ventricular end-diastolic pressure-segment length (LVEDP-SL) relation; $L_{0,es}$, zero pressure length intercept of the left ventricular end-systolic pressure-segment length (LVESP-SL) relation; LV, left ventricular; $LVdP/dt_{max}$, maximum rate of rise of LV pressure; $LVdP/dt_{min}$, maximum rate of fall of LV pressure; $MVO_{2\ beat}$, myocardial oxygen consumption per beat; NO, nitric oxide; PSS, post-systolic shortening; SS, systolic shortening; τ_e, time constant of regional myocardial elastance decay; τ_{LVP}, time constant of LV pressure decay

Introduction

Stunned myocardium is characterized by both a depressed systolic and diastolic function (Charlat *et al*., 1989; Ehring *et al*., 1993; Bolli & Marban, 1999). Since a decreased responsiveness of the myofilaments to calcium (Ca^{2+}) has been implicated in the mechanism underlying stunning, several groups of investigators have successfully employed 'Ca^{2+}-sensitizing agents' to restore systolic function of stunned myocardium in a variety of *in vivo* (Soei *et al*., 1994; Abe *et al*., 1995) and *in vitro* (Korbmacher *et al*., 1994; 1995; 1997) models. It has been suggested that the inotropic response to Ca^{2+}-sensitizers may differ between stunned and normal myocardium. For instance, we have shown that the EMD 60263-induced increase in regional systolic shortening in stunned myocardium of anaesthetized pigs was much more pronounced than that in normal myocardium (Soei *et al*., 1994). However, systolic shortening is strongly load-dependent, even more so in stunned myocardium, and does not necessarily reflect myocardial contractility (Fan *et al*., 1995). That also the lusitropic response to Ca^{2+}-sensitizers in stunned and normal myocardium may differ was suggested by Korbmacher *et al*. (1997), who showed that in response to high doses of EMD 60263 global diastolic function deteriorated more in normal than in globally stunned isolated rabbit hearts. However, data on diastolic functional responses of stunned myocardium to Ca^{2+}-sensitizing agents *in vivo* are currently lacking.

In view of these considerations, we studied the effects of EMD 57033 on regional systolic and diastolic function in an *in vivo* porcine model of stunned and normal myocardium. EMD 57033 was chosen because it is a thiadiazinone derivative, that lacks the inhibitory action on the delayed rectifier inward current that is exhibited by EMD 60263 and which may potentially modify systolic and diastolic responses by prolongation of the action potential duration (Ravens *et al*.,

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1996). However, EMD 57033 possesses phosphodiesterase III inhibiting properties (which EMD 60263 lacks) that could act to enhance systolic and diastolic function (Ravens *et al*., 1996). Therefore, we also studied EMD 57033 in the presence of α- and β-adrenergic receptor blockade. To circumvent the problem of load-dependency of systolic shortening we employed, in analogy to the time-varying elastance concept, regional LV end-systolic pressure-segment length relations to obtain a more load-independent measure of regional myocardial contractility (Aversano *et al*., 1986). Also based on the time-varying elastance concept we applied regional LV end-diastolic pressure-segment length relations to describe diastolic function. Since the latter is a measure of late diastolic function, we also determined the maximum rate of fall (dE/dt_{min}) and the time constant (τ_e) of the decay of myocardial elastance to describe early diastolic function.

Methods

Animal care

All experiments were performed in accordance with the 'Guiding Principles in the Care and Use of Laboratory Animals, as approved by the American Physiological Society and with prior approval of the Animal Care Committee of the Erasmus University Rotterdam.

Animal preparation

Cross-bred Landrace × Yorkshire pigs of either sex (28–36 kg) were sedated with ketamine i.m. (20–30 mg kg^{-1}, Apharmo, Huizen, The Netherlands) and anaesthetized with sodium pentobarbital i.v. (20 mg kg^{-1}, Sanofi, Paris, France), before they were intubated and connected to a respirator for intermittent positive pressure ventilation with a mixture (1 : 2 vol%) of oxygen and nitrogen. Arterial blood gas values were kept within the normal range (pH: 7.35–7.45; pCO_2: 35–45 mmHg; pO_2: 100–150 mmHg) by adjusting respiratory rate and tidal volume. Fluid-filled catheters were placed in the superior caval vein for administration of sodium pentobarbital (5–10 mg kg^{-1} h^{-1}) to keep a constant depth of anaesthesia and for administration of Haemaccel (Behringwerke A.G., Marburg, Germany) to maintain fluid balance. A fluid-filled catheter was positioned in the descending aorta to monitor arterial blood pressure, while a micromanometer-tipped catheter (B. Braun Medical B.V., Uden, The Netherlands) was advanced into the left ventricle to measure LV blood pressure and its first derivative (LVdP/dt). In order to construct the LV pressure-segment length relations a balloon catheter was positioned in the inferior caval vein for varying LV preload.

After administration of pancuronium bromide (4 mg, Organon Teknika, Oss, The Netherlands) a midsternal thoracotomy was performed and the heart suspended in a pericardial cradle. Then, an electromagnetic flow probe (Skalar, Delft, The Netherlands) was placed around the ascending aorta to measure ascending aortic blood flow (cardiac output). A proximal segment of the left anterior descending coronary artery (LADCA) was dissected free for placement of a Doppler flow probe (Crystal Biotech, Northboro, MA, U.S.A.) to measure coronary blood velocity and for placement of an atraumatic clamp for occlusion of the artery. The vein accompanying the LADCA was cannulated for collection of blood samples for determination of coronary venous O_2 content.

Regional myocardial function was measured using sonomicrometry (Triton Technology Inc., San Diego, CA, U.S.A.) by placing one pair of ultrasonic crystals in the distribution area of the LADCA and one pair in the distribution area of the left circumflex coronary artery (LCXCA). Each pair was implanted in the midmyocardial layer approximately 10 mm apart and parallel to the fibre direction.

Experimental protocols

After a 30–45 min stabilization period, baseline data of systemic haemodynamics and regional myocardial function were recorded, while arterial and coronary venous blood samples were collected. Preload was transiently reduced (<12 s, Aversano *et al*., 1986) by inflation of the balloon in the inferior caval vein for determination of the LV pressure-segment length relations. Subsequently, the LADCA was occluded for 15 min, and after 15 min of reperfusion the 21 animals were randomly allocated to one of three groups. The first two groups received either intravenous 0.5 ml min^{-1} propylene glycol ($n=7$) or 0.2 mg kg^{-1} min^{-1} EMD 57033 ($n=7$) during 60 min, starting at 30 min of reperfusion. In five animals of the latter group, blood samples were collected at 15 min intervals and the plasma stored at −25°C until determination of plasma levels of EMD 57033. Tissue samples were collected from various organs in two animals at the end of the experiment for determination of EMD 57033 levels. The third group ($n=7$) received EMD 57033 after adrenoceptor blockade to eliminate the putative dependency on adrenergic activity of the EMD 57033-induced changes. For this purpose, the α- and β-adrenoceptors were blocked after 15 min of reperfusion, i.e. 15 min before administration of EMD 57033, by intravenous infusion of 1 mg kg^{-1} phentolamine and 0.5 mg kg^{-1} propranolol (followed by 0.5 mg kg^{-1} h^{-1}), respectively. The adequacy of the doses of phentolamine and propranolol has been demonstrated previously (Verdouw *et al*., 1984; Duncker *et al*., 1987). Since we observed that the systemic vasodilation produced by EMD 57033 was unmitigated in the presence of combined α- and β-adrenoceptor blockade, we added a fourth group of pigs ($n=4$) in which EMD 57033 (0.2 mg kg^{-1} min^{-1} i.v.) was infused after 30 min of reperfusion in the presence of α- and β-adrenoceptor blockade and blockade of NO synthesis (Nankervis *et al*., 1994). NO synthesis was inhibited with N^{ω}-nitro-L-arginine (20 mg kg^{-1} i.v.) (Duncker *et al*., 1997).

In the first three groups of animals, regional myocardial blood flows were determined by intra-atrial injection of 1–2×10^6 radioactive microspheres [15 ± 1 μm (s.d.) in diameter] labelled with either ^{46}Sc, ^{95}Nb, ^{103}Ru, ^{113}Sn or ^{141}Ce (NEN Company, Dreieich, Germany), using the arterial reference sampling technique (Duncker *et al*., 1986). At the end of each experiment, the LADCA was ligated at the site of occlusion and the area perfused by the LADCA was identified by intracoronary injection of patent blue violet (Sigma Chemical Co., St. Louis, U.S.A.). Immediately thereafter, the animals were killed with an overdose of pentobarbital and the heart excised and handled as described earlier in order to obtain regional myocardial blood flow data in the LADCA and non-LADCA regions (Duncker *et al*., 1986; Sassen *et al*., 1990).

Data acquisition and analysis

Systolic and post-systolic shortening All segment length data were normalized to an end-diastolic length (EDL) of 10 mm at baseline to correct for variability in the implantation distance between the crystals. Systolic shortening (SS) was computed as

100% • (EDL-ESL)/EDL, in which EDL and ESL (end-systolic length) are the segment length at the onset of the rapid increase in LV pressure ($LVdP/dt = 250$ mmHg s^{-1}) and at the end of LV ejection, respectively. Post-systolic segment shortening (PSS) was calculated as 100% • ($ESL-L_{min}$)/EDL, in which L_{min} is the minimum segment length after closure of the aortic valves.

Regional myocardial elastance using the LV pressure-segment length relations Regional myocardial end-systolic and end-diastolic elastance were determined from the LV pressure-segment length relations which were obtained by varying preload (Figure 1A). Using linear regression analysis end-systolic elastance (E_{es}) and the segment length at zero pressure intercept ($L_{0,es}$) were obtained applying the iterative method described by Van der Velde *et al.* (1991), while end-diastolic elastance (E_{ed}) and the segment length at zero pressure intercept ($L_{0,ed}$) were determined using the time point at which LVdP/dt had increased to 250 mmHg s^{-1}.

To describe early diastolic function in the LADCA and LCXCA regions we determined the time course of instantaneous regional elastance using the corresponding $L_{0,es}$ of the LADCA and LCXCA regions, obtained during the preload reduction (Figure 1B). From the time course of elastance (in analogy to the global LV indices of relaxation) we determined the time constant (τ_e) of the elastance decay between the occurrence of the maximum rate of fall of regional myocardial elastance (dE/dt_{min}) and the time point at which regional elastance had decreased to 20% of the peak systolic elastance at baseline. For this purpose the decay in elastance was fitted to $E(t) = E_0\, e^{-t/\tau_e}$ where E(t) is the instantaneous elastance at time point t, and E_0 is the elastance at dE/dt_{min}. Finally, to study alterations in the onset of relaxation, we measured the duration of regional myocardial systole in the LADCA ($T_{systole,\ LADCA}$) and LCXCA ($T_{systole,\ LCXCA}$) perfused regions, which was defined as the time interval between the onset of the rapid increase in regional elastance and the occurrence of dE/dt_{min}, in analogy to the determination of the duration of global LV systole.

To complement these regional myocardial measurements we also determined the time constant of global LV pressure decay (τ_{LVP}) during the time-interval between the occurrence of $LVdP/dt_{min}$ and the timepoint when LVP had reached 5 mmHg above LV end-diastolic pressure (Harkin *et al.*, 1995). The duration of global LV systole ($T_{systole,\ LV}$) was defined as the time interval between the onset of the rapid increase in LV pressure ($LVdP/dt = 250$ mmHg s^{-1}) and end-ejection.

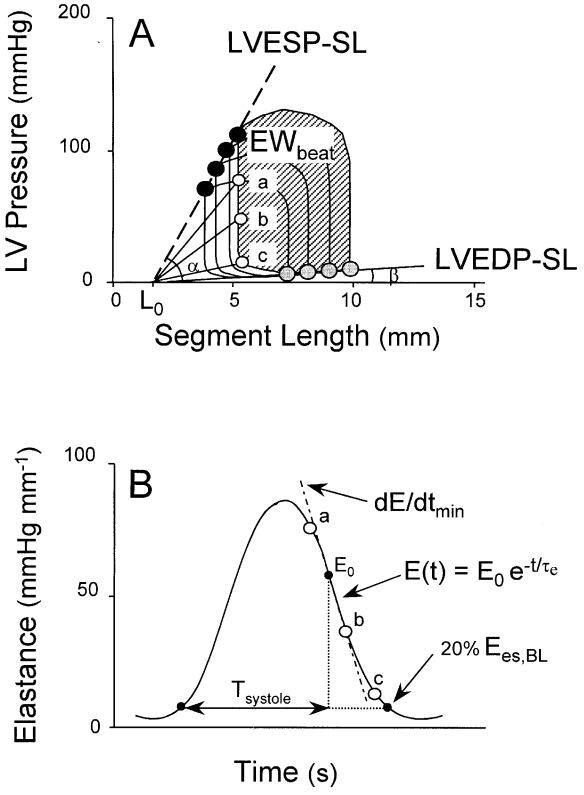

Figure 1 Left ventricular (LV) pressure-segment length relations during transient decrease of preload (A). External work (EW_{beat}) was determined by integrating the area enclosed by the pressure-segment length loop over a full cardiac cycle. Regional end-systolic and end-diastolic elastance were defined as the slopes ($\tan \alpha$ and $\tan \beta$) of the LV end-systolic pressure-segment length (LVESP-SL) and LV end-diastolic pressure-segment length (LVEDP-SL) relations, respectively. From the time-elastance curve the duration of systole ($T_{systole}$) and the maximum rate of fall of elastance (dE/dt_{min}) were determined (B). The time constant (τ_e) of the elastance decay between the occurrence of the maximum rate of fall of regional myocardial elastance (dE/dt_{min}) and the time point at which regional elastance had decreased to 20% of the peak systolic elastance at baseline was determined by fitting the decay in elastance to $E(t) = E_0\, e^{-t/\tau_e}$ where E is elastance, t is time, and E_0 is the elastance at dE/dt_{min}.

Myocardial oxygen consumption, external work and mechanical efficiency Myocardial oxygen consumption (MVO_2) of the perfusion territory of the LADCA was calculated as the product of local transmural myocardial blood flow and the difference in the oxygen content of the arterial and local coronary venous blood. The area inside the LV pressure-segment length loop (Figure 1) was taken as an index of external work per beat (EW_{beat}) (Morris *et al.*, 1987; Vinten-Johansen *et al.*, 1991; Krams *et al.*, 1993), while mechanical efficiency was defined as the ratio of EW_{beat} and $MVO_{2\ beat}$. Because EW reflects mechanical work but does not have the dimensions of work, the changes in mechanical efficiency have been expressed as percentage of baseline.

Determination of plasma and tissue concentrations of EMD 57033

To 600 μl of plasma or homogenized tissue 500 μl of water saturated ethylether was added and mixed. After the organic and aqueous phases were separated in an Eppendorff table centrifuge, the organic top layer was removed and collected in an Eppendorff vial. This extraction procedure was repeated five times and the ether phases were collected separately. Thereafter the ether was evaporated in a speed vac centrifuge and the residuals were resuspended and dissolved in 300 μl acetonitril. The amount of EMD 57033 in a given plasma or tissue sample was determined on a HPLC system. To this end 30 μl of the acetonitril solutions was injected on a LiChrosorb RP 8 (5 μm) RT 125-4 column (Merck KGaA), which was equipped with a Hibar LiChroCart 4-4 precolumn (Merck KGaA). The column was equilibrated and developed in a buffer composed of 35% acetonitril and 65% 0.1 M sodium phosphate, pH 6.0 at a flow rate of 1 ml/min. The elution was monitored at a wavelength of 320 nm. The concentration of EMD 57033 was determined from the area of the peaks eluting at the appropriate time from the column by comparison with the values determined for identically treated standard samples. The plasma concentration of a given blood or tissue sample was determined by adding the peak areas of the ether extraction samples.

Statistical analysis

All data have been presented as mean ± s.e.mean. Statistical significance ($P<0.05$, two-tailed) of the changes within each group was tested using one-way analysis of variance for repeated measures followed by Dunnet's test. Comparison between the changes produced by the different interventions was assessed by two-way analysis of variance for repeated measures.

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; Merck KGaA, Darmstadt, Germany) was dissolved in propylene glycol so that an infusion rate of 0.5 ml min^{-1} corresponded to 0.2 mg kg^{-1} min^{-1} EMD 57033. Propranolol hydrochloride (ICI-Pharma, Rotterdam, The Netherlands) and phentolamine-methanosulfonide (CIBA-Geigy, Basel, Switzerland) were dissolved in saline. N^{ω}-nitro-L-arginine (Sigma Chemical Co., St Louis, U.S.A.) was dissolved in deionized water. Fresh solutions were prepared on the day of each experiment.

Results

Systemic haemodynamics

During the 15 min LADCA occlusion, mean arterial pressure had decreased from 100 ± 2 mmHg at baseline to 92 ± 2 mmHg ($n=21$, $P<0.05$; Table 1). Since the decrease in mean arterial pressure was accompanied by a similar fall in cardiac output, it follows that systemic vascular resistance had remained unchanged. $LVdP/dt_{max}$ decreased by 14 ± 3%, but $T_{systole}$ was not affected. However, $LVdP/dt_{min}$ decreased from −2000 ± 70 to −1590 ± 70 mmHg s^{-1} ($P<0.05$), while τ_{LVP} and LV end-diastolic pressure increased (Table 1). After 15 min of reperfusion there was no recovery in any of the haemodynamic variables, except for LV end-diastolic pressure which had returned to baseline values. In the following 15 min the changes were negligible in the 14 animals which were left untreated, but in the other seven animals adrenergic blockade caused marked decreases in mean arterial pressure, heart rate, cardiac output, $LVdP/dt_{max}$ and $LVdP/dt_{min}$ (to −1340 ± 140 mmHg s^{-1}, $P<0.05$), and caused a further increase in τ_{LVP}, while $T_{systole}$ was maintained.

Infusion of the vehicle had minimal effects on systemic haemodynamics and global LV relaxation parameters (Table

Table 1 Effect of EMD on systemic haemodynamics in anaesthetized pigs with stunned myocardium

	Baseline ($n=21$)	*15 min occlusion* ($n=21$)	*15 min reperfusion* ($n=21$)	*Adrenergic Blockade*	*30 min reperfusion*	*Infusion*	*Δabs by Infusion 30 min*	*60 min*
MAP, mmHg	100 ± 2	92 ± 2*	91 ± 3*	−	94 ± 4	PG	5 ± 3	1 ± 3
						EMD	2 ± 6	−18 ± 7‡¶
				+	68 ± 6†	EMD	11 ± 3‡	−3 ± 2
CO, 1 min^{-1}	2.7 ± 0.1	2.4 ± 0.1*	2.5 ± 0.1*	−	2.5 ± 0.1	PG	0.2 ± 0.1‡	0.2 ± 0.1‡
						EMD	0.6 ± 0.1‡¶	0.3 ± 0.2‡
				+	2.0 ± 0.1†	EMD	0.9 ± 0.1‡¶	0.9 ± 0.2‡¶§
SVR, mmHg min l^{-1}	37 ± 1	39 ± 1	37 ± 2	−	39 ± 1	PG	−1.1 ± 1.5	−2.8 ± 1.2
						EMD	−7.5 ± 3.0‡	−11.6 ± 3.0‡¶
				+	33 ± 1	EMD	−5.9 ± 0.8‡¶	−11.3 ± 0.9‡¶
HR, b.p.m	117 ± 4	121 ± 4	124 ± 5	−	119 ± 6	PG	−7 ± 2	−8 ± 4
						EMD	6 ± 4¶	16 ± 8¶
				+	89 ± 7†	EMD	11 ± 2‡¶	19 ± 4‡¶
LVSP, mmHg	114 ± 2	105 ± 2*	104 ± 3*	−	108 ± 4	PG	7 ± 3	3 ± 3
						EMD	5 ± 7	−14 ± 6‡¶
				+	84 ± 5	EMD	16 ± 3‡¶	5 ± 3§
$LVdP/dt_{max}$, mmHg s^{-1}	1850 ± 90	1620 ± 80*	1470 ± 70*	−	1630 ± 100	PG	−10 ± 100	−130 ± 90
						EMD	760 ± 140‡¶	1230 ± 200‡¶
				+	1060 ± 80†	EMD	880 ± 110‡¶	1380 ± 210‡¶
$T_{systole}$, ms	306 ± 7	308 ± 8	305 ± 9	−	316 ± 11	PG	20 ± 3‡	19 ± 5‡
						EMD	−16 ± 7‡¶	−27 ± 14‡¶
				+	340 ± 9†	EMD	−26 ± 5‡¶	−35 ± 9‡¶
τ_{LVP}, ms	49 ± 2	52 ± 2*	50 ± 2*	−	53 ± 3	PG	2.3 ± 0.7	2.3 ± 1.4
						EMD	4.7 ± 1.7	11.0 ± 2.6‡¶
				+	57 ± 4†	EMD	2.0 ± 5.4	−6.3 ± 5.1§
LVEDP, mmHg	6.8 ± 0.6	10.1 ± 0.7*	7.6 ± 0.7*	−	7.2 ± 0.8	PG	0.7 ± 0.3	0.3 ± 0.3
						EMD	−2.0 ± 0.4‡¶	−1.9 ± 0.7‡¶
				+	9.0 ± 1.2	EMD	−2.5 ± 0.6‡¶	−3.9 ± 0.7‡¶

MAP, mean arterial pressure; CO, cardiac output; SVR, systemic vascular resistance; HR, heart rate; left ventricular systolic pressure; $LVdP/dt_{max}$, maximal rate of rise in left ventricular pressure; LVEDP, left ventricular end-diastolic pressure; PG, propylene glycol ($n=7$); EMD, EMD 57033 (0.2 mg kg^{-1}, $n=7$ in each group);-, no α and β blockade; +, α and β blockade; Values are mean ± s.e.mean; *$P<0.05$ vs baseline (only for 15 min occlusion and 15 min reperfusion); †$P<0.05$ vs corresponding 15 min rep (only for 30 min reperfusion); ‡$P<0.05$ vs 30 min reperfusion; ¶$P<0.05$ vs change in vehicle group; §$P<0.05$ vs EMD 57033-induced change in animals without adrenoceptor blockade.

1). In contrast, infusion of EMD 57033 caused a marked decrease in mean arterial pressure due to a decrease in systemic vascular resistance. LV systolic pressure did not change during the first 30 min (113±8 mmHg), but fell to 94±7 mmHg during the following 30 min. There was a gradual increase in $LVdP/dt_{max}$ up to 186±19% of its stunning value (thereby exceeding the baseline value), which reflected an increase in global contractility as preload (LV end-diastolic pressure) and afterload (LV systolic pressure) both decreased. $LVdP/dt_{min}$ and τ_{LVP} remained unchanged initially, but after 60 min $LVdP/dt_{min}$ became less negative by 430±190 mmHg s^{-1} and τ_{LVP} had increased by 19±4% ($P<0.05$ vs vehicle) at a time when heart rate had increased and LV systolic pressure had decreased slightly. The increase in heart rate was also likely to be responsible for the decrease in $T_{systole}$. The effects of EMD 57033 did not depend on the activity of the adrenergic system, as the increases in heart rate and $LVdP/dt_{max}$ and the decrease in systemic vascular resistance were not affected when EMD 57033 was administered in the presence of adrenoceptor blockade. $LVdP/dt_{min}$, however, became more negative initially by −430±110 mmHg s^{-1} ($P<0.05$) but had returned to stunning values at 60 min of infusion, possibly because LV systolic pressure was maintained during the infusion. Finally, τ_{LVP} remained unchanged.

Additional blockade of NO synthesis by N^{ω}-nitro-L-arginine, resulted in an elevated systemic vascular resistance (52±8 mmHg min l^{-1}) compared to adrenoceptor blockade alone (33±1 mmHg min l^{-1}, $P<0.05$). However, NO was not involved in the vasodilating actions of EMD 57033 as the decrease in systemic vascular resistance (−14±5 mmHg min l^{-1}) was unmitigated at 60 min of infusion of EMD 57033. Similarly, additional blockade of NO also did not modify the responses of the other haemodynamic variables to EMD 57033 (not shown).

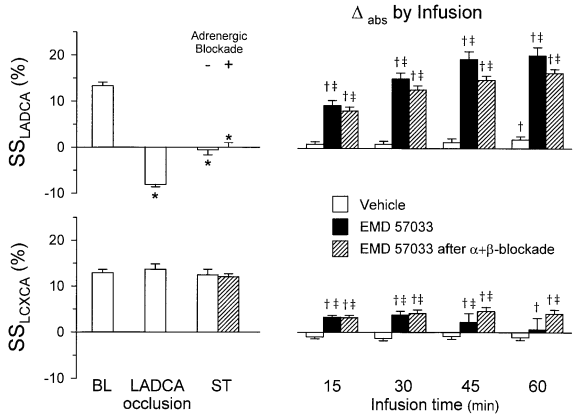

Figure 2 Effect of EMD 57033 (0.2 mg kg^{-1} min^{-1}, i.v.) on regional systolic shortening (SS) in the area perfused by the LADCA and the area perfused by the LCXCA. Absolute values are shown at baseline (BL, $n=21$), during ischaemia (after 15 min LADCA occlusion, $n=21$), and during stunning (ST) in the absence ($n=14$) and in the presence ($n=7$) of $\alpha+\beta$-adrenoceptor blockade. The effects of the infusions of vehicle ($n=7$) and EMD 57033 ($n=7$ in both groups) have been presented as absolute changes (Δ_{abs}) from their respective stunning values. Data are mean±s.e.mean; *$P<0.05$ stunning vs baseline, †$P<0.05$ vs stunning, ‡$P<0.05$ vs change in vehicle group, ¶$P<0.05$ vs EMD 57033-induced change in animals without adrenoceptor blockade.

Regional systolic function

Systolic shortening After production of stunning, there was a complete loss of SS, and the appearance of a pronounced PSS (9.0±0.5% vs 1.5±0.3% at baseline) in the distribution area of the LADCA, while SS of the normal myocardium remained unchanged (Figure 2). Infusion of vehicle had a negligible effect on SS and PSS of both normal and stunned myocardium. During infusion of EMD 57033, SS in the stunned myocardium increased up to 19±2%, while PSS disappeared. In the normal myocardium, SS increased from 14±2% to 18±3% ($P<0.05$). Adrenoceptor blockade also did not modify the responses to EMD 57033 of regional wall motion in either stunned or normal myocardium.

End-systolic elastance Stunning produced a rightward shift of the LV end-systolic pressure-segment length relation in the LADCA area with an 18±7% decrease in E_{es} ($P<0.05$, $n=14$; Figure 3; Table 2). Infusion of vehicle had no effect on this relation, but EMD 57033 produced a leftward shift with a progressive increase in E_{es} to four times above its stunning value (both $P<0.05$). Adrenoceptor blockade blunted the EMD 57033-induced increase in E_{es}.

In the LCXCA area, stunning and the subsequent infusion of vehicle had no effect on the LV end-systolic pressure-segment length relation. Infusion of EMD 57033 caused a tripling of E_{es} without a change in $L_{0,es}$. Adrenoceptor blockade had no effect on the responses of E_{es} and $L_{0,es}$ to EMD 57033.

Regional diastolic function

Onset of relaxation Stunning did not alter the onset of relaxation as the duration of regional LV systole in either LADCA ($T_{systole,\ LADCA}$) or LCXCA ($T_{systole,\ LCXCA}$) perfused myocardium remained unchanged (Figure 4). The small decrease in heart rate that occurred during infusion of the vehicle was associated with small increases in $T_{systole,\ LADCA}$ and $T_{systole,\ LCXCA}$. Conversely, the EMD 57033-induced increase in heart rate resulted in reductions of $T_{systole,\ LADCA}$ and $T_{systole,\ LCXCA}$, that reached levels of statistical significance in the presence of adrenoceptor blockade.

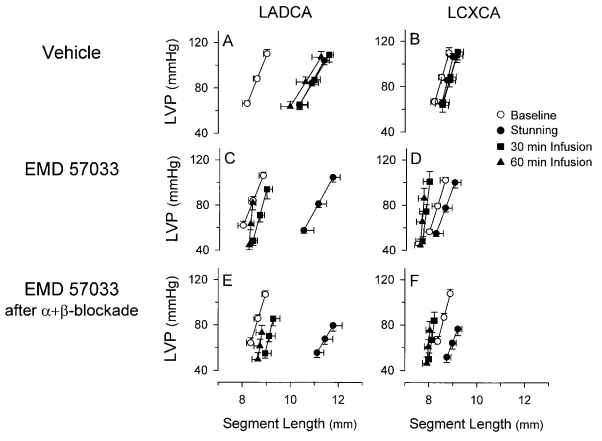

Figure 3 LV end-systolic pressure-segment length relations in the LADCA (left panels) and LCXCA (right panels) perfusion territories at baseline, during stunning and after 30 min and 60 min infusion of vehicle (A and B), or EMD 57033 (0.2 mg kg^{-1} min^{-1}, i.v.) in the absence (C and D) and in the presence (E and F) of adrenoceptor blockade. LVP = left ventricular pressure. For statistical analysis see Table 2.

Table 2 Effect of EMD 57033 on regional left ventricular end-systolic elastance in anaesthetized pigs with stunned myocardium

	Baseline (n=*21*)	*Reperfusion* *Adrenergic blockade*	*30 min*		Δ_{abs} *by infusion* *30 min*	*60 min*
LADCA perfusion territory						
E_{es}, mmHg mm^{-1}	67±7	–	46±5*	PG	−7±4	−8±4
				EMD	100±52	239±41†‡
		+	49±11	EMD	60±23†‡	126±29†‡¶
$L_{0,es}$, mm	7.0±0.2	–	8.9±0.4*	PG	−0.2±0.3	−0.7±0.3
				EMD	−1.2±0.5†	−1.0±0.5
		+	9.7±0.3*	EMD	−1.3±0.4†	−1.4±0.4†
LCXCA perfusion territory						
E_{es}, mmHg mm^{-1}	80±7	–	76±7	PG	−18±7	−14±9
				EMD	165±49†‡	204±28†‡
		+	52±6*	EMD	119±26†‡	234±56†‡
$L_{0,es}$, mm	7.4±0.1	–	7.5±0.2*	PG	−0.2±0.1	−0.2±0.2
				EMD	0.1±0.1	0.1±0.2
		+	7.8±0.1	EMD	−0.1±0.1	0.1±0.1

LADCA, left anterior descending coronary artery; LCXCA, left circumflex coronary artery; E_{es}, end-systolic elastance; $L_{0,es}$, intercept at zero pressure of the LV end-systolic pressure-segment length relation; PG, propylene glycol ($n=7$); EMD, EMD 57033 (0.2 mg kg^{-1} min^{-1}, iv., $n=7$ in each group); −, in the absence of α-and β-blockade; +, in the presence of α-and β-blockade; Values are mean±s.e.mean; *$P<0.05$ vs baseline (only for 30 min reperfusion); †$P<0.05$ vs 30 min reperfusion; ‡$P<0.05$ vs change in vehicle group; ¶$P<0.05$ vs EMD 57033-induced change in animals without adrenoceptor blockade.

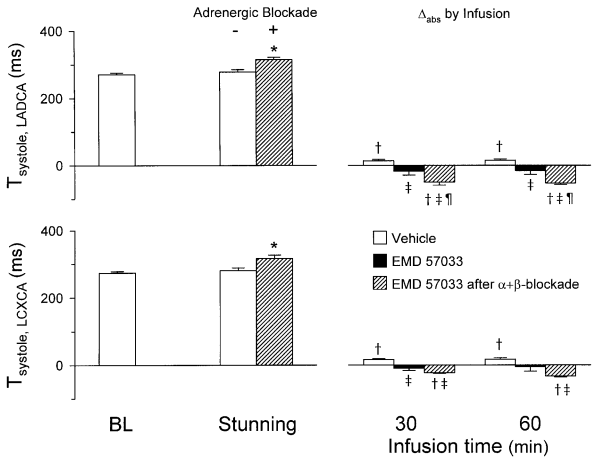

Figure 4 The effect of EMD 57033 (0.2 mg kg^{-1} min^{-1}, i.v.) on the duration of systole ($T_{systole}$) in the LADCA (top) and the LCXCA (bottom) perfusion territories. Absolute values are shown at baseline (BL, $n=21$), and during stunning in the absence ($n=14$) and in the presence of ($n=7$) of α+β-adrenoceptor blockade. The effects of the infusions of vehicle ($n=7$) and EMD 57033 ($n=7$ in both groups) have been presented as absolute changes (Δ_{abs}) from their respective stunning values. Data are mean±s.e.mean; *$P<0.05$ stunning vs baseline, †$P<0.05$ vs stunning, ‡$P<0.05$ vs change in vehicle group, ¶$P<0.05$ vs EMD 57033-induced change in animals without adrenoceptor blockade.

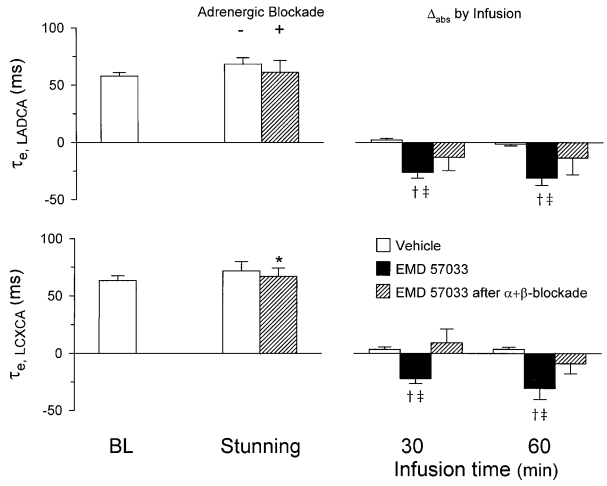

Figure 5 From top to bottom are shown the time constants of decay of regional elastance (τ_e) during early diastole of the LADCA ($\tau_{e, LADCA}$) and LCXCA ($\tau_{e, LCXCA}$) perfusion territories. Absolute values are shown at baseline (BL, $n=21$) and during stunning in the absence ($n=14$) and in the presence ($n=7$) of α+β-adrenoceptor blockade. The effects of the infusions of vehicle ($n=7$) and EMD 57033 (0.2 mg kg^{-1} min^{-1}, i.v., $n=7$ in both groups) have been presented as absolute changes (Δ_{abs}) from their respective stunning values. Data are mean±s.e.mean; *$P<0.05$ stunning vs baseline, †$P<0.05$ vs stunning, ‡$P<0.05$ vs change in vehicle group, ¶$P<0.05$ vs EMD 57033-induced change in animals without adrenoceptor blockade.

Maximum rate of fall of elastance and time constant of decay of elastance Stunning resulted in a less negative dE/dt_{min} (from −850±100 mmHg mm^{-1} s^{-1} at baseline to −280±20 mmHg mm^{-1} s^{-1}) of the LADCA perfused area, but had no effect on dE/dt_{min} of the LCXCA perfused area (−890±80 mmHg mm^{-1} s^{-1} at baseline). Vehicle had no effect on dE/dt_{min} in either region but in the presence of EMD 57033, dE/dt_{min} became more negative by 3380±640 and 1800±650 mmHg mm^{-1} s^{-1} in the LADCA and LCXCA areas, respectively. Although adrenoceptor blockade tended to blunt the effects of EMD 57033, this did not reach levels of statistical significance (not shown).

In view of the dependency of dE/dt_{min} on maximum elastance (E_{es}), we also determined the time constants of regional myocardial elastance decay (τ_e, Figure 5). Stunning increased τ_e in the LADCA area, but had no effect on τ_e in the LCXCA area. Vehicle had no effect on τ_e of either area, while EMD 57033 decreased τ_e in both areas ($P<0.05$). Adrenoceptor blockade blunted the EMD 57033-induced decrease in both stunned and normal myocardium.

End-diastolic elastance In the LADCA area stunning had no effect on E_{ed}, but increased $L_{0,ed}$ from 8.7±0.3 to 9.9±0.4 mm (Table 3, Figure 6). Vehicle had no effect on E_{ed} or $L_{0,ed}$. In contrast, EMD 57033, which also had no effect on E_{ed}, tended to decrease $L_{0,ed}$ ($P=0.098$). Adrenoceptor blockade had no significant effect on the responses of E_{ed} to EMD 57033, but

Table 3 Effect of EMD 57033 on regional left ventricular end-diastolic elastance in anaesthetized pigs with stunned myocardium

	Baseline (n = *21*)	*Reperfusion* *Adrenergic blockade*	*30 min*		Δ_{abs} *by infusion* *30 min*	*60 min*
LADCA perfusion territory						
E_{ed}, mmHg mm^{-1}	4.2±0.4	–	3.9±0.4	PG	−0.4±0.5	−0.7±0.5
				EMD	−0.7±0.6	−0.6±1.3
		+	4.4±0.4	EMD	−0.8±0.5	−1.0±0.9
$L_{0,ed}$, mm	8.7±0.3	–	9.9±0.4*	PG	0.0±0.1	−0.3±0.2
				EMD	−0.3±0.4	−1.9±1.4
		+	9.8±0.9*	EMD	−0.4±0.2	−0.6±0.3
LCXCA perfusion territory						
E_{ed}, mmHg mm^{-1}	3.7±0.2	–	4.6±0.4*	PG	0.4±0.4	0.5±0.5
				EMD	−0.6±1.3	1.9±2.1
		+	6.2±1.1	EMD	1.9±0.8‡	−2.3±1.5
$L_{0,ed}$, mm	9.1±0.4	–	9.6±0.7*	PG	0.2±0.2	0.1±0.2
				EMD	−2.3±1.8†	−2.8±1.5†
		+	10.1±0.8*	EMD	−0.5±0.1†‡¶	−0.7±0.2†‡¶

LADCA, left anterior descending coronary artery; LCXCA, left circumflex coronary artery; E_{ed}, end-diastolic elastance; $L_{0,ed}$, intercept at zero pressure of the LV end-diastolic pressure-segment length relation; PG, propylene glycol ($n=7$); EMD, EMD 57033 (0.2 mg kg^{-1} min^{-1}, iv., $n=7$ in each group); −, in the absence of α- and β-blockade; +, in the presence of α- and β-blockade; values are mean±s.e.mean; *$P<0.05$ vs baseline (only for 30 min reperfusion); †$P<0.05$ vs 30 min reperfusion; ‡$P<0.05$ vs change in vehicle group; ¶$P<0.05$ vs EMD 57033-induced change in animals without adrenoceptor blockade.

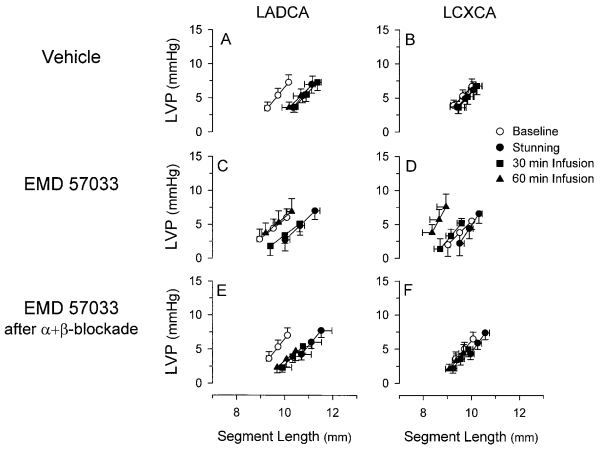

Figure 6 LV end-diastolic pressure-segment length relations in the LADCA (left panels) and LCXCA (right panels) perfusion territories at baseline, during stunning and after 30 min and 60 min of infusion of vehicle (A and B), or EMD 57033 (0.2 mg kg^{-1} min^{-1}, i.v.) in the absence (C and D) and in presence (E and F) of adrenoceptor blockade. LVP = left ventricular pressure. For statistical analysis see Table 3.

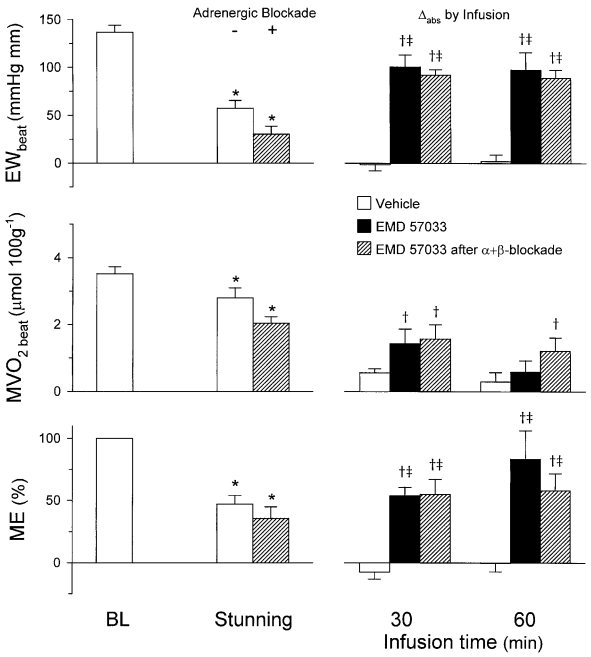

Figure 7 EW_{beat}, $MVO_{2\ beat}$ and ME of the LADCA perfused area. Absolute values are shown at baseline (BL, $n=21$), and during stunning in the absence ($n=14$) and in the presence ($n=7$) of α- and β-adrenoceptor blockade. The effects of the infusions of vehicle ($n=7$) and EMD 57033 (0.2 mg kg^{-1} min^{-1}, i.v.) in the absence ($n=7$) or presence ($n=7$) of adrenoceptor blockade have been presented as absolute changes (Δ_{abs}) from their respective stunning values. Data are mean±s.e.mean; *$P<0.05$ stunning vs baseline, †$P<0.05$ vs stunning, ‡$P<0.05$ vs change in vehicle group, ¶$P<0.05$ vs EMD 57033-induced change in animals without adrenoceptor blockade.

prevented the decrease in $L_{0,ed}$.

In the adjacent LCXCA area, the stunning protocol and subsequent infusion of vehicle had no effect on the LV end-diastolic pressure-segment length relation (Table 3, Figure 6). Infusion of EMD 57033 also had no effect on E_{ed}, but caused a leftward shift of the LV end-diastolic pressure-segment relation. Adrenoceptor blockade did not modify the responses of E_{ed} to EMD 57033, but blunted the decrease in $L_{0,ed}$.

Mechanical efficiency of the stunned myocardium

Stunning caused a 68±5% decrease ($P<0.05$) in EW_{beat} but only a 12±6% decrease in the $MVO_{2\ beat}$ of the LADCA-perfused myocardium, so that mechanical efficiency (EW_{beat}/$MVO_{2\ beat}$) decreased by 66±6% (Figure 7). Infusion of vehicle had no effect on mechanical efficiency as both EW_{beat} and $MVO_{2\ beat}$ remained unchanged. However, during infusion of EMD 57033 both EW_{beat} and $MVO_{2\ beat}$ returned to baseline values and consequently also mechanical efficiency was normalized. The increase in MVO_2 was accompanied by an equivalent increase in myocardial blood flow, so that myocardial O_2 extraction (62±6% at stunning and 62±6% at 60 min of infusion) and coronary venous PO_2 (27±3 mmHg at stunning 28±3 mmHg at 60 min of infusion) remained unchanged. In the pigs in which EMD 57033 was infused in the presence of adrenoceptor blockade, the responses of EW_{beat},

$MVO_{2\ beat}$, mechanical efficiency and coronary venous PO_2 were not different from the responses to EMD 57033 in the absence of adrenoceptor blockade.

Plasma and tissue levels of EMD 57033

EMD 57033 could not be detected in the pre-drug samples. During EMD 57033 infusion, the plasma levels increased time-dependently to 3.54 ± 0.13, 5.03 ± 0.63, 6.53 ± 0.74 and 7.23 ± 0.67 μg ml^{-1} at 15, 30, 45 and 60 min, respectively. In two of these experiments it was shown that at the end of the 60 min infusion EMD 57033 had accumulated in the left ventricle (26.6 and 29.5 μg per g of wet weight) and the liver (29.4 and 26.2 μg g^{-1}), while tissue levels in the stomach (6.7 and 6.6 μg g^{-1}) and skeletal muscle (13.5 ± 5.3 μg g^{-1}) were in the range of the plasma concentrations. EMD 57033 concentrations in the cerebellum (4.3 and 4.2 μg g^{-1}) and cerebrum (4.4 ± 5.3 μg g^{-1}) were below plasma levels, possibly due to the blood-brain barrier.

Discussion

The major findings in this *in vivo* porcine model of regional myocardial stunning are (1) EMD 57033 increased systolic shortening in stunned myocardium more than in the adjacent normal myocardium; (2) EMD 57033 also increased end-systolic elastance in normal and stunned myocardium, but with similar responses in both regions; (3) EMD 57033 did not delay the onset of relaxation, but improved the maximum rate of fall of regional myocardial elastance and decreased the time constant of early diastolic regional myocardial elastance decay; EMD 57033 had no effect on end-diastolic elastance in either normal and stunned myocardium; (4) EMD 57033 restored mechanical efficiency of the stunned myocardium; (5) the effects on regional systolic and diastolic function were only slightly modified by pretreatment with α- and β-adrenoceptor blockade, indicating that at the dose used phosphodiesterase III inhibition contributes only minimally to the actions of EMD 57033; (6) finally, the EMD-induced systemic vasodilation was not amenable to either adrenoceptor blockade or additional inhibition of NO synthesis.

Systolic function

EMD 57033 resulted in a time-dependent (i.e. plasma concentration-dependent) restoration of SS in stunned myocardium, while producing only a small increase in SS in the remote normal region. This finding is in accordance with previous observations in our laboratory with EMD 60263 in a similar model of myocardial stunning in the *in vivo* pig heart (Soei *et al*., 1994). These studies could be interpreted to suggest a relatively preferential effect of Ca^{2+}-sensitizing drugs on systolic function of stunned myocardium. However, SS is a load-sensitive index of systolic function and does not necessarily reflect the contractile state of the myocardium (Krams *et al*., 1993), particularly under conditions of stunning when its load-dependency is even greater (Fan *et al*., 1995). Indeed, EMD 57033 produced similar increments in E_{es} in stunned and normal myocardium. This suggests that the greater increase in SS in stunned myocardium resulted principally from a greater sensitivity of regions with a lower E_{es} for positive inotropic interventions. This is supported by the observation that the β-adrenoceptor agonist dobutamine also produces a preferential increase in systolic shortening of stunned myocardium, whereas E_{es} responses were similar in normal and stunned region (McFalls *et al*., 1992; Fan *et al*., 1995). Similar to the present study, Korbmacher *et al*. (1997) observed that EMD 60263 at an optimal dose of 3 μM produced similar increments in $LVdP/dt_{max}$ in normal (from 1415 to 1885 mmHg s^{-1}) and globally stunned (from 845 to 1300 mmHg s^{-1}) isolated rabbit hearts in which afterload was held constant. Also with EMD 57033, these authors reported similar increments in LV systolic pressure in stunned and normal isolated rabbit hearts at constant afterload. Interestingly, we observed that the increase in E_{es} in the stunned myocardium was time-dependent whereas the E_{es} in normal myocardium at 30 min of infusion of EMD 57033 had not increased further after 60 min of infusion. In view of the progressive increase in plasma levels over time, this observation suggests that the maximum effect in normal myocardium was reached at a lower dose than that in stunned myocardium. Indeed, Korbmacher *et al*. (1997) observed in their *in vitro* study that a further increase in dose of EMD 57033 to 10 μM did not further increase $LVdP/dt_{max}$ in stunned hearts, while it slightly decreased $LVdP/dt_{max}$ in normal myocardium. These findings suggest that the normal myocardium is more sensitive to adverse effects on systolic function than stunned myocardium in which the Ca^{2+} responsiveness is lower (Bolli & Marban, 1999).

It has been proposed that *in vitro* EMD 57033 exerts, besides its Ca^{2+}-sensitizing effects, phosphodiesterase inhibiting actions (Solaro *et al*., 1993; Ravens *et al*., 1996). In the present study, blockade of α- and β-adrenoceptors did not modulate the inotropic responses to EMD 57033 in normal myocardium *in vivo*. Thus, the increase in E_{es} produced by EMD 57033 in normal myocardium was not altered by adrenoceptor blockade, while the index of global LV contractility $LVdP/dt_{max}$ was also unmitigated in the presence of adrenoceptor blockade. Hence the EMD-57033-induced actions appeared to be primarily the result of an increase in Ca^{2+} responsiveness which is in accordance with previous studies in awake pigs from our laboratory (Stubenitsky *et al*., 1997). However, there was a slight blunting of the increase in E_{es} in stunned myocardium, which is difficult to explain by a phosphodiesterase III inhibitory action, as it seems unlikely that a pharmacological property such as phosphodiesterase III inhibition could differ in the stunned from normal myocardium. Moreover, the duration of global LV systole, and $T_{systole,\ LADCA}$ and $T_{systole,\ LCXCA}$ which were not altered by EMD 57033 in the presence of intact adrenoceptor activity, were not increased but were slightly shortened by EMD 57033 in the presence of adrenoceptor blockade. Taken together the present study suggests that the EMD-57033-induced systolic actions *in vivo* are not the result of phosphodiesterase III inhibition.

Stunning reduced mechanical efficiency likely due to a decrease in E_{es} which not only decreases external work but also increases potential work, resulting in a relatively high MVO_2 (Krams *et al*., 1993). EMD 57033 restored external work while producing only a small increase in MVO_2, thereby restoring efficiency of stunned myocardism. This favourable effect is most likely the result of positive inotropism, rather than a unique feature of EMD 57033, because we previously observed a similar restoration of mechanical efficiency of stunned myocardium during infusion of the β-adrenoceptor agonist dobutamine (McFalls *et al*., 1992).

Diastolic function

It has been suggested that increased Ca^{2+} responsiveness can lead to maintained contraction at Ca^{2+} levels at which normally relaxation occurs (Palmer & Kentish, 1997). In rat skinned right ventricular trabeculae these authors have shown that EMD 57033 can even cause contraction in the absence of

Ca^{2+}. Consequently, contraction could be prolonged thereby delaying the onset of relaxation (Solaro *et al*., 1993; Ravens *et al*., 1996), the rate of relaxation could be attenuated (Hajjar *et al*., 1997; Korbmacher *et al*., 1997) and end-diastolic stiffness could be increased (Hgashiyama *et al*., 1995; Hajjar *et al*., 1997) even when Ca^{2+} concentrations are normal.

Early diastolic function appeared to be well preserved during EMD 57033 infusion in the present study. $T_{systole}$ slightly decreased in both normal and stunned myocardium and in the left ventricle as a whole, possibly due to the small increase in heart rate. Thus, the onset of relaxation was not delayed by EMD 57033 either in the presence or absence of intact adrenoceptor activity, indicating that phosphodiesterase III inhibition did not mask the potential contraction prolongation produced by the Ca^{2+}-sensitizing actions of EMD 57033. $LVdP/dt_{min}$ is often used as an index of global early LV relaxation, despite its dependency on, in particular, LV systolic pressure (Weisfeldt *et al*., 1974; Gaasch *et al*., 1986). In the present study, $LVdP/dt_{min}$ improved slightly during the first 30 min of EMD 57033 infusion but fell to 75% of the value at stunning during the following 30 min. The decrease in $LVdP/dt_{min}$ may have been due to the 20% reduction of LV systolic pressure, and does not necessarily reflect an impairment of global LV relaxation. This is even more so, as in the adrenoceptor blocked animals, both $LVdP/dt_{min}$ and LV systolic pressure were not affected by EMD 57033. This strongly suggests that phosphodiesterase III inhibition did not contribute significantly to the maintenance of $LVdP/dt_{min}$. Inspection of the less load-sensitive time constant τ_{LVP} supports this notion. Nevertheless, both indexes are global variables and do not discriminate between the effects of EMD 57033 on normal and stunned myocardium, which is also why regional indexes of ventricular relaxation were employed. In both stunned and normal myocardial regions, the maximum rate of fall of regional myocardial elastance (dE/dt_{min}) increased, which is not surprising in view of the marked increments in E_{es}. However, even after correction for the load-dependency of dE/dt_{min}, i.e. by calculating the time constants of elastance decay τ_e, the data indicated no untoward effects of EMD 57033 on early diastolic function. Importantly, even in the presence of adrenoceptor blockade, EMD 57033 did not exert detrimental actions on dE/dt_{min} or the relaxation time constants, suggesting that the lack of adverse effects were not the result of masking of untoward effects of Ca^{2+}-sensitization by concomitant phosphodiesterase III inhibitory effects of the compound. In the present study we also failed to find evidence of a negative effect of EMD 57033 on late diastolic function, as E_{ed} was maintained during EMD 57033 infusion, even in the presence of adrenoceptor blockade.

The importance of abnormalities in diastolic function is that these could compromise systolic pump function *via* reduced LV filling and *via* impairment of myocardial perfusion by reducing the effective diastolic perfusion time of the coronary bed. However, in the present study EMD 57033, either with or without adrenoceptor blockade, restored regional systolic shortening, external work and mechanical efficiency, while myocardial oxygen supply was unimpeded.

Conclusions

The Ca^{2+}-sensitizing agent EMD 57033, at a dose of 0.2 mg kg^{-1} min^{-1}, restored regional systolic and diastolic function of stunned myocardium in the *in vivo* porcine heart. In normal myocardium, quantitatively similar improvements in systolic and diastolic function were observed. Phosphodiesterase III inhibition contributed minimally to these actions. The results of the present study suggest that Ca^{2+}-sensitizing agents are prime candidates for complementing the current inodilator therapeutic arsenal in the clinical setting for acute states of heart failure, because they are powerful enhancers of systolic performance *in vivo* at doses that do not appear to exert adverse effects on diastolic function.

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