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Long-Term Effects of Beta-Adrenergic Blocking Agent Treatment on Hemodynamic Function and Left Ventricular Remodeling in Rats With Experimental Myocardial Infarction

Importance of Timing of Treatment and Infarct Size

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Objectives. **This study was designed to assess the long-term effects of a beta$_1$-selective beta-adrenergic blocking agent on mortality, in vivo hemodynamic function, left ventricular volume and wall stress in post-myocardial infarction (MI) rats.**

Background. **Beta-blockers have shown beneficial results in clinical studies after MI. However, the underlying mechanism is not yet understood, and experimental studies have shown conflicting results.**

Methods. **Bisoprolol (60 mg/kg body weight per day) was given 30 min or 14 days after MI or sham operation.**

Results. **The mortality rate was reduced only in early bisoprolol-treated rats (29% vs. 46% in untreated rats, $p < 0.05$). Heart rate was equally reduced in all treatment groups, and the maximal rate of rise of left ventricular systolic pressure (dP/dt_{max}) decreased in sham rats and in rats with a small to moderate infarct size. Stroke volume index was unchanged in sham rats and in rats with a small to moderate infarct with early or late bisoprolol treatment and increased in rats with a large infarct in the late bisoprolol group. Left ventricular volume was increased by bisoprolol in sham rats and rats with a small infarct but not in rats with a large infarct.**

Conclusions. **Treatments starting early (30 min) or late (14 days) after coronary artery ligation with bisoprolol increased left ventricular volume in sham rats and in rats with a small infarct but not in rats with a large infarct. Late bisoprolol treatment improved stroke volume index, and early bisoprolol treatment reduced diastolic wall stress, in rats with a large myocardial infarct. Thus, bisoprolol effects on remodeling and cardiac performance after myocardial infarction strongly depend on infarct size and timing of treatment. This finding may explain previous controversial results that did not consider infarct size and timing of treatment.**

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Activation of neurohumoral systems may play an important role in the development of progressive cardiac dysfunction (1,2). Angiotensin-converting enzyme inhibitors have been proven beneficial in the prevention of heart failure after myocardial infarction (MI) both in animal models (3,4) and clinical studies (5,6). The effect of short-term beta-adrenergic blockage after MI was tested on, and a substantial reduction in mortality from the use of oral propranolol was found (7). A meta-analysis of ~29,000 patients (8) revealed a 13% reduction in mortality when intravenous beta-blockade was started in the early hours of MI. Anti-ischemic or antiarrhythmic effects of beta-blockers may be important in clinical studies. However, the underlying mechanism is not yet completely understood. Fishbein et al. (9) found that long-term propranolol treatment blunted myocardial hypertrophy and increased left ventricular cavity dimensions in the rat model of experimental MI. Oh et al. (10) reported similar observations in rats exercising after MI and reperfusion. Hochman and Wong (11) showed that atenolol had no significant effect on myocardial infarct expansion in the rat model. In summary, these observations were in conflict with the beneficial effects of beta-blockers reported in clinical studies. Various factors may have influenced the experimental studies available so far: beta-blocker type, degree of left ventricular dysfunction or size of infarction and time to start of treatment after MI. In addition, detailed hemodynamic studies have not been performed to determine the long-term effect of beta-blockers after MI. Therefore, the present study aimed to determine the effects of early or late use of the beta-blocker bisoprolol on mortality, hemodynamic function and left ventricular remodeling in rats with infarcts of various sizes.

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Abbreviations and Acronyms

CI	=	cardiac index
CK	=	creatine kinase
dP/dt_{max}	=	maximal rate of rise of left ventricular systolic pressure
MAP	=	mean arterial pressure
MI	=	myocardial infarction
RAP	=	right atrial pressure
SVI	=	systemic vascular index
TPRI	=	total peripheral resistance index

Methods

Animals, experimental MI and pharmacologic interventions. Adult male Wistar rats weighing 300 to 320 g were used. Coronary artery ligation was performed as previously described by Pfeffer et al. (3). In brief, rats were anesthetized by ether, intubated and ventilated by a volume-constant rodent ventilator (UB 7025 rodent ventilator, Hugo Sachs Elektronik, Germany), and a left thoracotomy was performed. The heart was exteriorized from the thorax, and the left main coronary artery was ligated using a 5-0 Prolene suture between the pulmonary artery outflow tract and left atrium. The heart was then returned to its normal position and the thorax closed. All procedures conformed to the guiding principles of the American Physiological Society and were approved by the institutional animal research committee.

Dose-finding study. Dose-response curves of the effect of bisoprolol on heart rate and mean arterial pressure (MAP) were obtained. Rats were divided into four groups: group 1 (n = 5) included untreated control rats; groups 2, 3 and 4 (n = 5 for each group) were treated with bisoprolol (30, 60, 120 mg/kg body weight per day of drinking water, respectively) for 1 week. On the day of study, rats were anesthetized with ether, and polyethylene (PE50) catheters were inserted into the right carotid artery and connected to a microtip manometer (Millar) through a three-way stopcock for blood pressure and heart rate measurements (Fig. 1). A dose of 60 mg/kg per day was chosen for the main study on the basis of these experiments.

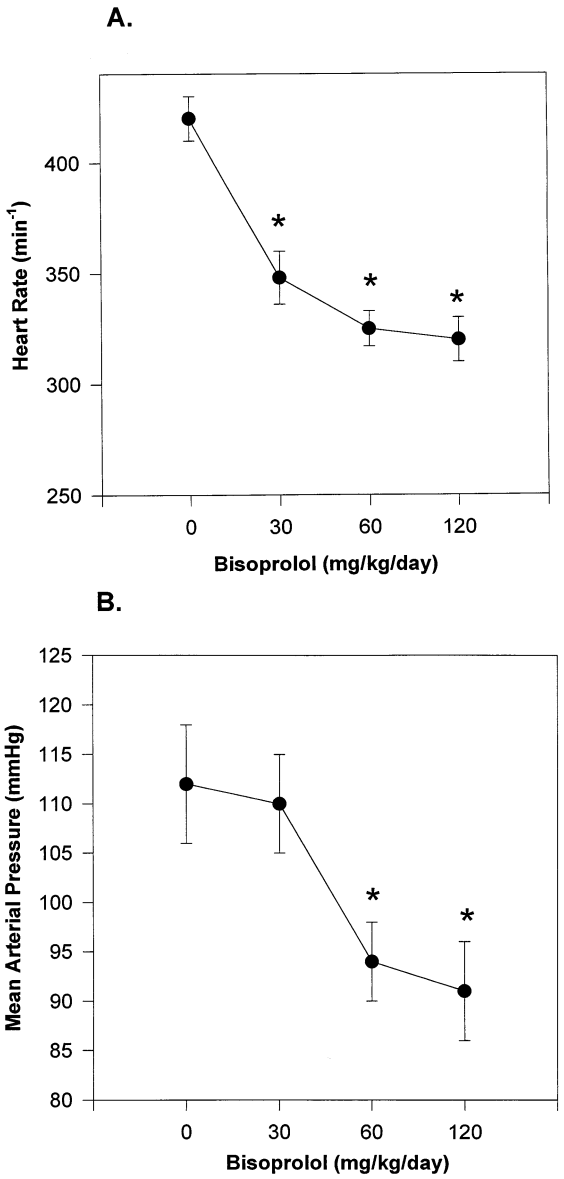

Figure 1. Dose–response curves for heart rate **(A)** and MAP **(B)** with various doses of bisoprolol. Data shown are mean value ± SEM. *p < 0.05 versus bisoprolol, 0 mg/kg per day.

Main study. Rats were housed in polyethylene cages (two rats/cage) in climatized rooms with a 12-h light-dark cycle, fed with standard laboratory food and had free access to water. Rats were weighed, and their water consumption was measured at weekly intervals. Drug concentration in drinking water was adjusted every week. Twelve groups of rats were studied: the three treatment groups included untreated control rats; early-B rats, in which bisoprolol (60 mg/kg per day) treatment was started 30 min after coronary artery ligation, with the first dose given per gavage followed by bisoprolol dissolved in drinking water; and late-B rats, in which bisoprolol (60 mg/kg per day) treatment was started 14 days after coronary artery ligation. According to the extend of histologic infarct size, four additional subgroups in each treatment group were established. These included rats in which failure of ligation of the coronary artery occurred (*sham*) or in which infarct size was <30% (*small*), ranged from 30% to <45% (*moderate*) or was ≥45% (*large*).

Hemodynamic studies. Eight weeks after coronary ligation, rats were reanesthetized with ether. Polyethylene cannulas were inserted into the trachea for artificial ventilation and into the right carotid artery and jugular vein for pressure measurements. Pressures were measured through a short segment of a fluid-filled PE50 tubing connected to a microtipped manometer (Millar) through a three-way stopcock, with zero adjusted to midchest level. The carotid cannula was briefly advanced into the left ventricle and then withdrawn to the aortic arch while pressure were recorded. The jugular vein cannula was advanced to the right atrium. Left ventricular systolic and

end-diastolic pressures, the maximal rate of rise of left ventricular systolic pressure (dP/dt_{max}), MAP, heart rate and mean right atrial pressure (RAP) were measured under light ether anesthesia and spontaneous respiration.

During positive pressure ventilation and after midsternal thoracotomy, a calibrated flow probe (2.5 mm, Gould Statham Instruments, Hato Rey, Puerto Rico) was placed around the ascending aorta for continuous measurement of aortic blood flow. Mean aortic blood flow was obtained electronically and taken as the cardiac index (CI) (ml/min per kg body weight) (3). Systemic vascular resistance index (SVRI) was calculated as (MAP − RAP)/CI.

After a stable state was established, baseline measurements were carried out over a 10-min period. Warmed (39 to 40°C) Tyrode's solution was then infused into a femoral vein at the rate of 40 ml/kg per min for 45 s or until maximal flow was achieved (12). This infusion produces an increase in cardiac output to peak values, followed by a plateau, despite further elevation of right atrial pressure. Maximal cardiac performance was defined as peak values of cardiac index and stroke volume index during this Tyrode's infusion.

Ten to 15 min after the volume load, when all hemodynamic variables had returned to baseline levels, the flow probe was removed, and the arterial catheter was advanced into the left ventricle. The ascending aorta was briefly occluded around the catheter by a suture to produce contractions that are isovolumetric except for coronary flow. Measurements were made of left ventricular peak systolic and end-diastolic pressures. Maximal left ventricular developed pressure was calculated as peak systolic minus end-diastolic pressure during aortic occlusion. These measurements defined the maximal pressure-generating ability of the left ventricle, as previously described (13).

Left ventricular volume. The passive pressure–volume characteristics of the left ventricle were obtained as previously described (14). The heart was arrested by potassium chloride and a double-lumen catheter (PE 50 inside PE 200) was inserted into the left ventricle through the ascending aorta. The right ventricular free wall was incised to avoid fluid accumulation. The atrioventricular groove was ligated, and isotonic saline was infused at a rate of 0.76 ml/min through one lumen while intraventricular pressure was continuously recorded through the other lumen from negative pressure to 30 mm Hg. At least three reproducible pressure–volume curves were obtained within 10 min of cardiac arrest, well before the onset of rigor mortis. Stiffness constants (K_0, K_1, K_2, K_3 and K_4) were defined as previously described by Fletcher et al. (13). Ventricular volumes at pressures of 0, 2.5, 5, 10, 15, 20 and 30 mm Hg were calculated from the pressure–volume curve. The operating left ventricular end-diastolic volume was derived from the left ventricular pressure–volume curve (3) and was defined as the volume on the pressure–volume curve corresponding to a filling pressure equal to in vivo end-diastolic pressure.

Infarct size. The method used to process the heart for the measurement of infarct size was similar to that described by Pfeffer et al. (3). After the pressure–volume data had been recorded, the hearts were fixed in the distended form in 10% buffered formalin for 24 h and then dissected into the left ventricle plus interventricular septum and right ventricular free wall, which were weighed separately. The whole left ventricle was dehydrated in alcohol, cleared in xylene and embedded in paraffin. Transverse serial sections of 10-μm thickness were obtained in 1-mm intervals from apex to base and mounted and stained according to the phosphotungstic acid hematoxylin method in that necrotic infarct tissue stained red and noninfarcted myocardium blue. Ten to 12 sections were obtained from each heart. The slices were projected, and the lengths of infarct and total left ventricle on both epicardial and endocardial surfaces of each section were measured using a calibrated digitizer (Numonics Digitizer 2200). Infarct size was calculated by dividing the sum of the planimetered endocardial and epicardial circumferences of the infarcted area by the sum of the total epicardial and endocardial circumferences of the left ventricle.

Left ventricular shape. The method used for measurement of left ventricular shape was previously described (14). Left ventricular diameter was measured as the maximal distance from the endocardial surface of the septum to the endocardial surface of the free left ventricular wall, along with a line perpendicular to the septum and, accordingly, was used as a measure of aneurysmal shape distortion. Left ventricular free wall thickness, which represents scar thickness in rats with infarction, was measured at the point where the left ventricular diameter reached the free left ventricular wall. Average septal thickness was determined as the septal area enclosed by two lines originating from the center of gravity of the endocardial circumference that connected the two origins of right ventricular free wall divided by the average of right and left ventricular surface length. These measurements were performed by using a calibrated digitizing tablet (Numonics Digitizer 2200).

Data analysis. Results are expressed as mean value ± SEM. We used multiple comparisons to find differences between the various infarct and treatment groups; the comparisons were corrected by the Bonferroni test. Differences in mortality among the various treatment groups were determined by chi-square and Fisher exact test; $p < 0.05$ was considered to indicate statistical significance.

Results

A total of 289 rats underwent the surgical procedure: 89 in the control group, 93 in the early-B group and 107 in the late-B group. A total of 107 rats died before hemodynamic measurements (102 rats died within 24 h after coronary ligation). Differences were found in total mortality between the control (46%) and early-B (29%) groups ($p < 0.05$). The mortality rate was 34% in the late-B group (p = NS vs. control or early-B

Table 1. Myocardial Infarct Size in Untreated and Treated Rats

Treatment	Sham Operation	Infarct Size: Small (<30%)	Infarct Size: Moderate (≥30% to <45%)	Infarct Size: Large (≥45%)
Untreated				
MI (%)	0	18 ± 3	39 ± 1	51 ± 2
No. of rats	15	9	8	11
Early-B				
MI (%)	0	17 ± 2	38 ± 1	50 ± 2
No. of rats	16	11	14	8
Late-B				
MI (%)	0	21 ± 1	37 ± 1	54 ± 1
No. of rats	22	17	13	9

Data presented are mean value ± SEM, unless otherwise indicated. Early-B = early bisoprolol treatment; Late-B = late bisoprolol treatment; MI = myocardial infarction.

rats). Infarct size was similar among the various treatment groups (Table 1).

Body weight and ventricular weights. Table 2 shows body and heart weights for the various groups. Body weight was somewhat lower in rats with a large infarct than in sham rats and in bisoprolol-treated animals with a moderate infarct size versus untreated rats. Left ventricular weight was reduced in untreated rats with a large infarct versus sham rats but was not significantly different from early-B or late-B group rats.

Hemodynamic measurements before thoracotomy. Table 3 shows hemodynamic variables obtained before thoracotomy. Left ventricular systolic pressure and MAP as well as dP/dt_{max} were in general reduced by bisoprolol, although this reduction did not achieve statistical significance in each individual group. Heart rate was consistently reduced by ~20% in all groups. Large infarcts were characterized by lower arterial and higher left ventricular end-diastolic pressures.

Hemodynamic measurements at baseline after thoracotomy. As shown in Table 4, CI was decreased by bisoprolol in sham rats and in rats with a small infarct but not in rats with a large infarct. Stroke volume index (SVI) was increased in this group by late bisoprolol treatment. Total peripheral resistance index (TPRI) was increased in rats with a large infarct in the untreated group and in the early-B group. Both early and late bisoprolol treatment significantly increased TPRI in sham but not in infarcted rats.

Peak cardiac performance. These effects of bisoprolol treatment in general persisted during volume loading (Table 5). Peak CI was reduced by early or late bisoprolol treatment because of the reduced heart rate. Peak SVI was again improved by late bisoprolol treatment in rats with a large infarct. In contrast, peak developed pressure was even reduced in this group by late bisoprolol treatment. In general, rats with a large infarct had a reduced peak CI, SVI and developed pressure but increased TPRI and left ventricular end-diastolic pressure.

Table 2. Body and Heart Weights

	Sham Operation	Infarct Size: Small (<30%)	Infarct Size: Moderate (≥30% to <45%)	Infarct Size: Large (≥45%)
BW (g)				
Untreated	522 ± 10	510 ± 16	552 ± 14	467 ± 15*
Early-B	508 ± 17	519 ± 13	490 ± 12†	428 ± 18*
Late-B	491 ± 13	496 ± 13	499 ± 14	474 ± 16
LVW (g)				
Untreated	0.945 ± 0.017	0.946 ± 0.051	0.976 ± 0.027	0.839 ± 0.044*
Early-B	0.929 ± 0.031	0.946 ± 0.030	0.968 ± 0.029	0.840 ± 0.050
Late-B	0.909 ± 0.025	0.936 ± 0.028	0.898 ± 0.016*	0.908 ± 0.035
LVW/BW (g/kg)				
Untreated	1.812 ± 0.026	1.855 ± 0.072	1.769 ± 0.030	1.811 ± 0.113
Early-B	1.827 ± 0.021	1.828 ± 0.055	1.980 ± 0.054†	1.993 ± 0.148
Late-B	1.862 ± 0.038	1.890 ± 0.042	1.813 ± 0.051	1.927 ± 0.081
RVW (g)				
Untreated	0.262 ± 0.014	0.267 ± 0.019	0.291 ± 0.024	0.297 ± 0.028
Early-B	0.262 ± 0.016	0.250 ± 0.012	0.270 ± 0.012	0.267 ± 0.036
Late-B	0.247 ± 0.008	0.234 ± 0.012	0.260 ± 0.012	0.276 ± 0.021
RVW/BW (g/kg)				
Untreated	0.501 ± 0.026	0.520 ± 0.027	0.530 ± 0.049	0.647 ± 0.071
Early-B	0.513 ± 0.024	0.484 ± 0.024	0.554 ± 0.030	0.626 ± 0.087
Late-B	0.508 ± 0.017	0.473 ± 0.024	0.522 ± 0.020	0.599 ± 0.069

*$p < 0.05$ versus sham-operated rats in same treatment group. †$p < 0.05$ versus untreated rats with comparable infarct size. Data presented are mean value ± SEM. BW = body weight; LVW = left ventricular weight; RVW = right ventricular weight; other abbreviations as in Table 1.

Table 3. Hemodynamic Variables Before Thoracotomy

		Infarct Size		
	Sham Operation	Small (<30%)	Moderate (≥30% to <45%)	Large (≥45%)
LVSP (mm Hg)				
Untreated	133 ± 2	135 ± 3	131 ± 4	127 ± 3
Early-B	127 ± 4	123 ± 4	125 ± 3	116 ± 3
Late-B	124 ± 3	124 ± 3	121 ± 2	119 ± 4
MAP (mm Hg)				
Untreated	107 ± 2	109 ± 2	107 ± 4	107 ± 2
Early-B	104 ± 3	98 ± 3†	103 ± 3	97 ± 2†
Late-B	100 ± 2	105 ± 2	101 ± 2	97 ± 4
dP/dt_{max} (× 1,000 mm Hg/s)				
Untreated	16.8 ± 1.0	13.5 ± 0.7	16.5 ± 1.8	8.5 ± 1.0*
Early-B	8.5 ± 2.6†	9.5 ± 0.9†	8.5 ± 0.6†	7.1 ± 1.1
Late-B	9.3 ± 0.4†	9.5 ± 0.5†	8.7 ± 0.4†	7.1 ± 0.5*
LVEDP (mm Hg)				
Untreated	3.5 ± 0.9	3.6 ± 0.4	9.0 ± 4.0	22.2 ± 4.0*
Early-B	5.5 ± 0.7	6.5 ± 1.4	10.8 ± 2.9	13.8 ± 2.9
Late-B	6.0 ± 0.7	5.9 ± 0.4†	8.7 ± 1.6	19.5 ± 1.7*
RAP (mm Hg)				
Untreated	0.7 ± 0.3	0.4 ± 0.2	0.4 ± 0.3	2.4 ± 0.8
Early-B	0.9 ± 0.3	0.9 ± 0.5	0.6 ± 0.3	1.4 ± 0.8
Late-B	0.2 ± 0.2‡	0.1 ± 0.1	0.7 ± 0.3	1.7 ± 0.7
HR (beats/min)				
Untreated	387 ± 11	392 ± 12	406 ± 11	386 ± 14
Early-B	299 ± 14†	318 ± 11†	317 ± 8†	322 ± 9†
Late-B	312 ± 7†	315 ± 7†	323 ± 8†	323 ± 6†

*$p < 0.05$ versus sham-operated rats in same treatment group. †$p < 0.05$ versus untreated rats with comparable infarct size. ‡$p < 0.05$ versus rats with early bisoprolol treatment and comparable infarct size. Data presented are mean value ± SEM. dP/dt_{max} = maximal rate of rise of left ventricular systolic pressure; HR = heart rate; LVEDP = left ventricular end-diastolic pressure; LVSP = left ventricular systolic pressure; MAP = mean arterial pressure; RAP = right atrial pressure; other abbreviations as in Table 1.

Table 4. Baseline Cardiac Performance

		Infarct Size		
	Sham Operation	Small (<30%)	Moderate (≥30% to <45%)	Large (≥45%)
MAP (mm Hg)				
Untreated	98 ± 1	99 ± 2	99 ± 2	98 ± 2
Early-B	95 ± 2	91 ± 2	96 ± 2	91 ± 1
Late-B	94 ± 2	96 ± 1	91 ± 2	89 ± 2†
HR (beats/min)				
Untreated	419 ± 8	416 ± 13	413 ± 11	401 ± 16
Early-B	336 ± 17†	328 ± 15†	338 ± 11†	329 ± 7†
Late-B	319 ± 5†	326 ± 9†	320 ± 8†	315 ± 7†
CI (ml/min per kg)				
Untreated	258 ± 11	234 ± 14	225 ± 9	193 ± 12*
Early-B	193 ± 9†	183 ± 15	193 ± 12	151 ± 19
Late-B	195 ± 11†	212 ± 19	193 ± 18	211 ± 26
SVI (ml/kg)				
Untreated	0.62 ± 0.03	0.56 ± 0.03	0.55 ± 0.03	0.49 ± 0.03*
Early-B	0.58 ± 0.03	0.56 ± 0.05	0.57 ± 0.04	0.45 ± 0.05
Late-B	0.61 ± 0.03	0.65 ± 0.05	0.59 ± 0.05	0.66 ± 0.07
TPRI (mm Hg/ml per min per kg)				
Untreated	0.37 ± 0.01	0.43 ± 0.03	0.43 ± 0.02*	0.50 ± 0.03*
Early-B	0.50 ± 0.03†	0.53 ± 0.06	0.51 ± 0.04	0.66 ± 0.09
Late-B	0.49 ± 0.02†	0.49 ± 0.04	0.53 ± 0.07	0.47 ± 0.07

*$p < 0.05$ versus sham-operated rats in same treatment group. †$p < 0.05$ versus untreated rats with comparable infarct size. Data presented are mean value ± SEM. CI = cardiac index; SVI = stroke volume index; TPRI = total peripheral resistance index; other abbreviations as in Tables 1 and 3.

Left ventricular volume. The complete pressure–volume curves of sham rats and rats infarcts of various sizes in the untreated, early-B and late-B groups are shown in Figure 2. A rightward shift of the pressure–volume curves was found with increasing infarct size. Accordingly, histologic left ventricular diameter and cavity (endocardial area) were increased (not shown). Bisoprolol treatment tended to increase left ventricular volume in sham rats and in rats with a small and moderate infarct (Fig. 2). In rats with a large MI, this effect was not significant, and pressure–volume curves were similar, irrespective of treatment. Stiffness constants (Table 6, K_0 to K_4) were consistently reduced by a large infarct. Most important, bisoprolol reduced stiffness constants in rats with a small to moderate infarct but not in those with a large infarct. Free wall (scar) thickness decreased with increasing infarct size in the control, early-B and late-B groups and was not affected by bisoprolol treatment (not shown). Septal thickness tended to be increased in control rats with a large infarction and was not affected by bisoprolol treatment.

As shown in Table 7, left ventricular operating volume and volume/weight ratio tended to be increased by bisoprolol treatment in sham rats and rats with a small to moderate infarct but not in rats with a large infarct.

Discussion

Mortality. The mortality rate was reduced by 22% in rats with early treatment with bisoprolol versus untreated rats. Similar findings were reported by Smith et al. (15) who used the vasodilator/beta-blocker carvedilol. Late treatment could not be expected to reduce mortality because most animals died before late therapy was established. Clinical studies have shown that beta-blockers may reduce post-MI mortality (16), with emphasis on an effect most prominent during the first 24 h (17). Opitz et al. (18) studied the arrhythmia pattern in this model using continuous electrocardiographic recording by an implanted telemetry system, and two active arrhythmogenic periods were found from 0 to 0.5 h and 1.5 to 9 h after coronary ligation. Most deaths in that study were arrhythmic. In the present study, mortality was calculated beginning from 30 min after coronary ligation. Most rats died within 24 h, and it is conceivable, although not proven, that the reduction of ventricular fibrillation by bisoprolol was responsible for its benefit (19).

Importance of infarct size. A major result of the present study was that the effect of bisoprolol strongly depended on

Table 5. Peak Cardiac Performance

	Sham Operation	Infarct Size: Small (<30%)	Infarct Size: Moderate (≥30% to <45%)	Infarct Size: Large (≥45%)
MAP_{max} (mm Hg)				
Untreated	93 ± 3	89 ± 4	85 ± 3	85 ± 3
Early-B	82 ± 3†	76 ± 4	78 ± 3	76 ± 4
Late-B	78 ± 2†	84 ± 3	74 ± 3†	74 ± 4
HR_{max} (beats/min)				
Untreated	404 ± 9	390 ± 15	389 ± 12	385 ± 13
Early-B	317 ± 7†	320 ± 14†	329 ± 10†	313 ± 10†
Late-B	312 ± 5†	313 ± 7†	316 ± 7†	297 ± 10†
CI_{max} (ml/min per kg)				
Untreated	446 ± 15	363 ± 29*	307 ± 25*	271 ± 17*
Early-B	323 ± 15†	278 ± 23	289 ± 19	241 ± 26*
Late-B	329 ± 12†	324 ± 20	305 ± 25	265 ± 23*
SVI_{max} (ml/kg)				
Untreated	1.11 ± 0.03	0.94 ± 0.07	0.80 ± 0.08*	0.71 ± 0.04*
Early-B	1.02 ± 0.05	0.86 ± 0.05	0.88 ± 0.05	0.77 ± 0.08*
Late-B	1.06 ± 0.04	1.03 ± 0.06	0.97 ± 0.08	0.89 ± 0.07
$TPRI_{max}$ (mm Hg/ml per min per kg)				
Untreated	0.20 ± 0.01	0.24 ± 0.03	0.26 ± 0.01*	0.28 ± 0.02*
Early-B	0.24 ± 0.01	0.26 ± 0.02	0.26 ± 0.02	0.31 ± 0.04
Late-B	0.21 ± 0.01	0.24 ± 0.02	0.24 ± 0.03	0.26 ± 0.03
Dev P_{max} (mm Hg)				
Untreated	226 ± 6	209 ± 10	187 ± 8*	163 ± 6*
Early-B	221 ± 4	182 ± 10*	166 ± 6*	150 ± 6*
Late-B	201 ± 5†‡	190 ± 3	174 ± 5*	142 ± 4*†
$LVEDP_{max}$ (mm Hg)				
Untreated	20.8 ± 0.8	17.5 ± 1.6	20.9 ± 1.3	26.5 ± 3.2
Early-B	20.1 ± 0.8	19.8 ± 1.7	22.9 ± 1.6	24.5 ± 2.4
Late-B	20.0 ± 0.9	20.5 ± 0.9	21.1 ± 0.7	26.4 ± 1.5*

*$p < 0.05$ versus sham-operated rats in same treatment group. †$p < 0.05$ versus untreated rats with comparable infarct size. ‡$p < 0.05$ versus rats with early bisoprolol treatment and comparable infarct size. Data presented are mean value ± SEM. max = maximal; Dev P = developed pressure; other abbreviations as in Tables 1, 3 and 4.

infarct size. Bisoprolol consistently reduced dP/dt_{max} in sham-operated rats and in rats with a small or moderate infarct size and reduced CI and increased TPRI in sham-operated rats and in rats with a small infarct. Thus, blood pressure was maintained at reduced cardiac output by peripheral vasoconstriction. In contrast, bisoprolol did not reduce dP/dt_{max} in rats with a large infarct. In addition, these animals, when treated late with bisoprolol, showed a substantial increase in stroke volume at rest and during volume loading and preservation of CI, whereas peak developed pressure was reduced. In this group, bisoprolol did not induce an increase in TPRI.

A rightward shift by bisoprolol of the pressure–volume curves occurred in sham-operated rats and in rats with a small infarct. This observation was in accordance with previous experimental studies (9,10) but was not significant in rats with a moderate infarct size and was virtually absent in rats with a large infarct. The "operating left ventricular volume" calculated for in vivo end-diastolic pressure was 0.62 ± 0.10 in untreated, 0.57 ± 0.11 in early-treated and 0.69 ± 0.08 in late-treated rats with a large infarct (p = NS) (Table 7). Left ventricular end-diastolic pressure tended to be increased, whereas the volume/mass ratio was more than doubled by early or late beta-blockade in rats with a small infarct. In contrast, left ventricular end-diastolic pressure tended to be decreased after beta-blockade in animals with a large infarct, and the volume/mass ratio remained unchanged. These data strongly suggest that diastolic wall stress was increased in sham-operated rats and in rats with a small infarct but was reduced by early beta-blockade in rats with a large infarct. Finally, left ventricular stiffness was consistently reduced by bisoprolol only in sham-operated rats and in rats with a small or moderate infarct. However, there was no significant effect of bisoprolol in rats with a large infarct. In the rat model, quantitative micromorphologic analysis demonstrated that propranolol could increase left ventricular cavity (9,10). However, those studies tested beta-blockers only in rats with an infarct size <30% of the left ventricle and therefore do not contradict the present results.

Reduction of wall stress may explain the hemodynamic effects in rats with a large infarct. Although individual variables contributing to wall stress (volume, pressure, mass) were not significantly changed by bisoprolol, their changes were combined in favor of reduced wall stress. In addition, reduction of heart rate and, thus, of "minute wall stress" by beta-blockade may have contributed to improve myocardial energy balance in rats with a large infarct. A previous study (20) used papillary muscles isolated from rat heart 21 days after a large myocardial infarction. These morphologically intact but hypertrophied papillary muscles showed reduced developed tension, impaired beta-adrenoceptor density and reduced adenylate cyclase activity after beta-receptor stimulation, respectively. Long-term propranolol treatment improved developed tension and increased beta-adrenoceptor density but did not improve adenylate cyclase activity or isoproterenol-stimulated muscle function. Thus, the effect of long-term beta-blockade on myocardial function appeared unrelated to the beta-receptor–adenylate cyclase system. The improvement in myocardial function was also unrelated to hypertrophy because the cross-sectional area of papillary muscle that was increased after MI was unchanged by propranolol (20). Recently, Laser et al. (21) showed that bisoprolol therapy preserved total creatine kinase (CK) activity and prevented the shift of CK isoenzyme toward the B isoenzymes and depletion of mitochondrial CK and creatine. The increase in anaerobic lactic dehydrogenase isoenzymes observed in untreated infarcted rats did not occur during bisoprolol treatment. Thus, beta-blockade prevented in part the changes in CK and lactate dehydrogenase that are typical for myocardial hypertrophy and failure (22).

Most post-MI changes depend on infarct size (23,24), and the effect of angiotensin-converting enzyme inhibitors was also

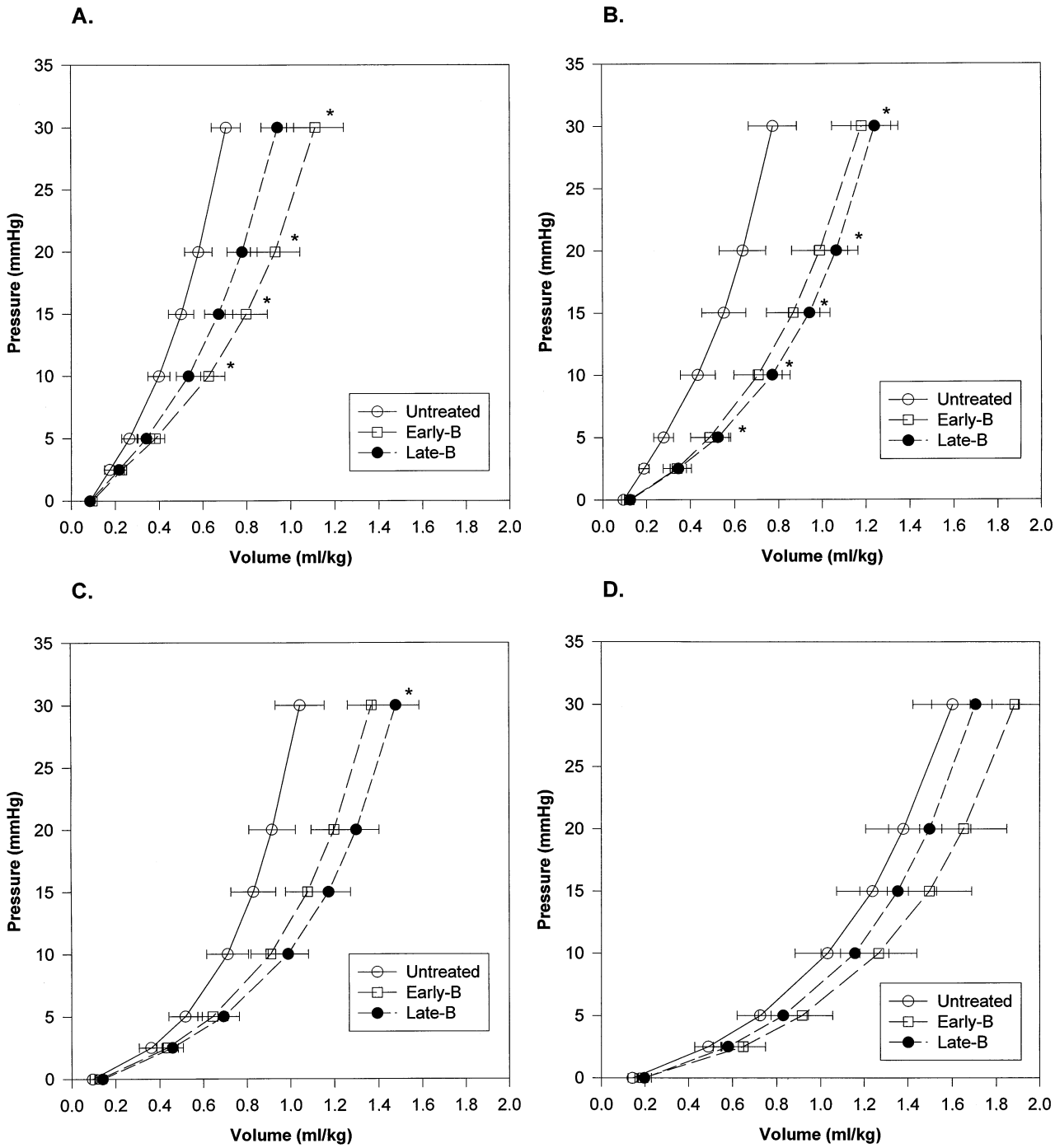

Figure 2. Passive pressure–volume curves in sham-operated rats (**A**) and in rats with a small (**B**), moderate (**C**) or large MI (**D**) in the untreated (**open circle**), early bisoprolol (Early-B) treatment (**open squares**) and late bisoprolol (Late-B) treatment (**solid circles**) groups. Data shown are mean value ± SEM. *$p < 0.05$ versus untreated rats with a comparable infarct size.

found to be related to infarct size (3). The observation of the present study that rats with a large infarct responded better to beta-blockade than those with a small infarct is in good accordance with clinical observations that showed that post-MI patients with the highest risk and most severe dysfunction benefited from beta-blocker therapy (2). In addition, numerous studies (2) in patients with severe chronic heart failure have documented that long-term beta-blocker treatment may improve cardiac index and left ventricular ejection fraction and may reduce cardiac filling pressure. It is conceivable that neurohumoral systems are most activated in these animals and patients (25–27) but not in rats with a small infarct (28). Activation of the sympa-

Table 6. Stiffness Values

	Sham Operation	Infarct Size: Small (<30%)	Infarct Size: Moderate (≥30% to <45%)	Infarct Size: Large (≥45%)
K_0 (2.5–30 mm Hg)				
Untreated	4.0 ± 0.3	3.6 ± 0.3	3.4 ± 0.2	2.1 ± 0.2*
Early-B	2.8 ± 0.3†	2.5 ± 0.2†	2.6 ± 0.2	1.9 ± 0.1*
Late-B	3.0 ± 0.2†	2.7 ± 0.3	2.4 ± 0.2†	2.2 ± 0.2
K_1 (0–3 mm Hg)				
Untreated	21.9 ± 2.6	20.4 ± 2.5	8.1 ± 1.1*	6.5 ± 0.9*
Early-B	16.6 ± 3.6	10.1 ± 1.5†	6.8 ± 0.8	5.0 ± 0.7*
Late-B	18.8 ± 3.1	9.9 ± 1.5*†	6.9 ± 1.3*	5.9 ± 1.0*
K_2 (3–10 mm Hg)				
Untreated	6.5 ± 0.6	5.9 ± 0.7	4.1 ± 0.5*	3.0 ± 0.5*
Early-B	4.3 ± 0.8	3.9 ± 0.4†	3.2 ± 0.4	2.2 ± 0.2
Late-B	4.8 ± 0.6	4.0 ± 0.7	2.8 ± 0.4*	2.6 ± 0.4*
K_3 (10–20 mm Hg)				
Untreated	4.1 ± 0.3	3.6 ± 0.3	3.5 ± 0.3	2.2 ± 0.2*
Early-B	2.8 ± 0.4†	2.6 ± 0.2†	2.6 ± 0.2†	1.8 ± 0.1
Late-B	3.1 ± 0.2†	2.7 ± 0.3	2.4 ± 0.2†	2.2 ± 0.2
K_4 (20–30 mm Hg)				
Untreated	3.4 ± 0.2	3.0 ± 0.2	3.0 ± 0.2	1.9 ± 0.2*
Early-B	2.5 ± 0.2†	2.3 ± 0.2†	2.5 ± 0.2	1.8 ± 0.1*
Late-B	2.6 ± 0.1†	2.5 ± 0.2	2.3 ± 0.1†	2.1 ± 0.2

*$p < 0.05$ versus sham-operated rats in same treatment group. †$p < 0.05$ versus untreated rats with comparable infarct size. Data presented are mean value ± SEM. Abbreviations as in Table 1.

thoadrenal system might be a prerequisite for certain effects of beta-blockade.

Early versus late treatment. Only late treatment improved SVI at baseline and during volume loading in rats with a large infarct, whereas early treatment had no consistent effect. Operating left ventricular volume was not different between sham-operated rats or rats treated by bisoprolol early or late; therefore, the effect could not be explained by different left ventricular geometry or afterload. Left ventricular end-diastolic pressure tended to be lower, and an increased preload and recruitment of the Frank-Starling reserve could be responsible. However, mortality was different between the early and late treatment groups, creating some selection bias. In addition, differences were small, and biologic relevance must be questioned.

Table 7. Operating Volume and Volume/Left Ventricular Weight Ratio

	Sham Operation	Infarct Size: Small (<30%)	Infarct Size: Moderate (≥30% to <45%)	Infarct Size: Large (≥45%)
Vol (ml)				
Untreated	0.11 ± 0.02	0.12 ± 0.02	0.31 ± 0.08	0.62 ± 0.10*
Early-B	0.20 ± 0.03	0.27 ± 0.06	0.41 ± 0.06*	0.57 ± 0.11*
Late-B	0.17 ± 0.02	0.28 ± 0.03*†	0.45 ± 0.07*	0.69 ± 0.08*
Vol/LV wt (ml/g)				
Untreated	0.12 ± 0.02	0.12 ± 0.02	0.32 ± 0.08	0.79 ± 0.11*
Early-B	0.21 ± 0.03	0.28 ± 0.05†	0.42 ± 0.07*	0.66 ± 0.11*
Late-B	0.19 ± 0.03	0.30 ± 0.03*†	0.49 ± 0.07*	0.77 ± 0.09*

*$p < 0.05$ versus sham-operated rats in same treatment group. †$p < 0.05$ versus untreated rats with comparable infarct size. Data presented are mean value ± SEM. LV wt = left ventricular weight; Vol = volume; other abbreviations as in Table 1.

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