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Ca^{2+} sensitization and diastolic function of normal and stunned porcine myocardium

Loe Kie Soei, Sandra de Zeeuw, Rob Krams, Dirk J. Duncker, Pieter D. Verdouw *

Experimental Cardiology, Thoraxcenter, Cardiovascular Research Institute COEUR, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, Netherlands

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Abstract

Ca^{2+} sensitizers prolong myofibrillar force development in vitro and might therefore aggravate relaxation abnormalities of stunned myocardium. This is the first in vivo study of the effects of the thiadiazinone derivative EMD 60263 ((+)-5-(l-(alpha-ethylimino-3,4-dimethoxybenzyl)-1,2,3,4-tetrahydroquinoline-6-yl)-6-methyl-3,6-dihydro-2H-1,3,4-thiadiazine-2-on), a Ca^{2+}-sensitizing agent with negligible phosphodiesterase III inhibitory activity, on diastolic function of regionally stunned myocardium. After producing stunning by two sequences of 10-min coronary artery occlusion and 30 min of reperfusion, anaesthetised pigs received either saline ($n = 7$) or 1.5 and 3.0 mg/kg of EMD 60263 ($n = 8$) or its enantiomer EMD 60264 ($n = 6$), which lacks the Ca^{2+}-sensitizing properties but shares the bradycardiac action via inhibition of the delayed inward rectifier K^+ current. In stunned myocardium, systolic shortening was reduced to $46 \pm 4\%$ of baseline ($P < 0.05$) and mean rate of half end-diastolic segment lengthening, an index for diastolic function, to $35 \pm 4\%$; systolic shortening and mean rate of half end-diastolic lengthening of remote normal myocardium remained unchanged. Saline did not affect these parameters in stunned or normal myocardium. EMD 60264 did not affect systolic shortening but decreased mean rate of half end-diastolic lengthening in normal myocardium to $61 \pm 8\%$ and in stunned myocardium to $16 \pm 5\%$ of baseline. During saline and EMD 60264, normal and stunned segments started to lengthen immediately after minimal segment length was reached ($\Delta T = 0$). Low dose EMD 60263 restored systolic shortening of the stunned region with no effect on ΔT. The high dose increased systolic shortening above baseline and ΔT to 210 ± 30 ms in both regions. Consequently, mean rate of half end-diastolic lengthening increased to $66 \pm 11\%$ in stunned, while decreasing to $55 \pm 3\%$ in normal myocardium. After elimination of bradycardia, ΔT and hence mean rate of half end-diastolic lengthening recovered in stunned myocardium, but in normal myocardium the latter remained depressed because ΔT persisted. In conclusion, both doses of EMD 60263 improved systolic as well as diastolic function of stunned myocardium. The high dose delayed relaxation of normal myocardium without adversely affecting systolic function.

Keywords: Ca^{2+} sensitizer; Myocardial oxygen consumption; Diastolic function, regional; Systolic shortening, regional; Systemic haemodynamics

1. Introduction

In regionally stunned myocardium both systolic and diastolic function are depressed (Charlat et al., 1989; Ehring et al., 1993; Bolli and Marban, 1999). Since a decreased responsiveness of the myofilaments to Ca^{2+} is one of the plausible mechanisms underlying stunning (Bolli and Marban, 1999), it is not surprising that the effects of Ca^{2+}-sensitizing agents on regional systolic function of stunned myocardium have been investigated. Indeed, several studies in in vivo (Soei et al., 1994; Abe et al., 1995) and in vitro (Korbmacher et al., 1994, 1995, 1997) models have shown that this class of drugs is capable of completely restoring systolic function. However, since Ca^{2+}-sensitizing agents may prolong force development of the myofibrils (Solaro et al., 1993; Ravens et al., 1996), there is some concern that an increase in the myofibrillar responsiveness to Ca^{2+} might delay the onset of left ventricular relaxation, thereby impairing early left ventricular filling and ultimately systolic function. This may be of particular importance under conditions in which diastolic Ca^{2+} concentrations are elevated such as heart failure (Hajjar and Gwathmey, 1991) and possibly myocardial stunning (Kusuoka et al., 1990; Gao et al., 1995), although in the latter case this is not an ubiquitous finding (Steenbergen et al., 1987; Marban et al., 1990; Amende et al., 1992;

* Corresponding author. Tel.: +31-10-4088029; fax: +31-10-4089494.
E-mail address: verdouw@tch.fgg.eur.nl (P.D. Verdouw)

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Carrozza et al., 1992; Harada et al., 1994). Indeed, there are in vitro studies that reported a Ca^{2+} sensitization-induced impairment of relaxation in myocardium obtained from normal (White et al., 1993; Hgashiyama et al., 1995; Hajjar et al., 1997), stunned (Korbmacher et al., 1997) and failing hearts (Hajjar et al., 1997). However, in several studies no adverse effects of Ca^{2+}-sensitizing agents on diastolic function of normal myocardium in vitro (Simnett et al., 1993; Korbmacher et al., 1994; Haikala et al., 1995; Palmer and Kentish, 1997) and in vivo (Pagel et al., 1994, 1995; Teramura et al., 1997; Stubenitsky et al., 1997) or stunned myocardium in vitro (Korbmacher et al., 1994, 1995) could be demonstrated. Importantly, the agents used in these studies (EMD 53998, EMD 57033, CGP 48506, MCI-154 and levosimendan) do not only possess Ca^{2+}-sensitizing but also phosphodiesterase III inhibiting properties (Ravens et al., 1996). It can therefore not be excluded that a potentially adverse effect of increased Ca^{2+} sensitivity on diastolic function was blunted by the positive lusitropic effect of phosphodiesterase III inhibition.

Currently, there are no in vivo studies in which the effect of Ca^{2+}-sensitizing agents without phosphodiesterase inhibiting properties on diastolic function of regional stunned myocardium has been evaluated. In the present study, we therefore assessed the effect of the thiadiazinone derivative EMD 60263 ((+)-5-(l-(alpha-ethylimino-3,4-dimethoxybenzyl)-1,2,3,4-tetrahydroquinoline-6-yl)-6-methyl-3,6-dihydro-2H-1,3,4-thiadiazine-2-on), a Ca^{2+}-sensitizing agent with negligible phosphodiesterase III inhibiting properties (Solaro et al., 1993; Ravens et al., 1996), on regional left ventricular diastolic function of stunned and adjacent normal myocardium. Since in vitro experiments suggest that the nature of the response of normal as well as stunned myocardium may be dose-dependent (Korbmacher et al., 1997) two doses were chosen, one that is sufficient and one that is in excess of that required to restore systolic function (Soei et al., 1994). As an index of regional left ventricular diastolic function the mean rate of half end-diastolic lengthening was employed (Tilton et al., 1985; Charlat et al., 1989). Since EMD 60263 produces profound bradycardia (Soei et al., 1994) that potentially could mask untoward effects on diastolic function, we also studied the actions of EMD 60263 after its bradycardic action was abolished by atrial pacing. In addition, we compared the actions of EMD 60263 to those of its (−) enantiomer EMD 60264, a compound that lacks the Ca^{2+}-sensitizing properties of EMD 60263 but shares its inhibitory action on the delayed rectifier inward K^+ current, that is most likely responsible for the bradycardic action of EMD 60263 (Ravens et al., 1996). EMD 60264 shows some weak phosphodiesterase III inhibitory activity with a concentration for half maximum inhibition of this enzyme (1.4 μM),which is approximately one twentieth of that of EMD 60263 (see Ravens et al., 1996). Finally, disturbances in diastolic function are more likely to be aggravated and thereby to affect systolic function when the diastolic period is shortened. Therefore, we also studied whether adverse effects emerged or were aggravated after heart rate was increased above baseline levels in the presence of EMD 60263.

2. Material and methods

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 prior approval of the Animal Care Committee of the Erasmus University Rotterdam.

2.1. Surgical preparation

Cross-bred Landrace × Yorkshire pigs of either sex (n = 21, 28–30 kg) were sedated with ketamine i.m. (20–30 mg/kg, Apharmo, Huizen, The Netherlands) and anaesthetised with pentobarbital i.v. (20 mg/kg followed by 5–10 mg/kg per h, Sanofi, Paris, France) before they were intubated and connected to a respirator for intermittent positive pressure ventilation with a mixture of oxygen and nitrogen; arterial blood gas values were kept within the normal range by adjusting respiratory rate and tidal volume. Haemaccel (Behringwerke, Marburg, FRG) was administered via an intravenous infusion to maintain fluid balance. A micromanometer-tipped catheter (B. Braun Medical, Uden, The Netherlands) was inserted into the left carotid artery and advanced into the left ventricular cavity for recording of left ventricular blood pressure and its first derivative (LV$dP/dt$), while a fluid-filled catheter was positioned in the descending aorta for monitoring arterial blood pressure (Sassen et al., 1990; Soei et al., 1994).

After administration of 4 mg pancuronium bromide (Organon Teknika, Oss, The Netherlands), a midsternal thoracotomy was performed and the heart was suspended in a pericardial cradle. An electromagnetic flow probe (Skalar, Delft, The Netherlands) was then positioned around the ascending aorta for measurement of aortic blood flow (cardiac output) and pacing leads were attached to the right atrial appendage and connected to a pacemaker. A proximal segment of the left anterior descending coronary artery was dissected free for placement of an atraumatic clamp, while the anterior interventricular vein draining the left anterior descending coronary artery perfusion territory was cannulated for collection of local coronary venous blood (Bier et al., 1991).

One pair of ultrasonic crystals was inserted inside the distribution territory of the left anterior descending coronary artery and another pair inside the distribution territory of the left circumflex coronary artery for measurement of regional myocardial function by sonomicrometry (Triton Technology, San Diego, CA, USA). Crystals were positioned in the midmyocardial layer approximately 10 mm apart and parallel to fibre direction.

2.2. Experimental protocol and groups

Baseline values of systemic haemodynamics and regional myocardial function were recorded after haemodynamic variables had been stable for at least 30 min following completion of instrumentation. After arterial and coronary venous blood, samples were collected for determination of haemoglobin, oxygen saturation and blood gas values, a batch ($1–2 \times 10^6$) of radioactive microspheres (15 ± 1 μm (S.D.), labeled with either ^{46}Sc, ^{95}Nb, ^{103}Ru, ^{113}Sn or ^{141}Ce (NEN; Dreieich, FRG), was injected into the left atrium, and a reference blood sample was withdrawn at a rate of 10 ml/min for determination of regional myocardial blood flow (Sassen et al., 1990). Subsequently, the distribution area of the left anterior descending coronary artery was stunned by two sequences of 10-min occlusion and 30 min of reperfusion (Brand et al., 1992). At the end of the second 30-min reperfusion period, the animals received either two doses (1.5 and 3.0 mg/kg administered intravenously over 3 min) of EMD 60263 ($n = 8$) or EMD 60264 ($n = 6$) or two equivalent volumes of the vehicle (3 and 6 ml of saline, $n = 7$), separated by 15 min. Since both EMD 60263 and EMD 60264 produce bradycardia, measurements after the second dose of these compounds were repeated after heart rates were restored to pre-drug levels observed following stunning (HR_{st}) and additionally after heart rate was raised to 30 beats/min above HR_{st} (HR_{st+30}). In the animals that received EMD 60264, regional myocardial blood flows were not determined. Furthermore, in these animals, heart rate could not be raised to HR_{st+30} due to development of atrio-ventricular block during pacing.

At the end of each experiment, normal and stunned myocardium were identified by a left atrial injection of patent blue violet (Sigma, St. Louis, MO, USA) after the left anterior descending coronary artery had been reoccluded. The animals were then killed with an overdose of pentobarbital, the heart excised and the left ventricle handled as described earlier to obtain regional myocardial blood flow data (Sassen et al., 1990).

2.3. Data analysis

Myocardial oxygen consumption of the perfusion territory of the left anterior descending coronary artery was calculated as the product of local transmural myocardial blood flow (radioactive microsphere data) and the difference in the oxygen contents of the arterial and local coronary venous blood, while the area inside the left ventricular pressure-segment length loop was taken as an index of external work (Morris et al., 1987; Vinten-Johansen et al., 1991; Soei et al., 1994). Mechanical efficiency was defined as the ratio of external work and myocardial oxygen consumption per beat. The area inside the left ventricular pressure-segment length loop reflects mechanical work but does not have the dimensions of work and changes in mechanical efficiency have therefore been expressed as percentage of baseline (Soei et al., 1994). Mechanical efficiency data are only presented for the distribution area of the left anterior descending coronary artery, because myocardial oxygen consumption of the adjacent normal myocardium could not be calculated as local coronary venous blood was not sampled from that area.

Systolic shortening was computed as $100\% \cdot (EDL - ESL)/EDL$, in which EDL (end-diastolic length) and ESL (end-systolic length) are the segment length at the start and the end of left ventricular ejection, respectively. Post-systolic shortening was calculated as $100\% \cdot (ESL - L_{min})/EDL$, in which L_{min} is the minimum segment length after closure of the aortic valves. All segment length data were normalized to an EDL of 10 mm at baseline. Analo-

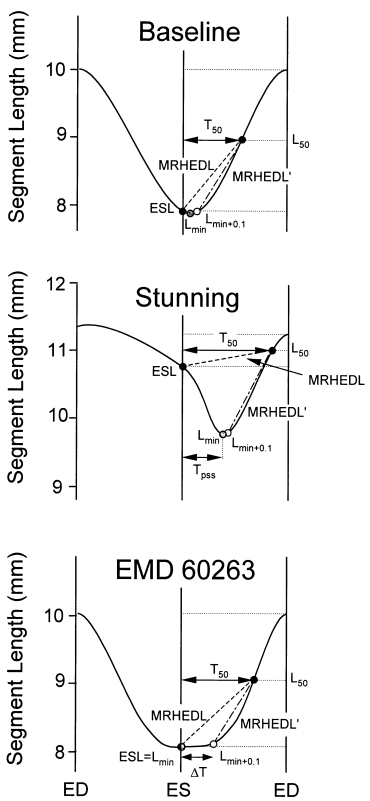

Fig. 1. Definition of the mean rate of half end-diastolic lengthening (MRHEDL) during baseline conditions, stunning and after the higher dose of EMD 60263. Notice that at baseline and during stunning the segment started to lengthen immediately after L_{min} (●) was reached, but that the onset of lengthening was delayed by ΔT after the higher dose of EMD 60263 (see Results). ΔT is the time interval in which the segment length increased from L_{min} to $L_{min} + 0.1$ mm (○). L_{50} is the point midway between ESL and the following EDL. MRHEDL (- - -) is the mean rate of lengthening between ESL and L_{50}, while MRHEDL′ (-·-·-·-) is defined as the mean rate of lengthening between $L_{min} + 0.1$ mm and L_{50}. EDL and ESL are the end-diastolic and end-systolic length, respectively; L_{min} = minimal segment length; T_{PSS} = duration of post-systolic shortening; T_{50} = the time interval between ESL and L_{50}.

gous to the studies by Tilton et al. (1985) and Charlat et al. (1989), we used as index for early diastolic function the mean rate of half-end diastolic lengthening, which was defined as the slope of the line drawn from ESL to L_{50}, which is the point midway between ESL and the following EDL ($(L_{50} - \mathrm{ESL})/T_{50}$, Fig. 1). We also determined the time interval (ΔT) between L_{min} and the onset of segment lengthening ($L_{min} + 0.1$ mm) and calculated mean rate of half end-diastolic lengthening as the mean rate of segment lengthening during the time interval between $L_{min} + 0.1$ mm and L_{50}.

All data have been presented as means ± S.E.M. Statistical significance of the changes produced by stunning were evaluated by paired *t*-test. The effects of the administered agents were assessed by two-way analysis of variance for repeated measures followed by paired *t*-test with Bonferroni adjustment. Statistical significance was accepted for $P < 0.05$ (two-tailed).

Table 1
Systemic haemodynamics during intravenous infusion of EMD 60263 and its (−) enantiomer EMD 60264 in anaesthetized pigs with stunned myocardium D_1 and D_2 are 3 and 6 ml of saline for control and 1.5 and 3.0 mg/kg for EMD 60264 and EMD 60263, respectively. HR_{st}, pacing at heart rate observed during stunning; HR_{st+30}, pacing at 30 beats/min above HR_{st}. Data are mean ± S.E.M.; $n = 7$ (Saline); $n = 8$ (EMD 60263); $n = 6$ (EMD 60264).

	Baseline	Stunning	D_1	D_2	HR_{st}	HR_{st+30}
Cardiac output (l / min)						
Saline	2.8 ± 0.2	2.4 ± 0.1[a]	2.4 ± 0.2	2.4 ± 0.1	2.3 ± 0.2	2.4 ± 0.2
EMD 60264	2.6 ± 0.2	2.1 ± 0.2[a]	1.9 ± 0.1[b]	1.9 ± 0.2	1.8 ± 0.2	
EMD 60263	3.0 ± 0.2	2.7 ± 0.2	2.5 ± 0.2	2.4 ± 0.2[b,c]	2.5 ± 0.2	2.2 ± 0.3[d]
Heart rate (beats / min)						
Saline	107 ± 2	104 ± 7	105 ± 6	102 ± 6	106 ± 4	138 ± 6[b]
EMD 60264	108 ± 4	109 ± 4	91 ± 3[b,c]	80 ± 5[b,c]	108 ± 4	
EMD 60263	109 ± 5	100 ± 4	73 ± 5[b,c]	54 ± 5[b,c,e]	100 ± 4	130 ± 5[b]
Left ventricular systolic pressure (mm Hg)						
Saline	111 ± 3	104 ± 4	104 ± 3	108 ± 3	109 ± 5	104 ± 3
EMD 60264	111 ± 4	102 ± 3	93 ± 4	79 ± 3[b,c]	74 ± 5[b]	
EMD 60263	113 ± 2	108 ± 3	106 ± 3	109 ± 4[e]	103 ± 8	6 ± 6[b,c]
Left ventricular end-diastolic pressure (mm Hg)						
Saline	9 ± 1	9 ± 1	9 ± 1	10 ± 1	8 ± 1	7 ± 1[b]
EMD 60264	7 ± 1	6 ± 1	7 ± 1	8 ± 1	7 ± 1	
EMD 60263	10 ± 1	10 ± 1	11 ± 1	14 ± 1[b,c]	10 ± 1	13 ± 2[d]
$LVdP/dt_{max}$ (mm Hg / s)						
Saline	2110 ± 190	1660 ± 170[a]	1670 ± 160	1700 ± 150	1660 ± 140	1790 ± 160
EMD 60264	2030 ± 240	1560 ± 120[a]	1470 ± 140	1210 ± 100[b]	1120 ± 110[b]	
EMD 60263	2130 ± 130	1640 ± 120[a]	1620 ± 130	1720 ± 130	1870 ± 120	1670 ± 160
$LVdP/dt_{min}$ (mm Hg / s)						
Saline	−2120 ± 150	−1780 ± 210[a]	−1730 ± 150	−1930 ± 300	−2010 ± 300	−2230 ± 260[b,c]
EMD 60264	−2450 ± 350	−2060 ± 310[a]	1240 ± 140[b,c]	−870 ± 70[b,c]	−870 ± 90[b]	−1320 ± 160[c]
EMD 60263	−2110 ± 90	−1740 ± 80[a]	1140 ± 120[b,c]	−890 ± 110[b,c]	−1220 ± 130[b,c]	
Mean arterial pressure (mm Hg)						
Saline	89 ± 3	84 ± 4	83 ± 2	86 ± 2	89 ± 3	87 ± 3
EMD 60264	92 ± 5	85 ± 3	75 ± 4[b]	61 ± 4[b,c]	61 ± 5[b]	
EMD 60263	91 ± 2	87 ± 3	79 ± 3[b]	77 ± 3[b,c,e]	84 ± 7	73 ± 6[b]
Stroke volume (ml)						
Saline	27 ± 2	23 ± 1	23 ± 1	24 ± 1	22 ± 1	17 ± 1[b]
EMD 60264	24 ± 1	19 ± 2	21 ± 1	24 ± 1[b]	17 ± 2	
EMD 60263	27 ± 1	27 ± 1	34 ± 2[b,c]	45 ± 3[b,c,e]	25 ± 2	17 ± 2[b,d]
Systemic vascular resistance (dynes · s / cm^5)						
Saline	2640 ± 240	2880 ± 160	2800 ± 160	2880 ± 240	3200 ± 400	3040 ± 320
EMD 60264	2960 ± 240	3360 ± 160	3280 ± 240	2640 ± 240[b]	2880 ± 240	
EMD 60263	2560 ± 160	2720 ± 240	2720 ± 240	2720 ± 240	2880 ± 320	2960 ± 320

[a] $P < 0.05$ Stunning vs. Baseline.
[b] $P < 0.05$ vs. Stunning.
[c] Changes from Stunning are different ($P < 0.05$) vs. changes from Stunning in the Saline group.
[d] Changes from HR_{st} are different ($P < 0.05$) vs. changes from HR_{st} in the Saline group (only for data at HR_{st+30}).
[e] Changes from Stunning are different ($P < 0.05$) vs. changes from Stunning in the EMD 60264 group.

2.4. Drugs

EMD 60263 and EMD 60264 (E. Merck Darmstadt, Germany) were dissolved in saline. Fresh solutions were prepared on the day of each experiment.

3. Results

3.1. Systemic haemodynamics

At the end of the second occlusion–reperfusion cycle, mean arterial blood pressure ($6 \pm 3\%$), cardiac output ($15 \pm 4\%$), stroke volume ($11 \pm 6\%$), $\mathrm{LVd}P/\mathrm{d}t_{max}$ ($21 \pm 5\%$) and $\mathrm{LVd}P/\mathrm{d}t_{min}$ ($16 \pm 3\%$) had decreased from their respective baseline levels (all $P < 0.05$, $n = 21$), while heart rate, left ventricular systolic pressure and left ventricular end-diastolic pressure had remained unchanged (Table 1).

Infusion of saline did not produce any change in global systemic haemodynamic variables of the control group, demonstrating the haemodynamic stability of the preparation. Increasing heart rate to HR_{st+30} via pacing decreased left ventricular end-diastolic pressure, stroke volume and $\mathrm{LVd}P/\mathrm{d}t_{min}$ (all $P < 0.05$), while $\mathrm{LVd}P/\mathrm{d}t_{max}$ was not affected. Administration of EMD 60264 produced dose-dependent decreases in mean arterial blood pressure, heart rate, $\mathrm{LVd}P/\mathrm{d}t_{max}$ and $\mathrm{LVd}P/\mathrm{d}t_{min}$. A decrease in systemic vascular resistance, most likely secondary to the phosphodiesterase III inhibitory activity of EMD 60264, was responsible for the decrease in mean arterial blood pressure, as cardiac output was not altered. The lower dose of EMD 60264 had no effect on stroke volume, but the higher dose produced an increase ($25 \pm 8\%$, $P < 0.05$),

Table 2
Myocardial perfusion and oxygen consumption of regionally stunned myocardium during intravenous infusion of EMD 60263 in anaesthetized pigs with stunned myocardium
LADCA = left anterior descending coronary artery; LCXCA = left circumflex coronary artery; D_1 and D_2 are 3 and 6 ml of saline for the saline group and 1.5 and 3.0 mg/kg for the EMD 60263 group, respectively. HR_{st+30} = pacing at 30 beats/min above the heart rate observed during stunning; $\Delta(aO_2$ cont-cvO_2 cont) = difference in the O_2 contents of the arterial and coronary venous blood; MVO_2 = regional myocardial O_2 consumption; EW = external work. Data are expressed as means ± S.E.M.; $n = 7$ (Saline); $n = 8$ (EMD 60263) except for the measurements at HR_{st+30},which were for six and seven animals, respectively.

	Baseline	Stunning	D_1	D_2	HR_{st+30}
Saline					
LADCA perfusion territory					
Transmural flow (ml/min/100g)	156 ± 6	121 ± 10[a]	111 ± 10	123 ± 8	119 ± 7
Endo/Epi	1.14 ± 0.05	1.18 ± 0.08	1.17 ± 0.04	1.19 ± 0.05	1.13 ± 0.11
$\Delta(aO_2$ cont-cvO_2 cont) (μmol/ml)	2.85 ± 0.18	2.95 ± 0.11	2.98 ± 0.12	3.04 ± 0.22	3.01 ± 0.25
$MVO_{2\,beat}$ (μmol/100g)	441 ± 26	353 ± 26[a]	331 ± 29	362 ± 12	353 ± 7
MVO_2 (μmol/min/100g)	4.1 ± 0.3	3.4 ± 0.2	3.2 ± 0.3	3.6 ± 0.2	2.6 ± 0.1
EW_{beat} (mm Hg · mm)	190 ± 17	95 ± 20[a]	100 ± 18	108 ± 23	63 ± 18[b]
Mechanical efficiency (%)	100	57 ± 9[a]	68 ± 11	60 ± 10	53 ± 13
LCXCA perfusion territory					
Transmural flow (ml/min/100g)	168 ± 7	160 ± 15	143 ± 15	148 ± 8	146 ± 9
Endo/Epi	1.05 ± 0.05	0.98 ± 0.06	0.94 ± 0.07	0.95 ± 0.06	0.91 ± 0.04
EW_{beat} (mm Hg · mm)	136 ± 10	120 ± 14	115 ± 12	114 ± 14	84 ± 21[b]
EMD 60263					
LADCA perfusion territory					
Transmural flow (ml/min/100g)	167 ± 5	122 ± 8[a]	119 ± 11	118 ± 10	110 ± 17
Endo/Epi	1.07 ± 0.09	1.16 ± 0.09[a]	1.13 ± 0.09	1.17 ± 0.08	1.07 ± 0.07
$\Delta(aO_2$ cont-cvO_2 cont) (μmol/ml)	2.91 ± 0.18	2.74 ± 0.20	2.97 ± 0.34	2.92 ± 0.18	3.10 ± 0.22
MVO_2 (μmol/min/100g)	484 ± 27	328 ± 19[a]	334 ± 27	334 ± 12	357 ± 35
$MVO_{2\,beat}$ (μmol/100g)	4.5 ± 0.3	3.3 ± 0.2[a]	4.7 ± 0.4[b,c]	6.4 ± 0.5[b,c]	2.7 ± 0.3[d]
EW_{beat} (mm Hg · mm)	185 ± 21	84 ± 11[a]	159 ± 1[b,c]	243 ± 21[b,c]	89 ± 9[d]
Mechanical efficiency (%)	100	64 ± 9[a]	89 ± 8[b,c]	100 ± 14[b,c]	80 ± 12
LCXCA perfusion territory					
Transmural flow (ml/min/100g)	180 ± 7	164 ± 10[a]	138 ± 13	129 ± 10[b]	116 ± 17[b]
Endo/Epi	1.08 ± 0.06	0.99 ± 0.05[a]	0.99 ± 0.04	0.97 ± 0.04	0.79 ± 0.05[b]
EW_{beat} (mm Hg · mm)	161 ± 16	145 ± 17[a]	161 ± 12	241 ± 15[b,c]	58 ± 8[b,d]

[a] $P < 0.05$ Stunning vs. Baseline.
[b] $P < 0.05$ vs. Stunning.
[c] Changes from stunning are different ($P < 0.05$) from changes in saline group.
[d] Changes from D_2 are different ($P < 0.05$) vs. changes from D_2 in saline group (only for data at HR_{st+30}).

which was a direct effect of the bradycardia as stroke volume returned to pre-EMD 60264 values after restoring heart rate to HR_{st}.

Administration of EMD 60263 lowered heart rate (up to $46 \pm 3\%$), mean arterial blood pressure ($12 \pm 4\%$) and cardiac output ($10 \pm 4\%$), but increased stroke volume ($69 \pm 7\%$) and left ventricular end-diastolic pressure (from 10 ± 1 to 14 ± 1 mm Hg) (all $P < 0.05$). Despite the bradycardia, $LVdP/dt_{max}$ was maintained but $LVdP/dt_{min}$ showed a response similar to that observed with EMD 60264. After elevating heart rate to HR_{st}, left ventricular end-diastolic pressure, mean arterial blood pressure and stroke volume returned to pre-EMD 60263 values. $LVdP/dt_{min}$ recovered partially, while the increase in $LVdP/dt_{max}$ did not reach levels of statistical significance. However, it is important that at corresponding time points and heart rates, EMD 60264 produced decreases in $LVdP/dt_{max}$. The further increase of heart rate to HR_{st+30} resulted in a decrease in cardiac output due to a further decrease in stroke volume from 25 ± 2 to 17 ± 2 ml/min, while left ventricular end-diastolic pressure increased from 10 ± 1 to 13 ± 2 mm Hg ($P < 0.05$ vs. saline). $LVdP/dt_{max}$ as well as $LVdP/dt_{min}$ did not change.

3.2. Myocardial oxygen consumption per beat and mechanical efficiency

In stunned myocardium transmural blood flow was decreased by $23 \pm 3\%$ ($n = 21$) without affecting the endo–epi blood flow ratio. In the saline-treated animals, no changes were observed during infusion of saline or after heart rate was increased to HR_{st+30}. Because the difference in arterial and coronary venous oxygen contents remained unchanged, myocardial oxygen consumption of stunned myocardium decreased in parallel with transmural blood flow (Table 2). In normal myocardium transmural

Table 3
Effects of EMD 60263 and its (−) enantiomer EMD 60264 on regional systolic shortening in anaesthetized pigs with stunned myocardium
D_1 and D_2 are 3 and 6 ml of saline for the saline group and 1.5 and 3.0 mg/kg for the EMD 60264 and EMD 60263 groups, respectively. HR_{st} = pacing at heart rate observed during stunning; HR_{st+30} = pacing at 30 beats/min above HR_{st}. Data are expressed as means ± S.E.M.; $n = 7$ (Saline); $n = 6$ (EMD 60264); $n = 8$ (EMD 60263).

	Baseline	Stunning	D_1	D_2	HR_{st}	HR_{st+30}
LADCA perfusion territory						
End-diastolic length (mm)						
Saline	11.0 ± 0.7	12.0 ± 0.6^{a}	11.8 ± 0.7^{b}	11.8 ± 0.7	11.4 ± 0.7^{b}	10.9 ± 0.7^{b}
EMD 60264	9.8 ± 0.9	10.7 ± 1.1^{a}	10.8 ± 1.1	10.7 ± 1.1	10.4 ± 1.1^{b}	nm
EMD 60263	12.0 ± 1.2	13.0 ± 1.4^{a}	12.8 ± 1.3	12.6 ± 1.3^{b}	$11.7 \pm 1.2^{b,e}$	10.8 ± 1.1^{b}
Systolic shortening (%)						
Saline	17 ± 1	8 ± 1^{a}	8 ± 1	9 ± 2	9 ± 2	6 ± 2
EMD 60264	19 ± 1	8 ± 1^{a}	7 ± 1	6 ± 2	4 ± 3	nm
EMD 60263	17 ± 1	9 ± 1^{a}	$15 \pm 1^{b,c,d}$	$24 \pm 2^{b,c,d}$	$17 \pm 1^{b,c,d,e}$	13 ± 1
Post systolic shortening (%)						
Saline	3.1 ± 0.9	7.2 ± 0.8^{a}	6.2 ± 0.8	5.6 ± 1.1	5.1 ± 0.7^{e}	4.9 ± 0.6^{b}
EMD 60264	1.5 ± 0.6	9.0 ± 1.1^{a}	9.0 ± 1.2	7.3 ± 1.1	7.2 ± 1.4	nm
EMD 60263	1.3 ± 0.4	7.0 ± 0.8^{a}	$3.2 \pm 0.4^{b,c,d}$	$0.5 \pm 0.2^{b,c,d}$	$0.8 \pm 0.3^{b,c,d}$	$0.3 \pm 0.2^{b,c}$
LCXCA perfusion territory						
End-diastolic length (mm)						
Saline	9.9 ± 0.8	10.1 ± 0.7	10.0 ± 0.7	10.1 ± 0.8	9.5 ± 0.6	9.2 ± 0.6^{b}
EMD 60264	10.5 ± 0.8	10.3 ± 0.8	10.4 ± 0.8	10.7 ± 0.8	10.4 ± 0.8	nm
EMD 60263	11.4 ± 1.1	11.6 ± 1.2	11.6 ± 1.2	11.5 ± 1.2	10.2 ± 0.9^{b}	$9.4 \pm 1.0^{b,f}$
Systolic shortening (%)						
Saline	12 ± 1	11 ± 2	11 ± 2	11 ± 1	11 ± 2	9 ± 2
EMD 60264	14 ± 2	13 ± 1	13 ± 1	13 ± 1	12 ± 2	nm
EMD 60263	14 ± 1	14 ± 1	$17 \pm 1^{b,c,d}$	$22 \pm 1^{b,c,d}$	12 ± 1^{e}	$8 \pm 1^{b,f}$
Post systolic shortening (%)						
Saline	1.5 ± 0.5	2.5 ± 1.0	2.2 ± 0.5	2.2 ± 0.4	2.7 ± 0.4	2.5 ± 0.8
EMD 60264	0.4 ± 0.2	0.6 ± 0.3	0.6 ± 0.3	1.0 ± 0.4	0.4 ± 0.3	nm
EMD 60263	2.1 ± 0.5	1.5 ± 0.7	0.6 ± 0.4^{b}	0.5 ± 0.3	0.8 ± 0.4	0.6 ± 0.2

[a] $P < 0.05$ Stunning vs. Baseline.
[b] $P < 0.05$ vs. Stunning.
[c] Changes from Stunning are different ($P < 0.05$) vs. changes from Stunning in saline group.
[d] Changes from Stunning are different ($P < 0.05$) vs. changes from Stunning in the EMD 60264 group.
[e] Changes from D_2 are different ($P < 0.05$) vs. changes from D_2 in Saline group.
[f] Changes from HR_{st} are different ($P < 0.05$) vs. changes from HR_{st} in Saline group, nm = not measured.

blood flow and the endo–epi flow ratio were not significantly affected.

Administration of EMD 60263 had no effect on myocardial blood flow or the difference in arterial and coronary venous oxygen content of stunned myocardium. Consequently, myocardial oxygen consumption also remained unchanged. Raising heart rate to HR_{st+30} had no effect on transmural myocardial blood flow, the endo–epi blood flow ratio or arterio-coronary venous oxygen content difference. In normal myocardium, the endo–epi blood flow ratio decreased from 0.97 ± 0.04 to 0.79 ± 0.05 ($P < 0.05$) due to a decrease in subendocardial blood flow, when the pacing rate was increased to HR_{st+30}.

External work (per beat) of the left anterior descending coronary artery perfusion territory had decreased by as much as 50% after production of stunning. While infusion of saline had no effect on external work, the latter recovered almost completely during administration of the lower dose of EMD 60263 and increased to 130% of baseline ($P < 0.05$ vs. baseline) following administration of the higher dose. In the left circumflex coronary artery perfusion territory, external work had decreased slightly at the end of the second ischaemia–reperfusion cycle. External work was not affected by the lower dose of EMD 60263, but showed a similar increase as in the stunned myocardium during the higher dose. Raising heart rate to HR_{st+30} resulted in a significant fall of external work of both normal and stunned myocardium, which was accompanied by a smaller decrease in myocardial oxygen consumption per beat of stunned myocardium.

In view of the larger decrease in external work than in myocardial oxygen consumption per beat, it follows that mechanical efficiency had decreased (to approximately 60% of baseline) in stunned myocardium. This decrease remained unchanged during infusion of saline, but administration of EMD 60263 completely restored mechanical efficiency. When heart rate was raised to HR_{st+30} mechanical efficiency tended to decrease ($P = 0.60$).

3.3. Segment shortening during systole and post-systole

At the end of the occlusion–reperfusion sequences, systolic shortening had decreased to $46 \pm 4\%$ of baseline in stunned myocardium, while systolic shortening of normal myocardium remained unchanged. Infusion of saline had no effect on systolic shortening of either the stunned or normal myocardium, while the increase in heart rate to HR_{st+30} resulted in a decrease in systolic shortening of stunned myocardium ($P < 0.05$) and tended to decrease systolic shortening of normal myocardium. Infusion of EMD 60264 and subsequent restoration of heart rate to HR_{st} had no effect on systolic shortening in either the normal or stunned myocardium (Table 3,Figs. 2 and 3).

After infusion of 1.5 mg/kg of EMD 60263, systolic shortening of stunned myocardium increased from $9 \pm 1\%$ to $15 \pm 1\%$, while systolic shortening of the normal myocardium increased from $14 \pm 1\%$ to $17 \pm 1\%$ (both $P < 0.05$). Administration of 3.0 mg/kg of EMD 60263 increased systolic shortening of both the stunned and normal myocardium even further to $24 \pm 2\%$ and $22 \pm 1\%$, respectively (both $P < 0.05$ vs. pre-stunning baseline). When heart rate was raised to HR_{st}, systolic shortening decreased to baseline values in both the stunned and normal my-

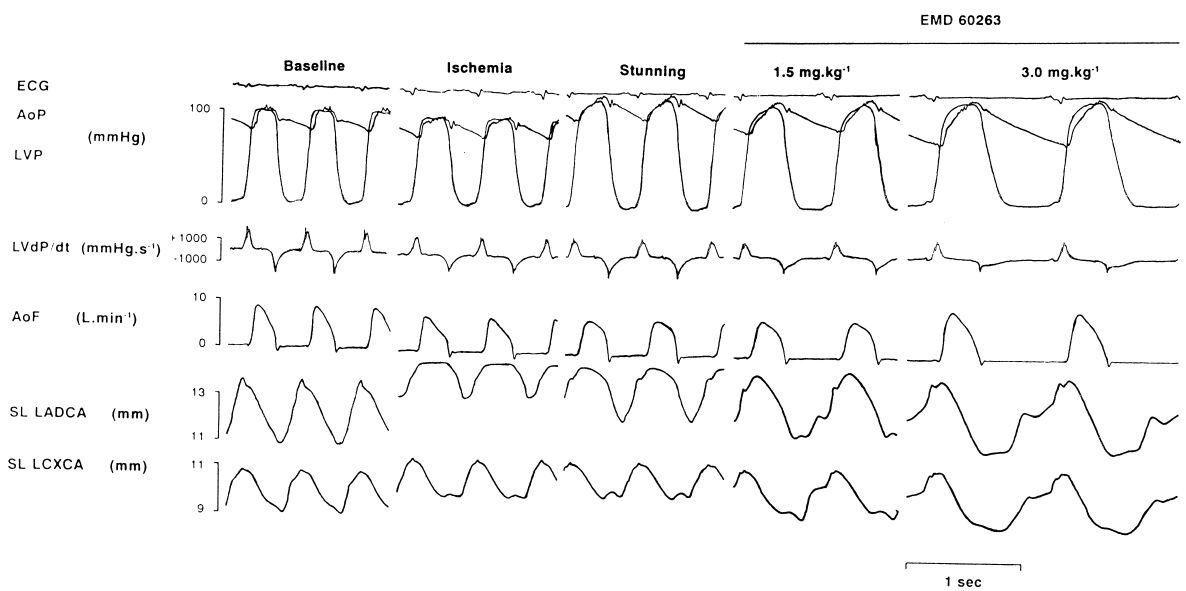

Fig. 2. This recording of a representative experiment with EMD 60263 shows that systolic shortening already recovered during infusion of the lower dose of EMD 60263 and that in both stunned (LADCA) and normal (LCXCA) myocardium the onset of segment lengthening was delayed after administration of the higher dose. ECG, electrocardiogram; AoP, aortic pressure; LVP, left ventricular pressure; LV$dP/dt$, left ventricular dP/dt; AoF, aortic blood flow; SL, segment length, LADCA, left anterior descending coronary artery; LCXCA, left circumflex coronary artery.

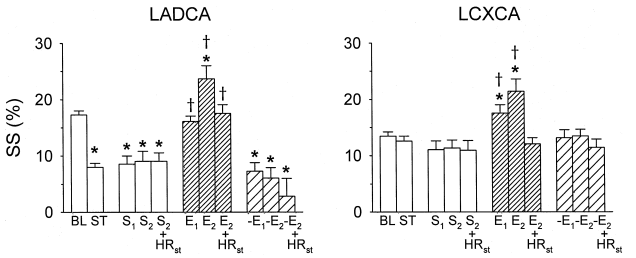

Fig. 3. Systolic shortening (SS) at baseline (BL), during stunning (ST) and after infusion of two volumes of saline (3 ml [S_1] and 6 ml [S_2]) or two doses of EMD 60263 (1.5 mg/kg [E_1] and 3.0 mg/kg [E_2]) or EMD 60264 (1.5 mg/kg [$-E_1$] and 3.0 mg/kg [$-E_2$]). After administration of the high dose, the heart rate was increased to heart rate at stunning (HR_{st}) to eliminate the effect of bradycardia. $^{*}P < 0.05$ vs. baseline; $^{\dagger}P < 0.05$ vs. stunning; LADCA = left anterior descending coronary artery distribution area; LCXCA = left circumflex coronary artery distribution area.

ocardium and to levels below baseline when heart rate was further increased to HR_{st+30}.

Post-systolic shortening was negligible under baseline conditions, but became apparent during coronary artery occlusion (Fig. 2) and persisted during reperfusion. Neither infusion of saline nor that of EMD 60264 affected post-systolic shortening. However, post-systolic shortening was markedly attenuated after infusion of the lower dose of EMD 60263 and was completely abolished after administration of the higher dose. Atrial pacing had no effect on post-systolic shortening in any of the three experimental groups.

3.4. Rate of segment lengthening during diastole

The stunning protocol reduced the mean rate of half end-diastolic lengthening in the territory perfused by the left anterior descending coronary artery to $35 \pm 4\%$ of baseline ($n = 21$), but had no effect on the mean rate of half end-diastolic lengthening of the adjacent normal myocardium ($96 \pm 7\%$ of baseline). Infusion of saline and the subsequent increase in heart rate to 30 bpm above baseline did neither affect the mean rate of half end-diastolic lengthening of the stunned nor that of the normal myocardium. Administration of EMD 60264 caused a dose-dependent decrease in the mean rate of half end-diastolic lengthening of both the stunned and normal myocardium to $16 \pm 5\%$ and $61 \pm 8\%$ of baseline, respectively (both $P < 0.05$ vs. stunning). During pacing at HR_{st}, mean rate of half end-diastolic lengthening remained unchanged in both stunned and normal myocardium (Fig. 4).

Administration of EMD 60263 caused a dose-dependent increase in the mean rate of half end-diastolic lengthening of the stunned myocardium (Fig. 4). However, at variance with systolic shortening, recovery of mean rate of half end-diastolic lengthening was only partial (to $66 \pm 11\%$ of baseline; $P < 0.05$ vs. baseline). The major reason was that, while under baseline conditions the segment started to lengthen immediately after L_{min} had been reached ($\Delta T = 0$), there was a delay ($\Delta T = 210 \pm 40$ ms) in the onset of segment lengthening during administration of the higher dose of EMD 60263. Once the segment started to lengthen, the rate of lengthening was not different from control. At HR_{st}, mean rate of half end-diastolic lengthening increased to $92 \pm 16\%$ of baseline predominantly because of a reduction in ΔT to 60 ± 20 ms. The further increase in heart rate to HR_{st+30} did not affect mean rate of half end-diastolic lengthening ($93 \pm 18\%$ of baseline). In contrast to the stunned myocardium, mean rate of half end-diastolic lengthening of the normal myocardium decreased dose-dependently to $55 \pm 3\%$ of baseline ($P < 0.05$), due to a doubling of T_{50}. The latter was again caused by an in-

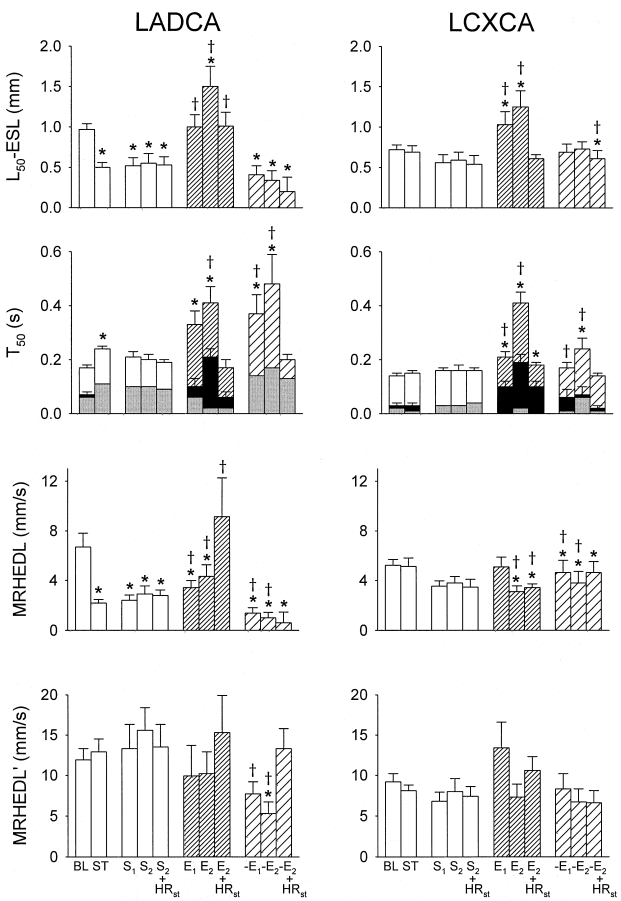

Fig. 4. From top to bottom are shown the effects of EMD 60263 on (L_{50} −ESL), T_{50}, mean rate of half end-diastolic lengthening (MRHEDL) and mean rate of half end-diastolic lengthening (MRHEDL) at baseline (BL), during stunning (ST) and after infusion of two volumes of saline (3 ml [S_1] and 6 ml [S_2]) or two doses of EMD 60263 (1.5 mg/kg [E_1] and 3.0 mg/kg [E_2]) or EMD 60264 (1.5 mg/kg [$-E_1$] and 3.0 mg/kg [$-E_2$]). After administration of the high dose, the heart rate was increased to heart rate at stunning (HR_{st}) to eliminate the effect of bradycardia. Notice that T_{50} includes T_{PSS} (grey bars) and ΔT (solid bars) (see Fig. 1). $^{*}P < 0.05$ vs. baseline; $^{\dagger}P < 0.05$ vs. stunning; LADCA = left anterior descending coronary artery distribution area; LCXCA = left circumflex coronary artery distribution area.

crease of ΔT to 210 ± 40 ms. Increasing heart rate to HR_{st+30} increased mean rate of half end-diastolic lengthening to $77 \pm 11\%$ as ΔT decreased to 130 ± 20 ms in the normal myocardium; this decrease in ΔT of the normal myocardium was, however, significantly less than in the stunned myocardium ($P < 0.05$).

4. Discussion

The use of Ca^{2+}-sensitizing agents in the treatment of cardiovascular conditions is still under debate. While there is a general consensus that Ca^{2+} sensitizers may be superior positive inotropic agents compared to beta sympathomimetic drugs as they can increase force production with no (or negligible) increments in energy cost (De Tombe et al., 1992; Hata et al., 1992; Gross et al., 1993), considerable concern has been expressed that the same characteristics could slow relaxation and elevate diastolic tension (White et al., 1993; Hgashiyama et al., 1995; Hajjar et al., 1997; Korbmacher et al., 1997). Several studies have examined the effects of Ca^{2+}-sensitizing agents on diastolic function of myocardium (Table 4). These studies differ with respect to species, myocardial tissue localization (right vs. left ventricle), state of tissue (normal or diseased), type of agent used (''pure'' Ca^{2+} sensitizer or mixed Ca^{2+} sensitizer/phosphodiesterase III inhibitor), doses used and the diastolic functional parameter that is studied. Studies in isolated intact or skinned muscle fibers obtained from right or left ventricle using EMD 53998, its (+) enantiomer EMD 57033, levosimendan, MCI-154 or CGP 48506 have yielded equivocal results with either no change or a reduction in the rate of relaxation (Table 4), while one study reported that the Ca^{2+}-sensitizing substance caffeine improved relaxation (Palmer and Kentish, 1997). Moreover, studies in isolated or in situ hearts have shown that compounds such as EMD 57033, MCI-154 and levosimendan do not exert a negative effect on diastolic function of both normal and globally stunned myocardium, while MCI-154 enhanced relaxation in failing human (Mori et al., 1995; Takaoka et al., 1997) and canine myocardium (Teramura et al., 1997). However, these agents also possess phosphodiesterase III inhibiting properties and it has been argued that the beneficial effect of phosphodiesterase III inhibition on diastolic function may have counterbalanced any adverse effect of the increased Ca^{2+} sensitivity. This argument cannot be dismissed as in none of the aforementioned studies of the agents were administered in the presence of adrenergic blockade. This is also corroborated by the observation that when levosimendan was administered in the presence of the selective bradycardic agent zatebradine to prevent the tachycardia by levosimendan, the increase in the maximum rate of fall of LV pressure and the decrease in the relaxation time constant tau were no longer observed, indicating that the improved relaxation depended entirely on the tachycardia, which is a feature of phosphodiesterase III inhibition (Pagel et al., 1995). In a recent study in awake pigs, we observed that EMD 57033 either in the absence or presence of β-adrenoceptor blockade did not alter $LVdP/dt_{min}$ or LV end-diastolic pressure (Stubenitsky et al., 1997). Those findings suggest that the lack of effect of EMD 57033 on diastolic function is not simply the result of simultaneous myocardial phosphodiesterase III inhibition. However, to allow the selective study of Ca^{2+}-sensitization on diastolic function in the present study we employed EMD 60263, a compound that is devoid of phosphodiesterase III inhibitory actions (Solaro et al., 1993; Ravens et al., 1996).

In the present study, EMD 60263 produced a decrease in $LVdP/dt_{min}$ (i.e., became less negative), suggesting that it slowed the early diastolic relaxation of the left ventricle as a whole. However, this decrease was at least in part mediated by the bradycardic effect of EMD 60263, as it was partially restored when the hearts were paced at pre-drug levels. In the EMD 60264 group elevating heart rate to pre-drug levels did not increase $LVdP/dt_{min}$, furthermore suggesting an adverse effect of inhibition of delayed inward rectifier K^+ current on early relaxation. It must therefore be assumed that the EMD 60263-induced decrease in $LVdP/dt_{min}$ is at least in part due to the effect on the delayed inward rectifier K^+ current.

As index of ''early'' regional diastolic function we used analogous to Tilton et al. (1985) and Charlat et al. (1989) the mean rate of half end-diastolic lengthening. The latter group of authors (Charlat et al, 1989) has shown that in conscious dogs this index of early diastolic function is as severely depressed as systolic function. Moreover, the time course of rate of recovery during the early hours of reperfusion was faster for systolic than for diastolic function, although complete recovery of diastolic function preceded that of systolic function. In the present study, the lower dose of EMD 60263 resulted in a complete recovery of systolic shortening, but in only a partial recovery of the mean rate of half end-diastolic lengthening. With the higher dose of EMD 60263, systolic shortening of the post-ischaemic myocardium exceeded baseline values, but the mean rate of half end-diastolic lengthening remained depressed at $66 \pm 11\%$ of baseline.

The depressed mean rate of half end-diastolic lengthening associated with the high dose was the result of a delay in the onset of segment lengthening ($\Delta T \neq 0$), because once the segment started to lengthen, the rate of lengthening was not different from baseline. To exclude that the effect of EMD 60263 on the delayed inward rectifier K^+ current and on heart rate contributed to the increase in ΔT, we not only included a group of animals treated with EMD 60264, but we also studied the actions of EMD 60263 after eliminating its bradycardic action by atrial pacing. Since EMD 60264 did not cause an increase in ΔT, this suggests that the increase in ΔT with the higher dose of EMD 60263 was not the result of an effect on the delayed inward rectifier K^+ current. In addition, retrospective in-

Table 4
Current studies of effects of Ca^{2+} sensitizers on diastolic function
IHD = ischemic heart disease; $T_{1/2}$ = time to 50% peak tension or peak pressure; $T_{80\%}$ = time to 80% peak tension; τ = time constant of tension or pressure decay; LV(SP) = left ventricular (systolic pressure); RV = right ventricular; DF; diastolic force; $LVdP/dt_{min}$ = maximum rate of fall of LV pressure; PCWP = pulmonary capillary wedge pressure; ANS = autonomic nervous system.

Authors	Model	Myocardium	Ca^{2+} sensitizer	Dose	Relaxation-parameters	Remarks
White et al. (1993)	isolated ferret papillary muscle stimulated electrically	normal	EMD 57033	0.1–20 μM	$T_{1/2}$ ↑	at doses > 1 μM
Simnett et al. (1993)	skinned fibers of guinae pig right ventricle	normal	EMD 57033	10 μM	τ ↔	
Pagel et al. (1994)	anesthetized dogs	normal	levosimendan	0.5–4 μg kg^{-1} min^{-1} i.v.	$LVdP/dt_{min}$ ↔, τ ↔	ANS blockade
	awake dogs	normal	levosimendan	0.5–4 μg kg^{-1} min^{-1} i.v.	$LVdP/dt_{min}$ ↔, τ ↔	ANS blockade
Korbmacher et al. (1994)	isolated rabbit hearts	normal	EMD 57033	30 μM	$LVdP/dt_{min}$ ↑, LVEDP ↔	LVSP increased
		stunned	EMD 57033	30 μM	$LVdP/dt_{min}$ ↑, LVEDP ↔	LVSP increased
Pagel et al. (1995)	awake dogs	normal	levosimendan	0.5–4 μg kg^{-1} min^{-1} i.v.	$LVdP/dt_{min}$ ↑, τ ↓	
	awake dogs	normal	levosimendan	0.5–4 μg kg^{-1} min^{-1} i.v.	$LVdP/dt_{min}$ ↔, τ ↔	HR held constant by zatebradine
Mori et al. (1995)	awake humans	IHD	MCI-154	1.5 μg/kg + 0.12 μg kg^{-1} min^{-1} i.v.	τ ↓, $dV/dt/_{max}$ ↔	
Korbmacher et al. (1995)	isolated rabbit hearts	stunned	EMD 57033	10–30 μM	LVEDP ↔, dP/dt_{min} ↑ LVSP↑	
Hgashiyama et al. (1995)	isolated ejecting rabbit hearts	normal	EMD 57033	5–5.8 μM	$LVdP/dt_{min}$ ↔, $T_{1/2}$ ↑, LVEDP ↑	
Haikala et al. (1995)	paced guinae pig papillary muscle	normal	levosimendan	0.03–3 μM	''relaxation time'' ↔	
			EMD 53998	0.1–3 μM	''relaxation time'' ↑	
Hajjar et al. (1997)	human LV trabeculae	normal failing	EMD 57033	1–50 μM	DF ↑, $T_{80\%}$ ↑	effect greater in failing than in normal hearts
Palmer and Kentish (1997)	rat skinned RV trabeculae	normal	Caffeine	20 mM	τ ↓	
			CGP 48506	10 μM	τ ↔	
Takaoka et al. (1997)	humans	IHD	MCI-154	1.5–3 μg/kg + 16.6–33.2 μg/min i.v.	PCWP↓	
Korbmacher et al. (1997)	isolated rabbit hearts	normal and stunned	EMD 60263	3 μM	$LVdP/dt_{min}$ ↑	Detrimental effects less in stunned than normal hearts
				10 μM	$LVdP/dt_{min}$ ↔	
				30 μM	$LVdP/dt_{min}$ ↓	
Teramura et al. (1997)	open-chest dogs	normal	MCI-154	1 μg kg^{-1} min^{-1} i.v.	$T_{1/2}$ ↔	
		failing	MCI-154	1 μg kg^{-1} min^{-1} i.v.	$T_{1/2}$ ↓	
Stubenitsky et al. (1997)	awake swine	normal	EMD 57033	0.2–0.8 mg kg^{-1} min^{-1} i.v.	$LVdP/dt_{min}$ ↔, LVEDP ↔	Similar response after β-blockade

spection of diastolic function of normal and stunned myocardium during infusion of the selective bradycardic agent zatebradine, which reduced heart rate to 55 bpm (Soei et al., 1994), indicates that bradycardia per se cannot also explain the delay in the onset of relaxation. Interestingly, pacing blunted the delay in onset of relaxation, but this effect was more pronounced in the stunned than in the normal myocardium.

The EMD 60263-induced increase in ΔT is consistent with in vitro reports that thiadiazinone derivatives prolong the duration of force development by enhancing the cross-bridge interaction between actin and myosin (Solaro et al., 1993; Ravens et al., 1996). This increase in ΔT may have an adverse effect on global left ventricular function, as it could impair left ventricular filling of which the largest fraction normally occurs during early diastole. Although after the higher dose of EMD 60263 left ventricular end-diastolic pressure increased, we failed to observe an impairment in left ventricular function as both the rate of segment lengthening and the end-diastolic segment length were not affected. Furthermore, systolic function was not impaired as stroke volume even increased to above baseline values. In a previous study using the same model, induction of bradycardia to similar heart rates with the specific bradycardic agent zatebradine increased left ventricular end-diastolic pressure to the same extent without affecting end-diastolic segment length (unpublished data). Similarly, zatebradine increased stroke volume although significantly less than EMD 60263 (Soei et al., 1994). These observations suggest that left ventricular filling and global systolic left ventricular function were not adversely affected by an increase in myofibrillar sensitivity to Ca^{2+} during the EMD 60263-induced bradycardia.

In the present study restoration of heart rate to pre-drug levels (HR_{st}) in the presence of EMD 60263, decreased systolic shortening in both stunned and normal myocardium, while the effects on regional diastolic segment dynamics differed between stunned and normal myocardium. The different response of the delay of relaxation to pacing in stunned and normal myocardium was unexpected, particularly in view of evidence that pacing can induce an increase in intracellular Ca^{2+} concentration (Langer, 1968; Frampton et al., 1991) during both systole and diastole, which would tend to augment the EMD 60263-induced diastolic relaxation abnormalities. On the other hand, Kusuoka et al. (1986) reported that pacing increases intracellular inorganic phosphate levels, which could reduce the myofibrillar sensitivity to Ca^{2+}. The latter observation may explain why despite an increased diastolic intracellular Ca^{2+} concentration pacing improved relaxation in the stunned myocardium (i.e., reduced the time delay to onset of segment lengthening). The persisting delay in segment lengthening in normal myocardium during pacing at a time when diastolic function of stunned myocardium was normal is supported by in vitro observations of Korbmacher et al. (1997) in isolated rabbit hearts. This finding suggests that, despite the presence of EMD 60263, a difference in myofibrillar Ca^{2+} sensitivity remains between stunned and normal myocardium.

The prolonged development of force could be most detrimental when diastole is shortened, i.e., at higher heart rates. Thus, to study the physiological significance of the observed delay in diastolic lengthening, we paced the hearts at 30 beats/min above stunning level (HR_{st+30}). Pacing at HR_{st+30} decreased the duration of diastole to below baseline value, which in the saline-treated group decreased end-diastolic segment length and systolic shortening of both stunned and normal myocardium. The delay in diastolic lengthening produced by EMD 60263 further shortened the period of diastolic lengthening and resulted in a decrease in EDL of both stunned and normal myocardium and an impairment of systolic shortening. However, only the changes in normal myocardium and not in stunned myocardium of the EMD 60263 treated group were statistically significant compared to the changes in the saline-treated group. This finding suggests that diastolic function of normal myocardium is more susceptible to increases in myofibrillar Ca^{2+} sensitivity than that of stunned myocardium, which is supported by observations in normal and post-ischaemic isolated rabbit hearts (Korbmacher et al., 1997).

Another important point to consider in the assessment of the effect of Ca^{2+}-sensitizers on diastolic function is the dose required to normalize systolic function relative to the dose at which Ca^{2+}-sensitizers exert a negative effect on diastolic function. In the present study, the higher dose (3.0 mg/kg, i.v.) is in excess of that needed to restore systolic shortening of stunned myocardium (Soei et al., 1994). Indeed, in the present study, 1.5 mg/kg already normalized systolic segment shortening but had no adverse effect on the onset of relaxation. Similarly, previous studies have also indicated that the impairment of diastolic function by Ca^{2+}-sensitizers is likely a dose-related finding. Thus, Hajjar et al. (1997) reported that EMD 57033 exerted its negative lusitropic effects in normal human myocardium at doses higher than 10 μM. Korbmacher et al. (1997) observed, however, that at 3 μM EMD 57033 increased $LVdP/dt_{max}$ in post-ischaemic isolated rabbit hearts by 50% while improving early relaxation. A further increase to 10 μM had no additional effect on $LVdP/dt_{max}$ but tended to decrease the maximum rate of relaxation. Finally, at 30 μM and consistent with the report of Hajjar et al. (1997), diastolic function was seriously impaired resulting in deterioration of systolic function.

In conclusion, in the present study, both doses of EMD 60263 improved systolic function (shortening and mechanical efficiency) as well as diastolic function (mean rate of half end-diastolic lengthening) of stunned myocardium. The high dose delayed relaxation of normal myocardium but without adversely affecting systolic function in the presence and absence of the EMD 60263 induced-bradycardia.

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