Carvedilol Retards Sudden Loss of Contraction during Early Regional Myocardial Ischemia in Feline Hearts

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ABSTRACT
The purpose of our study was to investigate whether loss of myocardial contraction immediately after coronary occlusion was nonuniform, and if pretreatment with carvedilol, a vasodilating nonselective beta-adrenoceptor antagonist, could retard loss of contraction after coronary artery occlusion. Feline hearts were subjected to acute regional ischemia by total occlusion of the left anterior descending coronary artery. The animals were either treated with vehicle (control group) or with carvedilol 1 mg/kg i.v. before left anterior descending coronary artery occlusion (n = 9 in each group). Regional contraction in the left anterior descending coronary artery perfused region of the heart was studied by cross-oriented sonomicrometry. In control animals, circumferential (subepicardial) contraction ceased after 10 sec, whereas longitudinal (subendocardial) contraction ceased immediately after left anterior descending coronary artery occlusion. Loss of contraction in animals treated with carvedilol was significantly slower compared to controls. Circumferential contraction ceased between 30 sec and 1 min, whereas longitudinal contraction ceased after 20 sec. In conclusion, loss of contraction during the first seconds after coronary occlusion was nonuniform, with most rapid dysfunction in the subendocardium. Pretreatment with carvedilol retarded loss of contraction in both axes.

It has been known for many years that myocardial contraction ceases in the ischemic myocardium within just a few seconds after a coronary artery occlusion has occurred (Tennant and Wiggers, 1935; Tyberg et al., 1974). This sudden loss of contraction may lead to myocardial pump failure, with severe consequences for the affected individual after coronary artery occlusion. However, even if contractile failure during ischemia is well recognized, there are important deficiencies in current knowledge on details in the pattern of loss of contraction, as well as consequences of cardioprotective treatment on loss of contraction.

The subendocardium is more susceptible to ischemic injury than the subepicardium. Myocardial infarction starts in the subendocardium and spreads like a wavefront toward the subepicardium (Jennings and Reimer, 1991). Whether there are differences in segment shortening between layers during myocardial ischemia has been debated. Some studies have found a more pronounced reduction in segment shortening in the inner than outer layers (Gallagher et al., 1982; Prinzen et al., 1986), whereas others have not found such a difference (Weintraub et al., 1981; Hattori et al., 1982). In our laboratory regional myocardial function has been assessed by two-dimensional sonomicrometry. The longitudinal segment is aligned parallel to subendocardial fiber direction, whereas the circumferential segment aligns parallel to outer wall fibers, orientated perpendicular to the longitudinal segment (Hexeberg et al., 1989, 1991). We have demonstrated that shortening in the longitudinal axis is sensitive to subendocardial function (Hexeberg et al., 1989, 1991), as reduced performance is seen during subendocardial ischemia (Birkenland et al., 1992a, b, Hexeberg et al., 1992), and abolished function is sustained after reperfusion, when subendocardial infarction is evident (Brunvand et al., 1995). The circumferential axis seems to reflect mainly subepicardial function (Hexeberg et al., 1991).

The protective effects of beta adrenoceptor antagonists on acute myocardial infarction are well established, particularly for lipophilic beta adrenoceptor antagonists without intrinsic sympathomimetic effects (Hjalmarson and Olsson, 1991). Carvedilol is a new lipophilic vasodilating beta adrenoceptor antagonist that has been shown to possess very potent anti-ischemic properties, superior to conventional beta adrenoceptor antagonists (Hamburger et al., 1991; Bril et al., 1992; Feuerstein et al., 1992). We have shown that carvedilol reduces infarct size and improves posts ischemic regional dysfunction more than a combination of propranolol and doxazo-

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ABBREVIATIONS: LAD, left anterior descending coronary artery; EDL, end diastolic length; ESL, end systolic length; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end diastolic pressure; RPP, rate pressure product.
sin after ischemia and reperfusion in cats (Brunvand et al., 1996a, b). Based on the potent effect of carvedilol on infarct reduction and improved postischemic dysfunction, we wanted to investigate if carvedilol treatment before coronary artery occlusion could delay loss of contraction during acute myocardial ischemia.

To examine these questions, we applied cross-oriented sonomicrometry in the anterior wall of the left ventricle (supplied by LAD) and studied regional function before and immediately after LAD occlusion. Control animals were compared with animals treated with carvedilol before LAD occlusion.

Our primary purpose was to test the hypothesis that carvedilol could retard loss of contraction after coronary artery occlusion. Furthermore, we wanted to examine whether loss of contraction immediately after coronary artery occlusion was nonuniform in the anterior wall of the left ventricle.

**Methods**

**Experimental preparation.** The experimental protocol was approved by the Norwegian Committee for Research on Animals. Eighteen outbred male cats (Iifa Credo, L’Arbresle, France) weighing 3.7 to 5.0 kg were anesthetized with sodium pentobarbital (40 mg · kg\(^{-1}\) i.p.), tracheotomized and ventilated with a positive pressure ventilator delivering 50% \(\text{N}_2\text{O}\), 47.5% \(\text{O}_2\) and 2.5% \(\text{CO}_2\) (Loosco Infant Ventilator MK2, Amsterdam, Holland). Body temperature was held constant with a heat blanket regulated by a rectal thermometer. Midline thoracotomy and pericardiotomy provided access to the heart. A pressure tip transducer (Millar MPC 500, Houston, TX) was introduced into the left ventricular cavity through the apex for continuous recording of left ventricular pressure, heart rate and dP/dt. The left atrium was cannulated with a short 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 LAD 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 LAD perfused anterior wall of the left ventricle. One pair (longitudinal segment) was positioned 15 degrees clockwise to an axis from the main stem of the left coronary artery to the apex, and thus parallel to subendocardial fibers in that region. The other pair (circumferential segment) was positioned perpendicular to the longitudinal segment and aligned parallel to mid and outer wall fibers. Segment lengths were measured with a Sonomicrometer 102.2 (Triton Technology, San Diego, CA). Left ventricular pressure and the two segment length signals were recorded (Instrumentation tape recorder 3694A, Hewlett-Packard, Waltham, MA), 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 analyzed by a program developed in our laboratory.

**Experimental protocol.** The cats were randomized into two groups \((n = 9\) in each group). The control group received vehicle (100 \(\mu\)l dimethylformamide 10% acidified with HCl and diluted in 0.9% NaCl to a total volume of 10 ml, the final solution being pH neutral), whereas the other group was treated with carvedilol (1 mg/kg) dissolved in vehicle. This dose is previously well described in myocardial ischemia (Hamburger et al., 1991; Bril et al., 1992; Feuerstein et al., 1992, Brunvand et al., 1996a). Vehicle or carvedilol was administered at a rate of 1 ml/min for 10 min, starting 15 min before LAD occlusion. Intravenous infusion of 0.9% saline at a rate of 15 ml · kg\(^{-1}\)hr was continued throughout the experiment. Preocclusion hemodynamic recordings were performed after a 30 min period of stabilization, before and after drug administration. The first microsphere injection was performed immediately after drug administration. LAD was then occluded by a nontraumatic vessel clamp, with simultaneous recording of hemodynamics and regional function during the first minute of ischemia. Mean values of five consecutive beats with 5-sec intervals during the first 30 sec of ischemia were analyzed and compared within and between groups. During the ischemic period further hemodynamic recordings were performed after 60 sec and thereafter every 5 min. The final microsphere injection and hemodynamic recording were performed 30 min after LAD occlusion in both groups. The experiment was terminated after 40 min of ischemia. At the end of the experiment, LAD was reoccluded and 1 ml fluorescein was injected into the aortic root for demarcation of the ischemic area, the animals were killed by cardiac arrest and the hearts excised for measurement of regional myocardial blood flow.

**Myocardial blood flow and cardiac output.** Regional tissue blood flow in myocardial specimens and cardiac output were determined with carbonized microspheres (15.5 ± 0.1 \(\mu\)m, Du Pont, Wilmington, DE) labeled with \(^{46}\text{Sc}\), \(^{51}\text{Cr}\), \(^{85}\text{Sr}\) or \(^{141}\text{Ce}\). Microspheres (approximately \(10^9\) spheres) were injected into the left atrium in a randomized sequence. During injection, reference blood samples were withdrawn with a constant-rate extraction pump (Sage instruments 351, Cambridge, MA) from the abdominal aorta. Specimens, reference blood, residuals and standards were counted for \(\gamma\)-emission (Compugamma 1282, LKB-Wallac Company, Turku, Finland) and tissue blood flow rate and cardiac output were calculated according to Heyman et al. (1977).

**Analysis of hemodynamics.** End diastole was defined as the timepoint where dP/dt > 100 mmHg · sec\(^{-1}\) and end systole as 20 msec before peak negative dP/dt (Abel, 1981). Segment length at end diastole and end systole are EDL and ESL respectively. Segment lengths were normalized by defining EDL at preocclusion state as 10 mm. Normalization of segment lengths is helpful for comparison of data with base-line hemodynamics, particularly for evaluation of relative changes due to interventions. Systolic shortening \([\text{EDL}-\text{ESL}] / \text{EDL} \times 100\%\] was calculated. Comparison between circumferential and longitudinal segments was performed based on preocclusion values set to 100%. The rate-pressure product was calculated as the product of heart rate and LVSP. dP/dt was presented relative to LVSP to correct for the afterload reduction in carvedilol treated animals. This was done by dividing dP/dt by LVSP.

**Statistical analysis.** Hemodynamics, regional function and tissue blood flow were analyzed by two-way analysis of variance with repeated measurements. Newman-Keul contrast tests were used when appropriate, and \(P < .05\) was regarded as statistically significant. Values are mean ± S.E.M.

**Results**

**Hemodynamics.** All hemodynamic results are presented in table 1. All values within groups were compared to preocclusion recordings before administration of vehicle or carvedilol. In the control group, heart rate did not change during the experiment. LVSP and rate-pressure product were reduced after 10 sec of ischemia, and remained reduced after 30 min of ischemia. LVEDP increased after 15 sec of ischemia, and continued to increase during the first minute of ischemia. Treatment with carvedilol led to a significant fall in heart rate, LVSP and RPP. The reduced values remained low during the ischemic period. LVEDP was not altered during the first minute of ischemia, but was elevated after 30 min of ischemia. dP/dt divided by LVSP as a correction for reduced afterload in carvedilol-treated animals did not differ within or between groups.

**Regional myocardial tissue blood flow and cardiac output.** Treatment with carvedilol led to a significant drop in blood flow in the normal myocardium. LAD occlusion was
successful in both groups with evidence of total ischemia by measurement of blood flow in the ischemic tissue (table 2). We did not find evidence of significant collateral blood flow in either group. Preocclusion blood flow was performed after treatment with either vehicle or carvedilol to observe any effect of carvedilol on preocclusion blood flow (compared to blood flow in vehicle-treated animals). Compared to preocclusion values, cardiac output fell in both groups after ischemia (table 2).

**Regional function.** Administration of carvedilol before LAD occlusion did not alter systolic shortening in the normally perfused LAD region; 9.97 ± 0.60 vs. 11.11 ± 0.61 (NS) in circumferential segments and 6.28 ± 0.84 vs. 5.77 ± 0.78 (NS) in longitudinal segments. Because loss of contraction in both groups occurred within the first minute after LAD occlusion, with no further significant changes beyond 1 min of LAD occlusion, we present data from regional function only during the first minute of regional ischemia. Systolic shortening in circumferential and longitudinal segments during the first minute of ischemia is presented in figure 1 with emphasis on comparison between groups for each segment. Shortening in the circumferential segment was gradually reduced during the first 10 sec of ischemia in the control group, until total loss of function occurred 15 sec after LAD occlusion, and systolic bulging became evident as negative shortening. In the longitudinal segment, loss of function occurred immediately after LAD occlusion in the control group. In contrast, animals treated with carvedilol had a significantly slower loss of function, and active contraction was evident in circumferential segments for the first 30 sec of ischemia in the group treated with carvedilol. In the longitudinal axis, carvedilol-treated animals had a significantly slower reduction of contraction compared to control animals until loss of function occurred after 25 sec of ischemia. Figure 2 demonstrates differences between circumferential and longitudinal segment shortening, with normalization of data. In both groups, longitudinal segment shortening was lost significantly sooner than circumferential shortening.

After 1 min of ischemia, there was total loss of contraction in both groups and both axes, with no difference between groups. This loss of function was constant throughout the experiment with no difference between groups. Thus we only present data from the first 60 sec of the ischemic period.

**Discussion**

Our major findings were that loss of contraction was non-uniform with more rapid dysfunction in longitudinal fibers than circumferential fibers immediately after LAD occlusion in feline hearts. Loss of contraction was retarded after pretreatment with carvedilol in both axes.

**Nonuniform regional contraction.** We have previously demonstrated that regional function is nonuniform both in the normal myocardium and in the stunned myocardium (Hexeberg et al., 1989, 1991; Brunvand et al., 1995; Rynning et al., 1993). We have also shown a close relation between loss
of function in longitudinal segments after reperfusion and manifest subendocardial infarction (Brunvand et al., 1995). Furthermore, we have shown that protection against the development of subendocardial necrosis by carvedilol leads to recovery of active shortening in longitudinal segments (Brunvand et al., 1996a, b). Taken together, these studies strongly indicate that longitudinal systolic shortening reflects contraction in the subendocardium. In our study a more rapid loss of function in longitudinal fibers compared to circumferential fibers after LAD occlusion in control experiments indicated that subendocardial contraction was lost immediately after coronary occlusion, whereas subepicardial contraction was more slowly reduced. This study using cross-oriented sonomicrometry, demonstrated that subendocardial contraction was more susceptible to ischemic injury than the subepicardium from the onset of an ischemic episode, and the findings support previous studies suggesting that subendocardial contraction is lost before subepicardial contraction after coronary artery occlusion (Gallagher et al., 1982; Prinzen et al., 1986).

Impaired mechanical function after coronary occlusion is thought to be of metabolic origin (Hearse, 1979). During myocardial ischemia transmural gradients have been shown for high energy phosphates (Schaefer et al., 1989; Brunvand et al., 1992), intracellular pH (Schaefer et al., 1989), lactate (Griggs et al., 1972) and calcium levels (Figueredo et al., 1993). Prinzen et al. (1986) suggested that a rapid depletion of creatine phosphate stores or acidosis in the subendocardium could explain the more rapid loss of function in the subendocardium. Thus, the more dysfunctional subendocardium may be explained by a more rapid alteration of subendocardial metabolism. In addition, sustained subepicardial active contraction may directly inhibit subendocardial contraction due to tethering between layers with compression of subendocardial fibers during subepicardial contraction (Hexeberg et al., 1995).

In species with significant collateral blood flow, subendocardial flow is more restricted than in the subepicardium during myocardial ischemia, suggesting that more severe subendocardial flow restriction may lead to a more rapid subendocardial functional loss. However, the cat does not have significant collateral coronary blood flow as demonstrated in our study and several previous studies (Brunvand et al., 1992, 1995; Rynning et al., 1993). Thus, a more rapid loss of subendocardial contraction seemed not to be explained by transmural gradients in blood flow during ischemia, making metabolic explanations more likely.

**Carvedilol and regional function.** Carvedilol is a new lipophilic vasodilating beta adrenoceptor antagonist which has shown to be a very potent antiischemic drug compared to
other beta adrenoceptor antagonists (Bril et al., 1992; Brunvand et al., 1996b). We have previously shown that carvedilol can reduce infarct size and improve regional postischemic dysfunction in cats, even to a greater extent than a combination of propranolol and doxazosin (Brunvand et al., 1996a, b). In our study, treatment with carvedilol before LAD occlusion led to a significant delay in loss of contraction compared to control experiments in both circumferential and longitudinal segments. Loss of contraction was more rapid in longitudinal segments compared to circumferential segments adding further evidence to the finding that contractile failure is nonuniform.

Treatment with carvedilol led to a decrease in RPP through both reduced LVSP and heart rate, indicating reduced oxygen demand. Previous studies have shown less severe depletion of high energy phosphates during ischemia after treatment with beta adrenoceptor antagonists (Stangeland et al., 1984). We suggest that reduced oxygen demand may lead to reduced energy requirements and less severe metabolic alterations and thereby delayed contractile dysfunction. However, RPP as a measure of oxygen consumption is an indirect method (Ball et al., 1981) and does not give as accurate information as do direct methods. It indicates global oxygen consumption and not regional oxygen consumption. However, RPP is easy to measure and readily available based only on heart rate and LVSP, because these two parameters are the main determinants of oxygen consumption.

Another major difference between the control and carvedilol group was reduction in afterload after treatment with carvedilol. Lower afterload may directly affect loss of contraction both by reducing oxygen consumption and by reducing the workload on the ischemic muscle fibers. A significant effect of low afterload on delayed loss of function may be particularly true because the difference in systolic shortening between groups occurred despite a similar LVEDP, thus making it unlikely that the effect of carvedilol was due to preload effects. However, in the normal myocardium lower afterload due to carvedilol treatment did not affect segment shortening compared to control experiments. Furthermore, we have previously shown that carvedilol improves postischemic regional dysfunction significantly more than a combination of propranolol and doxazosin despite the same degree of afterload reduction (Brunvand et al., 1996b). Altogether, these findings indicate that carvedilol could retard early loss of contraction in the ischemic myocardium through reduction of oxygen demand through reduced load, wall tension and heart work. Its efficiency may also be due to rapid effects on the receptor level as a result of the highly lipophilic property (Ruffolo et al., 1992).

During the first minute of ischemia after treatment with carvedilol, LVEDP did not increase significantly. Intracellular calcium overload during myocardial ischemia has been suggested to play a role in the development of LVEDP (Paulus et al., 1982). Previous studies have demonstrated that calcium antagonists with the ability to prevent intracellular calcium accumulation during myocardial ischemia, may attenuate ischemia-induced increase in LVEDP (Vandeplasche et al., 1991). Based on these findings, we suggest that carvedilol may retard loss of regional function by preventing calcium overload, possibly as a result of reduced oxygen demand.

**Limitations.** This study has not investigated mechanisms underlying the observed effect of carvedilol on systolic shortening in the ischemic myocardium. Carvedilol works both as a nonselective beta adrenoceptor antagonist and an alpha1 adrenoceptor antagonist. However, we cannot conclude if both these effects may act to retard loss of function, or if these effects act separately. Furthermore, the effect may be due to the afterload reduction exerted by carvedilol. Further studies are needed to investigate the details of the effect of carvedilol observed in our study.

In conclusion loss of contraction immediately after coronary occlusion was nonuniform, with a more rapid loss of function in the subendocardium. Carvedilol retarded loss of contraction transmurally during the first minute of ischemia after coronary occlusion.

Clinically, carvedilol may prove beneficial by reducing the risk of sudden pump failure due to acute myocardial ischemia.

**References**


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