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Carvedilol improves function and reduces infarct size in the feline myocardium by protecting against lethal reperfusion injury

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Abstract

This study examined the effect of carvedilol, a vasodilating β-adrenoceptor antagonist and antioxidant, on lethal reperfusion injury in feline hearts subjected to 40 min of regional ischaemia and 180 min of reperfusion. 30 open chest anaesthetized cats were randomized into three groups. A control group ($n = 10$) was compared with a group given carvedilol before coronary artery occlusion ($n = 10$) and a group given carvedilol immediately before and during early reperfusion ($n = 10$). Regional myocardial function was measured by sonomicrometry. Infarct size was determined by staining the left ventricle with triphenyl tetrazolium chloride. Myocardial blood flow was measured by radiolabeled microspheres. Tissue levels of glutathione were measured after reperfusion. Infarct size was significantly reduced compared to control both when carvedilol was given before ischaemia (0.2 ± 0.1 vs. $17.6 \pm 3.6\%$, $P < 0.05$) and when given immediately before reperfusion (3.7 ± 1.3 vs. $17.6 \pm 3.6\%$, $P < 0.05$). Regional shortening improved significantly and the incidence of ventricular fibrillation during early reperfusion was reduced in both groups treated with carvedilol compared to control. Oxidized glutathione did not differ between groups in the post-ischaemic myocardium. This study supports that lethal reperfusion injury is a significant phenomenon. Furthermore, carvedilol reduces infarct size and reperfusion arrhythmias, and improves post-ischaemic regional myocardial function by protecting against both ischaemic and lethal reperfusion injury. The present study does not answer whether it is the non-selective β- or α_1-adrenoceptor antagonism, the antarrhytmic or the antioxidant actions of carvedilol that is responsible for the protective effect.

Keywords: Carvedilol; (Cat); Myocardial ischaemia; Reperfusion injury, lethal

1. Introduction

Reperfusion of the ischaemic myocardium is an absolute necessity to limit infarct size during early stages of evolving myocardial necrosis (Reimer et al., 1993). However, the early phase of reperfusion may be harmful to the myocardium by aggravating cellular injury caused by ischaemia. There is strong evidence that reperfusion injury following reversible ischaemia contributes to reversible post-ischaemic contractile dysfunction (Bolli, 1990, 1991). It has also been suggested that reperfusion of the ischaemic myocardium may induce irreversible damage to myocytes that are reversibly injured at the end of an ischaemic period, a phenomenon named lethal reperfusion injury (Becker and Ambrosio, 1987; Engler and Gilpin, 1989; Kloner, 1993; Reimer et al., 1993; Simpson and Lucchesi, 1987). Review articles discussing the relevance of lethal reperfusion injury conclude that there is no consensus on whether or not lethal reperfusion injury is a significant phenomenon (Bolli, 1991; Jeroudi et al., 1994; Kloner, 1993; Reimer et al., 1993; Willerson and Buja, 1990). Recently, Zahger et al. (1995) proposed direct evidence against the existence of lethal reperfusion injury in a single-canine-heart model of ischaemia and reperfusion.

Most of the studies aiming at protection against lethal reperfusion injury have used antioxidative therapy, but results are inconsistent (Bolli, 1991; Willerson and Buja, 1990). Studies have suggested that invasion of leukocytes play a role in the pathogenesis of lethal reperfusion injury

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(Litt et al., 1989), and that inhibition of hypercontracture protects against lethal reperfusion injury (Schlack et al., 1994). In a review, Opie (1989) stated that there are no data on effects of β-adrenoceptor antagonists given only during the reperfusion period. To our knowledge, there are no recent studies that have examined or discussed the role of the adrenergic system in lethal reperfusion injury.

Carvedilol is a vasodilating adrenoceptor antagonist possessing non-selective β- and α_1-adrenoceptor antagonism (Nichols et al., 1989a,b, 1991). In addition, it has been shown to possess antioxidative properties in vitro (Feuerstein et al., 1993; Yue et al., 1992, 1993). It is well known that β-adrenoceptor blockade during myocardial ischaemia can reduce infarct size by reducing oxygen demand in the myocardium through reduction of cardiac work and wall tension. Previous studies with carvedilol administered *before* an ischaemic episode have demonstrated a more potent infarct-reducing effect in several species compared to other non-selective β-adrenoceptor antagonists (Bril et al., 1992; Feuerstein et al., 1992; Hamburger et al., 1991). It has been speculated whether the antioxidative property of the drug in addition to adrenoceptor blockade may contribute to this potent cardioprotective effect (Feuerstein et al., 1993).

Based on the lack of information on effects of β-adrenoceptor antagonists on lethal reperfusion injury, the main aim of the present study was to investigate whether carvedilol could protect against lethal reperfusion injury. Since salvage of ischaemic myocardium by reperfusion is most effective during early stages of evolving necrosis, we chose a model with only subendocardial infarction, where the majority of myocytes in outer wall layers would be reversibly injured at the end of the ischaemic period (Brunvand et al., 1995).

2. Materials and methods

2.1. Animal preparation

The experimental protocol was approved by the Norwegian Committee for Research on Animals. 30 male cats (Iffa Credo, L'Arbresle, France) weighing 3.1–5.1 kg were anaesthetized with sodium pentobarbital (40 mg/kg i.p.), tracheotomized and ventilated with a positive pressure ventilator delivering 50% N_2O, 47.5% O_2 and 2.5% CO_2 (Loosco Infant Ventilator MK2, Amsterdam, The Netherlands). Body temperature was held constant with a heat blanket regulated via a rectal thermistor. Midline thoracotomy and pericardiotomy provided access to the heart. A pressure tip transducer (Millar MPC 500, Houston, TX, USA) was introduced into the left ventricle through the apex for recording of left ventricular pressure, its first derivative (dP/dt) and heart rate. The left atrium was cannulated with a polyethylene catheter for microsphere injections. A catheter was placed in the abdominal aorta via the left femoral artery for reference blood sampling. The left femoral vein was cannulated for infusion purposes. The proximal part of the left anterior descending coronary artery, supplying the anterior left ventricular wall, was dissected free for later occlusion. Two pairs of piezo-electric crystals (1.0 mm diameter, 5 MHz) were implanted in the midmyocardium of the anterior wall of the left ventricle. One pair (longitudinal segment) was positioned 15° clockwise to an axis from the main stem of the left coronary artery to the apex of the left ventricle and, thus, parallel to subendocardial fibres in that region (Hexeberg et al., 1989, 1991). The other pair (circumferential segment) was positioned perpendicular to the longitudinal segment and aligned mid and outer wall fibres. Segment lengths were measured with a Sonomicrometer 102.2 (Triton Technology, San Diego, CA, USA). Left ventricular pressure and segment length signals were recorded on magnetic tape (Instrumentation tape recorder 3694A; Hewlett-Packard, Waltham, MA, USA) and later digitized at a sampling rate of 200 Hz (CED 1401 Intelligent Data Interface; Cambridge Electronic Design, Cambridge, UK), transferred to a microcomputer (Acorn Archimedes 310, Cambridge, UK) and analysed by a program developed in our laboratory.

2.2. Experimental protocol

30 cats were randomized into three groups ($n = 10$ in each group), receiving either vehicle (100 μl dimethylformamide 10% acidified with HCl and diluted in 0.9% NaCl to a total volume of 10 ml, the final solution being pH neutral) or carvedilol (1 mg/kg) dissoluted in the vehicle i.v. in a volume of 1 ml/min for 10 min. This dosage was based on previous studies using carvedilol i.v. in a variety of animal models for comparing the effects of carvedilol and the β-adrenoceptor antagonist propranolol on ischaemic injury (Bril et al., 1992; Feuerstein et al., 1992, 1993; Hamburger et al., 1991; Yue et al., 1993). As non-selective β-adrenoceptor antagonist, 1 mg/kg of carvedilol is considered equipotent to 1 mg/kg of propranolol.

All animals were subjected to 40 min of left anterior decending coronary artery occlusion and 180 min of reperfusion. The control group was given vehicle both prior to coronary occlusion and prior to reperfusion. The two treatment groups were either given carvedilol before coronary occlusion and vehicle prior to reperfusion, or vehicle before occlusion and carvedilol immediately before and during early reperfusion (Fig. 1). The administration of the drug/vehicle was performed as follows: infusion of either vehicle or carvedilol was started 15 min before coronary artery occlusion and was terminated 5 min prior to occlusion. 2 min before onset of reperfusion, infusion of either vehicle or carvedilol was started and continued for the first 8 min of reperfusion. Intravenous infusion of 0.9% saline at a rate of 15 ml/kg per h was continued throughout the

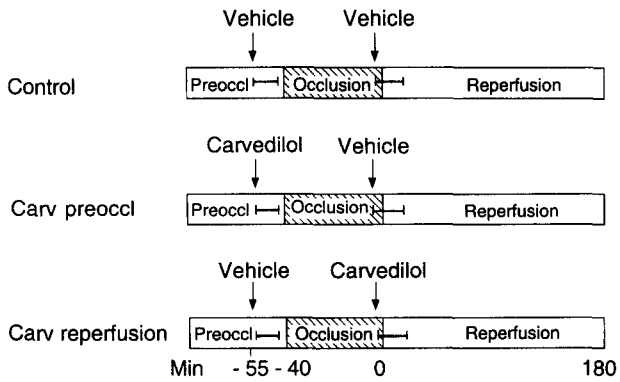

Fig. 1. Experimental protocol. Administration of vehicle and carvedilol is shown relative to the onset of coronary occlusion and reperfusion. Control, control group; Carv preoccl, animals treated with carvedilol before coronary occlusion; Carv reperf, animals treated with carvedilol during initial reperfusion. The infusion period (10 min) of carvedilol or vehicle is specifically marked.

experiment. Heparin (500 IU/kg) was given i.v. before coronary artery occlusion and followed by additional doses (250 IU/kg) every 30 min to prevent microthrombi.

Baseline haemodynamic recordings were performed before and after the administration of vehicle or carvedilol was ended. After the first administration of vehicle or carvedilol, pre-occlusion haemodynamics were recorded and the first microsphere injection was performed. The left anterior decending coronary artery was then occluded by a non-traumatic vessel clamp. The second microsphere injection and haemodynamic recording were performed 30 min after the onset of occlusion. The third and fourth microsphere injections were performed after 10 and 180 min of reperfusion. Haemodynamic recordings were performed after 5, 10, 30, 60, 120 and 180 min of reperfusion. The heart was arrested by injecting 50 ml of cold cardioplegic solution (Na^+ 120, K^+ 16, Ca^{2+} 1.2, Mg^{2+} 16, Cl^- 160 and HCO_3^- 10 mM, with 1 mmol procaine hydrochloride) via a catheter through the apex into the aortic root with partial occlusion of the ascending aorta. At the end of cardioplegic arrest, the coronary artery was re-occluded and yellow fluorescing zinc cadmium sulphide particles (1–10 μm; Duke Scientific, Palo Alto, CA, USA) suspended in saline (0.9%) were injected into the open coronary arteries. The heart was removed from the animal, and biopsies for measurements of glutathione levels were obtained from the left ventricle, frozen in liquid nitrogen and stored at −80°C for later analysis. The rest of the ventricle was frozen for later infarct size measurements.

2.3. Histochemical staining

The frozen ventricle was cut in horizontal slices from apex to base, each 2–3 mm thick. The slices were incubated at 37°C in 200 ml 1% phosphate-buffered 2,3,5-triphenyltetrazolium chloride at pH 7.4 (Sigma, St. Louis, MO, USA) for 20 min. Slices were then placed in 4% formaldehyde overnight, compressed to a standard 2 mm thickness between glass plates and illuminated with ultraviolet light. Area at risk was identified as non-fluorescing, viable tissue was stained brick red, whereas necrotic tissue appeared pale or brown. The slices were traced on plastic overlays, demarcating the whole area of the slice, area at risk and necrotic areas. The demarcated areas were determined by planimetry of the tracings. Infarcted tissue was expressed as a percentage of area at risk.

2.4. Determination of oxidized and reduced glutathione

2.4.1. Standards

Reduced glutathione (GSH) and oxidized glutathione (GSSG) (Sigma) were dissolved at a concentration of 500 μM in 5% 5-sulfosalisylic acid (Merck, Darmstadt, Germany) containing 50 μM dithioerythritol (Sigma).

2.4.2. Sample processing

Myocardial samples (stored at −80°C) were homogenized in 5% 5-sulfosalisylic acid and 50 μM dithioerythritol to a final 10% solution. All procedures were performed at 0–4°C. GSH was determined in 30 μl of protein-free myocardial homogenate added 30 μl of 5% 5-sulfosalisylic acid/50 μM dithioerythritol, 160 μl distilled water, 50 μl 1.0 M *N*-ethylmorpholine (Sigma) and 10 μl 20 mM monobromobimane (Calbiochem-Behring Diagnostics, La Jolla, CA, USA). After a 10-min dark incubation period in room temperature, 20 μl 10% perchloric acid was added. The samples were again stored at −80°C until analysed.

GSSG was determined by reducing glutathione with $NaBH_4$ (Fluka Chemie, Switzerland), producing total myocardial GSH. Free sulfhydryl groups of GSH were subsequently derivatized with monobromobimane. GSH was subtracted from total GSH giving the GSSG level. The determination of GSH was performed as earlier described (Svardal et al., 1990). Briefly, total GSH was determined from 30 μl protein-free myocardial homogenate added 1.4 M $NaBH_4$ in 50 mM NaOH, 160 μl 0.9% NaCl containing 140 mM hydrogen bromide and 44% dimethyl sulfoxide (Merck), 50 μl 1.0 M *N*-ethylmorpholine and 10 μl 20 mM monobromobimane in acetonitrile. After 10-min dark incubation in room temperature, 20 μl 10% perchloric acid was added. The samples were stored at −80°C until analysed.

Both GSH and total GSH were determined by reversed-phase high-pressure liquid chromatography (HPLC) using a Shimadzu RF-535 fluorometer detector (Shimadzu, Kyoto, Japan) and Spectraphysics P4000 gradient pump, AS3000 autosampler and SP4270 integrator (Spectra-Physics, San Jose, CA, USA). Determination of GSH was performed with a gradient system using a 3-μm HICHROM Hypersil ODS 150 × 4.6 column (Hichro, Reading, UK). The mobile phase was run as a gradient composed of 3 solvents. Solvent A: 43.5 mM acetic acid,

10 mM phosphoric acid, 10 mM tetrabutylammoniumhydroxide (Sigma) at pH 3.4; solvent B: 43.5 mM acetic acid/10 mM phosphoric acid/10 mM tetrabutylammoniumhydroxide/20% acetonitrile at pH 3.25; solvent C: acetonitrile/distilled water (75:25, v/v).

2.5. Myocardial blood flow and cardiac output

Regional tissue blood flow in non-fluorescent and fluorescent subepi- and subendocardium, and cardiac output were determined with carbonized microspheres (15.5 ± 0.1 μm; Du Pont, Wilmington, DE, USA) labeled with ^{46}Sc, ^{51}Cr, ^{85}Sr or ^{141}Ce. Microspheres were injected into the left atrium in a randomized sequence. Approximately 10^6 spheres were given at each injection. During injection of microspheres, reference blood samples were withdrawn with a constant-rate extraction pump (Sage instruments 351, Cambridge, MA, USA) from the abdominal aorta. Specimens, reference blood, residuals and standards were counted for γ-emission (Compugamma 1282; LKB-Wallac, Turku, Finland). Tissue blood flow and cardiac output were calculated (Heyman et al., 1977).

2.6. Analysis of haemodynamics

End-diastole was defined as the timepoint where dP/dt > 100 mm Hg/s. End-systole was defined as 20 ms prior to peak negative dP/dt (Abel, 1981). Segment length at end-diastole and end-systole are EDL and ESL, respectively. Systolic shortening (SS%) [(EDL-ESL)/EDL] · 100% was calculated.

2.7. Statistical analysis

Haemodynamics, regional function, blood flow and glutathione levels were analysed by two-way analysis of variance (ANOVA) with repeated measurements. Infarct size was analysed by one-way ANOVA. Newman-Keuls multiple contrast tests were used when appropriate. The incidence of ventricular fibrillations were compared between groups by χ^2-tests. $P < 0.05$ was regarded as statistically significant. Values are mean ± S.E.M.

3. Results

3.1. Haemodynamics and blood flow

Baseline registrations before interventions are presented in Table 1 as pre-occlusion values and did not differ between groups. Heart rate was reduced by carvedilol in

Table 1
Haemodynamic results

	Preocc	Occl	Reperfusion					
			5 min	10 min	30 min	60 min	120 min	180 min
Heart rate (beats / min)								
Control	196 ± 7	200 ± 6	203 ± 9	193 ± 8	196 ± 8	199 ± 8	199 ± 9	196 ± 11
Carv preocc	189 ± 5	178 ± 4 [a,b]	177 ± 5 [a,b]	180 ± 5 [a]	187 ± 5	182 ± 4	184 ± 4	182 ± 5
Carv reperf	193 ± 8	191 ± 7	175 ± 5 [a,b]	176 ± 4 [a]	177 ± 5 [a]	182 ± 5	183 ± 4	183 ± 5
LVSP (mm Hg)								
Control	141 ± 6	137 ± 3	104 ± 6 [a]	115 ± 5 [a]	121 ± 4 [a]	133 ± 4	140 ± 7	129 ± 4
Carv preocc	133 ± 10	81 ± 6 [a,b]	81 ± 6 [a,b]	83 ± 6 [a,b]	90 ± 6 [a,b]	99 ± 5 [a,b]	111 ± 4 [a,b]	111 ± 5 [a,b]
Carv reperf	136 ± 5	135 ± 6	66 ± 3 [a,b]	68 ± 4 [a,b]	77 ± 2 [a,b]	89 ± 2 [a,b]	105 ± 4 [a,b]	104 ± 3 [a,b]
LVEDP (mm Hg)								
Control	2.4 ± 0.8	11.3 ± 2.5 [a]	10.6 ± 2.6 [a]	11.7 ± 2.6 [a]	8.4 ± 2.0 [a]	5.3 ± 1.0 [a]	4.2 ± 0.7	4.2 ± 1.2
Carv preocc	1.5 ± 0.5	8.7 ± 2.7 [a]	7.0 ± 1.8 [a]	6.1 ± 1.8 [a,b]	5.5 ± 1.6 [a]	5.1 ± 1.4 [a]	4.7 ± 1.3 [a]	5.6 ± 1.3 [a]
Carv reperf	2.6 ± 0.7	9.6 ± 2.4 [a]	5.2 ± 1.5 [a,b]	5.4 ± 1.9 [a,b]	4.6 ± 1.2	4.5 ± 0.9	5.1 ± 0.8 [a]	4.4 ± 0.7
dP/dt_{max} (mm Hg / s)								
Control	3516 ± 226	3728 ± 128	2315 ± 183 [a]	2622 ± 219 [a]	3004 ± 158 [a]	3259 ± 137	3431 ± 165	3117 ± 93 [a]
Carv preocc	3048 ± 235	2083 ± 239 [a,b]	2050 ± 197 [a]	2099 ± 200 [a,b]	2221 ± 151 [a,b]	2507 ± 104 [a,b]	2948 ± 162	2948 ± 154
Carv reperf	3299 ± 177	3504 ± 229	1429 ± 91 [a,b]	1536 ± 110 [a,b]	1930 ± 99 [a,b]	2474 ± 119 [a,b]	2907 ± 150 [a,b]	2859 ± 137 [a]
RPP (mm Hg × beat / min × 10^{-2})								
Control	277 ± 16	272 ± 6	211 ± 16	222 ± 14	237 ± 11	263 ± 11	279 ± 21	251 ± 11
Carv preocc	250 ± 21	145 ± 12 [a,b]	144 ± 12 [a,b]	150 ± 13 [a,b]	167 ± 10 [a,b]	180 ± 10 [a,b]	204 ± 10 [a,b]	201 ± 9 [a,b]
Carv reperf	264 ± 17	258 ± 14	115 ± 5 [a,b]	120 ± 5 [a,b]	136 ± 3 [a,b]	161 ± 5 [a,b]	193 ± 9 [a,b]	191 ± 9 [a,b]
CO (ml / min)								
Control	378 ± 31	289 ± 18 [a]		260 ± 24 [a]				310 ± 39
Carv preocc	367 ± 25	312 ± 29 [a]		350 ± 34 [b]				401 ± 33 [b]
Carv reperf	403 ± 20	309 ± 24 [a]		264 ± 21 [a]				338 ± 18

[a] $P < 0.05$ vs. preocc, [b] $P < 0.05$ vs. control. Values are mean ± S.E.M. ($n = 10$ in each group).
Preocc, pre-occlusion; occl, occlusion. HR, heart rate; LVSP and LVEDP, left ventricular peak systolic and end-diastolic pressures, respectively. dP/dt_{max}, the maximal first derivative of left ventricular pressure; CO, cardiac output; RPP, rate-pressure product. Control, control group; Carv preocc, animals treated with carvedilol before left anterior decending coronary artery occlusion; Carv reperf, animals treated with carvedilol during initial reperfusion.

both treatment groups. During reperfusion, heart rate gradually increased in both treatment groups. Left ventricular peak systolic pressure was significantly reduced following treatment with carvedilol. The pressure reduction was sustained throughout the reperfusion period. Left ventricular end diastolic pressure increased significantly in all groups during coronary artery occlusion. During the first 10 min of reperfusion, left ventricular end-diastolic pressure was lower in both treatment groups compared to control. At the end of reperfusion, all groups tended towards normalisation. The maximum of the first derivative of left ventricular systolic pressure (dP/dt_{max}) was not altered after 30 min of coronary artery occlusion compared to pre-occlusion in the two groups not pre-treated with carvedilol. Pre-treatment with carvedilol induced a significant reduction of dP/dt_{max} during the ischaemic period. Whereas dP/dt_{max} tended towards normalisation in the control group, it remained depressed for 60 min in the group pre-treated with carvedilol and throughout the reperfusion period in the group treated with carvedilol during reperfusion. Cardiac output was significantly depressed during coronary artery occlusion in all groups. In the group pre-treated with carvedilol, cardiac output normalized upon reperfusion, and remaining low in the two other groups. Rate-pressure product was reduced throughout the experiment in both treatment groups after administration of carvedilol.

Regional myocardial blood flow in left ventricular myocardium perfused by the left anterior decending and the circumflex branch of the left coronary artery is presented in Table 2. Pre-occlusion blood flow rate was reduced in animals pre-treated with carvedilol. In all groups, blood flow was significantly reduced to minimal levels in the ischaemic region reflecting flow restriction by total coronary occlusion. There were no significant differences between ischaemic subepi- and subendocardial blood flow. 10 min after the onset of reperfusion, there was a significant transmural reactive hyperaemia in the reperfused myocardium of the control group. In the treatment groups, the reactive hyperaemia was significantly reduced compared with control. At the end of reperfusion, blood flow returned to pre-occlusion values in all groups.

3.2. Regional function and infarct size

Administration of carvedilol prior to coronary occlusion did not alter systolic shortening (SS%) in the non-ischaemic anterior wall of the left ventricle; 10.01 ± 0.54 vs. 10.98 ± 0.56 (N.S.) in circumferential segments and 5.94 ± 0.83 vs. 5.45 ± 0.77 (N.S.) in longitudinal segments. SS% in circumferential and longitudinal segments for all groups are shown in Fig. 2. Both groups treated with carvedilol exhibited a significant recovery of SS% in both segments during reperfusion, and more pronounced than control animals. Furthermore, 8 out of 10 animals developed reversible ventricular fibrillation during the first minutes of reperfusion in the control group. Treatment with carvedilol both before coronary artery occlusion and before reperfusion significantly reduced the incidence of ventricular fibrillation compared to control (1 out of 10 and 2 out of 10 animals ($P < 0.05$ for both), respectively). Ventricular fibrillation was electroconverted to normal rhythm within seconds (2 Joule DC shock).

Area at risk did not differ significantly between groups (43 ± 3 vs. 39 ± 3 vs. 49 ± 3%, control and both treatment groups, respectively). Infarct size presented as percent

Table 2
Regional myocardial blood flow (ml/min per g)

		Preoccl	Occlusion	Reperfusion	
				10 min	180 min
CFX region					
Control	EPI	2.12 ± 0.19	2.37 ± 0.13	2.13 ± 0.27	1.96 ± 0.20
	ENDO	2.51 ± 0.17	2.59 ± 0.09	2.27 ± 0.21	2.40 ± 0.23
Carv preoccl	EPI	1.32 ± 0.13 [b]	1.90 ± 0.18 [a]	1.99 ± 0.18 [a]	2.16 ± 0.23 [a]
	ENDO	1.67 ± 0.17 [b]	1.66 ± 0.21 [b]	2.06 ± 0.24 [a]	2.52 ± 0.21 [a]
Carv reperf	EPI	1.70 ± 0.14	2.02 ± 0.23	1.22 ± 0.09 [a]	1.92 ± 0.20
	ENDO	2.07 ± 0.16	2.07 ± 0.19	1.32 ± 0.11 [a]	2.30 ± 0.24
LAD region					
Control	EPI	2.07 ± 0.21	0.20 ± 0.03 [a]	3.91 ± 0.43 [a]	1.55 ± 0.20
	ENDO	2.47 ± 0.23	0.12 ± 0.02 [a]	4.50 ± 0.69 [a]	2.27 ± 0.39
Carv preoccl	EPI	1.40 ± 0.14 [b]	0.17 ± 0.04 [a]	2.22 ± 0.55 [b]	1.68 ± 0.16
	ENDO	1.60 ± 0.15 [b]	0.11 ± 0.02 [a]	2.91 ± 0.68 [b]	2.08 ± 0.19
Carv reperf	EPI	1.82 ± 0.09	0.25 ± 0.05 [a]	1.88 ± 0.18 [b]	1.53 ± 0.13
	ENDO	2.14 ± 0.15	0.11 ± 0.02 [a]	2.14 ± 0.32 [b]	2.36 ± 0.24

[a] $P < 0.05$ vs. preoccl, [b] $P < 0.05$ vs. control. Values are mean ± S.E.M.
CFX, left circumflex coronary artery; LAD, left anterior descending coronary artery. EPI, subepicardium; ENDO, subendocardium. Preoccl, pre-occlusion. Carv preoccl, animals treated with carvedilol before LAD occlusion; Carv reperf, animals treated with carvedilol during initial reperfusion.

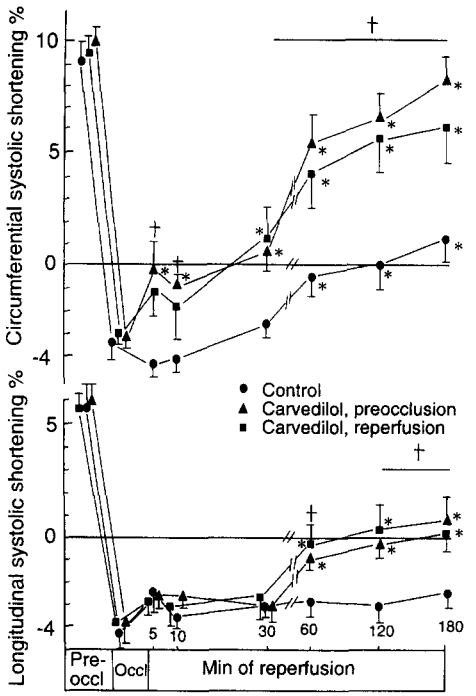

Fig. 2. Regional myocardial function. Shortening in the circumferential and longitudinal segments of the left ventricle are presented separately. Preoccl, pre-occlusion; occl, occlusion. * $P < 0.05$ vs. occlusion, † $P < 0.05$ vs. control. Bars indicate S.E.M.

necrosis of area at risk is shown in Fig. 3. Infarct size was significantly reduced in both treatment groups compared to control.

3.3. Reduced and oxidized glutathione

Reduced and oxidized glutathione (GSH and GSSG) are presented in Table 3. GSH was depleted in the region effected by coronary occlusion following reperfusion of the ischaemic tissue. The depletion was most extensive in the subendocardium compared to the subepicardium. There were no differences between groups, except a slightly higher GSH level in the group treated with carvedilol before coronary artery occlusion. In the reperfused anterior wall, there were no differences in GSSG levels between groups or layers.

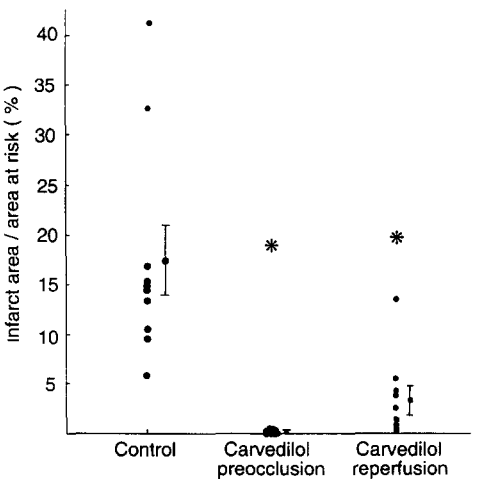

Fig. 3. Infarct size in all three groups is presented as percentage necrosis of area at risk. Each individual animal is presented in addition to mean for each group. * $P < 0.05$ vs. control. Bars indicate S.E.M.

Table 3
Myocardial reduced and oxidized glutathione (μmol/g)

	GSH		GSSG	
	EPI	ENDO	EPI	ENDO
CFX region				
Control	4.98±0.19	4.44±0.21	0.15±0.07	0.41±0.10 [a]
Carv preoccl	5.24±0.11	4.88±0.13	0.02±0.02	0.50±0.10 [a]
Carv reperf	5.19±0.13	4.83±0.12	0.05±0.04	0.45±0.07 [a]
LAD region				
Control	1.45±0.25	0.19±0.03 [a]	0.25±0.09	0.20±0.04
Carv preoccl	2.23±0.19 [b]	0.20±0.03 [a]	0.12±0.04	0.35±0.06
Carv reperf	1.79±0.17	0.33±0.01 [a]	0.21±0.11	0.29±0.06

[a] $P < 0.05$ vs. EPI. [b] $P < 0.05$ vs. control. Values are mean±S.E.M. GSH, reduced glutathione; GSSG, oxidized glutathione. EPI, subepicardium; ENDO, subendocardium. CFX region, area supplied by circumflex coronary artery; LAD region, area supplied by left anterior descending coronary artery. Carv preoccl, animals treated with carvedilol before LAD occlusion; Carv reperf, animals treated with carvedilol during initial reperfusion.

4. Discussion

The major findings of the present study were a marked reduction of infarct size together with improved regional function and reduced incidence of ventricular fibrillation following administration of carvedilol during early reperfusion. These findings indicate that lethal reperfusion injury is a significant phenomenon contributing to increased infarct size and post-ischaemic mechanical dysfunction in a model with subendocardial infarction.

Surprisingly, we have not been able to find studies on the effect of antiadrenergic treatment against lethal reperfusion injury. Prolonged coronary artery occlusion (30–60 min) in canine and feline models causes an up-regulation of β- and α-adrenoceptors on reversibly injured myocytes, and leakage of catecholamines from nerve endings during the same time period expose injured myocytes to high concentrations of catecholamines at the time of reperfusion, potentially resulting in arrhythmias, increased Ca^{2+} overloading and membrane injury (Kurz et al., 1991; Willerson and Buja, 1990). It would, thus, be reasonable to assume that adrenergic mechanisms could play a role in lethal reperfusion injury. To our knowledge, the present study is the first to demonstrate that a β-adrenoceptor antagonist (carvedilol) is able to protect against lethal reperfusion injury, and thereby reduce infarct size. Based

on findings that the longitudinal segment aligns subendocardial fibre direction, and that longitudinal segment shortening is sensitive to subendocardial ischaemia (Birkeland et al., 1992; Brunvand et al., 1995; Hexeberg et al., 1992), recovery of contractile function in longitudinal segments in animals treated with carvedilol is a consequence of reduced subendocardial necrosis, and, thus, supports the significance of infarct reduction offered by carvedilol.

4.1. Haemodynamic effects

Carvedilol treatment lead to a significant reduction of the rate-pressure product mainly due to a reduction of afterload, but also due to a moderate reduction in heart rate (Table 1). This is in accordance with previous findings that carvedilol mainly reduces systolic pressure, whereas cardiac output and heart rate are relatively unaffected, but is not asociated with reflex tachycardia (Ruffolo et al., 1993). The rate-pressure product has been used as an indirect measure of oxygen consumption (Baller et al., 1981), and demonstrated reduced oxygen consumption following carvedilol administration. Reactive hyperaemia seen in control animals during initial reperfusion is mainly due to the burst of vasoactive substances generated in the ischaemic myocardium during coronary artery occlusion. In addition, the high incidence of reperfusion arrhythmias support that heart work and oxygen demand is high during the first minutes of reperfusion. A marked reduction of reactive hyperaemia in both treatment groups (Table 2) also suggests reduced oxygen demand and alleviated effects of vasoactive substances during initial reperfusion. It has been shown that reduced blood flow rate during reperfusion may reduce infarct size, most likely through attenuation of sodium and calcium overload (Takeo et al., 1995). Thus, a lower blood flow rate during early reperfusion, as evidenced in animals treated with carvedilol, may also contribute to reduced potentiation of necrosis induced by lethal reperfusion injury.

β-Adrenoceptor blockade during impaired left ventricular function may aggravate pump failure due to negative inotropic properties. Despite reduced heart rate and afterload in carvedilol treated animals, cardiac output and coronary perfusion was not hampered at the end of reperfusion compared to controls.

These findings suggest that reduced heart work and wall tension mainly due to reduced afterload by adrenergic blockade during early reperfusion, may protect against lethal reperfusion injury through reduced oxygen consumption.

4.2. Receptor-mediated effects

Carvedilol offers non-selective β-adrenoceptor blockade which may be beneficial during early reperfusion by reducing heart work through reduced systolic pressure and heart rate, and possibly attenuating Ca^{2+} overload, thus, exerting several protective effects on the post-ischaemic myocardium. Even though non-selective β-adrenoceptor antagonism of carvedilol is 10–20-fold higher than the α_1-adrenoceptor antagonism (Nichols et al., 1989a,b; Ruffolo et al., 1993), the α_1-adrenoceptor blockade results in periferal vasodilation and thereby reduced afterload, suggesting that also α_1-adrenoceptor blockade may exert protection against lethal reperfusion injury. Other studies have shown that up-regulation of α_1-adrenoceptors persists during reperfusion and suggest an association with reperfusion injury (Kurz et al., 1991). This proposal may contradict the findings that α_1-stimulation may mimic pre-conditioning and act protective against ischaemic injury (Tsuchida et al., 1994). However, this is based on a low-grade α_1-adrenoceptor stimulation. It has been suggested that although low-grade α_1-adrenoceptor stimulation may be beneficial, high-grade is not (Kitakaze et al., 1991). Thus, the massive α_1-adrenoceptor stimulation that occurs during early reperfusion may in contrast be harmful. This hypothesis is supported by our findings that carvedilol offered significant protection against ventricular fibrillation during early reperfusion. α_1-Adrenoceptor recruitment and stimulation, and oxygen-derived free radicals have both been suggested as factors leading to arrhythmias and ventricular fibrillation induced by reperfusion (Bolli, 1991; Kurz et al., 1991). Carvedilol exerts both α_1-adrenoceptor antagonism and antioxidative properties, thereby being able to prevent both these potential sources of arrhythmogenesis. A recent study showed no effect of different antioxidants on reperfusion arrhythmias in dogs (Euler, 1995). In the present study, oxidative stress did not differ between groups as evaluated by glutathione levels. Thus, it is likely that α_1-adrenoceptor blockade protects against ventricular fibrillation. We have not examined the effects of separate β- and α_1-adrenoceptor blockade, and cannot conclude which of these effects play a major role, however, we propose that both β- and α_1-adrenoceptor blockade by carvedilol exerts important protection against lethal reperfusion injury.

It could be claimed that the high degree of reperfusion arrhythmias in the control group lead to larger infarct size in controls compared to treatment groups. However, ventricular fibrillation was cardioverted within seconds and did not lead to significant and persisting haemodynamic disturbances in affected animals.

4.3. Antioxidative effects

Despite the large number of studies performed with different antioxidants against lethal reperfusion injury, the results from these studies are inconsistent (Bolli, 1991; Jeroudi et al., 1994). Myocardial levels of GSH and GSSG have been used as indicators of oxidative stress in the myocardium (Ferrari et al., 1993). We confirmed findings that GSH is depleted with a simultaneous increase in GSSG following myocardial ischaemia. Levels of both

GSH and GSSG did not differ between groups in the post-ischaemic myocardium, except for slightly higher GSH levels in the subepicardium when carvedilol was given before coronary artery occlusion. The latter may be contributable to pure ischaemic protection exerted by carvedilol as described by others. Thus, judged by the levels of GSH and GSSG, carvedilol did not demonstrate any antioxidative effects in vivo, suggesting that the antioxidant effect of carvedilol did not protect against lethal reperfusion injury in our cat model. We administered carvedilol for 10 min during reperfusion. Failure of antioxidants to protect against lethal reperfusion injury might be due to short-term administration (Bolli, 1990). However, carvedilol has a half-life of approximately 5 h and will be present in therapeutic concentrations in the myocardium throughout the experiments. Furthermore, glutathione is primarily a cytosolic antioxidant, other endogenous antioxidants of lipophilic nature may play a role in protection of membranes.

It is well known that collateral blood flow may cause a wide variation in infarct size in dogs. In cats, we have demonstrated abscence of significant collateral blood flow in the ischaemic myocardium (Brunvand et al., 1995). In the present study, significant collateral blood flow was not present in any group during coronary artery occlusion. Neither were there any differences in area at risk between groups, demonstrating that differences in infarct size was not explained by extensive collateral circulation, nor by different risk zones. Finally, carvedilol itself has been shown not to influence triphenyl tetrazolium chloride staining (SmithKline Beecham Pharmaceuticals, unpublished results) making it unlikely that carvedilol disturbed the evaluation of infarct size.

In conclusion, we present new findings that carvedilol protected against lethal reperfusion injury as evidenced by reduced infarct size, improved regional contractile function and reduced incidence of ventricular fibrillation. The findings also argue in favour of lethal reperfusion injury as a significant phenomenon, since carvedilol improves post-ischaemic myocardial function and reduces infarct size also when administered only at the onset of reperfusion.

This may imply potential clinical use of carvedilol as adjuvant therapy to coronary revascularisation procedures, like thrombolysis, bypass surgery and balloon angioplasty. However, further studies are needed to elucidate the exact mechanisms underlying the cardioprotective effect of carvedilol.

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