Effects of Lidocaine on Ischemic Myocardial Metabolism Assessed by $^{31}$P-NMR in the Isolated Perfused Rat Heart

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SUMMARY

Using an isolated perfused rat heart preparation, the protective effects of lidocaine and diltiazem on ischemic derangements of myocardial energy metabolism were studied with $^{31}$P-nuclear magnetic resonance spectroscopy. The hearts were perfused with a solution containing lidocaine ($4.27 \times 10^{-5}$, $12.80 \times 10^{-5}$ M) or diltiazem ($2.22 \times 10^{-7}$, $2.22 \times 10^{-6}$ M) for 15 min prior to the induction of global ischemia. The decrease in myocardial oxygen consumption rate, assessed as the product of heart rate and left ventricular systolic pressure ($HR \times LVP$), was greater in diltiazem-treated than in lidocaine-treated hearts. Diltiazem and lidocaine significantly retarded the fall in myocardial pH during ischemia and improved ATP recovery after reperfusion. There was a good correlation between suppression of $HR \times LVP$ observed before induction of ischemia and decreased drop in pH during the early phase of ischemia in the diltiazem-treated groups ($r = -0.78$, $p < 0.01$), but not in the lidocaine-treated groups. These results indicate that the beneficial effects of diltiazem on the ischemic myocardium are due primarily to the cardiodepressant effects. The beneficial effects of lidocaine cannot, however, be explained solely on the basis of the depression of oxygen consumption.

Additional Indexing Words:
Lidocaine Ischemia Reperfusion Myocardial pH Adenosine triphosphate $^{31}$P-NMR

LIDOCAINE has been widely used to treat ventricular arrhythmias during the evolution of myocardial infarction. In experimental studies, lidocaine has been shown to suppress ischemic ventricular arrhythmia, and prevent ischemic derangement of myocardial ultrastructure.
and metabolism\(^7\),\(^8\) The mechanisms underlying the beneficial effects of lidocaine on the ischemic myocardium, however, remain unclear.

Diltiazem, a calcium antagonist, exerts a favorable action on the ischemic myocardium\(^9\)\(^-\)\(^14\); it also significantly preserves myocardial function,\(^15\) myocardial \(pH\)\(^16\) and high energy phosphate\(^17\) during ischemia and after reperfusion. To explain the protective effects seen in isolated perfused hearts,\(^18\),\(^19\) we investigated the depressant effects of diltiazem on myocardial mechanical function, expressed as the product of left ventricular pressure and heart rate. The purpose of this study was to compare the mechanisms involved in the protective effects of lidocaine and diltiazem on ischemic derangements of myocardial energy metabolism. The effects of these compounds on myocardial energy metabolism were studied using \(^31\)P-nuclear magnetic resonance (NMR).

**Materials and Methods**

**Preparation:** Male Wistar rats, weighing approximately 280 g each, were divided randomly into 5 experimental groups consisting of 5 rats each: control (no-drug) hearts, diltiazem (2.22\(\times\)10\(^-7\) M)-treated hearts, diltiazem (2.22\(\times\)10\(^-6\) M)-treated hearts, lidocaine (4.27\(\times\)10\(^-5\) M)-treated hearts, and lidocaine (12.80\(\times\)10\(^-5\) M)-treated hearts. These drug doses produced similar decreases in the heart rate (HR) in the experimental groups. The rats were lightly anesthetized with ether. Immediately after opening the thorax, the hearts were rapidly excised and immersed in ice-cold modified Krebs-Ringer bicarbonate solution to induce rapid cessation of the heartbeat. The adherent connective tissue was removed and the aortic root of the heart was attached to a cannula. Retrograde perfusion with a modified Krebs-Ringer bicarbonate solution was begun immediately from a reservoir 75 cm above the heart. The perfusion fluid, which was maintained at a temperature of 38°C, contained NaCl (127.2 mM), KCl (4.7 mM), CaCl\(_2\) (2.5 mM), KH\(_2\)PO\(_4\) (1.2 mM), and NaHCO\(_3\) (24.9 mM). The solution was oxygenated with 95% oxygen+5% carbon dioxide gas to ensure that \(P_O_2\) values exceeded 600 mmHg. Sodium pyruvate (2.0 mM) and glucose (5.5 mM) were added to the perfusion solution as substrates.

Coronary inflow was measured with an electromagnetic flowmeter probe (internal diameter=2 mm, Statham, Gould Inc., Oxnard, Calif.) placed in the perfusate inflow line; it was coupled with an electromagnetic flowmeter (Statham SP 2201, Gould Inc.). A latex balloon was inserted into the left ventricle via the left atrium. Left ventricular pressure (LVP) and left ventricular end-diastolic pressure (LVEDP) were measured by a pres-
sure transducer (Statham P 50, Gould Inc.) connected to the balloon by saline-filled polyethylene tubing. Care was taken to avoid the presence of any air in the balloon. HR was recorded using a cardiotachometer (Nihon Kohden AP-600G, Nihon Kohden Inc., Tokyo, Japan) triggered by LVP pulses. In this study the product of HR and LVP was measured to assess the myocardial oxygen consumption rate, since Suga et al.\(^20\)-\(^22\) demonstrated a linear relationship between the pressure-volume area and myocardial oxygen consumption per beat. In the case of isovolumic contractions, the end-systolic pressure represents the pressure-volume area. Therefore, the myocardial oxygen consumption rate was assessed by HR×LVP in this study.

**NMR studies:** \(^31\)P-NMR spectra were obtained using a JEOL FX-270 NMR spectrometer (JEOL Ltd., Tokyo, Japan) operating at 109.16 MHz. Spectra of 600 transients were accumulated in the Fourier transform mode at 0.5 sec intervals using a 45° flip angle (total acquisition time, 5 min). Hearts were inserted into a 15-mm diameter glass NMR tube and placed in the magnetic field. The detector chamber was maintained at 38°C by means of an integrated air flow system. Coronary effluent was evacuated from the NMR tube using a vacuum pump.

After a 30-min equilibration perfusion period, three control spectra (15 min) were obtained for each heart to ensure that stable levels of high-energy phosphate compounds (HEP) were present. Following acquisition of the control spectra, drugs were infused through the aortic cannula and three spectra (15 min) were obtained during the preischemic period. Global ischemia was then induced by cross-clamping the aortic perfusate inflow line for 40 min, and eight spectra were taken. At the end of the ischemic arrest, the aortic clamp was removed, and eight spectra were obtained during a 40-min reperfusion period. Drugs were infused for 5 min immediately after reperfusion. The distance between the peak of intracellular inorganic phosphate (Pi) and the peak of pH-independent creatine phosphate (Cr-P) was measured, and intracellular pH was determined using the pH titration curve of the Pi and Cr-P solutions at 38°C.

Quantitative analysis was performed on the relative intensity of beta-ATP (adenosine triphosphate). The alpha- and gamma-ATP peaks could not be quantified, because they were contaminated with interference resonance from ADP (adenosine diphosphate), NAD (nicotinamide adenine dinucleotide), and NADH (reduced form of nicotinamide adenine dinucleotide). The area under each peak was integrated with a digitizer (Oscon SQ-3100F, Photron Ltd., Tokyo) connected to a personal computer (NEC PC-9801, NEC Corp., Tokyo). To compare spectra from different hearts, a capillary filled with methylene diphosphonate (MDP) solution was placed
within the NMR tube as an external standard, and the intensity of the beta-ATP peak relative to the area for MDP was used for quantitative analysis. In our experiment, the interpulse delay of 0.5 sec and 45° pulse angle may cause saturation of the HEP peak. Since our HEP data were expressed as percentage change from control values obtained during the 10 to 15-min period just before drug treatment, no corrections for saturation were made.

**Analysis of data:** Data are expressed as percent change from values recorded just before drug treatment. Results were analyzed using one-way analysis of variance and Student’s t-test. In some instances, the Welch-Aspin method was used. Data are expressed as mean±standard error of the mean, and differences were considered statistically significant at a probability (p) value of less than 0.05. Some data were analyzed by linear regression to obtain linear equations and correlation coefficients (r).

**Results**

**Hemodynamic effects:** Control values for HR, LVP, the product of HR and LVP (HR×LVP) and coronary flow are shown in Table I. No significant differences were found among the groups with respect to any of these parameters. As shown in Fig. 1, diltiazem and lidocaine exerted a dose-dependent negative chronotropic effect before ischemia. The HR decreases just before ischemia were 14.2±3.4% for the 2.22×10⁻⁷ M diltiazem group, 39.2±6.5% for the 2.22×10⁻⁶ M diltiazem group, 16.1±0.7% for the 4.27×10⁻⁵ M lidocaine group, and 37.1±7.9% for the 12.80×10⁻⁵ M lidocaine group. No differences between the control group and the drug-treated groups were found with respect to the recovery of heart rate during the late phase of reperfusion.

Diltiazem significantly reduced HR×LVP in a dose-dependent manner (Fig. 2). Lidocaine caused almost no change in this parameter at the

<table>
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<th>Table I. Control Values for Each Group</th>
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<td><strong>Group</strong></td>
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</tr>
<tr>
<td>Control</td>
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<td>Diltiazem (2.22×10⁻⁷ M)</td>
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n = number of experiments; HR = heart rate; LVP = left ventricular pressure. Results are expressed as mean±SEM.
**Fig. 1.** Effect of diltiazem (A) and lidocaine (B) on heart rate in the isolated perfused rat heart. Each point represents the mean±SEM. The third value in the control period was taken as 100%. Symbols: open circle, control hearts (n=5); open triangle, 2.22×10⁻⁷ M diltiazem (n=5); solid triangle, 2.22×10⁻⁶ M diltiazem (n=5); open diamond, 4.27×10⁻⁵ M lidocaine (n=5); solid diamond, 12.80×10⁻⁵ M lidocaine (n=5). Single, double and triple asterisks denote statistically significant differences from the control group (p>0.05, p<0.01 and p<0.001, respectively). D.W.=distilled water.

**Fig. 2.** Effect of diltiazem (A) and lidocaine (B) on the product of heart rate and left ventricular systolic pressure (HR×LVP). Each point represents the mean±SEM. Asterisks are explained in the legend for Fig. 1.

lower dose, though it did produce a slight decrease at a higher dose. The recovery of HR×LVP after reperfusion was significantly depressed in the diltiazem-treated groups. The recovery of this parameter during the early phase of reperfusion was also reduced in the lidocaine-treated hearts, however, the final level of this parameter was significantly higher than in the control group (Fig. 2).

Figure 3 shows changes in LVEDP during ischemia and reperfusion. In the control hearts, LVEDP increased gradually after ischemic arrest and
Fig. 3. Effect of diltiazem (A) and lidocaine (B) on left ventricular end-diastolic pressure (LVEDP). Each point represents the mean±SEM. Asterisks are explained in the legend for Fig. 1.

Fig. 4. Effect of diltiazem (A) and lidocaine (B) on coronary flow. Each point represents the mean±SEM. A single asterisk denotes a statistically significant difference from the control (p<0.05).

decreased gradually from 5 min after reperfusion. The increase in LVEDP during ischemia was significantly attenuated only in the diltiazem (2.22×10^{-6} M)-treated hearts. The decrease in LVEDP after reperfusion differed significantly from controls only in the diltiazem (2.22×10^{-6} M)-treated hearts.

Coronary flow: Figure 4 summarizes the effects of diltiazem and lidocaine on coronary flow. An increase in coronary flow was observed just after treatment with diltiazem, and the level attained after reperfusion was higher than in the control group. There was no change in coronary flow after lidocaine administration, but the level after reperfusion was higher in the lidocaine-treated groups than in the control group. However, these differences were not statistically significant.

$^{31}$P-NMR: Figure 5 shows typical spectra for a control heart before
Fig. 5. Representative $^{31}$P-NMR spectra of a control heart. Each spectrum was obtained during an acquisition time of 5 min.

Fig. 6. A: Intramyocardial pH changes during ischemic arrest (40 min). B: Changes in adenosine triphosphate (ATP) during ischemic arrest and reperfusion. Each point represents the mean±SEM ($n=5$).
Fig. 7. Effect of diltiazem (A) and lidocaine (B) on changes in myocardial intracellular pH over time. Each point represents the mean±SEM. Single, double and triple asterisks denote statistically significant differences from the control group ($p<0.05$, $p<0.01$ and $p<0.001$, respectively).

Fig. 8. Effect of diltiazem (A) and lidocaine (B) on changes in ATP content over time. Each point represents the mean±SEM. Single and double asterisks denote statistically significant differences from the control group ($p<0.05$ and $p<0.01$, respectively).

and after ischemic arrest. During the ischemic period, the Pi peak intensity increased markedly and moved toward the Cr-P peak, while peak intensities of both Cr-P and ATP decreased. Myocardial intracellular pH values before and during ischemia are shown in Fig. 6A. pH decreased progressively starting immediately after induction of ischemia (Fig. 6A). Diltiazem and lidocaine significantly retarded the fall in myocardial intracellular pH during ischemia in a dose-dependent fashion (Fig. 7).

Figure 6B shows changes in ATP content during ischemia and reperfusion. In the control hearts, ATP levels decreased gradually after ischemic arrest and did not recover to preischemic levels after reperfusion (Fig. 6B). Diltiazem significantly suppressed the progression of ATP breakdown during ischemia and improved the ATP recovery after reperfusion. In the lidocaine-treated groups, the level of ATP in the later phase of reperfusion was
Fig. 9. The relationship between the myocardial depressant effect and the attenuation of the fall in intracellular pH produced by diltiazem (A) and lidocaine (B).

significantly higher than in the control group. No significant differences, however, were seen in the rate of ATP depletion during ischemia (Fig. 8).

Correlations between myocardial depressant effect and pH changes during ischemia: Figure 9A and B illustrate the relationship between the myocardial depressant effect observed just before the initiation of ischemia and the protective effect on myocardial intracellular pH during the 5 to 10-min period after induction of ischemia. In the diltiazem-treated hearts, there was a clear inverse correlation between the myocardial depressant effect and myocardial intracellular pH, expressed by the equation $Y = -0.0033X + 6.870$ ($r = -0.78, p<0.01, \text{Fig. 9A}$). However, no significant correlation was found in lidocaine-treated hearts (Fig. 9B).

DISCUSSION

This study demonstrated that both lidocaine and diltiazem can delay the evolution of the fall in myocardial pH during a 40-min period of ischemia and improve ATP recovery after reperfusion. The diltiazem- and lidocaine-treated groups differed with respect to the recovery of HR×LVP after reperfusion, although both drugs improved ATP recovery after reperfusion. Myocardial oxygen consumption (assessed by HR×LVP) before ischemia tended to be more depressed in diltiazem-treated hearts than in lidocaine-treated hearts. Because of the difference in washout rates between the 2 drugs, there is a possibility that the recovery of HR×LVP after reperfusion was more suppressed in diltiazem-treated groups.

Numerous studies have reported the beneficial effects of diltiazem on ischemic or hypoxic myocardium\(91-19\). These beneficial effects have been
explained in terms of a myocardial depressant effect, inhibition of ischemic damage to mitochondria, reduction of LV afterload, and improvement in collateral blood flow. Since right atrial pacing did not alter the protective action of diltiazem against enzyme release, Hamm and Opie concluded that the protective effects of diltiazem on the ischemic myocardium could not be attributed solely to a reduction in heart work; the possibility remains that diltiazem had a metabolic effect.

In the present study, the diltiazem-induced retardation of the drop in myocardial pH during ischemia was associated with its negative chronotropic and inotropic action before ischemia. There was also a significant linear relationship between the myocardial depressant effect observed before ischemia and pH level during the early phase of ischemia. This is in agreement with a previous report from this laboratory. In contrast, suppression of the fall in pH in lidocaine-treated hearts was greater relative to the magnitude of HR × LVP before ischemia, and there was no significant correlation between pH after ischemia and HR × LVP values before ischemia. This is in agreement with a recent report by Matsumura et al, which stated that the favorable effects of lidocaine on ischemic myocardial pH persist, even under conditions of constant HR by pacing. Thus, it is clear that the beneficial action of lidocaine cannot be explained solely on the basis of the cardiodepressant effects. The effects of lidocaine on ionic changes may also play a role. Lidocaine has no protective effect on myocardial calcium accumulation. It does, however, inhibit changes in sodium and potassium concentrations during ischemia and after reperfusion. Protective effects of lidocaine in cerebral ischemia have also been demonstrated, and Astrup has speculated that lidocaine reduces energy expenditure and delays the onset of irreversible structural damage by stabilizing the cell membranes. Lesnefsky et al have also reported that the protective effect of lidocaine in ischemic myocardium might be due to its membrane stabilizing effects, which may have protected the myocardial cell membrane from lipid peroxidation.

Recently, a controversy has arisen regarding the protective effect of lidocaine on ischemic-reperfused myocardial injury. Okamura et al and Nasser et al reported that lidocaine has a protective effect on the ischemic-reperfused myocardium and that it reduces myocardial infarct size. According to de Lorgeril et al, on the other hand, lidocaine does not reduce infarct size or myocardial neutrophil accumulation. Although these experiments were all done in open-chest dogs by occluding the left anterior descending coronary artery, there was a difference in the duration of the occlusion. Occlusion was performed for 40 min in the former 2 experiments...
but for 2 hours in the latter. In our investigation, there were no differences between the lidocaine-treated groups and the control with respect to myocardial intracellular pH or the level of ATP 40 min after ischemia. Thus, it is possible that the difference in duration of ischemia was the cause of the conflicting results obtained in these previous studies.\textsuperscript{7,8,21,33)}

Administration of lidocaine to the isolated rat heart retarded the progression of the drop in myocardial pH during ischemia and improved ATP recovery after reperfusion. If these results are verified by further studies in local ischemia models, it would indicate that a clinically relevant dose of lidocaine may be useful during early reperfusion in patients at risk of developing acute myocardial infarction, not only for dealing with arrhythmia, but also to minimize ischemic myocardial injury.

\textbf{REFERENCES}