The effect of two different diltiazem treatments on infarct size in ischemic, reperfused porcine hearts

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ABSTRACT  The effect of diltiazem on the development of infarcts was investigated in porcine hearts. The left anterior descending coronary artery was occluded in each of 32 anesthetized pigs for 75 min and was reperfused for 4 hr. Diltiazem (15 μg/kg-min) was infused in 11 pigs for 30 min before occlusion (therapy A) and in another eight pigs before reperfusion (therapy B). Eleven pigs served as controls. Tissue concentrations of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NAD) were determined in transmural needle biopsy samples taken from the ischemic apex after 70 min of ischemia. The infarct size, expressed as the ratio of the infarcted tissue over the area at risk of necrosis multiplied by 100, amounted to 79 ± 20% in the control group. Although there was no significant difference between hemodynamics in the control and the treated groups, pretreatment with diltiazem significantly reduced infarct size (53 ± 26%; p = .025). Reduction of infarct size by therapy B did not reach the required level of significance (66 ± 33%). The ischemic loss of ATP and NAD was significantly lower in the pretreated group, which further indicates that the beneficial effect of diltiazem was exerted primarily during ischemia and not during reperfusion.


IT IS well accepted that the speed at which ischemic myocytes die depends on the extent of the imbalance between oxygen supply and demand.1,2 Accordingly, a number of agents have been found that delay the development of infarcts by reducing myocardial oxygen consumption during ischemia.3 Among them are calcium antagonists, a heterogeneous group of compounds that can improve the myocardial oxygen supply-demand balance by depressing myocardial contractility, decreasing heart rate, and inducing coronary vasodilation.4 A beneficial effect of calcium antagonists on ischemic cellular injury has been demonstrated in a variety of different preparations,5–11 although in some studies this favorable influence has not been observed.12,13 Since the basic action of calcium antagonists is not only an inhibition of the slow channel in myocytes and pacemaker cells but also in all types of smooth muscle cells (which results in a systemic and coronary vasodilation14), it is difficult to distinguish in intact animals the beneficial effect that is due to an obviously improved oxygen supply-demand ratio (e.g., reduced blood pressure, heart rate, contractility, and augmented collateral blood flow) from that which may favorably influence myocardial metabolism. In this study we examined whether the calcium antagonist diltiazem beneficially affects the myocardial metabolism of pigs during ischemia and/or reperfusion, i.e., whether it reduces the infarct size after transient ischemia. We chose this animal preparation because it lacks significant collateral blood flow,15,16 thus facilitating the comparison between the control and the treated groups, which is known to be somewhat difficult in canine preparations.17 Furthermore, a beneficial effect of diltiazem on the development of infarcts in porcine hearts can primarily be attributed to an improved metabolism provided the hemodynamics of the different groups do not differ significantly.

Methods

Our experiments were performed in 32 young pigs (German country pigs) of both sexes weighing between 38 and 49 kg. Each was premedicated with intramuscular azaperon (7 mg/kg), metomidate hydrochloride (15 mg/kg), piripramide (15 mg), and atropine (0.5 mg iv). General anesthesia was maintained with the continuous infusion of metomidate hydrochloride and ventilation with nitrous oxide and oxygen by a Sulla 19 respirator (Dräger, W. Germany). Hexobarboclon bromide (6 mg iv) was used as a neuromuscular blocking agent. The infusion rate of metomidate hydrochloride (about 200 mg/hr) and additional injections of piripramide were adjusted to achieve a heart rate of 70 to 90 beats/min. A median thoracotomy was performed and

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the left anterior descending coronary artery (LAD) was prepared in its middle part. A No. 6F catheter was advanced through the right external jugular vein into the coronary sinus under manual control. Aortic pressure was measured with a fluid-filled catheter in the abdominal aorta with a Statham transducer. Left ventricular pressure and \( \frac{dp}{dt} \) were determined with a No. 6F Millar catheter-tipped manometer introduced through a carotid artery. A standard lead of the electrocardiogram and rectal body temperature were monitored throughout the experiments. The body temperature of each animal was kept constantly between 37° and 38°C by a temperature-controlled operating table. Arterial blood gas levels were determined frequently and adjusted to physiologic values. Before occluding the LAD, loading doses of heparin (20,000 IU) and lidocaine (100 mg) were injected intravenously. Lidocaine (0.5 mg/kg) was then given continuously during the first 45 min of ischemia, whereas heparin (2500 IU/hr) was given throughout the occlusion period. Left ventricular oxygen consumption was estimated by Breit- schneider’s equation \(^{18} \) at intervals of 5 min during ischemia. Diltiazem (15 \( \mu \)g/kg-min) was infused in 11 pigs for 30 min before occlusion (therapy A) and in another eight pigs for 30 min before reperfusion (therapy B). Eleven pigs served as controls. The LAD was occluded in its middle part with a parallel-jaw spring clip and was reperfused for 4 hr. Pigs that developed ventricular fibrillation and could not be resuscitated were discarded. The immediate effect of reperfusion was a significant increase in the lactate concentration in the coronary sinus. Myocardial tissue concentrations of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NAD, oxidized form) were determined in transmural needle biopsy samples taken from the ischemic apexes of six pigs from each group after 70 min of ischemia.

ATP was measured with a Boehringer test kit in a microcuvette with a 4 cm pathway at a wave length of 334 nm and with the use of an Eppendorf filter photometer. NAD was determined as described earlier. \(^{19} \) Before the hearts were excised the LADs were reocluded and 10 ml of a 10% solution of fluorescein sodium was injected intra-atrially to allow the postmortem delineation of the area at risk. The excised hearts were filled with a 2% agarose solution (40°C), cooled in crushed ice until the solution gelatinized, and cut into slices of 5 mm parallel to the atrioventricular groove with an electric rotary blade. Three to four slices of the ischemic region were photographed under ultraviolet light, which clearly distinguished the area at risk from the normally perfused myocardium. These sections were stained with nitro blue tetrazolium solution to assess the infarcted tissue \(^{20} \) and photographed once more, always at the same magnification. The areas at risk and the corresponding infarcted tissues were determined by planimetry of the photos by an investigator who did not know to which group of animals the photos belonged. Finally, the infarct size was expressed as the ratio of the infarcted tissue over the area at risk multiplied by 100. We compared the hemodynamics between the three groups with the Kruskal-Wallis test and within the three groups with the Wilcoxon test (two-tailed comparison). The Wilcoxon–Mann-Whitney rank-sum test was applied to compare the infarct sizes and the tissue concentrations of ATP and NAD in the two groups of treated animals with those of animals in the control group. Significance was accepted at the 5% probability level. All results are expressed as the mean ± SD.

## Results

### Rhythm disturbances

In the control group six of 11 pigs, in group A nine of 11 pigs, and in group B five of 10 pigs developed ventricular fibrillation. Two animals of each group had to be discarded because they could not be effectively resuscitated within 3 min. All analyses are therefore based on results in eight pigs in group B and nine pigs in the control group and group A. In the control group and in group B ventricular fibrillation occurred after 3 to 5 min and/or after 16 to 30 min of ischemia. The combined treatment with diltiazem and lidocaine in group A completely abolished very early ventricular fibrillation but was ineffective in controlling this rhythm disorder after 20 to 30 min of ischemia. The initiation of reperfusion was marked by a sudden onset of premature ventricular contractions and short runs of self-terminating ventricular tachycardias, but ventricular fibrillation never occurred at this time. We observed, however, intermittent atrioventricular rhythms with retrograde atrial activation in all pigs during reperfusion.

### Hemodynamics

The hemodynamic data from the three groups after thoracotomy, before LAD occlusion, and during ischemia are listed in table 1. After thoracotomy the measured hemodynamic parameters in the three groups did not differ significantly. In group A diltiazem was then infused into the right atrium. After the administration of only a third of the total dose

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>After thoracotomy</th>
<th>Before occlusion</th>
<th>During ischemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV peak</td>
<td>Control</td>
<td>85 ± 12</td>
<td>85 ± 12</td>
<td>82 ± 12</td>
</tr>
<tr>
<td>pressure (mm Hg) A</td>
<td>86 ± 10</td>
<td>86 ± 12</td>
<td>82 ± 9</td>
<td>77 ± 6</td>
</tr>
<tr>
<td>pressure (mm Hg) B</td>
<td>85 ± 12</td>
<td>86 ± 12</td>
<td>76 ± 6</td>
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<tr>
<td>LV enddiastolic pressure (mm Hg) A</td>
<td>8 ± 4</td>
<td>10 ± 3</td>
<td>13 ± 6</td>
<td></td>
</tr>
<tr>
<td>LV enddiastolic pressure (mm Hg) B</td>
<td>9 ± 4</td>
<td>10 ± 3</td>
<td>11 ± 3</td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min) A</td>
<td>82 ± 20</td>
<td>79 ± 21</td>
<td>91 ± 18</td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min) B</td>
<td>80 ± 19</td>
<td>74 ± 15</td>
<td>91 ± 18</td>
<td></td>
</tr>
<tr>
<td>dP/dt_{max} (mm Hg/sec) A</td>
<td>2185 ± 686</td>
<td>1600 ± 684^*</td>
<td>1078 ± 420</td>
<td></td>
</tr>
<tr>
<td>dP/dt_{max} (mm Hg/sec) B</td>
<td>2125 ± 939</td>
<td>2088 ± 1005</td>
<td>1183 ± 333^*</td>
<td></td>
</tr>
<tr>
<td>Systolic aortic pressure (mm Hg) A</td>
<td>85 ± 10</td>
<td>82 ± 9</td>
<td>79 ± 9</td>
<td></td>
</tr>
<tr>
<td>Systolic aortic pressure (mm Hg) B</td>
<td>85 ± 12</td>
<td>86 ± 12</td>
<td>77 ± 9^*</td>
<td></td>
</tr>
<tr>
<td>Diastolic aortic pressure (mm Hg) A</td>
<td>54 ± 6</td>
<td>54 ± 9</td>
<td>55 ± 9</td>
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<tr>
<td>Diastolic aortic pressure (mm Hg) B</td>
<td>51 ± 8</td>
<td>47 ± 9^*</td>
<td>49 ± 9</td>
<td></td>
</tr>
<tr>
<td>MVO2 (ml O2/100 g·min) A</td>
<td>4.9 ± 0.6</td>
<td>5.4 ± 0.8^*</td>
<td>5.3 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>MVO2 (ml O2/100 g·min) B</td>
<td>4.9 ± 0.9</td>
<td>5.2 ± 0.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( ^{*} \text{Significant hemodynamic changes (p \leq 0.05).} \) Comparisons within the groups: before occlusion vs after thoracotomy and during ischemia vs before occlusion (rank-sum test). No statistical differences exist between the three groups (Kruskal-Wallis test).
the negative chronotropic, negative inotropic, and vasodilating effect of this calcium antagonist was apparent and was observed until the LAD was ligated. The initiation of ischemia and the high dose of lidocaine caused a significant increase in heart rate, left ventricular oxygen consumption (p ≤ .05), and in left ventricular end-diastolic pressure (p ≤ .01) in the control group. A similar trend was observed in group A with regard to heart rate and left ventricular end-diastolic pressure (p = .02). In group B the combined effect of ischemia and treatment with lidocaine followed by diltiazem caused a small but significant decrease in systolic and diastolic blood pressures (p = .05) and maximum dP/dt (p = .01) as compared with preischemic values. In spite of these hemodynamic alterations within the three groups, no statistical difference was found between the values in the groups before and after occlusion of the LAD.

Effects of the different diltiazem treatments on infarct size. The influence of the two different diltiazem treatments on infarct size, expressed as the ratio of myocardial necrosis over the area at risk of necrosis, is illustrated in figure 1. Only pretreatment with diltiazem (group A) significantly reduced the mean infarct size (from 79% to 53%). The salvaged myocardium was mainly recruited from the outer layer of the ischemic anterior free wall and the inner part of the septum at risk. The mean infarct size of group B animals (66%) was smaller than in the control group but did not reach

FIGURE 1. Effect of the different diltiazem treatments on infarct size. Only the pretreatment with diltiazem (therapy A) significantly reduced infarct size (rank-sum test, mean ± SD).

FIGURE 2. Tissue concentrations of ATP and NAD after 70 min of ischemia. A = therapy A; B = therapy B; C = control group. The statistical comparison (rank-sum test) was performed between data from groups A and C and between that from groups B and C. Tissue concentrations of the two coenzymes were always significantly higher in group A (p < .025, mean ± SD).

the required level of significance. To further evaluate the period of the experiments during which the beneficial effect of the calcium antagonist occurred we determined the tissue concentrations of the two coenzymes in the ischemic/infarcted myocardium just before reperfusion was initiated. We found significantly higher tissue levels of ATP and NAD in group A (p < .025) and insignificantly higher coenzyme concentrations in group B compared with in the control group (figure 2). These results suggest that the beneficial effect of the pretreatment with diltiazem primarily occurred during ischemia and not during reperfusion since the tissue levels of the two measured coenzymes were already higher before reperfusion was started. However, we cannot exclude the possibility that the favorable influence of pretreatment with diltiazem was also exerted to a certain but smaller extent during reperfusion. In group B diltiazem was given during the last 30 min of ischemia. Although the collateral blood flow in porcine hearts is very low, at least a small amount of the calcium antagonist must have reached the ischemic myocardium and the drug must therefore be effective in the late stage of ischemia as well as during reperfusion. Whether or not the somewhat higher tissue levels of ATP and NAD before reperfusion and the insignificantly smaller mean infarct size in group B animals compared with in control animals is due to the action of the calcium antagonist remains an open question.

Discussion

Evaluation of the preparation. Despite the well-known rhythm instability in the porcine heart we chose it for
use in our preparation because it offers decided advantages over the canine heart. First, occlusion of the LAD caused an homogeneous ischemic region in all the animals studied because the pig lacks a significant collateral blood flow.\textsuperscript{15, 16} which furthermore has been shown to be insensitive to diltiazem treatment.\textsuperscript{21} Second, no areas of high flow ischemia exist, so that the area at risk of necrosis can accurately be delineated by an injected dye.

We determined the area at risk at the end of the experiments and not immediately before reperfusion because there was no substantial evidence that the region at risk might have changed during 4 hr of reperfusion. As cited above, the insignificant collateral blood flow in porcine hearts does not increase after treatment with diltiazem\textsuperscript{21} and a time-dependent diminution of the extent of the jeopardized porcine myocardium can only be expected after an ischemic period of about 24 hr, when collaterals actively start to grow.\textsuperscript{15} Demarcating the area at risk by the described dye technique offers the additional theoretical advantage that it allows the comparison of the two-dimensional extent of the risk region and the two-dimensional size of the infarcted tissue on the surface of the heart slices. In contrast, the delineation of the risk region by an autoradiographic method\textsuperscript{6} or by postmortem angiography can only be as precise as the dye technique in this preparation because the former two techniques demarcate the area at risk through the depth of the heart slice and this area is compared with the infarcted area of one of the two surfaces of a slice, which may differ considerably.

In our study the area of infarcted myocardium was assessed with a nitro blue tetrazolium staining technique; data obtained this way correspond very well with those from histologic and electromicroscopic evaluations of ischemic cell death, provided a reperfusion period of at least 90 min takes place.\textsuperscript{20, 22} In a previous study we determined the temporal and spatial development of infarcts in porcine hearts.\textsuperscript{23} Due to the insignificant collateral blood flow the whole area at risk became necrotic after only 90 to 120 min of ischemia. These results are supported by a histologic study in which transmural necrosis in porcine hearts was demonstrated after 120 min of ischemia.\textsuperscript{24} Most of the myocardial cells at risk die after 45 to 90 min of ischemia. We therefore ligated each coronary artery for 75 min since this is the most appropriate point in time to evaluate the effect of an intervention in this preparation. A high dose of heparin was administered to ensure that no blood coagulation could occur during ischemia.\textsuperscript{25} An extremely high dose of lidocaine was necessary to prevent ventricular fibrillation or to offer the possibility of fast resuscitation. The negative inotropic effect of this drug at high concentrations therefore had to be accounted for.

**Reduction of infarct size by pretreatment with diltiazem.** Although calcium influx is highest during reperfusion,\textsuperscript{9, 25} only intravenous treatment with diltiazem before ischemia reduced the infarct size significantly. This diminution of ischemic cell death took place even though the hemodynamic characteristics in the three groups did not differ. In particular, left ventricular oxygen consumption before occlusion and during ischemia in the control and the pretreated groups was almost identical.

To achieve comparable left ventricular oxygen consumption in the three groups we adjusted the piritramide dose (15 to 45 mg iv) to obtain a similar range of heart rates in the animals before initiating ischemia. Piritramide is a synthetic morphine-like drug that has been shown to slow the heart rate without having any other effect on the cardiovascular system, even when administered at very high doses.\textsuperscript{26, 27}

The reduction of infarct size was accompanied by higher levels of ATP and NAD in ischemic tissue before reperfusion was started. Since the tissue concentrations of these coenzymes correlate well with ultrastructural cell damage,\textsuperscript{19, 28} we assume that pretreatment with diltiazem reduced cellular injury during ischemia. An ATP-sparing effect of calcium antagonists (e.g., verapamil, diltiazem, and nifedipine) has also been demonstrated in isolated heart preparations\textsuperscript{9, 13} and in intact animals.\textsuperscript{29} The precise mechanism of this favorable action remains to be clarified. Several possibilities have to be taken into account. First, less calcium has to be pumped out of the cells if the intracellular calcium concentration is lowered by a calcium antagonist and this would lead to decreased ATP hydrolysis because the calcium efflux against a concentration gradient requires energy.\textsuperscript{30} Furthermore, a lower intracellular calcium concentration may reduce the amount of ATP consumed by the contractile system.\textsuperscript{14} Another possibility is that increased intracellular calcium may cause mitochondrial calcium overload, which can compete with ATP synthesis for respiratory energy.\textsuperscript{31} A fourth explanation is offered by Schwartz et al.,\textsuperscript{4} who demonstrated that diltiazem prevented the loss of mitochondrial oxidative phosphorylation that is caused by the increased intracellular phosphate concentration that occurs during ischemia. Although a great many experimental studies have demonstrated a beneficial effect of calcium antagonists on ischemic cell injury, nifedipine failed to reduce infarct
size in baboons. A possible explanation is that the administered dose of nifedipine for the most part influenced vascular smooth muscle and not myocardial metabolism since it is primarily a potent vasodilator. In this study dilatiazem did not reduce ultimate infarct size significantly when it was infused 30 min before reperfusion (group B). This time was chosen for treatment so that we could examine whether dilatiazem is able to manipulate ultimate infarct size during reperfusion when the sudden gain in intracellular calcium occurs. This issue is of great clinical importance because early reperfusion can be achieved in patients with acute myocardial infarction by thrombolytic therapy and there is evidence that infarct size can be reduced by diminution of the injury that takes place during reperfusion. The failure of dilatiazem to reduce infarct size at the time of reflow can mean that dilatiazem is not able to prevent intracellular calcium overload or that this sudden gain in intracellular calcium is only a consequence of profound myocardial damage and does not itself lead to myocardial necrosis. We favor the first assumption because it has been shown that two other calcium antagonists (verapamil and nifedipine) can not prevent intracellular calcium accumulation upon reperfusion in the isolated, arterially perfused septa of rabbit hearts.

Clinical implications. The results of this study can be extrapolated to the clinical situation only with some limitations. Many patients with slowly progressing coronary disease develop collaterals that can protect ischemic myocardium to some degree. Under these circumstances reduced left ventricular oxygen consumption can delay or even limit infarct size by improving the perfusion deficit in the area at risk of necrosis. Due to the insignificant collateral blood flow in porcine hearts a reduction of infarct size can only be expected in this preparation if reperfusion is achieved in time. We therefore did not investigate whether or not dilatiazem reduces ultimate infarct size without reperfusion. Our results indicate that pretreatment with dilatiazem delays the development of infarcts even without taking advantage of reduced left ventricular oxygen consumption or augmented collateral blood flow. The diminution of infarct size by reperfusion may therefore be even greater in pretreated patients with collaterals than in the animals in this study. Although we failed to demonstrate a favorable effect of dilatiazem on possible reperfusion damage, this drug may be used together with thrombolytic agents to improve myocardial oxygen supply-demand balance until reperfusion is initiated.

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